ISSN 2222-0682 (online)

World Journal of *Methodology*

Quarterly Volume 15 Number 3 September 20, 2025

Published by Baishideng Publishing Group Inc

World Journal of Methodology

Contents

Quarterly Volume 15 Number 3 September 20, 2025

EDITORIAL

OPINION REVIEW

REVIEW

MINIREVIEWS

World Journal of Methodology

Quarterly Volume 15 Number 3 September 20, 2025

Contents

SCIENTOMETRICS

CASE REPORT

Contents

ABOUT COVER

Peer Reviewer of *World Journal of Methodology*, Cristina Andreea Adam, MD, Assistant Professor, Medical I Department, Discipline of Medical Semiology, "Grigore T. Popa" University of Medicine and Pharmacy Iasi, 16 University Street, Iași 700115, Romania. adam.cristina93@gmail.com

AIMS AND SCOPE

The primary aim of *World Journal of Methodology* (*WJM*, *World J Methodol*) is to provide scholars and readers from various fields of methodology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJM mainly publishes articles reporting research results obtained in the field of methodology and covering a wide range of topics including breath tests, cardiac imaging techniques, clinical laboratory techniques, diagnostic self-evaluation, cardiovascular diagnostic techniques, digestive system diagnostic techniques, endocrine diagnostic techniques, neurological diagnostic techniques, obstetrical and gynecological diagnostic techniques, ophthalmological diagnostic techniques, otological diagnostic techniques, radioisotope diagnostic techniques, respiratory system diagnostic techniques, surgical diagnostic techniques, *etc*.

INDEXING/ABSTRACTING

The *WJM* is now abstracted and indexed in PubMed, PubMed Central, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Methodology

ISSN
ISSN 2222-0682 (online)

LAUNCH DATE
September 26, 2011

FREQUENCY
Quarterly

EDITORS-IN-CHIEF
Timotius Ivan Hariyanto

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2222-0682/editorialboard.htm

PUBLICATION DATE
September 20, 2025

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 98795

DOI: 10.5662/wjm.v15.i3.98795

ISSN 2222-0682 (online)

EDITORIAL

Fishing reviewing: A threat to research integrity and credibility

Mohammed Al-Beltagi

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade B, Grade C, Grade D

Novelty: Grade B, Grade B, Grade B, Grade C

Creativity or Innovation: Grade B, Grade B, Grade B, Grade D

Scientific Significance: Grade B, Grade B, Grade B, Grade C

P-Reviewer: Abdelsamad A; Lu R; Sulpiana S

Received: July 5, 2024

Revised: November 6, 2024

Accepted: December 2, 2024

Published online: September 20, 2025

Processing time: 243 Days and 16.2 Hours

Mohammed Al-Beltagi, Department of Pediatric, Faculty of Medicine, Tanta University, Tanta 31511, Alghrabia, Egypt

Mohammed Al-Beltagi, Department of Pediatric, University Medical Center, King Abdulla Medical City, Arabian Gulf University, Manama 26671, Bahrain

Corresponding author: Mohammed Al-Beltagi, MD, PhD, Department of Pediatric, Faculty of Medicine, Tanta University, Al-Bahr Street, the Medical Complex, Tanta 31511, Alghrabia, Egypt. mbelrem@hotmail.com

Abstract

The rise of the "fishing reviewer" phenomenon presents a significant threat to the integrity of academic publishing, undermining the credibility of the peer review process and eroding trust in scientific journals. This editorial explores the risk factors contributing to this troubling trend and identifies key indicators to recognize such reviewers. To address this issue, we propose strategies, including enhanced reviewer vetting, comprehensive training, and transparent recognition policies to foster a culture of accountability and ethical conduct in scholarly review. By implementing these measures, we can safeguard the quality and credibility of academic research.

Key Words: Fishing reviewer; Scientific publication; Academic research; Scholarly community; Scientific journal; Editor

Core Tip: "Fishing reviewers" conduct superficial peer reviews to gain recognition without providing meaningful feedback. This practice undermines the integrity of academic publishing by allowing substandard research to pass through the review process. Recognizing and addressing this issue is crucial for maintaining the credibility of scholarly communication. By implementing targeted strategies to identify and combat "fishing reviewers", we can preserve the quality and reliability of scientific journals.

Citation: Al-Beltagi M. Fishing reviewing: A threat to research integrity and credibility. *World J Methodol* 2025; 15(3): 98795

URL: https://www.wjgnet.com/2222-0682/full/v15/i3/98795.htm

DOI: https://dx.doi.org/10.5662/wjm.v15.i3.98795

INTRODUCTION

This article aims to spotlight a growing concern in academic publishing - the emergence of "fishing reviewers". These individuals engage in superficial peer reviews, offering little to no constructive feedback while seeking recognition for their efforts. The integrity and credibility of scientific research rely heavily on the peer review process, designed to ensure the validity, reliability, and overall merit of scholarly work[1]. The review process serves as a fundamental mechanism for ensuring the quality and validity of research before publication, promoting intellectual rigor, and fostering scholarly growth[2]. However, the rise of "fishing reviewers" threatens this essential system, potentially allowing substandard research to be published and eroding trust in academic journals. In this article, I will explore the risk factors contributing to this phenomenon, outline criteria for identifying such reviewers, and propose practical strategies to combat this issue. By addressing these challenges, we can safeguard the quality and credibility of scholarly communication, ensuring that the peer review process continues to serve as a pillar of academic integrity.

WHO IS THE "FISHING REVIEWER"?

I metaphorically use the term "fishing reviewers" to refer to reviewers who engage in a superficial review process devoid of genuine benefit to the authors or the journal editors. Pursuing recognition and appreciation drives them to accept the responsibility of reviewing an article merely to attain recognition and accolades for their efforts. Such actions ultimately undermine the credibility of the review process and harm the scholarly community, seriously threatening the integrity and efficacy of this critical evaluation process[3]. Their reviews lack substance, depth, and meaningful insights, rendering them inconsequential to the authors and the journal editors. This unethical practice not only sabotages the essence of scholarly review but also tarnishes the reputation of the journal and the academic community's reputation[4]. To underscore its unique impact, we should differentiate "fishing reviewers" from other unethical behaviors, like predatory or neglectful reviewing. Specifically, 'fishing reviewers' will be characterized by pursuing personal gain through superficial engagement in the review process without meaningful contribution, in contrast to neglectful reviewers who may lack adequate time or engagement for thorough reviews. It is essential to weed out these "fishing reviewers" and preserve the integrity of the review process for the benefit of the scientific and academic communities.

RISK FACTORS AND IDENTIFICATION CRITERIA

The emergence of the "fishing reviewer" phenomenon can be attributed to various risk factors within the academic and publishing landscapes. Understanding these factors is crucial for devising effective strategies to mitigate this concerning trend. To address the primary risk factors contributing to the rise of "fishing reviewers", comprehensive reviewer training, stringent selection processes, clearer guidelines, and enhanced oversight mechanisms can be implemented[5]. This will significantly reduce the prevalence of "fishing reviewers" and promote a more robust and reliable peer review system. I suggest specific criteria to identify potential "fishing reviewers".

Risk factors

The risk factors include: (1) Pressure to fulfill review commitments: Increasing pressure on scholars to meet the review's deadlines can lead to hasty, superficial reviews; (2) Inadequate reviewer vetting and selection: Lax selection processes may allow unqualified or unethical reviewers to participate; (3) Reviewer recognition and incentives: Emphasis on quantity over quality of reviews can incentivize superficial reviewing; (4) Lack of reviewer training and guidelines: Insufficient training on ethical reviewing practices; (5) Inadequate oversight and accountability: Poor monitoring of reviewer actions can enable unethical practices; (6) Lack of diversity and inclusivity in peer review: Limited diversity in reviewer pools can lead to exclusive and biased reviewing; and (7) Incentives for journal editors: Pressure on editors to maintain high acceptance rates may compromise review quality (Table 1)[6-8].

Identification criteria: Indicators for recognizing "fishing reviewers"

Indicators for identifying "fishing reviewers" include the following: (1) Diverse acceptance of articles: Reviewers accept articles outside their expertise regularly; (2) Short turnaround time: Extremely quick reviews indicating superficial assessment; (3) Non-specific and template-based replies: Use of generic feedback across multiple reviews; (4) Lack of constructive feedback: Providing vague, non-constructive feedback; (5) Bias based on author's attributes: Decisions influenced by the author's demographics; (6) Inconsistent review results: Reviews that significantly deviate from those of credible reviewers; (7) Consistently extreme ratings: Extreme ratings without nuanced consideration; (8) Inconsistencies in language proficiency: Fluctuating language quality in reviews; (9) Repetitive and overused phrases: Identical phrases across multiple reviews; (10) Unwillingness to engage in revision discussions: Refusal to provide further feedback during revisions; (11) Consistent acceptance of poor-quality manuscripts: Accepting substandard manuscripts without thorough critique; (12) Pattern of abrupt rejections: Immediate rejections without substantial review; and (13) Lack of engagement with related literature: Reviews that do not reference relevant literature (Tables 2 and 3)[2,9-12].

Table 1 Risk factors contributing to the emergence of "fishing reviewers"

Criteria	Description	Impact
Pressure to fulfill review commitments	Academic scholars face increasing pressure to fulfill review commitments in a limited time	This pressure may lead to superficial reviews, giving rise to the "fishing reviewer" phenomenon
Inadequate reviewer vetting and selection	Some journals may have less stringent vetting and selection processes for reviewers	Inadequate selection procedures can result in reviewers lacking the necessary expertise or commitment
Reviewer recognition and incentives	The academic community often values the number of reviews completed	This may incentivize quantity over quality in reviews
Lack of reviewer training and guidelines	Insufficient training for reviewers on best practices and ethical conduct	Reviewers may engage in careless or unethical reviewing practices
Inadequate oversight and accountability	Some journals lack robust systems to monitor reviewer actions	Reviewers may engage in unethical practices without appropriate checks and balances
Lack of diversity and inclusivity in peer review	Limited diversity in the reviewer pool	This can lead to an exclusive peer review system and promote "fishing" behavior
Incentives for journal editors	Editors may face pressure to publish a certain number of articles	This may lead to a less discerning selection of reviewers
Reviewer's country of origin	The country of origin of a reviewer may influence the risk of the "fishing reviewer" phenomenon	Different countries' cultural, institutional, and individual dynamics may contribute to this risk

Table 2 Integration between the risk factors and their identification criteria

Risk factor	Identification criteria	Description
Pressure to fulfill review commitments	Short turnaround time	Under pressure, reviewers may complete reviews rapidly without in-depth evaluation
Inadequate reviewer vetting and selection	Acceptance of unrelated articles	Lax selection processes lead to reviewers taking on articles outside their expertise
Reviewer recognition and incentives	Non-specific and template-based replies	Emphasis on quantity encourages superficial feedback, often repetitive or lacking depth
Lack of reviewer training and guidelines	Lack of constructive feedback, repetitive, and overused phrases	Untrained reviewers may provide vague feedback and rely on generic phrases
Inadequate oversight and accountability	Inconsistent review results, extreme ratings	Poor oversight allows reviewers to give inconsistent or biased assessments
Lack of diversity and inclusivity in peer review	Bias based on author's attributes	Limited diversity can lead to reviews biased by demographic or geographic factors
Incentives for journal editors	Consistent acceptance of poor-quality manuscripts	Editorial pressure for high acceptance rates can result in lenient reviews

This table integrates each risk factor with an observable identification criterion, which makes it easier to understand how specific weaknesses in the review system contribute to the emergence of "fishing reviewers".

SHORT AND LONG-TERM CONSEQUENCES OF FISHING REVIEW

The persistence of "fishing reviewers" has immediate and short-term consequences in addition to profound long-term effects on the academic community and weakens the integrity of scholarly communication. Both effects should be considered by editors and as well as the academic community. In the short term, the superficial reviews provided by "fishing reviewers" directly impact journal credibility. When reviewers fail to conduct in-depth evaluations, publishing lower-quality articles becomes more likely, tarnishing the journal's reputation among researchers and readers[13]. This immediate decline in quality also undermines peer review's core purpose, turning it from a rigorous quality control mechanism into a mere procedural formality. As a result, authors may become disillusioned and frustrated with the review process, particularly when they receive vague, non-specific feedback. This frustration can lead to reluctance among reputable authors to submit to journals where rigorous review standards are not upheld, potentially prompting them to explore alternative publication avenues outside of traditional academic channels[14]. Over the long term, the unchecked influence of "fishing reviewers" can erode scholarly trust, vital to the entire academic ecosystem. If high standards are not consistently maintained, researchers and the public may begin to doubt the validity of published research, weakening the foundation of academic discourse[15]. This lack of trust can lead to a proliferation of incorrect or unverified information, as flawed studies published due to inadequate peer review may form the basis for further research. This cumulative effect is particularly damaging in fields with progressive, cumulative research models, where subsequent studies build on previous findings[16]. Reversing these errors becomes challenging and resource-intensive,

Table 3 Criteria for recognizing "fishing reviewers"

Criteria	Description	Indicators
Diverse acceptance of articles	Reviewers accept articles beyond their specialized domain	Regularly accepting unrelated articles
Short turnaround time	The brief duration between review request acceptance and submission	Consistently short review times
Non-specific and template-based replies	Generic, non-specific feedback	Identical phrases across multiple reviews
Lack of constructive feedback	Vague feedback lacking specific suggestions	Primarily critical comments without actionable insights
Bias based on author's attributes	Decisions are based on the author's demographic details	Correlation of decisions with author's demographics
Inconsistent review results	Review outcomes differ substantially from others	Conflicts with evaluations from credible reviewers
Consistently extreme ratings	Extreme ratings for all manuscripts	Regularly providing highest or lowest ratings
Inconsistencies in language proficiency	Inconsistent language proficiency in reviews	Fluctuating levels of language proficiency
Repetitive and overused phrases	Overuse of specific phrases	Identifiable phrases in multiple reviews
Unwillingness to engage in revision discussions	Unwillingness to provide additional feedback	Declining requests for further clarification
Consistent acceptance of poor-quality manuscripts	Regularly accepting substandard manuscripts	Frequently accepting manuscripts with major flaws
Pattern of abrupt rejections	Immediate and outright rejections	Multiple swift rejections without comprehensive assessment
Lack of engagement with related literature	Failing to reference relevant literature	Reviews lacking discussion on related research

risking setbacks in advancing scientific knowledge.

In addition to trust erosion and misinformation, "fishing reviewers" also contribute to declining academic standards and journal impact. Journals that fail to monitor and control superficial reviews may experience a gradual decrease in rigor, potentially diminishing their impact factor, reputation, and readership. This, in turn, makes it difficult for journals to attract submissions from high-caliber authors and reviewers, creating a cycle that lowers academic quality across the field[17]. The consequences extend further to affect early-career researchers, who rely heavily on constructive peer review for skill development. Without thorough feedback, these authors miss valuable opportunities to refine their methodologies and critical thinking, resulting in knowledge gaps and hindering the scholarly growth essential for advancing the field[18]. Moreover, "fishing reviewers" effects are not uniformly distributed across disciplines and regions. Fast-paced fields like computer science and engineering, which often emphasize rapid publication, may be particularly vulnerable due to the pressure for quick reviews[19]. Similarly, regions with limited access to expert reviewers or lacking robust training for reviewers may be disproportionately affected, potentially amplifying disparities in research quality and reliability between global academic communities[20]. Recognizing and addressing both the short - and long-term impacts of "fishing reviewers" is thus critical. Proactive measures - such as rigorous reviewer vetting, clear guidelines, and active monitoring - are essential for preserving the integrity of academic publishing and fostering a resilient, trusted, and progressive scholarly ecosystem.

ADDRESSING THE "FISHING REVIEWER" ISSUE

The "fishing reviewer" phenomenon poses a significant threat to the integrity and reliability of the peer review process, a cornerstone of academic publishing. By understanding the risk factors and implementing targeted strategies to identify and mitigate this issue, we can uphold the standards of scholarly communication. Combatting the "fishing reviewer" issue requires a comprehensive approach that addresses the root causes and encourages a more reliable and trustworthy peer review process. To achieve this, it's crucial to implement proactive measures at the editorial level[21-23]. Below are specific actions that can be implemented at the editorial level (Table 4).

Enhance reviewer vetting and selection process

Implement rigorous vetting procedures to ensure reviewers have the appropriate expertise and experience in the specific research area[2]. For example: Journals might require reviewers to submit a recent curriculum vitae and a list of relevant publications to verify their qualifications. Additionally, potential reviewers could be asked to complete a brief assessment or provide references from previous review experiences to confirm their expertise.

Table 4 The different mechanisms to combat "fishing reviewers" with their priority to the journals

Approach	Description	Priority	Justification
Enhance the reviewer vetting and selection process	Implement rigorous vetting procedures to ensure expertise and commitment	High	Preventing unqualified reviewers at the outset can greatly reduce superficial reviews and improve review quality
Establish clear reviewer guidelines and expectations	Provide detailed guidelines and encourage appropriate rejection of review invitations	High	Clear guidelines set standards from the start, leading to more consistent, reliable reviews
Monitor and evaluate reviewer performance	Establish systems to regularly assess and track reviewer performance	High	Directly impacts the identification and management of "fishing reviewers" by creating accountability
Encourage constructive and specific feedback	Encourage reviewers to provide specific, actionable feedback focused on manuscript improvement	High	Specific feedback significantly enhances the quality of peer review and author satisfaction
Strengthen editorial oversight and transparency	Introduce an additional review stage and enhance transparency about review expectations and standards	Medium	Increases accountability and quality control, though it requires some editorial resources
Offer comprehensive reviewer training and resources	Develop training programs to educate reviewers on best practices and ethical standards	Medium	Training reinforces guidelines but may require additional resources
Implement transparent reviewer recognition policies	Recognize reviewers for quality contributions, not just quantity	Medium	Improves reviewer motivation, particularly for high-quality reviews
Address potential bias and discrimination	Implement policies to prevent bias based on author characteristics	Medium	Prevents biases that may compromise the fairness of reviews, ensuring an equitable review process
Promote responsible research evaluation	Advocate for responsible, constructive evaluation practices	Low	Indirectly impacts review quality; helpful but not urgent for managing "fishing reviewers"
Leverage technology and tools	Use artificial intelligence and machine learning tools to analyze review patterns and identify superficial reviewers	Low	Valuable for large journals, but often costly and complex for smaller journals to implement
Foster a culture of academic integrity	Encourage integrity and ethics across the academic community	Low	Benefits long-term review culture but has less immediate impact on preventing "fishing reviewers"
Collaborative efforts and knowledge sharing	Encourage journals, societies, and researchers to share strategies for combating poor review practices	Low	Useful for industry-wide improvements, though it may have a slower impact on individual journals

This prioritization framework helps journals focus on high-impact strategies that directly address quality and accountability in peer review, while also providing medium priority and low priority actions to implement as resources allow. High priority: These actions are foundational to maintaining the quality of peer review and addressing "fishing reviewers". Implementing these strategies provides immediate and substantial benefits to the peer review process by ensuring reviewers are qualified, accountable, and focused on providing constructive feedback. High-priority actions are recommended for all journals, regardless of size or resources, as they have the greatest direct impact on the quality and credibility of scholarly publishing. Medium priority: These actions enhance the effectiveness and consistency of the review process. While not as critical as high-priority actions, medium-priority strategies further improve reviewer performance and foster a fairer and more ethical review environment. Journals with sufficient resources or editorial capacity should consider these strategies to build upon their foundational practices. Low priority: These strategies contribute to long-term improvements in review culture and process but may require more resources or time to implement. They are often indirect in their impact on reviewer quality, focusing instead on broader cultural shifts, technological investments, or collaborative efforts. Low-priority actions are valuable for journals seeking to make more extensive, sustainable changes once higher-impact measures are in place.

Establish clear reviewer guidelines and expectations

Provide detailed guidelines outlining expectations for review quality, thoroughness, and ethical conduct[24]. For example: Develop a comprehensive reviewer handbook that covers best practices for providing constructive feedback, the importance of ethical behavior, and the consequences of failing to meet the required standards. This handbook could be supplemented with periodic training sessions or webinars to reinforce these guidelines.

Offer comprehensive reviewer training and resources

Develop training programs to educate reviewers on best practices, ethical conduct, and the peer review process[25]. For example: Create an online training module that new reviewers must complete before participating in the review process. This module could include interactive case studies illustrating common pitfalls in reviewing and how to avoid them. Additionally, journals could offer regular refresher courses to keep reviewers updated on evolving standards and practices.

Implement transparent reviewer recognition policies

Define clear and transparent policies for recognizing reviewers, focusing on the quality and depth of their reviews rather than the quantity[26]. For example: Instead of merely acknowledging the number of reviews completed, journals could highlight reviewers who provide particularly insightful and constructive feedback. This recognition could take the form of awards, certificates, or public acknowledgments on the journal's website or in its annual report.

Monitor and evaluate reviewer performance

Establish a system to monitor reviewer performance and adherence to guidelines, identifying potential "fishing reviewers" through regular assessments[27]. For example: Journals could introduce a peer feedback system where editors and authors rate the quality of the reviews they receive. These ratings could be tracked over time to identify patterns indicative of "fishing reviewers", such as consistently low scores or generic feedback.

Encourage constructive and specific feedback

Encourage reviewers to provide detailed and constructive feedback to authors, focusing on the manuscript's strengths, weaknesses, and areas for improvement[28]. For example: Journals could provide examples of well-constructed reviews as part of the training materials, highlighting the difference between vague criticism and specific, actionable suggestions. Reviewers could also be encouraged to cite relevant literature when making recommendations, thereby strengthening their feedback.

Address potential bias and discrimination

Implement policies to prevent reviewer bias based on author characteristics such as nationality, race, or institutional affiliation[29]. For example: Adopt double-blind review practices, where both the authors and reviewers remain anonymous. This approach can help minimize bias and ensure that manuscripts are evaluated solely on their academic merit.

Strengthen editorial oversight and transparency

Enhance editorial oversight by introducing an additional review stage where editors critically evaluate the quality and appropriateness of reviews[30]. For example: Editors could conduct random audits of completed reviews, assessing them for thoroughness, relevance, and adherence to the journal's standards. If issues are identified, editors could provide feedback to the reviewer and offer additional training or guidance.

Promote responsible research evaluation

Advocate for responsible research evaluation practices, emphasizing the importance of unbiased, constructive reviews [31]. For example: Journals could adopt the San Francisco Declaration on Research Assessment principles, which call for a more balanced approach to evaluating research, considering not just publication metrics but also the quality of peer reviews and the contribution to scientific knowledge.

Leverage technology and tools

Explore the use of artificial intelligence and machine learning tools to identify potential "fishing reviewers" based on review patterns and behaviors[32]. For example: Develop algorithms that analyze review length, depth, and consistency across different manuscripts to detect patterns indicative of superficial or template-based reviews. Such tools could flag potential "fishing reviewers" for further human evaluation.

Foster a culture of academic integrity

Cultivate a culture within the academic community that values integrity, transparency, and ethical behavior in all scholarly activities, including the peer review process[33]. For example: Academic institutions and societies could organize workshops and panel discussions on the ethics of peer review, encouraging open dialogue about reviewers' responsibilities and the impact of unethical practices.

Collaborative efforts and knowledge sharing

Encourage collaboration between journals, publishers, academic societies, and researchers to share best practices, experiences, and strategies to combat the "fishing reviewer" phenomenon[34]. For example: Journals could partner with academic societies to host webinars on improving the peer review system. These events could feature experienced reviewers and editors who share insights and advice. They could also be recorded and made available as resources for future training. Our recommendations aim to foster a culture of accountability, transparency, and ethical conduct among reviewers and editors alike. However, some of these recommendations may not apply to resource-limited journals. Table 5 suggests some recommendations for these journals to help fight fishing reviewer syndrome.

CONCLUSION

The rise of "fishing reviewer" incidents posed a significant threat to the credibility and dependability of the peer review system, a fundamental aspect of academic publishing. By identifying the risk factors and employing specific strategies to

Table 5 Shows practical guidelines for smaller or resource-limited journals to manage "fishing reviewers"

Recommendation	Description	Practical action for smaller journals
Refining reviewer guidelines	Clearly outline expectations for review quality, constructive feedback, and ethics	Develop a basic reviewer handbook emphasizing quality over quantity, accessible to all reviewers
Basic vetting measures	Verify reviewer expertise without advanced vetting tools	Request a curriculum vitae or relevant publications from reviewers to confirm expertise in the subject area
Utilizing author feedback for assessment	Use author feedback to assess reviewer performance and identify "fishing reviewers"	Include a simple author feedback form on the quality and relevance of the review to identify recurring superficial reviews
Prioritizing high-impact, actionable steps	Focus on measures that significantly impact review quality with minimal resources	Conduct spot checks on some reviews and offer brief reviewer training sessions to reinforce good practices
Encouraging constructive reviewer feedback	Guide reviewers on delivering specific and actionable feedback	Share high-quality and poor feedback examples with reviewers to clarify expectations for thorough reviews

This table format helps smaller journals quickly identify feasible actions they can implement without advanced technology or significant financial investment, ensuring a more manageable approach to improving peer review quality.

recognize and address this problem, we can maintain the integrity of scholarly communication. This collective effort will ensure that academic research continues to be evaluated rigorously and ethically, upholding the standards of scholarly communication.

FOOTNOTES

Author contributions: Al-Beltagi M conceptualized and developed the manuscript, performed all literature reviews, and formulated the arguments presented; Al-Beltagi M independently wrote, revised, and refined the content, including the development of new terminology and framing of ideas; Al-Beltagi M responded to peer review feedback and made all necessary adjustments to enhance clarity, structure, and readability.

Conflict-of-interest statement: The author reports no relevant conflicts of interest for this article.

Country of origin: Bahrain

ORCID number: Mohammed Al-Beltagi 0000-0002-7761-9536.

S-Editor: Bai Y
L-Editor: A
P-Editor: Guo X

REFERENCES

1 **Tennant JP**, Ross-Hellauer T. The limitations to our understanding of peer review. *Res Integr Peer Rev* 2020; **5**: 6 [PMID: 32368354 DOI: 10.1186/s41073-020-00092-1]
2 **Tumin D**, Tobias JD. The peer review process. *Saudi J Anaesth* 2019; **13**: S52-S58 [PMID: 30930722 DOI: 10.4103/sja.SJA_544_18]
3 **Riley BJ**, Jones R. Peer review: acknowledging its value and recognising the reviewers. *Br J Gen Pract* 2016; **66**: 629-630 [PMID: 27884912 DOI: 10.3399/bjgp16X688285]
4 **El-Guebaly N**, Foster J, Bahji A, Hellman M. The critical role of peer reviewers: Challenges and future steps. *Nordisk Alkohol Nark* 2023; **40**: 14-21 [PMID: 36793486 DOI: 10.1177/14550725221092862]
5 **Smith R**. Peer review: a flawed process at the heart of science and journals. *J R Soc Med* 2006; **99**: 178-182 [PMID: 16574968 DOI: 10.1177/014107680609900414]
6 **Hu X**. Prejudice, Interests, Jealousy: Inappropriate Peer Reviewers May Be Exacerbating Inequality in Academic Publication in Health Research. *J Korean Med Sci* 2023; **38**: e256 [PMID: 37605495 DOI: 10.3346/jkms.2023.38.e256]
7 **Haug CJ**. Peer-Review Fraud--Hacking the Scientific Publication Process. *N Engl J Med* 2015; **373**: 2393-2395 [PMID: 26488392 DOI: 10.1056/NEJMp1512330]
8 **Hauser M**, Fehr E. An incentive solution to the peer review problem. *PLoS Biol* 2007; **5**: e107 [PMID: 17439298 DOI: 10.1371/journal.pbio.0050107]
9 **Burgess A**, Roberts C, Lane AS, Haq I, Clark T, Kalman E, Pappalardo N, Bleasel J. Peer review in team-based learning: influencing feedback

literacy. *BMC Med Educ* 2021; **21**: 426 [PMID: 34384418 DOI: 10.1186/s12909-021-02821-6]
10 **Walker R**, Barros B, Conejo R, Neumann K, Telefont M. Personal attributes of authors and reviewers, social bias and the outcomes of peer review: a case study. *F1000Res* 2015; **4**: 21 [PMID: 26594326 DOI: 10.12688/f1000research.6012.2]
11 **Stephen D**. Peer reviewers equally critique theory, method, and writing, with limited effect on the final content of accepted manuscripts. *Scientometrics* 2022; **127**: 3413-3435 [PMID: 35431366 DOI: 10.1007/s11192-022-04357-y]
12 **Balyakina EA**, Kriventsova LA. Rejection rate and reasons for rejection after peer review: a case study of a Russian economics journal. *Eur Sci Editing* 2021; **47** [DOI: 10.3897/ese.2021.e51999]
13 **Mayden KD**. Peer Review: Publication's Gold Standard. *J Adv Pract Oncol* 2012; **3**: 117-122 [PMID: 25059293]
14 **Parresol J**. Frustration With the Submission Process/System: Results From Survey Data. *Sci Ed* 2022; 45: 81-83 [DOI: 10.36591/SE-D-4503-81]
15 **Livberber T**, Ayvaz S. The impact of Artificial Intelligence in academia: Views of Turkish academics on ChatGPT. *Heliyon* 2023; **9**: e19688 [PMID: 37809772 DOI: 10.1016/j.heliyon.2023.e19688]
16 **Ioannidis JP**. Why most published research findings are false. *PLoS Med* 2005; **2**: e124 [PMID: 16060722 DOI: 10.1371/journal.pmed.0020124]
17 **Ralph P**. Practical Suggestions for Improving Scholarly Peer Review Quality and Reducing Cycle Times. *Commun Assoc Inf Syst* 2016; **38**: 274-283 [DOI: 10.17705/1CAIS.03813]
18 **Hasanein AM**, Sobaih AEE. Drivers and Consequences of ChatGPT Use in Higher Education: Key Stakeholder Perspectives. *Eur J Investig Health Psychol Educ* 2023; **13**: 2599-2614 [PMID: 37998071 DOI: 10.3390/ejihpe13110181]
19 **Becker RC**, Cotarlan V, Sadayappan S. The rapid proliferation of solicited content online journals: a quest to disseminate knowledge? *J Thromb Thrombolysis* 2019; **47**: 337-344 [PMID: 30806870 DOI: 10.1007/s11239-019-01827-8]
20 **Chen P**, Wu LN, Wang L. AI Fairness in Data Management and Analytics: A Review on Challenges, Methodologies and Applications. *Appl Sci* 2023; **13**: 10258 [DOI: 10.3390/app131810258]
21 **Jawaid SA**. Improving the quality of Peer Review and accelerating the peer review process. *Pak J Med Sci* 2023; **39**: 1-3 [PMID: 36694754 DOI: 10.12669/pjms.39.1.7236]
22 **Picciotto M**. New Reviewer Mentoring Program. *J Neurosci* 2018; **38**: 511 [PMID: 29343590 DOI: 10.1523/JNEUROSCI.3653-17.2017]
23 **Welsby PD**. Peer reviews. A peer reviewer's view. *Postgrad Med J* 2020; **96**: 725-727 [PMID: 32943475 DOI: 10.1136/postgradmedj-2020-138793]
24 **Allen H**, Cury A, Gaston T, Graf C, Wakley H, Willis M. What does better peer review look like? Underlying principles and recommendations for better practice. *Learned Publ* 2019; **32**: 163-175 [DOI: 10.1002/leap.1222]
25 **Weaver ML**, Sundland R, Adams AM, Faria I, Feldman HA, Gudmundsdottir H, Marmor H, Miles V, Ochoa B, Ruff SM, Tonelli C, Altieri MS, Cannada L, Dewan K, Etkin Y, Marmor R, Plichta JK, Reyna C, Tatebe L, Drudi LM, Hicks CW. The art of peer review: Guidelines to become a credible and constructive peer reviewer. *Semin Vasc Surg* 2022; **35**: 470-478 [PMID: 36414364 DOI: 10.1053/j.semvascsurg.2022.10.002]
26 **Ali PA**, Watson R. Peer review and the publication process. *Nurs Open* 2016; **3**: 193-202 [PMID: 27708830 DOI: 10.1002/nop2.51]
27 **Lane JA**, Wade J, Down L, Bonnington S, Holding PN, Lennon T, Jones AJ, Salter CE, Neal DE, Hamdy FC, Donovan JL; ProtecT Study Team. A Peer Review Intervention for Monitoring and Evaluating sites (PRIME) that improved randomized controlled trial conduct and performance. *J Clin Epidemiol* 2011; **64**: 628-636 [PMID: 21239142 DOI: 10.1016/j.jclinepi.2010.10.003]
28 **Chong SW**. Improving peer-review by developing reviewers' feedback literacy. *Learned Publ* 2021; **34**: 461-467 [DOI: 10.1002/leap.1378]
29 **Silbiger NJ**, Stubler AD. Unprofessional peer reviews disproportionately harm underrepresented groups in STEM. *PeerJ* 2019; **7**: e8247 [PMID: 31844596 DOI: 10.7717/peerj.8247]
30 **Akın Ateş M**, Luzzini D, Meehan J, Suurmond R. Editorial: From judge to jury: the potential for crowd reviewing. *J Purch Supply Manag* 2022; **28**: 100770 [DOI: 10.1016/j.pursup.2022.100770]
31 **Le Sueur H**, Dagliati A, Buchan I, Whetton AD, Martin GP, Dornan T, Geifman N. Pride and prejudice - What can we learn from peer review? *Med Teach* 2020; **42**: 1012-1018 [PMID: 32631121 DOI: 10.1080/0142159X.2020.1774527]
32 **Owan VJ**, Abang KB, Idika DO, Etta EO, Bassey BA. Exploring the potential of artificial intelligence tools in educational measurement and assessment. *Eurasia J Math Sci Tech Ed* 2023; **19**: em2307 [DOI: 10.29333/ejmste/13428]
33 **Zhaksylyk A**, Zimba O, Yessirkepov M, Kocyigit BF. Research Integrity: Where We Are and Where We Are Heading. *J Korean Med Sci* 2023; **38**: e405 [PMID: 38050915 DOI: 10.3346/jkms.2023.38.e405]
34 **Kupfer DJ**, Murphree AN, Pilkonis PA, Cameron JL, Giang RT, Dodds NE, Godard KA, Lewis DA. Using peer review to improve research and promote collaboration. *Acad Psychiatry* 2014; **38**: 5-10 [PMID: 24449224 DOI: 10.1007/s40596-013-0027-1]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 99162

DOI: 10.5662/wjm.v15.i3.99162

ISSN 2222-0682 (online)

EDITORIAL

Innovative forecasting models for nurse demand in modern healthcare systems

Kalpana Singh, Abdulqadir J Nashwan

Specialty type: Nursing

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade C
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Itoh K

Received: July 15, 2024
Revised: December 17, 2024
Accepted: December 23, 2024
Published online: September 20, 2025
Processing time: 234 Days and 0.8 Hours

Kalpana Singh, Abdulqadir J Nashwan, Department of Nursing and Midwifery Research, Hamad Medical Corporation, Doha 3050, Qatar

Co-first authors: Kalpana Singh and Abdulqadir J Nashwan.

Corresponding author: Abdulqadir J Nashwan, PhD, Department of Nursing and Midwifery Research, Hamad Medical Corporation, Rayyan Road, Doha 3050, Qatar. anashwan@hamad.qa

Abstract

Accurate prediction of nurse demand plays a crucial role in efficiently planning the healthcare workforce, ensuring appropriate staffing levels, and providing high-quality care to patients. The intricacy and variety of contemporary healthcare systems and a growing patient populace call for advanced forecasting models. Factors like technological advancements, novel treatment protocols, and the increasing prevalence of chronic illnesses have diminished the efficacy of traditional estimation approaches. Novel forecasting methodologies, including time-series analysis, machine learning, and simulation-based techniques, have been developed to tackle these challenges. Time-series analysis recognizes patterns from past data, whereas machine learning uses extensive datasets to uncover concealed trends. Simulation models are employed to assess diverse scenarios, assisting in proactive adjustments to staffing. These techniques offer distinct advantages, such as the identification of seasonal patterns, the management of large datasets, and the ability to test various assumptions. By integrating these sophisticated models into workforce planning, organizations can optimize staffing, reduce financial waste, and elevate the standard of patient care. As the healthcare field progresses, the utilization of these predictive models will be pivotal for fostering adaptable and resilient workforce management.

Key Words: Nurse demand prediction; Time-series analysis; Machine learning; Simulation-based methods; Predictive models

Core Tip: Accurate forecasting of nurse demand is critical for efficient workforce planning in healthcare. Leveraging advanced methods like time-series analysis, machine learning, and simulation models enables precise staffing predictions. These models address challenges posed by healthcare system complexities, seasonal fluctuations, and policy changes. By integrating these techniques, healthcare organizations can optimize resource allocation, reduce inefficiencies, and enhance patient care quality, ensuring adaptability in an evolving healthcare landscape.

Citation: Singh K, Nashwan AJ. Innovative forecasting models for nurse demand in modern healthcare systems. *World J Methodol* 2025; 15(3): 99162
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/99162.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.99162

INTRODUCTION

Accurate prediction of the demand for nurses plays a crucial role in the strategic planning of the healthcare workforce, enabling healthcare managers to ensure appropriate staffing levels for providing high-quality care to patients[1]. The intricate and diverse nature of contemporary healthcare systems and the continuously expanding patient population have significantly heightened the need for precise and sophisticated forecasting models to anticipate staffing needs effectively. The complexity of healthcare delivery has recently escalated owing to advancements in medical technology, the implementation of novel treatment protocols, and the increasing prevalence of chronic illnesses[2]. These elements have rendered the accurate estimation of nurse staffing requirements more challenging through conventional means. Consequently, there is an urgent need for innovative and resilient forecasting methodologies capable of considering these variables and delivering dependable predictions.

Numerous studies have investigated forecasting techniques, such as time-series analysis, machine learning models, and simulation-based methods[3]. Time-series analysis involves using historical data to detect patterns and trends that can guide future staffing requirements. Conversely, machine learning models employ intricate algorithms to examine extensive datasets, reveal concealed patterns, and generate precise forecasts. Simulation-based approaches enable healthcare administrators to simulate diverse scenarios and evaluate the potential impact of different factors on nurse demand[3,4]. Each of these methodologies presents distinct benefits and offers valuable insights into nurse workforce planning. Time-series analysis, for example, proves highly effective in detecting seasonal variations and recurring patterns, while machine learning models excel in managing large data sets and providing real-time forecasts. Simulation-based strategies offer a versatile framework for testing various assumptions and assessing their influence on staffing needs. Moreover, incorporating these forecasting techniques into healthcare workforce planning procedures has exhibited encouraging outcomes. By utilizing sophisticated predictive models, healthcare administrators can optimize nurse staffing levels, mitigate the risk of inadequate or excessive staffing, and ultimately enhance patient care quality. Additionally, precise forecasting can help alleviate the financial burden associated with staffing inefficiencies and ensure efficient resource allocation.

ADVANCED TECHNIQUES IN FORECASTING FOR NURSE WORKFORCE PLANNING

One of the primary challenges in nurse workforce planning is the variability in demand, driven by factors such as seasonal illnesses, demographic changes, and policy shifts[5]. Traditional methods, such as time series analysis, have been widely used to predict future nurse demand based on historical staffing data and patient admission rates[1,6]. For instance, Pfeifer *et al*[1] in 2024 developed a robust time series model that accurately projected nurse staffing needs up to five years into the future by analyzing these variables. This approach helps hospital administrators make informed staffing decisions, potentially reducing overtime costs and improving patient care[1].

Machine learning and artificial intelligence have emerged as a powerful tool in forecasting nurse demand, offering more precise and adaptable predictions than traditional statistical methods[2,3]. Lin *et al*[2] in 2024 demonstrated that machine learning algorithms, trained on diverse datasets from multiple healthcare facilities, could outperform traditional models in predicting nursing workforce requirements. Dynamic simulation models provide another innovative approach to forecasting nurse demand, continuously integrating real-time data to update staffing predictions[3,4]. MacKenzie *et al*[3] in 2019 introduced a dynamic simulation model incorporating patient acuity levels and staff turnover rates, offering a more responsive and flexible workforce planning solution. This method allows healthcare managers to adjust staffing levels proactively, addressing changes in patient care needs.

Policy changes can significantly impact nurse staffing projections, as demonstrated by Yi and Kim[4] in 2022. Their research utilized econometric modeling and scenario analysis to predict the long-term effects of policy adjustments, such as changes to nurse-to-patient ratio laws and funding for nursing education programs[4]. The study concluded that supportive policies could mitigate projected nursing shortages, emphasizing the importance of strategic interventions in workforce planning[4].

Regression analysis has also been employed to forecast nurse demand, particularly in rural healthcare settings where staffing needs differ significantly from urban areas[5,6]. Squires *et al*[5] in 2017 developed a regression model that accounted for population growth and healthcare accessibility, providing accurate staffing predictions over ten years[5]. This approach underscores the importance of considering regional differences in nurse workforce planning[5]. Agent-based modeling is another innovative method used to simulate the behavior of individual nurses and patients within a healthcare system[6]. Lopes *et al*[6] in 2018 demonstrated that this approach could provide detailed insights into workforce dynamics, helping planners anticipate and respond to complex changes in nurse staffing needs. Agent-based models can simulate various scenarios, offering a comprehensive tool for managing workforce fluctuations[6].

Technological advancements like telemedicine and automation have also influenced nurse demand forecasting[7]. Ramsey *et al*[7] in 2014 investigated the impact of these technologies on staffing needs, finding that while some technologies can reduce the need for specific nursing tasks, the overall demand for nurses remains strong due to the increasing complexity of patient care. Their study highlighted the need for adaptive workforce planning to address the evolving technological landscape in healthcare[7]. Seasonal variations and pandemics pose additional challenges to nurse staffing, as seen during influenza seasons and the corona virus infectious disease-2019 pandemic[8]. Dempsey and Batten[8] in 2022 developed models to predict spikes in nurse demand during such events, using historical data to inform flexible staffing strategies and emergency preparedness. Their findings underscore the importance of quickly adapting staffing levels to meet sudden increases in patient care needs[8].

CONCLUSION

Accurate forecasting of nurse demand plays a vital role in efficiently planning the healthcare workforce. Given the intricate nature of contemporary healthcare systems and the expanding patient populace, advanced prognostication models are indispensable for achieving optimal staffing levels. Techniques like time-series analysis, machine learning, and simulation methodologies provide distinct advantages and valuable insights. Integrating these methodologies enables healthcare administrators to optimize nurse staffing, minimizing understaffing and overstaffing occurrences. This, in turn, elevates the quality of patient care and ensures the efficient allocation of resources, thereby alleviating financial strains stemming from staffing inefficiencies. As the healthcare landscape progresses, integrating sophisticated predictive models into workforce planning will grow in significance. These models empower healthcare institutions to adjust to shifting demands, uphold superior standards of patient care, and cultivate a more resilient workforce equipped to confront forthcoming challenges.

FOOTNOTES

Author contributions: Singh K wrote the draft and critically reviewing the literature; Singh K and Nashwan AJ revised the draft for important intellectual content, they contributed equally to this article, they are the co-first authors of this manuscript; and all authors thoroughly reviewed and endorsed the final manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: Qatar

ORCID number: Abdulqadir J Nashwan 0000-0003-4845-4119.

S-Editor: Bai Y
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Pfeifer A**, Knaup J, Holst C, Lohweg V. Evaluation of Time Series Forecasting Strategies for Demand Management. IEEE 2024: 2024 IEEE 29th International Conference on Emerging Technologies and Factory Automation (ETFA) 2024 Sep 10-13; Padova, Italy. United States: IEEE, 2024: 01-08

2 **Lin CP**, Chen LA. [Application of Artificial Intelligence Models in Nursing Research]. *Hu Li Za Zhi* 2024; **71**: 14-20 [PMID: 39350705 DOI: 10.6224/JN.202410_71(5).03]

3 **MacKenzie A**, Tomblin Murphy G, Audas R. A dynamic, multi-professional, needs-based simulation model to inform human resources for health planning. *Hum Resour Health* 2019; **17**: 42 [PMID: 31196188 DOI: 10.1186/s12960-019-0376-2]

4 **Yi J**, Kim J. Impact evaluation of nurse staffing policy reform in Korea: A quasi-experimental study. *J Nurs Manag* 2022; **30**: 3457-3465

[PMID: 36181261 DOI: 10.1111/jonm.13815]

5 **Squires A**, Jylhä V, Jun J, Ensio A, Kinnunen J. A scoping review of nursing workforce planning and forecasting research. *J Nurs Manag* 2017; **25**: 587-596 [PMID: 28891258 DOI: 10.1111/jonm.12510]

6 **Lopes MA**, Almeida ÁS, Almada-Lobo B. Forecasting the medical workforce: a stochastic agent-based simulation approach. *Health Care Manag Sci* 2018; **21**: 52-75 [PMID: 27592211 DOI: 10.1007/s10729-016-9379-x]

7 **Ramsey KS**. Using Predictive and Descriptive Models to Improve Nurse Staff Planning and Scheduling. M.Sc. Thesis, University of Tennessee – Knoxville. 2014. Available from: https://trace.tennessee.edu/utk_gradthes/2749

8 **Dempsey C**, Batten P. Outcomes-Based Nurse Staffing During Times of Crisis and Beyond. *J Nurs Adm* 2022; **52**: 91-98 [PMID: 35025827 DOI: 10.1097/NNA.0000000000001114]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 99176

DOI: 10.5662/wjm.v15.i3.99176

ISSN 2222-0682 (online)

EDITORIAL

Interleukin 10 supplement to reduce episodes of recurrent aphthous stomatitis

Cinzia Casu, Angelo Michele Inchingolo, Germano Orrù

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade C
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Tovani-Palone MR

Received: July 15, 2024
Revised: January 3, 2025
Accepted: February 8, 2025
Published online: September 20, 2025
Processing time: 233 Days and 16.8 Hours

Cinzia Casu, Germano Orrù, Department of Surgical Science, Oral Biotechnology Laboratory, University of Cagliari, Cagliari 09124, Italy

Angelo Michele Inchingolo, Department of Interdisciplinary Medicine, School of Medicine, University of Bari "Aldo Moro", Bari 70124, Italy

Co-corresponding authors: Cinzia Casu and Germano Orrù.

Corresponding author: Cinzia Casu, PhD, Department of Surgical Science, Oral Biotechnology Laboratory, University of Cagliari, Via ospedale 54, Cagliari 09124, Italy. ginzia.85@hotmail.it

Abstract

Recurrent aphthous stomatitis (RAS) is a very frequent condition in developed countries whose basic symptom is a lesion referred to as an aphthous ulcer. High levels of interleukin (IL)-1 and IL-6 and low salivary levels of IL-10 are the basis of RAS pathogenesis. Sublingual supplements based on IL-10 can be very useful in reducing the phenomenon of aphthous recurrence in patients with RAS. An observational clinical experience with a group of 5 patients with RAS receiving a commercially available IL-10-based supplement was reported by the authors. The findings revealed a subsequent reduction in the incidence of mouth ulcers.

Key Words: Recurrent aphthous stomatitis; Interleukin 10; Low-dose medicine; Aphthosis; Interleukin

Core Tip: Aphthous lesions and recurrent aphthous stomatitis are among the most common oral diseases and are often very painful. In some patients, the frequency of new onset is greater than once a month. The pathogenetic mechanism involves high levels of interleukin (IL)-1 and IL-6 and low levels of IL-10. Although several aids that are able to reduce healing times and pain have been proposed in the scientific literature, there is still no treatment that drastically reduces the frequency of lesion onset. Supplements based on IL-10, administered at low doses for prolonged periods, referred to as low-dose medicine, could be effective in reducing relapses.

Citation: Casu C, Inchingolo AM, Orrù G. Interleukin 10 supplement to reduce episodes of recurrent aphthous stomatitis. *World J Methodol* 2025; 15(3): 99176
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/99176.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.99176

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is a very frequent condition in developed countries whose basic symptom is an aphthous ulcer, a lesion generally round or oval with an erythematosus halo[1]. Depending on the size of the aphthosis, three different clinical variants can be determined: aphthosis minor, with lesions smaller than 1 cm in maximum diameter (> 80% of cases); aphthosis major, with lesions greater than 1 cm (15% of cases); and aphthosis herpetiformis, with a multitude of very small lesions of 2-3 mm. Minor aphthosis is characterized by the presence of multiple lesions that heal within 7-14 days without scars, whereas in major aphthosis, patients may have single lesions that generally heal within 30-40 days and can leave scars[1-3]. There must be at least 3-4 episodes of aphthosis per year to be able to define it as RAS, and this is an extremely disabling condition in some cases. The pain and burning sensation associated with the onset of canker sores can lead to difficulty in eating food, resulting in malnutrition[1,2].

A percentage of cases can be associated with systemic diseases (celiac disease, Chron's disease) or syndromic conditions (Bechet syndrome, mouth and genital ulcers with inflamed cartilage syndrome, and periodic fever adenitis pharyngitis aphthosis syndrome)[3]. The pathogenetic process that leads to the development of canker sores is vasculitis, an autoimmune inflammation in which interleukin (IL)-1 and IL-6 participate and where low levels of IL-10 are found. The latter is a fundamental cytokine for stopping the inflammatory process, and several studies have shown that patients with RAS have low salivary levels of IL-10, a molecule that could reduce the vasculitis phenomena underlying canker sores[4].

Various treatments for canker sores, such as cortisone and topical immunosuppressants[5], gels based on aloe vera and hyaluronic acid[1,2], low-level laser therapy[6], and photodynamic therapy[7], are well documented in the literature; however, currently, there is no treatment that is capable of reducing severe relapses from aphthosis. There is a supplement based on IL-10 on the market (GUNA® IL-10, Milan, Italy), whose concentration of IL-10 is on the order of picograms and which can be purchased without any requirement for a medical prescription. The aim of this work was to report the findings of some patients with particularly RAS and to provide scientific evidence on the potential efficacy of IL-10-based supplements in patients with aphthous lesions.

OBSERVATION REPORT

A number of patients characterized by a history of recurrent relapsing aphthous stomatitis showing new episodes on a monthly basis gained access to the Department of Surgical Science in San Giovanni Hospital, University of Cagliari, for a routine dental visit. All patients were adults (3 women and 2 men) aged between 36 years and 75 years; none of these patients had syndromic symptoms associated with RAS, and the IL-10 supplement was recommended.

According to the parent company's directions, the authors recommended taking 20 drops under the tongue 2 times per day for 3 months. As part of routine dental care, the mucous membranes were also examined, and the patients were interviewed to determine whether any other mouth ulcers had appeared. In all 5 patients, a remarkable reduction in the number of canker sores affecting the mouth was observed after 3 months and after 6 months (3 months after the supplement). The reduction in the number of canker sores in the various subjects occurred after the first month following the administration of the IL-10 supplement. Additional patient details follow. Two patients reduced the number of canker sores from 3 (present at the first visit) to 1 at a 3 months follow-up appointment. One patient's canker sores were reduced from 3 to 0 in the second month of follow-up, and the reduction was maintained at 3 months. One patient with 2 canker sores presented at T0 with 0 canker sores. One patient had 2 canker sores at time T0 and presented 1 canker sore at 2 months; and again, 2 canker sores at a 3 months follow-up appointment. The average number of canker sores present in the 5 patients was 2.6, whereas the average posttreatment (T3) was 0.8 canker sores. Despite one patient being refractory, with an initial reduction and the presence of 2 new lesions at the final visit, the other 4 had a reduction in the number of canker sores.

At 6 months, only 2 out of 5 patients reported the onset of a new minor aphthous ulcer during the previous 3 months but did not report aphthae at the time of the follow-up visit. It would have been very interesting to carry out salivary measurements of IL-1, IL-6, and IL-10 in these patients before and after consuming this supplement. The treatment proposed for patients with aphthosis is shown in Figure 1.

CONCLUSION

Since this type of supplement available on the market contains a concentration of IL-10 on the order of picograms, this treatment falls within the concept of low-dose medicine, which is the administration of very low concentrations of a certain active ingredient (supplement in this case), and repeated consistently for long periods of time. Low dose medicine

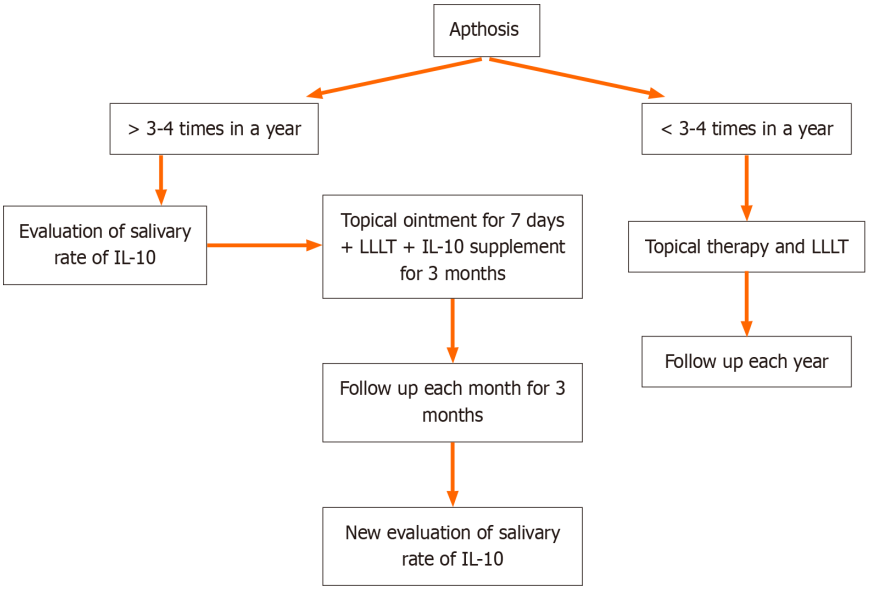


Figure 1 Flow chart of type of treatment proposed in patients with aphthosis. Interleukin 10 and low level laser therapy are proposed as new methods to reduce recurrent aphthous stomatitis. IL: Interleukin; LLLT: Low level laser therapy.

is able to create a new balance, and its effect is much longer lasting over time than that which would be achieved with an attack pharmacological therapy administered for much shorter periods of time (for example, cortisone and systemic immunosuppressants). This concept is highlighted very well in a randomized clinical trial[8], in which the same type of supplement recommended by the authors and purchased directly by the patient, administered following the same directions described by the authors, was used to determine a significant regression of lesions and flare-ups in patients with psoriasis vulgaris, another autoimmune phenomenon. The same commercial product, which is considered safe and without any side effects observed in the cited clinical trial, has also been administered for the management of chronic inflammatory diseases by other authors[9]. Other IL-2 supplements, which are always low-dose medicines, have recently been successfully tested in a clinical trial on bullous pemphigoid, an autoimmune disease that affects the oral cavity[10].

Based on this simple empirical observation of patients and analysis of the pathogenetic mechanisms underlying the phenomenon of canker sore formation, the use of IL-10-based supplements could be a new path to reduce the incidence of recurrences in patients with RAS, an already widespread phenomenon that is often somewhat underestimated, as it is not associated with malignant transformation but is capable of profoundly altering the quality of life of affected patients.

Limits

We observed a very small number of patients who spontaneously appeared to the Department of Surgical Sciences for dental care; it would be necessary to observe the effect of the suggested supplement on a much larger number of patients, with much longer observation times, to confirm these initial findings. In this work, we report our experience with patients who suffered from RAS and who appeared at the hospital outpatient clinic for routine dental care. The recommended product can be purchased without a prescription; therefore, it is not necessary to obtain approval from the Ethics Committee. The principles set out in the Declaration of Helsinki were respected.

FOOTNOTES

Author contributions: Casu C and Orrù G observed the patients, collected data, made maps, and consulted the literature, they contributed equally to this article, they are the co-corresponding authors of this manuscript; Casu C and Inchingolo AM wrote the manuscript; Inchingolo AM and Orrù G revised the manuscript and the literature citations; and all authors have read and approved the final manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: Italy

ORCID number: Cinzia Casu 0000-0002-7962-2712.

S-Editor: Bai Y
L-Editor: A
P-Editor: Guo X

REFERENCES

1 **Conejero Del Mazo R**, García Forcén L, Navarro Aguilar ME. Recurrent aphthous stomatitis. *Med Clin (Barc)* 2023; **161**: 251-259 [PMID: 37357066 DOI: 10.1016/j.medcli.2023.05.007]
2 **Bilodeau EA**, Lalla RV. Recurrent oral ulceration: Etiology, classification, management, and diagnostic algorithm. *Periodontol 2000* 2019; **80**: 49-60 [PMID: 31090148 DOI: 10.1111/prd.12262]
3 **Milia E**, Sotgiu MA, Spano G, Filigheddu E, Gallusi G, Campanella V. Recurrent aphthous stomatitis (RAS): guideline for differential diagnosis and management. *Eur J Paediatr Dent* 2022; **23**: 73-78 [PMID: 35274547 DOI: 10.23804/ejpd.2022.23.01.14]
4 **Chen L**, Ke Z, Zhou Z, Jiang X, Zhao Y, Zhang J. Associations of IL-1, 6, and 10 Gene Polymorphisms with Susceptibility to Recurrent Aphthous Stomatitis: Insights from a Meta-Analysis. *Genet Test Mol Biomarkers* 2018; **22**: 237-245 [PMID: 29641282 DOI: 10.1089/gtmb.2017.0072]
5 **Lau CB**, Smith GP. Recurrent aphthous stomatitis: A comprehensive review and recommendations on therapeutic options. *Dermatol Ther* 2022; **35**: e15500 [PMID: 35395126 DOI: 10.1111/dth.15500]
6 **Amorim Dos Santos J**, Normando AGC, de Toledo IP, Melo G, De Luca Canto G, Santos-Silva AR, Guerra ENS. Laser therapy for recurrent aphthous stomatitis: an overview. *Clin Oral Investig* 2020; **24**: 37-45 [PMID: 31720851 DOI: 10.1007/s00784-019-03144-z]
7 **Casu C**, Mannu C. Atypical Afta Major Healing after Photodynamic Therapy. *Case Rep Dent* 2017; **2017**: 8517470 [PMID: 29085681 DOI: 10.1155/2017/8517470]
8 **Roberti ML**, Ricottini L, Capponi A, Sclauzero E, Vicenti P, Fiorentini E, Savoia C, Scornavacca G, Brazioli D, Gaio L, Giannetti R, Ignazzi C, Meloni G, Chinni LM. Immunomodulating treatment with low dose interleukin-4, interleukin-10 and interleukin-11 in psoriasis vulgaris. *J Biol Regul Homeost Agents* 2014; **28**: 133-139 [PMID: 24750799]
9 **Mancini F**, Milardi D, Carfagna P, Grande G, Miranda V, De Cicco Nardone A, Ricciardi D, Pontecorvi A, Marana R, De Cicco Nardone F. Low-dose SKA Progesterone and Interleukin-10 modulate the inflammatory pathway in endometriotic cell lines. *Int Immunopharmacol* 2018; **55**: 223-230 [PMID: 29272819 DOI: 10.1016/j.intimp.2017.12.008]
10 **Xue R**, Li G, Zhou Y, Wang B, Xu Y, Zhao P, Teng L, Zheng J, Liu H, Ji S, Elston DM, Liang Y. Efficacy and safety of low-dose interleukin 2 in the treatment of moderate-to-severe bullous pemphigoid: A single center perspective-controlled trial. *J Am Acad Dermatol* 2024; **91**: 1113-1117 [PMID: 39182680 DOI: 10.1016/j.jaad.2024.08.033]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 100332

DOI: 10.5662/wjm.v15.i3.100332

ISSN 2222-0682 (online)

EDITORIAL

Interaction between gut virome and microbiota on inflammatory bowel disease

Xiao-Long Li, Mueen Megdadi, Humair S Quadri

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade B, Grade B

Novelty: Grade A, Grade B, Grade B

Creativity or Innovation: Grade A, Grade B, Grade B

Scientific Significance: Grade A, Grade B, Grade B

P-Reviewer: Gugulothu D; Wen R; Zhou C

Received: August 13, 2024

Revised: December 31, 2024

Accepted: January 15, 2025

Published online: September 20, 2025

Processing time: 204 Days and 17.4 Hours

Xiao-Long Li, Mueen Megdadi, Humair S Quadri, Department of Surgery, Ascension St Agnes Hospital, Baltimore, MD 21009, United States

Co-corresponding authors: Xiao-Long Li and Humair S Quadri.

Corresponding author: Xiao-Long Li, MD, PhD, Surgeon, Department of Surgery, Ascension St Agnes Hospital, 900 S Caton Ave, Baltimore, MD 21009, United States.
li.xiaolong@ascension.org

Abstract

Inflammatory bowel disease (IBD), encompassing Crohn's disease and ulcerative colitis, is a chronic condition marked by recurring gastrointestinal inflammation. While immune, genetic, and environmental factors are well-studied, the gut virome has received less attention. This editorial highlights the work which investigates the gut virome's role in IBD and its interactions with the bacterial microbiome and host immune system. The gut virome consists of bacteriophages, eukaryotic viruses, and endogenous retroviruses. Among these, Caudovirales bacteriophages are predominant and influence bacterial communities *via* lysogenic and lytic cycles. Eukaryotic viruses infect host cells directly, while endogenous retroviruses impact gene regulation and immune responses. In IBD, the virome shows distinct alterations, including an increased abundance of Caudovirales phages and reduced Microviridae diversity, suggesting a pro-inflammatory viral environment. Dysbiosis, chronic inflammation, and aberrant immune responses contribute to these changes by disrupting microbial communities and modifying virome composition. Phages affect bacterial dynamics through lysis, lysogeny, and horizontal gene transfer, shaping microbial adaptability and resilience. Understanding these interactions is crucial for identifying novel therapeutic targets and restoring microbial balance in IBD.

Key Words: Virome; Microbiota; Inflammatory bowel disease; Lysogeny; Horizontal gene transfer

Core Tip: The gut virome plays a crucial role in inflammatory bowel disease (IBD) pathogenesis, bacterial community dynamics, and immune responses. Alterations in virome composition in IBD patients, characterized by increased Caudovirales and decreased Microviridae phages, contribute to dysbiosis and inflammation. Understanding these interactions may reveal novel therapeutic targets for IBD.

Citation: Li XL, Megdadi M, Quadri HS. Interaction between gut virome and microbiota on inflammatory bowel disease. *World J Methodol* 2025; 15(3): 100332
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/100332.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.100332

INTRODUCTION

This editorial highlights the work which investigates the gut virome's role in inflammatory bowel disease (IBD) and its interactions with the bacterial microbiome and host immune system[1]. IBD is a chronic condition characterized by recurrent inflammation of the gastrointestinal tract. The two primary forms of IBD, Crohn's disease (CD) and ulcerative colitis (UC), differ in their clinical presentations, pathophysiology, and affected regions of the gastrointestinal tract. However, both conditions share common features, including immune responses, genetic alterations, and environmental factors[2,3]. The etiology of IBD remains elusive. Recent research revealed that the microbiota of the gut plays a crucial role in IBD[4].

The human gut microbiota, a complex community of microorganisms, has been implicated in numerous physiological and pathological processes. Recent bloom in next-generation sequencing technologies have expanded our understanding of the gut microbiota, revealing its intricate composition and dynamic nature[5,6]. Among the microbiota, bacteria have been the primary focus of research. However, the virome—the collection of viruses within the microbiome—has received comparatively less attention. This oversight is particularly significant given the unique and potentially pivotal roles viruses may play in modulating the immune system, influencing microbial ecology, and contributing to disease pathogenesis[7].

The gut virome consists of diverse viral populations, including bacteriophages, eukaryotic viruses, and endogenous retroviruses. Bacteriophages, or phages, are viruses that infect bacteria, and they represent the most abundant viral entities in the gut[8]. Phages can influence the composition and function of bacterial communities through lysogeny or lytic cycles[9]. This phage-bacteria interaction can modulate the microbiome's structure and metabolic activities, potentially impacting host health[10]. Eukaryotic viruses, although less prevalent, can directly infect host cells and contribute to inflammation and immune dysregulation[11]. Endogenous retroviruses, remnants of ancient viral infections integrated into the human genome, may also play a role in gene regulation and immune responses[12].

Emerging evidence suggests that the gut virome is altered in individuals with IBD, characterized by changes in viral diversity, composition, and abundance[13]. Several studies have reported an increase in the relative abundance of Caudovirales bacteriophages in IBD patients, alongside a decrease in Microviridae bacteriophages[14]. These alterations in the virome are thought to influence the bacterial microbiome, potentially exacerbating dysbiosis - a disrupted microbial community associated with disease states[15]. Dysbiosis has been implicated in the pathogenesis of IBD, with shifts in the microbial community structure leading to enhanced immune activation, impaired mucosal barrier function, and increased production of pro-inflammatory mediators[16].

The potential mechanisms through which the gut virome may contribute to IBD pathogenesis are multifaceted. Phages may affect bacterial community dynamics, thereby influencing the overall microbiota composition and its metabolic profiles[17]. For instance, the lytic activity of phages can lead to the release of bacterial antigens and other immunogenic components, which can potentially trigger an immune response[18]. Moreover, phage-derived proteins and genetic material may directly interact with the host immune system, modulate immune responses, and contribute to the inflammation[19].

Given the emerging recognition of the virome's importance in gut health and disease, there is a need to elucidate the specific contributions of the virome to IBD. Understanding the interactions between the virome, bacterial microbiome, and the host immune system may uncover novel insights into IBD pathogenesis and identify potential therapeutic targets.

VIROME COMPOSITION IN HEALTHY INDIVIDUALS AND IBD PATIENTS

In healthy individuals, the gut virome is characterized by a balanced and relatively stable composition, dominated by temperate phages from the Caudovirales and Microviridae families[7]. Caudovirales and Microviridae contribute to intestinal microbiota diversity through bacterial population control, gene transfer, co-evolution, ecological stabilization, and functional modulation. This stability contributes to maintaining a diverse and resilient microbiota, which is crucial for gut homeostasis and immune modulation[9]. In contrast, patients with IBD, encompassing both CD and UC, exhibit notable alterations in their gut virome. Several studies have reported a marked increase in the relative abundance of Caudovirales phages, particularly members of the Siphoviridae family, in IBD patients compared to healthy controls[13]. Caudovirales phages act as predators of specific bacterial species, preventing overgrowth and maintaining microbial

balance. Additionally, a reduction in the diversity of Microviridae phages has also been observed[15]. Normally, Microviridae phages induced lysis of host bacteria releases nutrients and genetic material that can be utilized by other microbes in the ecosystem. The above findings suggest a shift towards a more pro-inflammatory viral community. These changes in the virome composition are often accompanied by an overall decrease in viral diversity, which may reflect a disrupted ecological balance within the gut[20].

The observed differences in the gut virome between healthy individuals and IBD patients has multiple mechanisms. One key contributor is the alteration of the bacterial microbiota in IBD, which in turn affects the virome. The gut microbiota in IBD patients is often characterized by dysbiosis, with reduced diversity and an overrepresentation of specific bacterial taxa, such as *Enterobacteriaceae* and *Bacteroidaceae*[21]. The altered bacterial landscape may provide a conducive environment for the proliferation of specific bacteriophages, particularly those capable of lysing pathogenic bacteria. This shift could result in an increased presence of lytic phages, contributing to the observed changes in the virome composition[18].

Another contributing factor may be the inflammatory environment characteristic of IBD. Chronic inflammation can lead to increased intestinal permeability, allowing for greater translocation of microbial and viral components across the gut barrier[22]. This heightened exposure may trigger an immune response that selectively targets certain viral populations, leading to a decline in viral diversity. Moreover, the inflammatory milieu may favor the expansion of viruses with pro-inflammatory properties, such as certain lytic phages, further exacerbating the disease[23].

The role of the host immune response in shaping the virome composition is also critical. IBD is associated with an aberrant immune response, characterized by excessive activation of both innate and adaptive immune pathways[3]. This dysregulated immune response can influence the virome by selectively targeting certain viral strains. For example, an increase in IgA-coated viruses has been reported in IBD patients, suggesting a targeted immune response against these viral populations[24]. The selective pressure exerted by the immune system may thus contribute to the observed virome alterations.

VIROME-MICROBIOTA INTERACTIONS

Recent studies have demonstrated the critical role of phages in modulating bacterial populations through mechanisms such as lysis and lysogeny[9]. Phages can selectively infect and lyse specific bacterial hosts, by which they can influence bacterial species within the microbiota. This predator-prey dynamic can lead to the elimination of dominant bacterial populations and the subsequent proliferation of less dominant or rare taxa, resulting in alterations of microbial diversity and ecosystem resilience[25].

In addition to direct effects on bacterial populations, phages can also impact bacterial community dynamics through horizontal gene transfer (HGT). Phages can facilitate HGT by transducing bacterial genes, including those involved in antibiotic resistance, virulence, and metabolic functions[26]. This genetic exchange enhances the adaptability and functional capabilities of bacterial communities, improving their response to environmental challenges and host immune pressures. Furthermore, one study identified several phage-associated genes linked to metabolic pathways, suggesting that phages may play a role in the metabolic versatility of the gut microbiota[10]. The mechanisms through which the virome influences microbiota composition are multifaceted. One potential mechanism is the modulation of bacterial community structure *via* lysogeny. In lysogenic cycles, phages integrate their genetic material into the host bacterial genome. It often provides the host with beneficial genes that can enhance fitness under specific conditions[27]. It can also lead to the emergence of lysogenic bacteria with altered phenotypes, which can potentially impact their interactions with other microbial species and the host[28]. The presence of prophages within bacterial genomes may also serve as a reservoir of genetic diversity, leading to rapid adaptation to environmental changes. Prophages are dormant bacteriophage genomes integrated into bacterial chromosomes or maintained as plasmids. They arise during lysogenic cycles when a phage infects a bacterium and inserts its DNA into the host genome, allowing it to replicate with the bacterium. Prophages can influence bacterial fitness, virulence, and evolution by carrying genes that confer advantages, such as toxin production, antibiotic resistance, or stress tolerance.

Another mechanism is the induction of microbial dysbiosis. Dysbiosis, characterized by an imbalance in microbial communities, has been implicated in various gastrointestinal disorders such as IBD and irritable bowel syndrome[29]. Phage-mediated lysis of beneficial bacteria can disrupt the equilibrium of the microbiota, leading to the overgrowth of pathogenic or opportunistic bacteria. This shift in microbial balance can compromise the gut's barrier function, promote inflammation, and exacerbate disease symptoms[14].

CONCLUSION

The interplay between the virome and microbiota has far-reaching implications for understanding gut health and disease. The virome's ability to influence bacterial diversity and function suggests that it may play a crucial role in maintaining gut ecosystem homeostasis. Moreover, the virome's involvement in HGT highlights its potential contribution to the dissemination of antibiotic resistance genes, a growing public health concern[30]. Understanding the dynamics of phage-bacteria interactions may provide new avenues for therapeutic interventions, such as phage therapy, which leverages phages' ability to target specific bacterial pathogens[31]. Future research should focus on elucidating the functional roles of specific phages within the gut ecosystem. Advanced metagenomic and metatranscriptomic approaches can provide insights into the active virome and its interactions with the microbiota at the gene expression level. Additionally, longit-

udinal studies are needed to investigate the temporal dynamics of the virome and microbiota, particularly in response to dietary changes, antibiotic treatments, and disease states. Understanding the complex interactions between the virome and microbiota is crucial for developing targeted therapies and interventions aimed at modulating the gut ecosystem to promote health and prevent disease. An example for virome therapy is faecal virome transplantation (FVT). FVT significantly altered overall bacteriome compositions, however, most of the studies were conducted *via* mouse models in small sample size. We are hoping to see more promising data in virome targeted therapy in IBD.

ACKNOWLEDGEMENTS

We thank Dr. Thomas Kang for providing research opportunity and approving necessary fundings.

FOOTNOTES

Author contributions: Li XL designed the overall concept and outline of the manuscript; Quadri HS contributed to the discussion of the manuscript; Li XL and Quadri HS contributed equally as co-corresponding authors; Li XL and MM contributed to the writing, and editing the manuscript, illustrations, and review of literature.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest.

Country of origin: United States

ORCID number: Xiao-Long Li 0000-0002-1130-7986.

S-Editor: Lin C
L-Editor: A
P-Editor: Wang WB

REFERENCES

1 **Hetta HF**, Ahmed R, Ramadan YN, Fathy H, Khorshid M, Mabrouk MM, Hashem M. Gut virome: New key players in the pathogenesis of inflammatory bowel disease. *World J Methodol* 2025; **15** [DOI: 10.5662/wjm.v15.i2.92592]
2 **Podolsky DK**. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429 [PMID: 12167685 DOI: 10.1056/NEJMra020831]
3 **Kaser A**, Zeissig S, Blumberg RS. Inflammatory bowel disease. *Annu Rev Immunol* 2010; **28**: 573-621 [PMID: 20192811 DOI: 10.1146/annurev-immunol-030409-101225]
4 **Xavier RJ**, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007; **448**: 427-434 [PMID: 17653185 DOI: 10.1038/nature06005]
5 **Qin J**, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J; MetaHIT Consortium, Bork P, Ehrlich SD, Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**: 59-65 [PMID: 20203603 DOI: 10.1038/nature08821]
6 **Human Microbiome Project Consortium**. Structure, function and diversity of the healthy human microbiome. *Nature* 2012; **486**: 207-214 [PMID: 22699609 DOI: 10.1038/nature11234]
7 **Norman JM**, Handley SA, Baldridge MT, Droit L, Liu CY, Keller BC, Kambal A, Monaco CL, Zhao G, Fleshner P, Stappenbeck TS, McGovern DP, Keshavarzian A, Mutlu EA, Sauk J, Gevers D, Xavier RJ, Wang D, Parkes M, Virgin HW. Disease-specific alterations in the enteric virome in inflammatory bowel disease. *Cell* 2015; **160**: 447-460 [PMID: 25619688 DOI: 10.1016/j.cell.2015.01.002]
8 **Reyes A**, Haynes M, Hanson N, Angly FE, Heath AC, Rohwer F, Gordon JI. Viruses in the faecal microbiota of monozygotic twins and their mothers. *Nature* 2010; **466**: 334-338 [PMID: 20631792 DOI: 10.1038/nature09199]
9 **Gogokhia L**, Buhrke K, Bell R, Hoffman B, Brown DG, Hanke-Gogokhia C, Ajami NJ, Wong MC, Ghazaryan A, Valentine JF, Porter N, Martens E, O'Connell R, Jacob V, Scherl E, Crawford C, Stephens WZ, Casjens SR, Longman RS, Round JL. Expansion of Bacteriophages Is Linked to Aggravated Intestinal Inflammation and Colitis. *Cell Host Microbe* 2019; **25**: 285-299.e8 [PMID: 30763538 DOI: 10.1016/j.chom.2019.01.008]
10 **Patel SK**, Singh SK. Pyroglutamylated RFamide peptide (QRFP): Role in early testicular development in relation to Sertoli cell maturation in prepubertal mice. *Neuropeptides* 2022; **91**: 102215 [PMID: 34883413 DOI: 10.1016/j.npep.2021.102215]
11 **Virgin HW**, Wherry EJ, Ahmed R. Redefining chronic viral infection. *Cell* 2009; **138**: 30-50 [PMID: 19596234 DOI: 10.1016/j.cell.2009.06.036]
12 **Zhang K**, Rana F, Silva C, Ethier J, Wehrly K, Chesebro B, Power C. Human immunodeficiency virus type 1 envelope-mediated neuronal death: uncoupling of viral replication and neurotoxicity. *J Virol* 2003; **77**: 6899-6912 [PMID: 12768009 DOI:

10.1128/jvi.77.12.6899-6912.2003]
13 **Norman JM**, Handley SA, Virgin HW. Kingdom-agnostic metagenomics and the importance of complete characterization of enteric microbial communities. *Gastroenterology* 2014; **146**: 1459-1469 [PMID: 24508599 DOI: 10.1053/j.gastro.2014.02.001]
14 **Clooney AG**, Sutton TDS, Shkoporov AN, Holohan RK, Daly KM, O'Regan O, Ryan FJ, Draper LA, Plevy SE, Ross RP, Hill C. Whole-Virome Analysis Sheds Light on Viral Dark Matter in Inflammatory Bowel Disease. *Cell Host Microbe* 2019; **26**: 764-778.e5 [PMID: 31757768 DOI: 10.1016/j.chom.2019.10.009]
15 **Sheflin AM**, Melby CL, Carbonero F, Weir TL. Linking dietary patterns with gut microbial composition and function. *Gut Microbes* 2017; **8**: 113-129 [PMID: 27960648 DOI: 10.1080/19490976.2016.1270809]
16 **Mirzaei MK**, Maurice CF. Ménage à trois in the human gut: interactions between host, bacteria and phages. *Nat Rev Microbiol* 2017; **15**: 397-408 [PMID: 28461690 DOI: 10.1038/nrmicro.2017.30]
17 **Song L**, Jia J, Peng X, Xiao W, Li Y. The performance of the SEPT9 gene methylation assay and a comparison with other CRC screening tests: A meta-analysis. *Sci Rep* 2017; **7**: 3032 [PMID: 28596563 DOI: 10.1038/s41598-017-03321-8]
18 **Garzilli I**, Itzkovitz S. Design principles of the paradoxical feedback between pancreatic alpha and beta cells. *Sci Rep* 2018; **8**: 10694 [PMID: 30013127 DOI: 10.1038/s41598-018-29084-4]
19 **Young GR**, Mavrommatis B, Kassiotis G. Microarray analysis reveals global modulation of endogenous retroelement transcription by microbes. *Retrovirology* 2014; **11**: 59 [PMID: 25063042 DOI: 10.1186/1742-4690-11-59]
20 **Caetano MJD**, Menant JC, Schoene D, Pelicioni PHS, Sturnieks DL, Lord SR. Sensorimotor and Cognitive Predictors of Impaired Gait Adaptability in Older People. *J Gerontol A Biol Sci Med Sci* 2017; **72**: 1257-1263 [PMID: 27573810 DOI: 10.1093/gerona/glw171]
21 **Frank DN**, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A* 2007; **104**: 13780-13785 [PMID: 17699621 DOI: 10.1073/pnas.0706625104]
22 **Turner JR**. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* 2009; **9**: 799-809 [PMID: 19855405 DOI: 10.1038/nri2653]
23 **Shkoporov AN**, Hill C. Bacteriophages of the Human Gut: The "Known Unknown" of the Microbiome. *Cell Host Microbe* 2019; **25**: 195-209 [PMID: 30763534 DOI: 10.1016/j.chom.2019.01.017]
24 **Sattentau QJ**, Stevenson M. Macrophages and HIV-1: An Unhealthy Constellation. *Cell Host Microbe* 2016; **19**: 304-310 [PMID: 26962941 DOI: 10.1016/j.chom.2016.02.013]
25 **Suttle CA**. Marine viruses--major players in the global ecosystem. *Nat Rev Microbiol* 2007; **5**: 801-812 [PMID: 17853907 DOI: 10.1038/nrmicro1750]
26 **Penadés JR**, Chen J, Quiles-Puchalt N, Carpena N, Novick RP. Bacteriophage-mediated spread of bacterial virulence genes. *Curr Opin Microbiol* 2015; **23**: 171-178 [PMID: 25528295 DOI: 10.1016/j.mib.2014.11.019]
27 **Fogg PC**, Westbye AB, Beatty JT. One for all or all for one: heterogeneous expression and host cell lysis are key to gene transfer agent activity in Rhodobacter capsulatus. *PLoS One* 2012; **7**: e43772 [PMID: 22916305 DOI: 10.1371/journal.pone.0043772]
28 **Touchon M**, Moura de Sousa JA, Rocha EP. Embracing the enemy: the diversification of microbial gene repertoires by phage-mediated horizontal gene transfer. *Curr Opin Microbiol* 2017; **38**: 66-73 [PMID: 28527384 DOI: 10.1016/j.mib.2017.04.010]
29 **Khan MT**, Nieuwdorp M, Bäckhed F. Microbial modulation of insulin sensitivity. *Cell Metab* 2014; **20**: 753-760 [PMID: 25176147 DOI: 10.1016/j.cmet.2014.07.006]
30 **Young R**. Phage lysis: do we have the hole story yet? *Curr Opin Microbiol* 2013; **16**: 790-797 [PMID: 24113139 DOI: 10.1016/j.mib.2013.08.008]
31 **Abedon ST**, García P, Mullany P, Aminov R. Editorial: Phage Therapy: Past, Present and Future. *Front Microbiol* 2017; **8**: 981 [PMID: 28663740 DOI: 10.3389/fmicb.2017.00981]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 100534

DOI: 10.5662/wjm.v15.i3.100534

ISSN 2222-0682 (online)

EDITORIAL

Gut virome and its emerging role in inflammatory bowel disease

Rahat Khatoon Khokhar, Abdulqadir J Nashwan

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade C
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade C

P-Reviewer: Xu C

Received: August 19, 2024
Revised: January 20, 2025
Accepted: February 6, 2025
Published online: September 20, 2025
Processing time: 199 Days and 3.9 Hours

Rahat Khatoon Khokhar, Department of Medicine, People's University of Medical and Health Sciences, Nawabshah 67450, Pakistan

Abdulqadir J Nashwan, Department of Nursing & Midwifery Research, Hamad Medical Corporation, Doha 3050, Qatar

Corresponding author: Abdulqadir J Nashwan, PhD, Researcher, Department of Nursing & Midwifery Research, Hamad Medical Corporation, Rayyan Road, Doha 3050, Qatar. anashwan@hamad.qa

Abstract

Inflammatory bowel disease (IBD) is a progressive multifactorial inflammatory disease of the gut. The cause of IBD is yet unknown. Some researchers have shown that genetic factors, environmental factors, and the gut microbiome are significant considerations. Our gut contains gut virome and gut bacteria, which vary among individuals due to some factors. The gut virome is a substantial component of the microbiome. This editorial explores the emerging role of gut virome in IBD.

Key Words: Inflammatory bowel disease; Pathogenesis; Gut virome; Bacteriophage; Eukaryotic viruses

Core Tip: Inflammatory bowel disease (IBD) is a chronic multifactorial inflammatory disease involving the gastrointestinal tract. The exact etiopathogenesis is unknown, but gut microbiome dysbiosis is believed to be a cornerstone in triggering disease progression. The gut virome forms a significant part of the microbiome and participates in health and disease conditions. Until 2015, researchers paid little attention to their role in IBD. Subsequently, numerous studies have followed this line of inquiry, using advanced techniques to clarify this role. Herein, we emphasize the viral populations in the gut and their predicted roles in the etiopathogenesis of IBD based on current studies.

Citation: Khokhar RK, Nashwan AJ. Gut virome and its emerging role in inflammatory bowel disease. *World J Methodol* 2025; 15(3): 100534
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/100534.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.100534

INTRODUCTION

Inflammatory bowel disease (IBD), which is also known as ulcerative colitis (UC) or Crohn's disease (CD), is present in 1% of the population[1]. According to Le Berre *et al*[2], UC tends to have a genetic predisposition. At birth, the gut microbiome is not fully developed, but as the baby grows, it increases in number. The gut microbiota contains bacteria, fungi, viruses, and archaea. A narrative review by Manos[3] shows that disruption in gut microbiota plays a crucial role in the pathogenesis of multiple diseases like IBD and gastro-oesophageal reflux disease. It also reveals that dysregulation of imperative bacteria redesigns the constituents of bile acid that can exacerbate the progression of IBD. Some research has shown that gut bacteria help build mucosal immunity towards IBD, whereas an imbalance between good and bad bacteria leads to progressive tissue injury. Harmful bacteria such as *Escherichia coli* can lead to the production of inflammatory mediators[4]. The gut virome plays a crucial role in the gut microbiome. However, its imbalance can lead to the pathogenesis of IBD[5]. According to recent studies, the gut virome can be correlated with the etiology of diabetes mellitus[6]. Consequently, further studies reveal that gut virome plays a significant role in childhood diarrhea, and it eventually causes early stages of pediatric IBD[7]. Hetta *et al*[8] also showed that active phages that can kill the bacterial host cell are primarily seen in patients with IBD.

FACTORS INFLUENCING IBD

The human gastrointestinal tract (GIT) nurtures a complex community of bacteria, viruses, fungi, protists, and other microorganisms. Studies have shown that the virome is crucial in normal infant development[9]. Hetta *et al*[8] say in their research that the physical appearance of a human, like height, weight, age, and body mass index, can influence the gut virome. Other factors like nutrition, lifestyle, and medication are essential in determining gut virome abundance. It is also concluded that dysbiosis and inflammation related to IBD co-exist. Many factors like genetic makeup, environment, drugs, smoking, diet, mental health, and others influence the occurrence of IBD. It is also shown that a low-fiber diet switches the gut microbiome from digesting the fiber derived glycans to mucous-derived glycans, leading to the eruption of the mucous membrane and better infiltration of pathogens into the blood. Hetta *et al*[8], after going through multiple studies, found that gut phages can impact IBD pathogenesis. It can change the gut phage community so that some specific phages are more prevalent in patients with CD and UC than in healthy people. However, randomised controlled trial verification is still needed. It can cause changes in gut microbiota, leading to a decreased population of good bacteria and an elevated concentration of dangerous bacteria such as E. coli and Fusobacteria. Gut phages can even cause alterations in the immune response of the body. All this literature is supported by very scanty research.

Eukaryotic viruses reside in the human GIT and remain quiet for ages. Once reactivated by any stimuli tends to cause deterioration in gut microbiota, leading to IBD pathogenesis. Thus, there is a critical need for more research on the association between IBD and gut phages. Hetta *et al*[8] did a fantastic job describing the association between gut virome and IBD. Their work is commendable as it resolves many queries related to gut virome and IBD. However, it is tough to differentiate viral DNA in microbiological practice because viruses possess wide diversity, low genomes, and rapid rates of mutations. In addition to that, viruses are also difficult to grow as they rely on host cells for energy and multiplication. Hence, the host cell should also be extracted. Metagenomic analysis is a tricky procedure, but with recent technologies like VIP and VirFinder, it has become more doable. Also, problematic cultivations of GIT microorganisms, critical DNA sequencing, perplexing recognition, and differentiation of viral specimens have made this association very exhausting and laborious.

CONCLUSION

IBD is a chronic progressive inflammatory disease that consists of two parts: UC and CD. The precise cause of IBD is idiopathic. Hetta *et al*[8] described the correlation between gut virome dysbiosis and its impact on the progression of IBD. They also elaborate on the role of gut phages and eukaryotic viruses in the pathogenesis of IBD. Therefore, further innovative research is warmly welcomed.

FOOTNOTES

Author contributions: Khokhar RK and Nashwan AJ were responsible for writing the draft and critically reviewing the literature.

Conflict-of-interest statement: All the authors declare that they have no conflict of interest.

Country of origin: Qatar

ORCID number: Abdulqadir J Nashwan 0000-0003-4845-4119.

S-Editor: Lin C
L-Editor: A
P-Editor: Zheng XM

REFERENCES

1 **Bruner LP**, White AM, Proksell S. Inflammatory Bowel Disease. *Prim Care* 2023; **50**: 411-427 [PMID: 37516511 DOI: 10.1016/j.pop.2023.03.009]
2 **Le Berre C**, Honap S, Peyrin-Biroulet L. Ulcerative colitis. *Lancet* 2023; **402**: 571-584 [PMID: 37573077 DOI: 10.1016/S0140-6736(23)00966-2]
3 **Manos J**. The human microbiome in disease and pathology. *APMIS* 2022; **130**: 690-705 [PMID: 35393656 DOI: 10.1111/apm.13225]
4 **Quaglio AEV**, Grillo TG, De Oliveira ECS, Di Stasi LC, Sassaki LY. Gut microbiota, inflammatory bowel disease and colorectal cancer. *World J Gastroenterol* 2022; **28**: 4053-4060 [PMID: 36157114 DOI: 10.3748/wjg.v28.i30.4053]
5 **Tun HM**, Peng Y, Massimino L, Sin ZY, Parigi TL, Facoetti A, Rahman S, Danese S, Ungaro F. Gut virome in inflammatory bowel disease and beyond. *Gut* 2024; **73**: 350-360 [PMID: 37949638 DOI: 10.1136/gutjnl-2023-330001]
6 **Fang L**, Ning J. Gut virome and diabetes: discovering links, exploring therapies. *Arch Microbiol* 2024; **206**: 346 [PMID: 38976078 DOI: 10.1007/s00203-024-04068-3]
7 **Liang G**, Gao H, Bushman FD. The pediatric virome in health and disease. *Cell Host Microbe* 2022; **30**: 639-649 [PMID: 35550667 DOI: 10.1016/j.chom.2022.04.006]
8 **Hetta HF**, Ahmed R, Ramadan YN, Fathy H, Khorshid M, Mabrouk MM, Hashem M. Gut virome: New key players in the pathogenesis of inflammatory bowel disease. *World J Methodol* 2025; **15**: 92592 [DOI: 10.5662/wjm.v15.i2.92592]
9 **Lim ES**, Wang D, Holtz LR. The Bacterial Microbiome and Virome Milestones of Infant Development. *Trends Microbiol* 2016; **24**: 801-810 [PMID: 27353648 DOI: 10.1016/j.tim.2016.06.001]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 99874

DOI: 10.5662/wjm.v15.i3.99874

ISSN 2222-0682 (online)

OPINION REVIEW

Clinical inertia in sexual medicine practice

Arkiath Veettil Raveendran

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade D, Grade D

Novelty: Grade B, Grade C

Creativity or Innovation: Grade C, Grade C

Scientific Significance: Grade C, Grade C

P-Reviewer: Grizzi F; Smith JH

Received: August 1, 2024

Revised: October 20, 2024

Accepted: December 5, 2024

Published online: September 20, 2025

Processing time: 216 Days and 21.8 Hours

Arkiath Veettil Raveendran, Department of Internal Medicine, Former Assistant Professor of Medicine, Govt Medical College, Kozhikode 673010, Kerala, India

Corresponding author: Arkiath Veettil Raveendran, FRCP, MBBS, MD, Doctor, Department of Internal Medicine, Former Assistant Professor of Medicine, Govt Medical College, Medical College P.O., Kozhikode 673010, Kerala, India. raveendranav@yahoo.co.in

Abstract

Clinical inertia (CI) is common in clinical practice. Sexual health issues are common in society, and CI is ubiquitous in sexual medicine practice. CI influences all aspects of healthcare, including prevention, diagnosis, and treatment. In this short review, we briefly describe the various aspects of CI in sexual medicine practice and ways to tackle them

Key Words: Clinical inertia; Sexual medicine; Sexual dysfunction; Erectile dysfunction; Vaginismus

Core Tip: Clinical inertia (CI) occurs in various disease conditions and is very common in sexual medicine practices. It exists in all stages of healthcare such as prevention, diagnosis, and management. Various factors contributing to CI can be divided into physician or provider-, patient-, and system-related factors. It results in improper evaluation and treatment. Tackling CI helps to improve patient outcomes.

Citation: Raveendran AV. Clinical inertia in sexual medicine practice. *World J Methodol* 2025; 15(3): 99874

URL: https://www.wjgnet.com/2222-0682/full/v15/i3/99874.htm

DOI: https://dx.doi.org/10.5662/wjm.v15.i3.99874

INTRODUCTION

Clinical inertia (CI) denotes the physician's tendency to remain unchanged in the preventive, diagnostic, and or therapeutic aspects even when the changes are warranted[1]. CI occurs in various disease conditions, including both symptomatic and asymptomatic, acute and chronic[1,2]. In simple words, it is the 'recognition of the

problem, but failure to act'.

CI is very common in sexual medicine practices. In comparison with other diseases such as diabetes, hypertension, dyslipidemia, and rheumatoid arthritis where CI is well documented, there is significant delay in diagnosing and treating various sexual health issues, because of the stigma associated with sexual health problems, in addition to other usual causes of CI, which discourage people from seeking medical help[3-7]. CI exists in all stages of healthcare such as prevention, diagnosis, and management, and they are no well-demarcated compartments as there is overlap between all these types of inertia (Figure 1A). They are related to one another. For example, if there is diagnostic inertia, it will subsequently lead to a delay in the initiation of treatment (*i.e.* therapeutic inertia).

PREVENTIVE INERTIA

Inertia in preventing the development and progression of sexual dysfunction (SD) constitutes preventive inertia. It can be at any stage of prevention such as primordial prevention, primary prevention, secondary prevention, tertiary prevention, or quaternary prevention. The classical example of preventive inertia is erectile dysfunction (ED) due to atherosclerotic vascular disease, whereas the delay in preventing the development of lifestyle diseases such as obesity, diabetes, hypertension, and dyslipidemia (primordial prevention) and the delay in early diagnosis, treatment (primary prevention), and prevention of progression (secondary prevention) and complication (tertiary prevention) of these lifestyle disorders in those who already have it constitutes preventive inertia in the development of ED. Stages of prevention vary with the disease under consideration. In those with diabetes-related vascular ED, steps related to primary and secondary prevention of DM can be an example of primordial prevention of vascular ED. Usually, primordial prevention targets the community, whereas other stages of prevention target the individual. Hence, CI can be at the community or individual levels (Table 1). Childhood sexual trauma and sexual abuse can lead to vaginismus in women. Social and legal strategies to prevent childhood sexual abuse help to prevent the development of vaginismus. Our inertia in properly implementing these can lead to the development of various SD associated with childhood sexual trauma. This is an example of inertia at the community level in preventing SD.

DIAGNOSTIC INERTIA

Diagnostic inertia is very common in sexual medicine practice. People with SD are reluctant to disclose their sexual problems because of the associated stigma, and even if they disclose, they use vague terminologies to express their sexual health issues. For example, people with ED may say that they are feeling excessive tiredness or have weakness denoting ED, which may be difficult to recognize especially in a busy outpatient department, leading to delays in diagnosing ED. Similarly, even if the patient says that he has ED, the healthcare provider most of the time prescribes some multivitamins without properly paying attention and without proper evaluation of SD. Time constraints in busy out-patient departments (OPDs), stigma associated with sexual health issues, hesitation to discuss sexual problems, and lack of experience in the evaluation and treatment of SD are a few reasons behind this attitude[7].

THERAPEUTIC INERTIA

Inertia in initiating treatment of sexual health issues is widespread in day-to-day clinical practice. Clinicians are reluctant to discuss sexual health issues most of the time with the patient, even if the patient discloses their sexual problems, leading to barriers in the evaluation and specific management of sexual problems[7]. Healthcare providers usually buy time by prescribing multivitamins, antioxidants, or other placebos instead of doing a proper evaluation and starting specific treatment for SD (Figure 2).

Even after diagnosing underlying SD, specific therapy is often delayed especially due to multiple reasons such as time constraints, lack of training in sexual medicine, and lack of confidence to manage SD. Patients on polypharmacy receive delayed treatment for their SD because of the fear of aggravating the underlying disease and drug interaction. In people with ED and coronary artery disease (CAD) or heart failure, treatment of ED is often delayed. In patients with ED who fail to respond to phosphodiesterase inhibitors, there is a significant delay in initiating other treatments such as intracavernosal injections and penile prosthesis implantation.

INERTIA IN ADDRESSING CO-MORBIDITIES AND COMPLICATIONS

Associated co-morbidities and complications need to be properly addressed for optimal benefits in sexual medicine practice, as with any other branch of medicine. ED is considered a forerunner of future cardiovascular events, as people with atherosclerotic ED develop cardiovascular events 3 years to 5 years after the onset of ED[8,9]. Hence, treating ED without addressing the cardiovascular risk factors is a classic example of inertia in addressing the associated co-morbidities and complications. Similarly, females with diabetes and genital infection resulting in dyspareunia need optimal control of diabetes and other risk factors in addition to treatment of genital infection, for optimum results[10].

Table 1 Overview of clinical inertia in sexual medicine practice

CI at the population level
Primordial prevention
CI to follow a healthy lifestyle (*e.g.*, eating habits and physical activity to reduce the development of risk factors). Inertia in preventing sexual abuse in society
CI at the patient level
Primary prevention
CI in identifying and intervening susceptible individuals (*e.g.*, identifying people with diabetes with risk of ED at the earlier stage and intervening)
Secondary prevention
CI in identifying and intervening those with subclinical disease (*e.g.*, identifying people with diabetes with early ED at the earlier stage and intervening)
CI in identifying and intervening those with the clinical disease (*e.g.*, identifying people with diabetes with established ED and intervening)
Tertiary prevention
CI in appropriately treating those with clinical disease (*e.g.*, identifying people with diabetes with established ED and intervening)
Quaternary prevention
CI in appropriately down-titrating the dose of medication in those with the clinical disease who do not require (overmedication) (*e.g.*, identifying people with established ED on multiple medications for ED, which are ineffective in that particular patient and intervening by stopping ineffective medications)
Quinary prevention
CI in identifying and intervening misinformation/misconception regarding the clinical disease. CI in identifying and correcting misconceptions such as "there is no treatment for sexual problems" and "ED is a part of aging"

CI: Clinical inertia; ED: Erectile dysfunction.

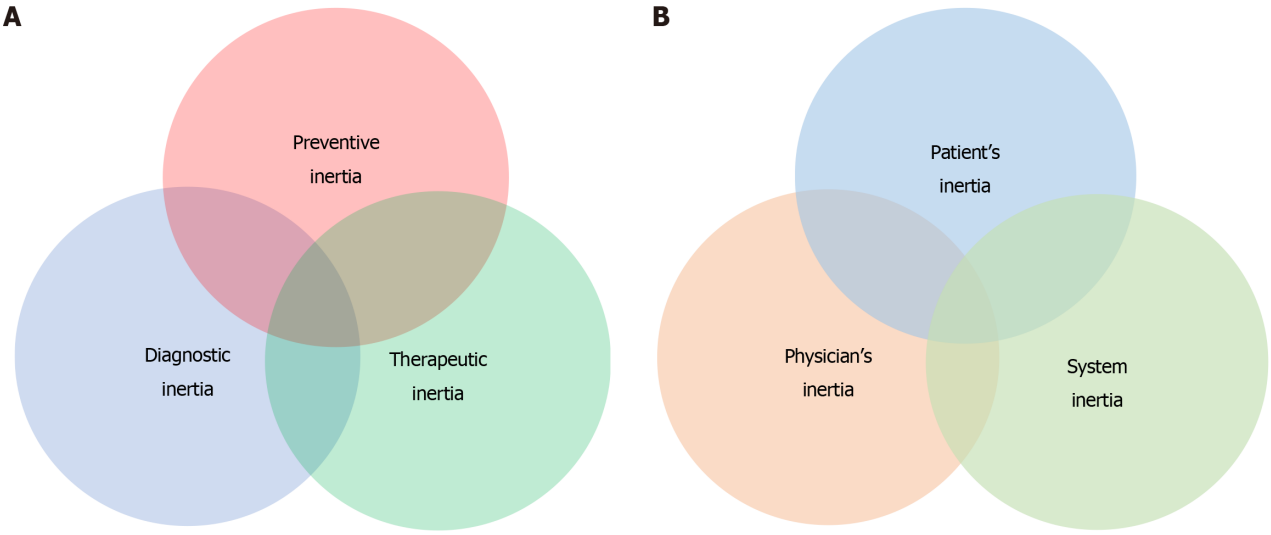


Figure 1 Interrelated and overlapping factors. A: Preventive, diagnostic, and therapeutic aspects of clinical inertia, which are interrelated and overlapping; B: Interrelated and overlapping factors in clinical inertia.

SPECIAL ISSUES IN SEMANTIC DEMENTIA PRACTICE

Sexual medicine is an evolving discipline and still, not a separate specialty to address sexual health issues under one roof. People approach different specialists such as psychiatrists, gynecologists, urologists, physicians, and endocrinologists [11]. They are all experts in their field but may not be in other dimensions of SD. Therefore, comprehensive training in sexual medicine, even from the undergraduate level, is the need of the hour[7,12,13].

Training in communication skills during the medical curriculum is important for proper sexual history taking and further evaluation[14]. It improves the level of comfort of healthcare providers.

Sex preference by the patient is another issue in dealing with sexual health issues[15]. Female patients usually prefer female healthcare providers, which is why most females initially consult an obstetrician and gynecologist for their sexual issues.

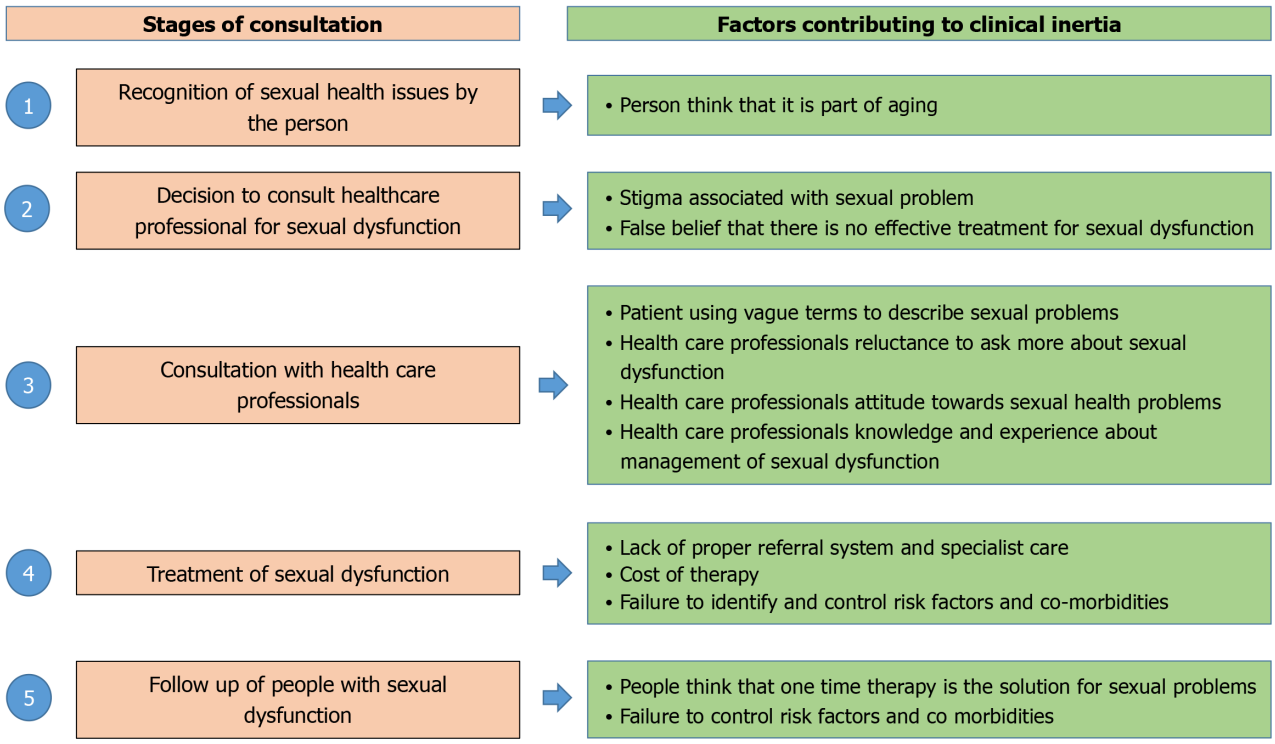


Figure 2 Clinical inertia at various stages of sexual medicine practice.

Taboos related to sexual matters; feeling uncomfortable, embarrassed, or anxious about discussing sexual health problems; beliefs and attitudes toward sexual health; cultural restrictions; and religious prohibitions add to CI in sexual medicine practice[7,16,17]. Lack of education about sexual health issues, lack of training programs, and lack of time in the busy OPD add to delays in the proper evaluation of SD, leading to CI[7,17-19].

Some people believe that SD is due to the normal aging process and hence do not seek medical help[7,20]. Patient's age, culture, sexual orientation, and comorbidities also influence their treatment-seeking behavior. Lack of guidelines regarding sexual assessment and management, lack of specialized referral clinics, lack of support from the institutions, lack of privacy, and work overload are important organizational barriers resulting in CI[21].

There are many sexual problems that interfere with a normal healthy life and relationships but are not described in the current nosography, which also cause limitations in diagnosing and managing SD. One study among males with SD classified such issues as "unmet needs" which included: (1) Lower penile rigidity; (2) Prolonged refractory period; (3) Increased threshold to stimuli; (4) Decreased frequency of spontaneous erections; (5) Delayed orgasm in the female partner; (6) Soft glans; (7) Perceived ejaculate volume reduction; and (8) Decreased force of ejaculation[22].

FACTORS CONTRIBUTING TO CI

Factors contributing to CI can be divided into physician or provider-related, patient-related, and system-related factors, also known as physician inertia, patient inertia, and system inertia, respectively. The stigma associated with sexual health issues, embarrassment, and discomfort in discussing sexual matters leads to delay (from the patient side, approaching healthcare provider) in seeking medical help. Even if the patient discloses their sexual health issues, healthcare providers are reluctant to explore the problem leading to delays in proper evaluation and treatment[7]. Healthcare providers are also embarrassed to discuss sexual issues, fearing that asking about sexual health issues may lead to the loss of patients from their practice[7]. In addition, a lack of proper training about sexual health issues in the medical curriculum adds to the lack of confidence in dealing with sexual health issues. Busy OPD and lack of time are other important factors contributing to physician-related CI. Lack of support, lack of availability of the multidisciplinary team and referral for specialist care, provider's ability to make appropriate care, and ambiguity in the existing guidelines also contribute to CI [6]. Patient characteristics such as patients with other comorbid diseases like CAD, heart failure, patients on poly-pharmacy, quality of the relation between patient and healthcare provider, concern about adverse reaction or drug interaction, health literacy, socioeconomic status of the patient, and their affordability all contribute to physician's decision to evaluate and treat sexual health issues. Patient attitudes and preferences, and lack of communication between patient and physician also influence therapeutic decision making. Non-adherence to treatment is an important factor that interferes with the physician's assessment of treatment response leading to undue delay in optimizing treatment[23]. System-related factors such as time concerns, inconsistencies between guidelines, poor planning, communication and coordination between members of the healthcare team, resource constraints, lack of team approach to care, and lack of decision support system contribute to CI[6] (Figure 3). Various factors such as physician or provider-, patient-, and

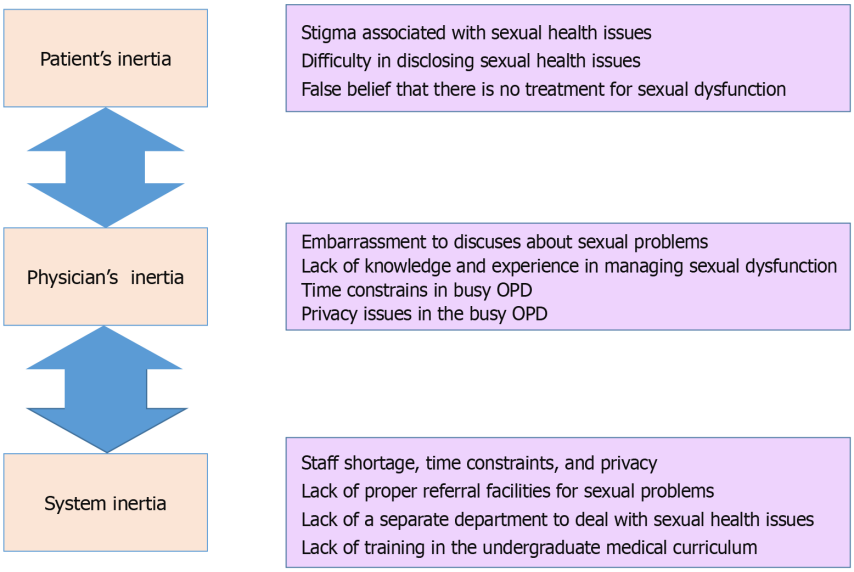


Figure 3 Physician-, patient-, and system-related factors contributing to clinical inertia in sexual medicine practice. OPD: Out-patient department.

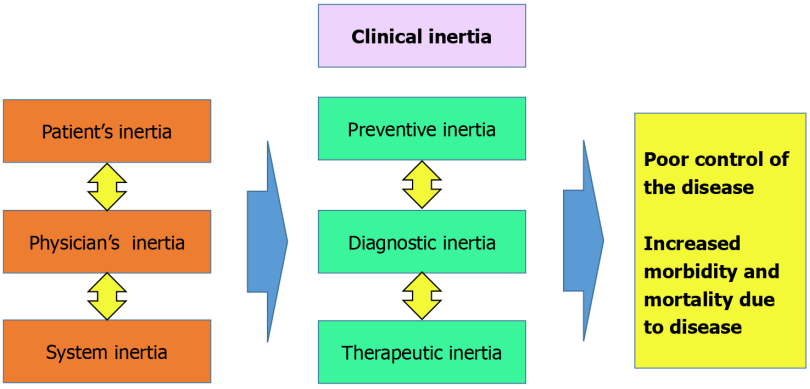


Figure 4 Impact of clinical inertia.

system-related factors are not isolated compartments but are interrelated and overlapping (Figure 1B).

HOW TO TACKLE CI SEXUAL MEDICINE PRACTICE

CI results in improper evaluation and treatment leading to failure to achieve treatment targets, poor control of the disease process, and increased risk of disease-associated complications (Figure 4). Improving awareness regarding CI among physicians, ongoing medical education and training programs, coordination between primary, secondary, and tertiary care, adapting current practice guidelines, self-examination of performance by healthcare professionals, use of computer-based decision support system, and patient education programs and improved communication helps to avoid CI[6]. In addition to that increased direct patient contact time, improvement in the system infrastructure, multi-disciplinary team approach help to tackle CI. A few special factors need to be considered regarding sexual medicine practice. Improving awareness regarding sexual problems in the public, educating the importance of treatment of sexual problems, and training regarding the management of sexual health problems to healthcare professionals, and ensuring privacy for patients with sexual health issues all help to overcome CI[24].

CONCLUSION

CI delays or even denies the best available treatment for the needy patient. CI exists in all aspects of sexual medicine practice. Identifying the factors contributing to CI and tackling it helps to improve patient outcomes.

FOOTNOTES

Author contributions: Raveendran AV designed the manuscript, collected the data, and wrote and revised the manuscript; The author read and approved the final version of the manuscript to be published.

Conflict-of-interest statement: The author has no conflicts of interest to declare.

Country of origin: India

ORCID number: Arkiath Veettil Raveendran 0000-0003-3051-7505.

S-Editor: Luo ML
L-Editor: Filipodia
P-Editor: Zheng XM

REFERENCES

1 **Phillips LS**, Branch WT, Cook CB, Doyle JP, El-Kebbi IM, Gallina DL, Miller CD, Ziemer DC, Barnes CS. Clinical inertia. *Ann Intern Med* 2001; **135**: 825-834 [PMID: 11694107 DOI: 10.7326/0003-4819-135-9-200111060-00012]
2 **Raveendran AV**. Clinical Inertia: A Wider Perspective and Proposed Classification Criteria. *Indian J Endocrinol Metab* 2023; **27**: 296-300 [PMID: 37867979 DOI: 10.4103/ijem.ijem_119_23]
3 **Mohan V**. Expanding the concept of 'Clinical Inertia' in diabetes. *J Diabetol* 2019; **10**: 1 [DOI: 10.4103/jod.jod_44_18]
4 **Shawahna R**, Odeh M, Jawabreh M. Factors Promoting Clinical Inertia in Caring for Patients with Dyslipidemia: A Consensual Study Among Clinicians who Provide Healthcare to Patients with Dyslipidemia. *J Natl Med Assoc* 2019; **111**: 18-27 [PMID: 30129490 DOI: 10.1016/j.jnma.2018.04.002]
5 **Lebeau JP**, Cadwallader JS, Aubin-Auger I, Mercier A, Pasquet T, Rusch E, Hendrickx K, Vermeire E. The concept and definition of therapeutic inertia in hypertension in primary care: a qualitative systematic review. *BMC Fam Pract* 2014; **15**: 130 [PMID: 24989986 DOI: 10.1186/1471-2296-15-130]
6 **Pallarés-Carratalá V**, Bonig-Trigueros I, Palazón-Bru A, Esteban-Giner MJ, Gil-Guillén VF, Giner-Galvañ V. Clinical inertia in hypertension: a new holistic and practical concept within the cardiovascular continuum and clinical care process. *Blood Press* 2019; **28**: 217-228 [PMID: 31023106 DOI: 10.1080/08037051.2019.1608134]
7 **Av R**, Lawrence T, Pv S. Doctors Attitude towards Sexual Health Problems - A Practise Survey. *J Assoc Physicians India* 2020; **68**: 24-27 [PMID: 32138478]
8 **Ponholzer A**, Temml C, Obermayr R, Wehrberger C, Madersbacher S. Is erectile dysfunction an indicator for increased risk of coronary heart disease and stroke? *Eur Urol* 2005; **48**: 512-518; discussion 517 [PMID: 15998563 DOI: 10.1016/j.eururo.2005.05.014]
9 **Vlachopoulos C**, Jackson G, Stefanadis C, Montorsi P. Erectile dysfunction in the cardiovascular patient. *Eur Heart J* 2013; **34**: 2034-2046 [PMID: 23616415 DOI: 10.1093/eurheartj/eht112]
10 **Rogoznica M**, Perica D, Borovac B, Belančić A, Matovinović M. Sexual Dysfunction in Female Patients with Type 2 Diabetes Mellitus—Sneak Peek on an Important Quality of Life Determinant. *Diabetology* 2023; **4**: 527-536 [DOI: 10.3390/diabetology4040046]
11 **Jannini EA**. Introduction: History of Sexual Medicine. Practical Clinical Andrology. Berlin: Springer, 2023: 1-12 [DOI: 10.1007/978-3-031-11701-5_1]
12 **Di Dionisio CM**, Bitzer J, Greil-Soyka M. A model curriculum in sexual medicine for undergraduate education in Europe. *Open Res Eur* 2023; **3**: 153 [PMID: 39318999 DOI: 10.12688/openreseurope.16146.2]
13 **Wylie K**, Hallam-Jones R, Daines B. Review of an undergraduate medical school training programme in human sexuality. *Med Teach* 2003; **25**: 291-295 [PMID: 12881053 DOI: 10.1080/0142159031000100382]
14 **Wylie K**. Assessment & management of sexual problems in women. *J R Soc Med* 2007; **100**: 547-550 [PMID: 18065705 DOI: 10.1177/0141076807100012011]
15 **Ryan KL**, Arbuckle-Bernstein V, Smith G, Phillips J. Let's Talk About Sex: A Survey of Patients' Preferences When Addressing Sexual Health Concerns in a Family Medicine Residency Program Office. *PRiMER* 2018; **2**: 23 [PMID: 32818195 DOI: 10.22454/PRiMER.2018.728252]
16 **Magnan MA**, Reynolds K. Barriers to addressing patient sexuality concerns across five areas of specialization. *Clin Nurse Spec* 2006; **20**: 285-292 [PMID: 17149019 DOI: 10.1097/00002800-200611000-00009]
17 **Bdair IAA**, Constantino RE. Barriers and Promoting Strategies to Sexual Health Assessment for Patients with Coronary Artery Diseases in Nursing Practice: A Literature Review. *Health* 2017; **9**: 473-492 [DOI: 10.4236/health.2017.93034]
18 **Beebe S**, Payne N, Posid T, Diab D, Horning P, Scimeca A, Jenkins LC. The Lack of Sexual Health Education in Medical Training Leaves Students and Residents Feeling Unprepared. *J Sex Med* 2021; **18**: 1998-2004 [PMID: 34711518 DOI: 10.1016/j.jsxm.2021.09.011]
19 **Hinchliff S**, Gott M. Seeking medical help for sexual concerns in mid- and later life: a review of the literature. *J Sex Res* 2011; **48**: 106-117 [PMID: 21409708 DOI: 10.1080/00224499.2010.548610]
20 **Hinchliff S**, Lewis R, Wellings K, Datta J, Mitchell K. Pathways to help-seeking for sexual difficulties in older adults: qualitative findings from the third National Survey of Sexual Attitudes and Lifestyles (Natsal-3). *Age Ageing* 2021; **50**: 546-553 [PMID: 33507242 DOI: 10.1093/ageing/afaa281]
21 **Doherty S**, Byrne M, Murphy AW, McGee HM. Cardiac rehabilitation staff views about discussing sexual issues with coronary heart disease

patients: a national survey in Ireland. *Eur J Cardiovasc Nurs* 2011; **10**: 101-107 [PMID: 20684891 DOI: 10.1016/j.ejcnurse.2010.05.002]

22 **Burgio G**, Giammusso B, Calogero AE, Mollaioli D, Condorelli RA, Jannini EA, La Vignera S. Evaluation of the Mistakes in Self-Diagnosis of Sexual Dysfunctions in 11,000 Male Outpatients: A Real-Life Study in An Andrology Clinic. *J Clin Med* 2019; **8**: 1679 [PMID: 31615034 DOI: 10.3390/jcm8101679]

23 **Yan X**, Mudiganti S, Husby H, Hudnut A, Gbotoe M, Jones JB. Medication non-adherence and therapeutic inertia independently contribute to poor disease control for cardiometabolic diseases. *Sci Rep* 2022; **12**: 18936 [PMID: 36344613 DOI: 10.1038/s41598-022-21916-8]

24 **Khalesi ZB**, Simbar M, Azin SA, Zayeri F. Public sexual health promotion interventions and strategies: A qualitative study. *Electron Physician* 2016; **8**: 2489-2496 [PMID: 27504163 DOI: 10.19082/2489]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 100490

DOI: 10.5662/wjm.v15.i3.100490

ISSN 2222-0682 (online)

OPINION REVIEW

Echo contrast medium: How the use of contrast echocardiography (ultrasound contrast agents) can improve patient care

Kevan English

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade B

Novelty: Grade B, Grade B

Creativity or Innovation: Grade B, Grade B

Scientific Significance: Grade B, Grade B

P-Reviewer: Zhao K

Received: August 18, 2024

Revised: December 14, 2024

Accepted: December 20, 2024

Published online: September 20, 2025

Processing time: 200 Days and 4.7 Hours

Kevan English, Department of Internal Medicine, University of Nebraska Medical Center College of Medicine, Omaha, NE 68198, United States

Corresponding author: Kevan English, MD, Department of Internal Medicine, University of Nebraska Medical Center College of Medicine, S 42nd & Emile St, Omaha, NE 68198, United States. keenglish@unmc.edu

Abstract

Conventional echocardiography can sometimes pose a challenge to diagnosis due to sub-optimal images. Ultrasound contrast agents (UCAs) have been shown to drastically enhance imaging quality, particularly depicting the left ventricular endocardial borders. Their use during echocardiography has become a valuable tool in non-invasive diagnostics. UCAs provide higher-quality images that may ultimately reduce the length of hospital stays and improve patient care. The higher cost associated with UCAs in many situations has been an impediment to frequent use. However, when used as an initial diagnostic test, UCA during rest echocardiogram is more cost-effective than the traditional diagnostic approach, which frequently includes multiple tests and imaging studies to make an accurate diagnosis. They can be easily performed across multiple patient settings and provide optimal images that allow clinicians to make sound medical decisions. This consequently allows for better diagnostic accuracies and improvement in patient care.

Key Words: Ultrasound contrast agents; Echocardiography; Myocardial perfusion; Ultrasound; Left ventricle; Optison; Definity; Sonazoid; Lumason

Core Tip: The use of ultrasound contrast agents (UCAs) during resting echocardiography can improve diagnostic accuracy by adequately assessing left ventricular (LV) function. Compared to standard echocardiograms, UCAs, when used, allow physicians to better assess regional wall motion abnormalities and LV ejection fraction. As a result, patients with LV apex disease can be accurately diagnosed without additional imaging. This consequently shortens hospital stays and improves patient outcomes.

Citation: English K. Echo contrast medium: How the use of contrast echocardiography (ultrasound contrast agents) can improve patient care. *World J Methodol* 2025; 15(3): 100490
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/100490.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.100490

INTRODUCTION

Ultrasound contrast agents (UCAs) are microspheres suspensions that consist of an outer albumin or phospholipid shell encompassing an inner high-molecular weight gas[1]. These are intravascular tracers that are similar in size to red blood cells, which allows for opacification of blood in the myocardial vessels and cavities[1,2]. UCAs are strong ultrasound reflectors, thereby producing intense echocardiographic signals that are stronger than standard ultrasound waves[2,3]. As a result, contrast echocardiograms serve as a tool to improve image quality[3,4]. They facilitate interpretability as more than 25% of stress echocardiograms and more than 5% of resting echocardiograms yield suboptimal images[4-6]. UCA during echocardiography is desirable as image improvement may redirect patient management, and its use improves the signal-to-noise ratio in spectral and color Doppler imaging[2,6,7]. When used as part of the initial diagnostic work-up, contrast echocardiography is highly beneficial due to better diagnostic accuracy than standard echocardiography[8]. This is particularly important as UCAs used during echocardiography may shorten hospitalization and improve patient care and mortality[8,9].

TYPES OF CONTRAST AGENTS

Contrast echocardiography has become a valuable tool in non-invasive cardiac imaging[10]. Currently, there are several available contrast agents (Table 1) as listed below.

Optison

Optison (perflutren protein-type A microspheres injectable suspension, USP) manufactured by General Electric Healthcare is a non-pyrogenic suspension consisting of microspheres with an Octafluoropropane (perflutren) gas core encapsulated by a 15 nm thick serum albumin shell[11,12]. Its reconstitution is done by mixing, and the concentration of the reconstituted suspension is approximately 5.0-8.0 × 10^8 microspheres/mL with an average diameter of 3.0-4.5 μm[13,14]. The albumin shell rigidity in Optison is 88.8 MPa with a shear viscosity of 0.177 Ns/m^2, derived from previous mathematical modeling and experimental measurements[15]. Based on data for Albunex, values for the shell elasticity and dilational viscosity can be estimated as 4 N/m and 3.2 × 10^{-8} kg/s, respectively[13-15]. *In vitro* assessment has indicated that the gas dissolution pressure for speedy destruction of Optison was 0.47 MPa when exposed to 3.5 MHz ultrasound, while a lower threshold was found to facilitate accelerated dissolution[14-16]. These innate features of the albumin shell make Optison an effective UCA for clinical use.

Optison was first introduced to the United States market in 1998[17]. More than 3 million patients have received Optison since it was approved by the Food and Drug Administration (FDA) in 1997[17,18]. It has a well-established safety profile, and it is used to opacify the left ventricle and improve the left ventricular (LV) endocardial border delineation in patients with suboptimal echocardiograms[12-14].

Definity

Definity (United States) is one of the second-generation microbubble contrast agents approved for LV endocardial border detection and LV opacification[19]. It was approved by the FDA in 2001 and the European Medicine Agency under the brand name Luminity in 2006 for patients with technically challenging or suboptimal echocardiograms[20]. Definity injectable suspension is sold as a single-use 2 mL vial containing a clear liquid and Octafluoropropane gas[21]. The liquid solution contains the three phospholipids DPPA (1,2-Dipalmitoyl-sn-glycero-3-phosphate, sodium salt), DPPC (1,2-Dipalmitoyl-sn-glycero-3-phosphocholine), and DPPE-$MPEG_{5000}$[21,22]. The shell properties for Definity microbubbles have been estimated at low (5-15 MHz) and high (12-29 MHz) ultrasound frequencies[23,24]. At lower frequencies, corresponding values for the shell elasticity and dilatational viscosity have been reported, which are significantly lower compared to those of Optison[24].

In addition to endocardial visualization, Definity is used to measure LV ejection fraction and volumes[22]. It also allows for the diagnoses of apical LV pathologies (*i.e.*, thrombus or apical variant of hypertrophic cardiomyopathy), post-myocardial infarction complications (*i.e.*, ventricular septal defect or LV pseudoaneurysm/rupture), and intracardiac masses, which are drastically enhanced[22-24]. Definity is approved for imaging of the kidney and liver in Australia and Canada[23-25].

SonoVue

SonoVue (sulfur hexafluoride microbubbles) (Italy) or Lumason (sulfur hexafluoride lipid-type A microspheres) (United States) is a UCA supplied as a kit containing a vial of phospholipid lyophilized powder and sulfur hexafluoride headspace, a mini-spike transfer system, and a pre-filled syringe with a 5 mL sodium chloride 0.9% diluent[26,27]. It is administered as an intravenous bolus injection followed by a 5-10 mL saline flush and should be shaken appropriately

Table 1 Commercially available ultrasound contrast agents

Name	Manufacturer	First approved for clinical use	Gas	Shell composition	Countries
Optison	General Electric Healthcare, Buckinghamshire (United Kingdom)	1998	Octafluoropropane	Cross-linked serum albumin	Unites States, Europe
Definity/luminity	Lantheus Medical Imaging Inc, North Billerica (Massachusetts, United States)	2001/2006	Octafluoropropane	Phospholipid	North America, Europe
SonoVue/lumason	Bracco Diagnostics Inc (New Jersey, United States), Bracco Imaging S.p.A (Milan, Italy)	2001/2014	Sulfur hexafluoride	Phospholipid	United States, Europe, China, Brazil
Sonazoid[1]	General Electric Healthcare, Buckinghamshire (United Kingdom), Daiichi Saniko, (Tokyo, Japan)	2007	Perfluorobutane	Hydrogenated egg yolk phosphatidyl serine (HEPS)	Japan, South Korea, Norway, Taiwan, China
Albunex	Molecular Biosystems Inc., (California, United States)	1993, withdrawn	Air	Sonicated serum albumin	United States, Japan
Imagent/imavist	Schering AG	2002, withdrawn	Perfluorohexane, Nitrogen	Phospholipid	United States
Echovist	Schering AG	1991, withdrawn	Air	Galactose microparticles	United Kingdom, Germany
Levovist	Schering AG	1995, withdrawn	Air	Galactose microparticles, palmitic acid	Europe, Canada, Japan, China

[1]Not approved by the Food and Drug Administration for any indication in the United States, but is approved for use in Japan, South Korea, Norway, and China for focal hepatic lesions depiction.

before administration[28]. The viscoelastic shell parameter values of 0.55 N/m for shell elasticity and 7.2 × 10^{-9} kg/s for dilational viscosity have been reported in the literature, which closely resemble the values of Definity[28,29].

SonoVue was approved for use in adult patients with suboptimal echocardiograms to opacify the LV chamber and improve the delineation of the LV endocardial border in China (2004) and Europe (2001)[29,30]. Lumason was subsequently approved in the United States (2014) for adult patients with suboptimal echocardiograms to improve delineation of the LV endocardial border[31]. The FDA approved it for characterizing focal hepatic lesions in adults and pediatric patients in 2016[30]. Lumason has also been recently approved for ultrasonography of the urinary tract for the evaluation of vesicoureteral reflux in pediatric patients in countries including the United States (2016), Europe (2017), and China (2018)[32,33].

Sonazoid

Sonazoid microspheres is a second-generation contrast agent approved in Japan, Norway, South Korea, Taiwan, and China for contrast-enhanced sonography of focal hepatic lesions[34,35]. It is compounded as a lyophilized powder for injection that consists of Perfluorobutane microspheres stabilized by a monomolecular membrane of hydrogenated egg yolk phosphatidyl serine[36]. The viscoelastic shell parameter values of 0.5-0.6 N/m for the shell elasticity and 1.2 × 10^{-8}-1.6 × 10^{-8} kg/s for dilational viscosity have been reported, with the dilational viscosity 2-3 times higher compared to SonoVue and Definity, respectively[36,37].

Sonazoid as a UCA in the liver produces two phases of contrast enhancement: A vascular phase followed by a Kupffer phase (post vascular phase)[38]. The normal liver parenchyma is enhanced during the post-vascular phase, and malignant lesions are visualized as clear contrast defects[38,39]. The pattern of vascular phase and post-vascular phase enhancements are used to better characterize focal hepatic lesions and detect the presence or absence of masses[38-40]. Recently, Sonazoid has been used off-label to detect sentinel lymph nodes in cancer[41].

SAFETY OF CONTRAST AGENTS

UCAs approved for clinical use are often well tolerated, and serious adverse effects are rarely observed[42]. Adverse reactions are minor and commonly include altered taste, sensation of heat, nausea, and headache, which are treated symptomatically[42]. In 2007, the FDA issued a "black box" warning with contraindications to several disease states, including ventricular arrhythmias, decompensated heart failure, acute myocardial infarction, among others, to several contrast agents due to four patient deaths and over 180 serious adverse events associated with UCA use[1,42]. Critics argued that there was no proof in relation to these adverse events and cited other reasons as an explanation. In 2008, the FDA deescalated the contraindications to warnings. Since then, safety studies have been done, particularly with Definity and Optison, which have demonstrated low rates of adverse events[43,44].

BUBBLE TO ULTRASOUND SIGNAL

Contrast agents induced changes in reflection pattern. They increase backscattered signals and appear nonlinear with increasing vibrational patterns when significant acoustic pressures are applied[1,2,44]. Differentiation of signal origin, whether UCA or tissue, is possible as tissue produces harmonic frequencies at a higher mechanical index[45]. With filter systems, several natural frequencies are received, allowing for a certain amount of background signal suppression[44,45]. High-pressure levels disrupt microbubbles and create nonlinear patterns that lead to ultrasound signals[1,43-45].

DISCUSSION

These contrast agents have achieved an established role in LV opacification and identification of the endocardial border [19]. When administered, UCAs can successfully yield LV ejection fraction and volume that correlate well with other imaging techniques, such as magnetic resonance imaging (MRI), in patients for whom the use of unenhanced imaging is technically difficult[19,46]. Additionally, UCAs have the ability to impact and alter disease management[47].

A large prospective cohort study by Kurt *et al*[48] evaluated the impact of UCAs on patient diagnosis and management. Over 600 patients with technically inadequate echocardiographic studies who received UCAs were enrolled. The quality of studies, estimated ejection fraction, number of LV segments visualized, presence of apical thrombus, and management decisions were compared before and after contrast. Results showed that technically difficult studies decreased from 86% to 9.8% ($P < 0.0001$), and uninterpretable studies declined from 11% to 0.3%. Before contrast, 11.6 +/- 3.3 of 17 LV segments were visualized, which improved to 16.8 +/-1.1 ($P < 0.0001$) after contrast. A major impact of UCAs on management was also observed, where additional diagnostic procedures were avoided in 32% of patients, and drug management was altered in 10% of patients. A cost-benefit analysis also showed an average saving of $122 per patient. The study revealed that contrast echocardiography improves endocardial visualization and diagnosis, resource utilization, and patient management. This was evident in both the reduction in the number of additional diagnostic procedures and in the drastic alteration in medical management.

UCAs possess the ability to limit unnecessary diagnostic imaging such as MRI, x-rays, and computed tomography scans to achieve an accurate diagnosis[49]. As a result, they can shorten hospitalization length, ultimately improving patient care and outcomes[49,50]. Conditions such as apical hypertrophic cardiomyopathy and cardiac masses, particularly ventricular thrombi, can be readily identified on contrast echo[51]. An accurate diagnosis saves time and potentially alters treatment decisions, which ultimately benefits the patient. In allowing fewer diagnostic tests to render a medical verdict, contrast echo provides added financial satisfaction to patients[50,52].

CONCLUSION

A compelling case can be made for using UCAs to depict the LV endocardial borders in patients undergoing echocardiography. They significantly improve diagnostic accuracy, reducing the need for additional imaging. Consequently, contrast echocardiography reduces both patient cost and healthcare burden. UCAs can also be used to accurately measure velocities from Doppler signal recordings, which may eliminate the need for cardiac catheterization. An additional case can be made for specialists to train in the use and interpretation of myocardial contrast echocardiography during stress and rest, which may equip the echocardiography laboratory with the ability to diagnose the presence of coronary artery disease in patients with normal regional LV function. Overall, UCAs can be used as a point of care tool to accurately assess myocardial disease, which may shorten hospitalization, reduce resource utilization, and improve patient care.

FOOTNOTES

Author contributions: English K wrote the original draft; English K contributed to conceptualization, writing, reviewing, and editing; English K read and approved the final version of the manuscript.

Conflict-of-interest statement: The author reports no relevant conflicts of interest for this article.

Country of origin: United States

ORCID number: Kevan English 0009-0006-8893-5696.

Corresponding Author's Membership in Professional Societies: American College of Physicians, No. 04437785.

S-Editor: Qu XL

L-Editor: A
P-Editor: Zhang YL

REFERENCES

1 **Ignee A**, Atkinson NS, Schuessler G, Dietrich CF. Ultrasound contrast agents. *Endosc Ultrasound* 2016; **5**: 355-362 [PMID: 27824024 DOI: 10.4103/2303-9027.193594]
2 **Calliada F**, Campani R, Bottinelli O, Bozzini A, Sommaruga MG. Ultrasound contrast agents: basic principles. *Eur J Radiol* 1998; **27** Suppl 2: S157-S160 [PMID: 9652516 DOI: 10.1016/s0720-048x(98)00057-6]
3 **Oglat AA**. A review of ultrasound contrast media. *F1000Res* 2023; **12**: 1444 [PMID: 38817410 DOI: 10.12688/f1000research.140131.2]
4 **Dietrich CF**, Averkiou M, Nielsen MB, Barr RG, Burns PN, Calliada F, Cantisani V, Choi B, Chammas MC, Clevert DA, Claudon M, Correas JM, Cui XW, Cosgrove D, D'Onofrio M, Dong Y, Eisenbrey J, Fontanilla T, Gilja OH, Ignee A, Jenssen C, Kono Y, Kudo M, Lassau N, Lyshchik A, Franca Meloni M, Moriyasu F, Nolsøe C, Piscaglia F, Radzina M, Saftoiu A, Sidhu PS, Sporea I, Schreiber-Dietrich D, Sirlin CB, Stanczak M, Weskott HP, Wilson SR, Willmann JK, Kim TK, Jang HJ, Vezeridis A, Westerway S. How to perform Contrast-Enhanced Ultrasound (CEUS). *Ultrasound Int Open* 2018; **4**: E2-E15 [PMID: 29423461 DOI: 10.1055/s-0043-123931]
5 **Schinkel AF**, Kaspar M, Staub D. Contrast-enhanced ultrasound: clinical applications in patients with atherosclerosis. *Int J Cardiovasc Imaging* 2016; **32**: 35-48 [PMID: 26206524 DOI: 10.1007/s10554-015-0713-z]
6 **Cotter B**, Raisinghani A, DeMaria AN. Established and emerging roles for ultrasound enhancing agents (contrast echocardiography). *Clin Cardiol* 2022; **45**: 1114-1122 [PMID: 36183366 DOI: 10.1002/clc.23924]
7 **Streeter JE**, Dayton PA. WE-C-218-01: Ultrasound Contrast Agents. *Med Phys* 2012; **39**: 3953 [PMID: 28520019 DOI: 10.1118/1.4736133]
8 **Eskandari M**, Monaghan M. Contrast echocardiography in daily clinical practice. *Herz* 2017; **42**: 271-278 [PMID: 28160033 DOI: 10.1007/s00059-017-4533-x]
9 **Motazedian P**, Marbach JA, Prosperi-Porta G, Parlow S, Di Santo P, Abdel-Razek O, Jung R, Bradford WB, Tsang M, Hyon M, Pacifici S, Mohanty S, Ramirez FD, Huggins GS, Simard T, Hon S, Hibbert B. Diagnostic accuracy of point-of-care ultrasound with artificial intelligence-assisted assessment of left ventricular ejection fraction. *NPJ Digit Med* 2023; **6**: 201 [PMID: 37898711 DOI: 10.1038/s41746-023-00945-1]
10 **Tang MX**, Mulvana H, Gauthier T, Lim AK, Cosgrove DO, Eckersley RJ, Stride E. Quantitative contrast-enhanced ultrasound imaging: a review of sources of variability. *Interface Focus* 2011; **1**: 520-539 [PMID: 22866229 DOI: 10.1098/rsfs.2011.0026]
11 **Paefgen V**, Doleschel D, Kiessling F. Evolution of contrast agents for ultrasound imaging and ultrasound-mediated drug delivery. *Front Pharmacol* 2015; **6**: 197 [PMID: 26441654 DOI: 10.3389/fphar.2015.00197]
12 **Clark LN**, Dittrich HC. Cardiac imaging using Optison. *Am J Cardiol* 2000; **86**: 14G-18G [PMID: 10997346 DOI: 10.1016/s0002-9149(00)00984-x]
13 **Hwang M**, Back SJ, Didier RA, Lorenz N, Morgan TA, Poznick L, Steffgen L, Sridharan A. Pediatric contrast-enhanced ultrasound: optimization of techniques and dosing. *Pediatr Radiol* 2021; **51**: 2147-2160 [PMID: 32955599 DOI: 10.1007/s00247-020-04812-z]
14 **Sridharan A**, Eisenbrey JR, Forsberg F, Lorenz N, Steffgen L, Ntoulia A. Ultrasound contrast agents: microbubbles made simple for the pediatric radiologist. *Pediatr Radiol* 2021; **51**: 2117-2127 [PMID: 34117892 DOI: 10.1007/s00247-021-05080-1]
15 **Coussios CC**, Holland CK, Jakubowska L, Huang SL, MacDonald RC, Nagaraj A, McPherson DD. In vitro characterization of liposomes and Optison by acoustic scattering at 3.5 MHz. *Ultrasound Med Biol* 2004; **30**: 181-190 [PMID: 14998670 DOI: 10.1016/j.ultrasmedbio.2003.10.015]
16 **Chen WS**, Matula TJ, Crum LA. The disappearance of ultrasound contrast bubbles: observations of bubble dissolution and cavitation nucleation. *Ultrasound Med Biol* 2002; **28**: 793-803 [PMID: 12113792 DOI: 10.1016/s0301-5629(02)00517-3]
17 **Cohen JL**, Cheirif J, Segar DS, Gillam LD, Gottdiener JS, Hausnerova E, Bruns DE. Improved left ventricular endocardial border delineation and opacification with OPTISON (FS069), a new echocardiographic contrast agent. Results of a phase III Multicenter Trial. *J Am Coll Cardiol* 1998; **32**: 746-752 [PMID: 9741522 DOI: 10.1016/s0735-1097(98)00311-8]
18 **Li P**, Armstrong WF, Miller DL. Impact of myocardial contrast echocardiography on vascular permeability: comparison of three different contrast agents. *Ultrasound Med Biol* 2004; **30**: 83-91 [PMID: 14962612 DOI: 10.1016/j.ultrasmedbio.2003.09.004]
19 **Kitzman DW**, Goldman ME, Gillam LD, Cohen JL, Aurigemma GP, Gottdiener JS. Efficacy and safety of the novel ultrasound contrast agent perflutren (definity) in patients with suboptimal baseline left ventricular echocardiographic images. *Am J Cardiol* 2000; **86**: 669-674 [PMID: 10980221 DOI: 10.1016/s0002-9149(00)01050-x]
20 **Mir T**, Uddin MM, Watson K, Meir EB, Abdo A. Definity, echo contrast, induced cardiac arrest: brief review of the literature. *BMJ Case Rep* 2021; **14** [PMID: 33875502 DOI: 10.1136/bcr-2020-240492]
21 **Salih M**, Ali SM, Jena N, Ananthasubramaniam K. Review of ultrasound contrast agents in current clinical practice with special focus on DEFINITY(®) in cardiac imaging. *Future Cardiol* 2021; **17**: 197-214 [PMID: 32897099 DOI: 10.2217/fca-2020-0049]
22 **Appis AW**, Tracy MJ, Feinstein SB. Update on the safety and efficacy of commercial ultrasound contrast agents in cardiac applications. *Echo Res Pract* 2015; **2**: R55-R62 [PMID: 26693339 DOI: 10.1530/ERP-15-0018]
23 **Shekhar H**, Smith NJ, Raymond JL, Holland CK. Effect of Temperature on the Size Distribution, Shell Properties, and Stability of Definity(®). *Ultrasound Med Biol* 2018; **44**: 434-446 [PMID: 29174045 DOI: 10.1016/j.ultrasmedbio.2017.09.021]
24 **Faez T**, Goertz D, De Jong N. Characterization of Definity™ ultrasound contrast agent at frequency range of 5-15 MHz. *Ultrasound Med Biol* 2011; **37**: 338-342 [PMID: 21257093 DOI: 10.1016/j.ultrasmedbio.2010.11.014]
25 **Lyshchik A**, Kono Y, Dietrich CF, Jang HJ, Kim TK, Piscaglia F, Vezeridis A, Willmann JK, Wilson SR. Contrast-enhanced ultrasound of the liver: technical and lexicon recommendations from the ACR CEUS LI-RADS working group. *Abdom Radiol (NY)* 2018; **43**: 861-879 [PMID: 29151131 DOI: 10.1007/s00261-017-1392-0]
26 **Westwood M**, Joore M, Grutters J, Redekop K, Armstrong N, Lee K, Gloy V, Raatz H, Misso K, Severens J, Kleijnen J. Contrast-enhanced ultrasound using SonoVue® (sulphur hexafluoride microbubbles) compared with contrast-enhanced computed tomography and contrast-enhanced magnetic resonance imaging for the characterisation of focal liver lesions and detection of liver metastases: a systematic review and cost-effectiveness analysis. *Health Technol Assess* 2013; **17**: 1-243 [PMID: 23611316 DOI: 10.3310/hta17160]

27 **Jingqi W**, Lu Z, Jun Z, Yuhong M, Wei Y, Lifeng R, Chengbing J, Dobromir DD, Hui Z, Kun Z. Clinical Usefulness of the Microbubble Contrast Agent SonoVue in Enhancing the Effects of High-Intensity Focused Ultrasound for the Treatment of Adenomyosis. *J Ultrasound Med* 2018; **37**: 2811-2819 [PMID: 29689636 DOI: 10.1002/jum.14638]

28 **Galema TW**, Geleijnse ML, Vletter WB, de Laat L, Michels M, Ten Cate FJ. Clinical usefulness of SonoVue contrast echocardiography: the Thoraxcentre experience. *Neth Heart J* 2007; **15**: 55-60 [PMID: 17612661 DOI: 10.1007/BF03085955]

29 **Gorce JM**, Arditi M, Schneider M. Influence of bubble size distribution on the echogenicity of ultrasound contrast agents: a study of SonoVue. *Invest Radiol* 2000; **35**: 661-671 [PMID: 11110302 DOI: 10.1097/00004424-200011000-00003]

30 **Zhang Q**, Liang X, Zhang Y, Nie H, Chen Z. A review of contrast-enhanced ultrasound using SonoVue® and Sonazoid™ in non-hepatic organs. *Eur J Radiol* 2023; **167**: 111060 [PMID: 37657380 DOI: 10.1016/j.ejrad.2023.111060]

31 **Barr RG**. Contrast enhanced ultrasound for focal liver lesions: how accurate is it? *Abdom Radiol (NY)* 2018; **43**: 1128-1133 [PMID: 28718042 DOI: 10.1007/s00261-017-1257-6]

32 **Velasquez M**, Emerson MG, Diaz E, Kennedy W, Rubesova E, Barth RA. The learning curve of contrast-enhanced 'microbubble' voiding urosonography-validation study. *J Pediatr Urol* 2019; **15**: 385.e1-385.e6 [PMID: 31133505 DOI: 10.1016/j.jpurol.2019.04.015]

33 **Filippone A**, Kirchin MA, Monteith J, Storto ML, Spinazzi A. Safety of Lumason® (SonoVue®) in special populations and critically ill patients. *Front Cardiovasc Med* 2023; **10**: 1225654 [PMID: 37600063 DOI: 10.3389/fcvm.2023.1225654]

34 **Maruyama H**, Sekimoto T, Yokosuka O. Role of contrast-enhanced ultrasonography with Sonazoid for hepatocellular carcinoma: evidence from a 10-year experience. *J Gastroenterol* 2016; **51**: 421-433 [PMID: 26694825 DOI: 10.1007/s00535-015-1151-3]

35 **Yao J**, Li K, Yang H, Lu S, Ding H, Luo Y, Li K, Xie X, Wu W, Jing X, Liu F, Yu J, Cheng Z, Tan S, Dou J, Dong X, Wang S, Zhang Y, Li Y, Qi E, Han Z, Liang P, Yu X. Analysis of Sonazoid contrast-enhanced ultrasound for predicting the risk of microvascular invasion in hepatocellular carcinoma: a prospective multicenter study. *Eur Radiol* 2023; **33**: 7066-7076 [PMID: 37115213 DOI: 10.1007/s00330-023-09656-3]

36 **Hwang JA**, Jeong WK, Min JH, Kim YY, Heo NH, Lim HK. Sonazoid-enhanced ultrasonography: comparison with CT/MRI Liver Imaging Reporting and Data System in patients with suspected hepatocellular carcinoma. *Ultrasonography* 2021; **40**: 486-498 [PMID: 33745266 DOI: 10.14366/usg.20120]

37 **Sontum PC**. Physicochemical characteristics of Sonazoid, a new contrast agent for ultrasound imaging. *Ultrasound Med Biol* 2008; **34**: 824-833 [PMID: 18255220 DOI: 10.1016/j.ultrasmedbio.2007.11.006]

38 **Jeong WK**. Diagnosis of hepatocellular carcinoma using Sonazoid: a comprehensive review. *J Liver Cancer* 2023; **23**: 272-283 [PMID: 37723641 DOI: 10.17998/jlc.2023.08.25]

39 **Inoue T**, Hyodo T, Korenaga K, Murakami T, Imai Y, Higaki A, Suda T, Takano T, Miyoshi K, Koda M, Tanaka H, Iijima H, Ochi H, Hirooka M, Numata K, Kudo M. Kupffer phase image of Sonazoid-enhanced US is useful in predicting a hypervascularization of non-hypervascular hypointense hepatic lesions detected on Gd-EOB-DTPA-enhanced MRI: a multicenter retrospective study. *J Gastroenterol* 2016; **51**: 144-152 [PMID: 26373860 DOI: 10.1007/s00535-015-1094-8]

40 **Kang HJ**, Lee JM, Yoon JH, Yoo J, Choi Y, Joo I, Han JK. Sonazoid™ versus SonoVue(®) for Diagnosing Hepatocellular Carcinoma Using Contrast-Enhanced Ultrasound in At-Risk Individuals: A Prospective, Single-Center, Intraindividual, Noninferiority Study. *Korean J Radiol* 2022; **23**: 1067-1077 [PMID: 36196767 DOI: 10.3348/kjr.2022.0388]

41 **Hao Y**, Sun Y, Lei Y, Zhao H, Cui L. Percutaneous Sonazoid-enhanced ultrasonography combined with in vitro verification for detection and characterization of sentinel lymph nodes in early breast cancer. *Eur Radiol* 2021; **31**: 5894-5901 [PMID: 33502555 DOI: 10.1007/s00330-020-07639-2]

42 **Jakobsen JA**, Oyen R, Thomsen HS, Morcos SK; Members of Contrast Media Safety Committee of European Society of Urogenital Radiology (ESUR). Safety of ultrasound contrast agents. *Eur Radiol* 2005; **15**: 941-945 [PMID: 15662495 DOI: 10.1007/s00330-004-2601-0]

43 **Aggeli C**, Giannopoulos G, Roussakis G, Christoforatou E, Marinos G, Toli C, Pitsavos C, Stefanadis C. Safety of myocardial flash-contrast echocardiography in combination with dobutamine stress testing for the detection of ischaemia in 5250 studies. *Heart* 2008; **94**: 1571-1577 [PMID: 18474538 DOI: 10.1136/hrt.2007.135145]

44 **Main ML**, Ryan AC, Davis TE, Albano MP, Kusnetzky LL, Hibberd M. Acute mortality in hospitalized patients undergoing echocardiography with and without an ultrasound contrast agent (multicenter registry results in 4,300,966 consecutive patients). *Am J Cardiol* 2008; **102**: 1742-1746 [PMID: 19064035 DOI: 10.1016/j.amjcard.2008.08.019]

45 **Şen T**, Tüfekçioğlu O, Koza Y. Mechanical index. *Anatol J Cardiol* 2015; **15**: 334-336 [PMID: 25880292 DOI: 10.5152/akd.2015.6061]

46 **Frinking P**, Segers T, Luan Y, Tranquart F. Three Decades of Ultrasound Contrast Agents: A Review of the Past, Present and Future Improvements. *Ultrasound Med Biol* 2020; **46**: 892-908 [PMID: 31941587 DOI: 10.1016/j.ultrasmedbio.2019.12.008]

47 **Tarighatnia A**, Fouladi MR, Nader ND, Aghanejad A, Ghadiri H. Recent trends of contrast agents in ultrasound imaging: a review of the classifications and applications. *Mater adv* 2022; **3**: 3726-3741 [DOI: 10.1039/D1MA00969A]

48 **Kurt M**, Shaikh KA, Peterson L, Kurrelmeyer KM, Shah G, Nagueh SF, Fromm R, Quinones MA, Zoghbi WA. Impact of contrast echocardiography on evaluation of ventricular function and clinical management in a large prospective cohort. *J Am Coll Cardiol* 2009; **53**: 802-810 [PMID: 19245974 DOI: 10.1016/j.jacc.2009.01.005]

49 **El Kadi S**, Porter TR, Verouden NJW, van Rossum AC, Kamp O. Contrast Ultrasound, Sonothrombolysis and Sonoperfusion in Cardiovascular Disease: Shifting to Theragnostic Clinical Trials. *JACC Cardiovasc Imaging* 2022; **15**: 345-360 [PMID: 34656483 DOI: 10.1016/j.jcmg.2021.07.028]

50 **Fraiche AM**, Strom JB. Impact of ultrasound enhancing agents on clinical management. *Curr Opin Cardiol* 2022; **37**: 389-393 [PMID: 35913366 DOI: 10.1097/HCO.0000000000000973]

51 **Bois JP**, Ayoub C, Geske JB, Wong YW, Abbasi MA, Foley TA, Mulvagh SL, Scott CG, Ommen SR, Pellikka PA. Ultrasound Enhancing Agents with Transthoracic Echocardiography for Maximal Wall Thickness in Hypertrophic Cardiomyopathy. *Mayo Clin Proc Innov Qual Outcomes* 2023; **7**: 309-319 [PMID: 37502339 DOI: 10.1016/j.mayocpiqo.2023.06.002]

52 **Chai SC**, Tan PJ, Tong KL. A review of the safety and clinical utility of contrast echocardiography. *Singapore Med J* 2020; **61**: 181-183 [PMID: 31820006 DOI: 10.11622/smedj.2019169]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 102709

DOI: 10.5662/wjm.v15.i3.102709

ISSN 2222-0682 (online)

REVIEW

Biological and translational attributes of mitochondrial DNA copy number: Laboratory perspective to clinical relevance

Deepak Parchwani, Ragini Singh, Digisha Patel

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade C, Grade C, Grade C

Novelty: Grade B, Grade B, Grade B

Creativity or Innovation: Grade B, Grade B, Grade B

Scientific Significance: Grade B, Grade C, Grade C

P-Reviewer: Anas M; Chhetri KB; Ebrahim NAA

Received: October 28, 2024

Revised: January 21, 2025

Accepted: February 8, 2025

Published online: September 20, 2025

Processing time: 131 Days and 2.8 Hours

Deepak Parchwani, Ragini Singh, Department of Biochemistry, All India Institute of Medical Sciences, Rajkot 360001, India

Digisha Patel, Department of Physiology, Shantabaa Medical College and General Hospital Amreli, Amreli 365601, Gujarāt, India

Corresponding author: Deepak Parchwani, PhD, Professor, Department of Biochemistry, All India Institute of Medical Sciences, AIIMS Rajkot, Village Khandheri, Tehsil Paddhari, Rajkot 360001, India. drdeepakparchwani@yahoo.com

Abstract

The mitochondrial DNA copy number (mtDNAcn) plays a vital role in cellular energy metabolism and mitochondrial health. As mitochondria are responsible for adenosine triphosphate production through oxidative phosphorylation, maintaining an appropriate mtDNAcn level is vital for the overall cellular function. Alterations in mtDNAcn have been linked to various diseases, including neurodegenerative disorders, metabolic conditions, and cancers, making it an important biomarker for understanding the disease pathogenesis. The accurate estimation of mtDNAcn is essential for clinical applications. Quantitative polymerase chain reaction and next-generation sequencing are commonly employed techniques with distinct advantages and limitations. Clinically, mtDNAcn serves as a valuable indicator for early diagnosis, disease progression, and treatment response. For instance, in oncology, elevated mtDNAcn levels in blood samples are associated with tumor aggressiveness and can aid in monitoring treatment efficacy. In neurodegenerative diseases such as Alzheimer's and Parkinson's, altered mtDNAcn patterns provide insights into disease mechanisms and progression. Understanding and estimating mtDNAcn are critical for advancing diagnostic and therapeutic strategies in various medical fields. As research continues to uncover the implications of mtDNAcn alterations, its potential as a clinical biomarker is likely to expand, thereby enhancing our ability to diagnose and manage complex diseases.

Key Words: Mitochondrial DNA copy number; Mitochondrial DNA; Quantitative polymerase chain reaction; Cancer; Neurodegenerative disease; Aging

Core Tip: This review highlights the critical biological and translational significance of mitochondrial DNA (mtDNA) copy number (mtDNAcn) variations and emphasizes their implications in both laboratory diagnostics and clinical settings. By exploring the mechanisms underlying mtDNA replication, the association of mtDNAcn with various diseases, and its potential as a biomarker for mitochondrial dysfunction, we underscore the need for standardized methodologies for measuring mtDNAcn. This perspective aims to bridge the gap between basic research and clinical applications by facilitating the integration of mtDNAcn assessments into routine diagnostic practices and therapeutic strategies for mitochondria-related diseases.

Citation: Parchwani D, Singh R, Patel D. Biological and translational attributes of mitochondrial DNA copy number: Laboratory perspective to clinical relevance. *World J Methodol* 2025; 15(3): 102709
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/102709.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.102709

INTRODUCTION

Mitochondrial DNA and mitochondrial DNA copy number

Mitochondria are essential organelles found in almost all cells, and are pivotal energy generators in mammalian cells. Endowed with their own genomes, referred to as mitochondrial DNA (mtDNA), mammalian cells maintain hundreds to thousands of mtDNA molecules per cell[1]. Mitochondria are organelles that reside in the cytoplasm of eukaryotic cells and are involved in energy production *via* oxidative phosphorylation (OXPHOS), which utilizes oxygen and nutrients to produce adenosine triphosphate (ATP) accompanied by harmful byproducts of reactive oxygen species[2]. Mitochondria also play central roles in various cellular processes such as apoptosis, calcium homeostasis, and reactive oxygen species metabolism[1,2]. OXPHOS is orchestrated by four large complexes embedded in the mitochondrial inner membrane, and defects in OXPHOS are associated with mitochondrial diseases. mtDNA copy number (mtDNAcn) indicates the relative abundance of mtDNA relative to the total amount of nuclear DNA (nDNA) in the genome, is an essential indicator of mitochondrial health and integrity, and has been suggested as a potential disease biomarker as it affects mitochondrial biogenesis and cellular oxidative stress[1,3,4].

Mitochondrial biogenesis is orchestrated by the concerted expression of nuclear and mitochondrial genes, which is regulated by various transcription factors, cofactors, and coactivators. Key transcription factors involved in the regulation of mitochondrial biogenesis include peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), nuclear respiratory factors (NRFs), and mitochondrial transcription factor A (TFAM)[3,5]. PGC-1α is a coactivator that controls the expression of mitochondrial and nuclear genes, thereby regulating mitochondrial biogenesis, energy substrate uptake, and OXPHOS activity in different tissues. PGC-1α activity is regulated by various signaling pathways that are activated in response to changes in cellular energy demand (*e.g.*, exercise, cold exposure, and starvation). After activation, PGC-1α increases mitochondrial biogenesis by promoting the expression of NRFs and TFAM, mitochondrial dynamics and motility, and the mitochondrial quality control system[5,6]. Experimental overexpression of PGC-1α has been associated with increased mitochondrial biogenesis and proliferation, whereas PGC-1α downregulation is associated with mitochondrial dysfunction, decreased mitochondrial biogenesis, and several pathologies[5]. However, in different tissues, mtDNAcn may also be affected by factors such as cell size, oxygen consumption, energy expenditure, and age[2,5]. Understanding the biological attributes of mtDNAcn will provide insights into the pathogenesis of various diseases.

mtDNA is a circular genome containing 16569 base pairs (15-17 kb in length), is characterized by covalent closed-loop structures, and is commonly present in multiple copies per mitochondrion[7,8]. The number of mtDNA per cell varies according to tissue specificity, ranging from several hundred in non-energy-demanding tissues, such as fibroblasts, to thousands in high-energy-demanding tissues, such as the heart, skeletal muscle, and brain. mtDNA is thought to encode 37 genes necessary for the correct functioning of mitochondria, including 13 proteins of the OXPHOS system, two ribosomal RNAs, and 22 transfer RNAs[7,9]. Among the 13 polypeptides encoded by mtDNA, 12 are subunits of OXPHOS complexes I, III, IV, and V, and awareness of their importance stems from genetic mutations that lead to mitochondrial disorders, which can affect post-mitotic cells rich in mitochondria, such as neurons, cardiac muscle, and skeletal muscle[9,10]. Reversible lesions within mtDNA have been implicated in a variety of disorders, including oxidative stress, energetics, and signaling dysregulations[10-12]. Accordingly, mtDNA alterations can differentially influence cellular homeostasis and behavior, facilitating either adaptation or sensitivity to external perturbations. Indeed, numerous studies have indicated that mitochondrial biogenesis is associated with an increased mtDNAcn, and a decrease in mtDNAcn correlates with mitochondrial dysfunction[10-14]. Notably, several nuclear-encoded genes are involved in the regulation of mtDNAcn, suggesting that the mtDNAcn is regulated by a highly coordinated process. Since ATP synthesis is linked to mitochondrial respiration, which involves the electron and proton gradients of the respiratory chain, these two processes are also involved in mtDNA replication, which regulates the number of mtDNA copies (Table 1)[10,13].

Alterations in mtDNAcn have been observed in aging and various disorders, including neurodegenerative, inflammatory, metabolic, and oncogenic diseases[10-14]. Aberrant changes in mtDNAcn are also believed to serve as potential biomarkers for early diagnosis of these diseases. It has been shown that mtDNAcn increases in a large number of human cancers, regardless of histological type, although the extent and distribution of mtDNAcn varies across tumors[15]. The

Table 1 Key genes regulating mtDNA copy number

Gene	Role in mtDNA regulation	Associated disorders	Ref.
PGC-1α	Master regulator of mitochondrial biogenesis	Neurodegeneration, cancer, metabolic syndrome	[3,5,6]
TFAM	Maintains mtDNA integrity and replication	Mitochondrial diseases, aging	[5,9]
POLγ	Essential for mtDNA replication	MELAS, Kearns-Sayre syndrome, cancer	[88-90]
NRFs (1 and 2)	Coordinate expression of mitochondrial genes	Neurodegeneration, metabolic disorders	[3,5]

mtDNA: Mitochondrial DNA; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; TFAM: Mitochondrial transcription factor A; POLγ: Polymerase gamma; NRFs: Nuclear respiratory factors; MELAS: Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes.

first mtDNA sequence decoded in humans in 1981 led to the discovery of more than 150-point mutations in the genes encoded by mtDNA in patients with Leber's hereditary optic neuropathy (LHON) and several large-scale deletions and duplications, mainly affecting mitochondrial coding genes and generating external frameshift mutations associated with mitochondrial myopathy and multiple mitochondrial dysfunction syndromes[15,16].

Unlike conventional nDNA quantification, mtDNA detection is based on polymerase chain reaction (PCR) amplification of a unique region of mtDNA, avoiding interference by nDNA[4-6,15]. Assessment of mtDNA and its structural alterations is emerging as a viable detection tool for various biological molecules, including cytosolic exosomes, body fluids, tissues, saliva, urine, and hair. The quantification of mtDNA content is underscored by the promise of a deeper understanding of cellular senescence, age-related cellular dysfunction, epigenetic regulation, tumorigenesis, and pharmacology. The emerging literature corroborates the correlation between mtDNAcn and its structural alterations (deletion and rearrangement) with the clinical progression of cancer, metabolic, and degenerative diseases. Studies have also suggested that alterations in mtDNAcn may be the earliest events in the progression of chronic diseases[4,12,15]. The modulation of mtDNAcn raises the possibility of a plausible contribution of mtDNAcn to cellular dysfunction, fate, and pathogenesis. Since mtDNAcn can be analyzed through non-invasive detection of peripheral blood, exploring the alterations of mtDNAcn in bodily cells and body fluids holds great promise for better understanding and improvement of the aforementioned diseases. Therefore, a comprehensive understanding of mtDNAcn regulation in cells is important for biomedical studies and has been substantiated. Recent studies have highlighted the clinical relevance of mtDNAcn in early diagnosis, prognosis of therapeutic intervention, and detection of disease recurrence.

Despite extensive research on mitochondrial function, the precise mechanisms regulating mtDNAcn and their quantification remain incompletely understood. Specifically, while various nuclear-encoded genes and transcription factors, such as PGC-1α, TFAM, and NRFs, have been identified as key regulators of mitochondrial biogenesis, significant gaps persist in the understanding of the intricate coordination of these factors in different tissues and the variability in mtDNAcn across tissues, its dynamic response to cellular energy demands, and its role in the pathogenesis of diverse diseases highlights a complex regulatory network that warrants further investigation. For example, the interplay between oxidative stress, mitochondrial dynamics, and mtDNA replication processes remains poorly characterized, particularly in the context of aging and chronic diseases[10,12]. These knowledge gaps underline the necessity of studying mtDNAcn as a potential biomarker of mitochondrial dysfunction. Addressing these unanswered questions could lead to improved diagnostic and therapeutic strategies, particularly for diseases for which mitochondrial health is critical.

METHODS FOR ASSESSING mtDNAcn

Methods for measuring mtDNAcn have proliferated alongside the growing application of mtDNA analysis. Because of the biased segregation of mtDNA mutations during cell division, some mutations in the mitochondrial genome become homoplasmic (existing in all copies) and persist at high copy numbers, while others remain heteroplasmic (existing only in a subset of copies) and diminish with time, indicative of cellular dysfunction[17,18]. All DNA in a diploid cell is referred to as its nuclear-equivalent DNA content, encompassing both nuclear and mitochondrial genomes[8,11,17]. Furthermore, all mtDNA in a diploid cell is referred to as its mtDNA content, which is directly measurable *via* quantitative PCR (qPCR) tests and other techniques[15,17,18].

Most methods for measuring mtDNAcn utilize qPCR in a real-time thermal cycler[19,20]. The assay can be performed using a two-target reference gene approach, normalized to one of the nuclear target genes, such as β-actin, or a single-target approach, where data are compared to a standard curve generated with known quantities of mtDNA input[19,20]. There is an implicit assumption that mtDNA and nDNA are under equal amplification conditions because of the use of the same PCR primer sets and reagents, with the difference being the number of target sequences[19,20]. Targeting and amplifying nDNA segments present in a chromosome with unequal pooling of homologs would confound any calculated ratio under equal amplification conditions. Although qPCR is the most common method employed, it has several caveats, particularly in the assessment of tissues with low-quality DNA, experiments with lower DNA concentrations, and heterogeneous samples. DNA preparation is also a rich source of systematic bias, and increased effort is needed to ensure that DNA samples are free of bias prior to mtDNAcn quantification[14,19,20].

Next-generation sequencing (NGS) platforms allow pyrosequencing of millions of individually sequenced DNA fragments in parallel[21]. NGS is a promising alternative mtDNAcn assessment tool that requires post-sequencing bioinformatic approaches that are currently either non-robust or non-widely adopted[8,14,21]. The design and optimization of the initial NGS experiments largely dictate read lengths and yields, amplicon sizes, primer dilution, PCR iterations, amplification conditions, and DNA concentrations in subsequent reactions using the same amplification primers for multiplexing, ProSEQ, resetting, and incorporating barcodes/indexes[20,21].

From a historical perspective, the southern blot (SB) hybridization has long been the gold standard for assessing the level and integrity of mtDNA in patient samples and model organisms[22]. Although SB techniques are highly reliable for determining mtDNA content, they are time-consuming, only semi-quantitative, and require a relatively large amount of DNA, which presents a significant drawback when studying human tissues[22]. Fluorescent in situ hybridization has also been used to spatially visualize mtDNA content with single-cell resolution; however, this method is only partially informative[23]. Its multi-step protocol is laborious and offers only a rough estimate of the changes in mtDNA levels.

Assessing mtDNAcn using qPCR

qPCR is the most widely used method for analyzing mtDNAcn[14,24]. It can be optimized to assess mtDNAcn in a high-throughput format, sensitive to low mitochondrial sample input, and conducive to sample pooling[24,25]. However, qPCR relies solely on specific DNA sequences. Thus, experimental design requires careful consideration and testing of qPCR primers to ensure robust, reproducible, and specific measurements, with low inter- and intra-assay variance being the most critical quality control step in this approach[24-26]. Traditional qPCR quantification methods typically analyze mtDNA and nDNA using a single primer set in two steps: The standard curve and the mtDNA/nDNA ratio[24,25]. However, for heterologous sample preparations containing nDNA genes homologous to mtDNA, this analysis will give rise to very large quantification errors in determining mtDNAcn[25-27]. This is attributed to the inability to monitor assay conditions and amplify efficiency with respect to specific sample preparation. As a result, such methods often lead to inconsistent data on mtDNAcns for the same sample preparation analyzed by different laboratories[25]. Quantification algorithms need more caution for qPCR assays to measure mtDNA-nDNA ratios and mtDNAcns, especially in samples containing *nDNA* genes with significant sequence similarity to mtDNA[26].

For sample preparation, all experiments must be performed while working on ice or at cold temperatures within a pre-cooled mini-cooled PCR cycler to prevent the unwarranted loss of fast-evolving wild-type genes[24-27]. The initial pre-preparation for the qPCR assay must avoid freezing and thawing of samples and resuspend all ultralow sample concentrations gently to produce a homogeneous distribution[24]. A low pipetting force should be used to prevent mtDNA shearing. As sample dilution and pooling may introduce batch effects and quenching of the qPCR signal, all samples to be pooled should be evenly split to avoid overconcentration of the same samples in specific wells[25,26]. An individual assay for each sample is advised for samples anticipated to fall below measurable concentrations.

mtDNAcn analysis using qPCR can be performed in either SYBR Green or TaqMan interrogation format, both of which target specific amplicon sequences[26-28]. The SYBR Green assay relies on the intercalation of a fluorescent dye into dsDNA, as it is amplified[27]. TaqMan assay is dependent on an oligonucleotide probe labeled with a fluorescent dye and a quencher dye. As amplification occurs, the 5′ nuclease activity of Taq polymerase cleaves the probe, releasing a fluorescent signal that is detected and proportional to the amount of PCR product generated[24,28]. When the experimental design requires the use of multiple primer pairs in the same plate layout and run, controls must be included on the same plate for each primer pair, including Q5 Hot Start High-Fidelity DNA Polymerase as a positive control, to verify the performance of the primer pairs used[26,27]. Compounds and probes should be included to test their effects on the protocol. This design allows for a validity check of the batch size. Preferably, qPCR analysis should be repeated for different batch sizes in a high-throughput qPCR setup[24-28].

Assessing mtDNAcn using NGS

Complementary to qPCR methods, NGS has been developed to quantify mtDNAcn in a single run in a genome-wide manner without prior knowledge of mitochondrial sequences[28]. Using read-depth normalization strategies, NGS data can be used to measure the total and/or specific mtDNAcn changes[28]. Furthermore, their integration with existing whole-genome sequencing and whole-exome sequencing (WES) is enabled by the almost universal implementation of NGS in most laboratories that perform large-scale genome analysis[28-30].

The process begins with the extraction of total DNA, including both nDNA and mtDNA, from cells or tissues. The next step involves library preparation, wherein the extracted DNA is fragmented into smaller pieces to facilitate efficient sequencing[28-30]. Sequencing adapters, which are short pieces of synthetic DNA, are attached to the fragmented DNA to allow binding to the sequencing platform. This step is critical for ensuring that DNA fragments can be correctly amplified and sequenced. During sequencing, both nuclear and mitochondrial genomes were sequenced simultaneously, providing a comprehensive overview of the entire cellular DNA content. The sequencing platform generates millions of reads, which are short DNA sequences that correspond to fragments of both nDNA and mtDNA. Once the sequencing data are generated, bioinformatics tools are used to align the reads to their respective reference genomes, allowing for precise identification of whether the reads originate from the nuclear genome or the mitochondrial genome[28-32].

The number of reads mapped to the mitochondrial genome was compared to the number of reads mapped to the nuclear genome[29,30]. Because the nuclear genome is present in two copies per diploid cell, this comparison allows for the normalization and accurate calculation of the mtDNAcn per cell[29,31]. The formula used for this calculation typically normalizes the number of mtDNA reads to the size of the mitochondrial genome and the number of nDNA reads to the ploidy of the nuclear genome[32]. This allows for the estimation of the number of copies of the mitochondrial genome present in each cell. Given that the nuclear genome is usually constant in copy number across cells, any variation in the ratio of mtDNA to nDNA reads reflects changes in mitochondrial content, providing insights into mitochondrial

biogenesis or degradation under different physiological or pathological conditions[30-33].

The diversity of experimental designs remains a potential source of bias, even in strictly controlled laboratory environments. Molecular experiments should be reported with the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines in mind, which would cover both laboratory knowledge and actively adopted best practices. Ideally, all design-specific factors should be reported so that all procedures leading to the normalization and quantification of results are transparent and open to independent validation.

For mtDNAcn assessment, technical considerations include qPCR-specific issues related to reaction volume, number of replicates or parallel reactions, reaction mixtures (acid/base ratio affecting amplification efficiency) leading to stricter biochemical requirements for samples, and reliance on qPCR properties, which can differ among thermocyclers. Systematic biases stemming from qPCR chemistry and thermocycling conditions are classically solved through plate-like replication of controls (standard/reference/sample) at a ratio of at least 1/8, flat-lined volumetric neutrality (classically 20 μL reaction volume), baseline filtering, and standard curve correction[34]. Controversies remain regarding the use of synthetic amplification additives (*e.g.*, surfactants) that act as smoothing agents to flatten amplification results[34]. Environmental sources that directly impact laboratory design are unavoidable (*e.g.*, geographical location, surrounding industry, and building architecture) (Table 2).

mtDNAcn IN HEALTH AND DISEASE

Changes in mtDNAcn can lead to, or be a consequence of, mitochondrial dysfunction. mtDNA deletions and depletion (mtDNA deletions refer to the loss of a specific segment of the mtDNA molecule that results in a shortened mtDNA molecule that lacks certain genes or regulatory regions, and mtDNA depletion refers to a significant reduction in the overall copy number of mtDNA within cells or tissues, while the remaining mtDNA molecules remain intact and structurally normal), and mutations can occur as a result of damage from free radicals, ionizing radiation, chemotherapeutic agents, or toxins[4,8,9,15]. Accumulation of these mutations can lead to mitochondrial dysfunction and energy deficiency[2,4,15]. This can be compensated, in part, by an increase in mtDNAcn; however, it is hypothesized that there will be a deleterious cumulative effect on function and health.

Recent studies have linked alterations in mtDNAcn to a number of human disorders, such as neurodegenerative diseases [*e.g.*, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease], diabetes, Fanconi's anemia, diseases of the skeletal muscle (*e.g.*, mitochondrial myopathy, congenital defects like Pearson's syndrome), cardiomyopathies, non-syndromic deafness, certain cancer types (*e.g.*, breast, colorectal, endometrial, gastric, annular pancreas, gliomas, prostate, cervical, bladder carcinoma), and complex diseases (like prion diseases)[7,15]. Trimethylation of histone H3 lysine 9 has been implicated in the silencing of promoters of nuclear-encoded respiratory complex subunit genes, resulting in a gradual increase in mtDNAcn in response to mitochondrial dysfunction in animal models[35]. In contrast, hypomethylation of H3 lysine 4 chromatin marks at the Pol I transcription start sites was shown to be involved in the switch of the mtDNA replication mechanism from legacy to prominent mode in metazoan species[35]. The mtDNAcn varies widely among cell types[15], and a common medical interest is to determine the mtDNAcn in cells for diagnostic or prognostic purposes. With aging, somatic mutations accumulate in mtDNA, and a gradual decrease in the relative number of full-length mtDNA is observed in various organs of older mice, suggesting an age-dependent decline in mitochondrial function, substantiated by an altered mtDNAcn in the tissues or blood of aging animals or humans[4,15]. Cell-free mtDNA has recently attracted attention, especially because of its potential use as a non-invasive biomarker for both mitochondrial dysfunction and cancer.

Etiology of mitochondrial mutations

Mutations in mtDNA can arise either from spontaneous errors during DNA replication or from incorrect repair of damaged DNA bases. Sequence analyses and experimental studies involving a large number of mtDNA genomes indicate that most mtDNA mutations result from spontaneous replication errors introduced by DNA polymerase gamma (POLγ) during mtDNA synthesis[36,37]. These pathogenic mtDNA mutations are commonly found in mitochondrial tRNA, rRNA, and protein-coding genes, leading to compromised mitochondrial gene expression and varying degrees of OXPHOS deficiency. Owing to the multicopy nature of the mitochondrial genome, mtDNA mutations may be homoplasmic or heteroplasmic. Many severe mtDNA mutations cannot be tolerated in the homoplasmic state, which makes their presence in the heteroplasmic state more common in patients. Typically, heteroplasmic pathogenic mtDNA mutations exhibit a functionally recessive nature, meaning that they only cause respiratory chain deficiencies when present above a certain threshold level[38]. Consequently, cells with mutated mtDNA levels exceeding this critical threshold develop respiratory chain dysfunction, whereas adjacent cells with lower mutation loads can maintain normal respiratory chain function. The threshold level is highly tissue-dependent and varies with the type of mtDNA mutation; for example, the threshold for single large deletions is approximately 50%-60%[39], while some tRNA gene point mutations may exceed 90% mutated mtDNA[39,40]. Throughout an individual's life, levels of heteroplasmy can fluctuate owing to mitotic segregation, a result of relaxed replication, and random mitochondrial partitioning between daughter cells. Additionally, deleterious mtDNA alleles may be actively eliminated in proliferating tissues through purifying selection, a phenomenon observed in the blood for certain mtDNA mutations[40-42]. It is well established that point mutations in tRNA genes or single large deletions in mtDNA lead to functional impairments or deficiencies in one or more tRNAs. However, these pathogenic mtDNA mutations typically lack dominant effects, allowing tolerance to high levels of mutated mtDNA. The recessive nature of most human pathogenic mtDNA mutations results in a deficiency of wild-type gene products, impairing OXPHOS, and contributing to disease phenotypes. Beyond their role as direct causes

Table 2 Methods for assessing mitochondrial DNA copy number

Method	Steps involved	Applications	Advantages	Limitations	Ref.
qPCR	DNA extraction → primer design → amplification → analysis	Widely used in clinical and research diagnostics	High sensitivity, high throughput, cost-effective, rapid	Susceptible to bias in low-quality DNA, issues with heterogeneous samples, requires careful primer design	[15, 24-26]
NGS	DNA extraction → library preparation → sequencing → bioinformatics analysis	Genome-wide studies, detects mtDNA mutations alongside copy number analysis	Genome-wide analysis, accurate quantification, detects mtDNA heteroplasmy	Requires advanced bioinformatics, high cost, computational complexity	[21, 28-32]
Southern blot hybridization	DNA extraction → gel electrophoresis → hybridization → quantification	Historically the gold standard, reliable for mtDNA integrity assessment	High reliability, detects large-scale deletions	Time-intensive, requires large DNA quantities, semi-quantitative	[22]
FISH	Sample preparation → probe hybridization → microscopy	Single-cell resolution studies, spatial visualization of mtDNA	Single-cell resolution, visualizes mtDNA distribution	Labor-intensive, provides only rough mtDNA estimates, technically demanding	[23]

qPCR: Quantitative polymerase chain reaction; NGS: Next-generation sequencing; FISH: Fluorescent in situ hybridization; mtDNA: Mitochondrial DNA.

of primary mitochondrial diseases, which are characterized by severe mitochondrial impairment in multiple tissues[42,43], mtDNA mutations have also been implicated in the pathophysiology of common age-associated diseases[15,44-46] and in the natural aging process[15].

Aging

The mtDNAcn during aging has been assessed in most tissues from humans to rodents. However, currently, there is no consensus regarding the relevance of mtDNAcn analysis. The impact of mtDNAcn changes on different tissues may not be equivalent. In a seminal paper, mtDNAcn was shown to decrease with age in the brain, heart, liver, skeletal muscle, and blood of humans[47,48] but increased in the skin, adipose tissue, peripheral blood mononuclear cells, and urine[15,49]. mtDNAcn levels were increased in the lymphocytes of TWAY mice, a model for human aging and age-related pathologies. Transient decreases in mtDNAcn were observed during early postnatal development in the brain, heart, liver, and skeletal muscle but not in the skin[50].

The amount of mtDNA in the tissues of people of various ages has been measured in numerous investigations[8,15]. During aging, mtDNA levels vary among different tissues. In lymphocytes and blood, there is a notable decrease in the mtDNAcn, as revealed by whole-genome sequencing and qPCR[51,52]. This decline in mtDNA has also been observed in skeletal muscle, where one study using NGS and digital droplet PCR reported a reduction[49]. However, other investigations in skeletal muscle using qPCR and southern blotting have shown that mtDNA levels remain unchanged[47,48]. Similarly, the heart showed no significant change in mtDNA levels, as confirmed by qPCR and SB analyses[47,48].

In contrast, certain tissues display increased mtDNA levels with age. For example, the liver exhibits elevated mtDNA content, based on NGS and digital droplet PCR data[49]. Likewise, mtDNA levels are higher in the substantia nigra (SN), as determined by qPCR[53]. Interestingly, some brain regions, such as the caudate nucleus, frontal lobe cortex, and cerebellar cortex, show no change in mtDNA levels during aging, as evidenced by SB analysis[47]. These findings suggest a complex and tissue-specific pattern of mtDNA regulation during the aging process, with some tissues experiencing a decline, others remaining stable, and some exhibiting increased mitochondrial content.

A significant reduction in mtDNAcn was observed in blood samples, with the decline becoming more noticeable in individuals in their 50 seconds. This decrease in mtDNA levels intensifies with age, particularly in older populations where a more dramatic reduction is evident[54,55]. The mtDNAcn has been estimated to decrease by a few percent per decade[56]. In individuals over 58 years of age, a low mtDNAcn in peripheral blood has been linked to higher mortality rates and poor health outcomes, including decline in both cognitive and physical performance[55]. However, studies conducted on long-lived families, including nonagenarians and centenarians, have produced puzzling and often contradictory results, complicating our understanding of mtDNA dynamics during extreme longevity[55,57].

The aging process is accompanied by a decline in mitochondrial function, along with noticeable changes in mitochondrial morphology, content (both in terms of number and protein levels), and OXPHOS[15,57,58]. Pathogenic mutations in mtDNA, including both large deletions and point mutations, have been identified in various tissues of aged individuals, affecting both post-mitotic and proliferating cells[59]. Experimental evidence has suggested that somatic mtDNA mutations play a role in driving certain age-related phenotypes. For example, in mice, a deficiency in the proofreading function of mitochondrial POLγ leads to progressive accumulation of mtDNA mutations, resulting in premature aging syndrome. This syndrome is characterized by reduced lifespan, decreased fertility, anemia, hair greying, hair loss, hearing loss, and stem cell dysfunction[15,47,60].

Diminished bioenergetic function and increased oxidative damage are hallmarks of aging in mammals. Mitochondria are susceptible to oxidative damage owing to their proximity to reactive oxygen species, resulting in mtDNA lesions. Autophagic clearance of damaged mitochondria decreases with age in various species. Overall, mtDNAcn dynamics should be explored more thoroughly across different tissues in animal models of aging.

mtDNAcn as a biomarker

mtDNAcn can be employed as a quantifiable biomarker, thereby providing a measurement that offers information related to cellular changes in disease diagnosis, severity prediction, and treatment efficacy evaluation in a multitude of human diseases, especially in cancers, neurodegenerative diseases, infections, maternal-fetal diseases, and inherited diseases. Advances in technology have enabled the assessment of mtDNAcn as part of genomic profiling in a high-throughput, rapid, and cost-effective manner because unlike nDNA, mtDNA is present in numerous copies, up to thousands per cell, and its copy number is subject to regulation[9,10,29]. Individuals vary in their mtDNAcn, and while they are relatively stable across tissues and over time, they may be modulated by diet, lifestyle, and exposure to various environmental factors[15,61]. mtDNAcn also appears to be regulated during mitochondrial biogenesis and in response to changes in cellular energy demand[11,13]. Preclinical studies involving cell lines and animal models, along with epidemiologic studies concerning human subjects with various diseases, have collectively revealed that the mtDNAcn is altered in a disease-dependent manner. As a result, there is a compelling interest in the clinical application of mtDNAcn as a diagnostic biomarker for potential use in clinical laboratories. To date, the diagnostic applications of mtDNAcn have been evaluated and assessed across multiple disciplines, including oncology, metabolic disorders, and infectious diseases, using different detection techniques in various tissues, including peripheral blood, buccal mucosa, saliva, lymphocytes, urine, breast tissues, tumor tissues, neurons in post-mortem brain samples, hair follicle tissues, and cultivated cells.

The focus of oncology is on cell-free mtDNAcn found in the plasma or serum of patients with various cancers. The rationale for investigating cell-free mtDNA is that it is released from dead or damaged cells, thereby creating an abundance of mtDNA in the circulatory system. The same is substantiated by the studies corroborating a link between reduced cell-free mtDNA in plasma or serum and the onset or progression of cancers, particularly with respect to lung, breast, ovarian, endometrial, colorectal, prostate, and hepatitis virus-induced liver cancer (Table 3)[15,62,63].

mtDNAcn in neurodegenerative disorders

Mitochondrial dysfunction has emerged as a potential contributor to neurodegenerative disorders such as AD and PD[64,65]. Initial studies on mtDNAcn in AD focused solely on brain tissue. Several studies have used a more systematic approach to evaluate mtDNAcn levels in the peripheral tissues and biofluids. Follow-up studies have assessed mtDNAcn levels in a diverse array of tissues. Most studies have found that mtDNAcn is reduced in the brains of patients diagnosed with AD[66,67]. In support of these findings, decreased mtDNAcn is associated with increased phosphorylated tau levels in the cerebral spinal fluid of several AD cohorts[68,69]. Interestingly, contradictory findings have been reported for other tissues[70]. In peripheral blood and arteries, mtDNAcn has been primarily observed to be increased in patients with AD. As an important consideration, most of these studies used 12S rRNA to quantify mtDNAcn. Previous studies have shown that nDNA-located mitochondrial ribosomal RNA genes can be lost with age, potentially confounding the nature of the differences between groups.

In Parkinson's, gene expression in various brain regions and biological fluids shows distinct patterns. In the geniculate nucleus region, expression is upregulated, whereas in the SN and blood, it is downregulated, as detected by qPCR techniques[69,71,72]. The cerebellum and cerebellar cortex remained unchanged based on WES analysis[73]. The frontal cortex also showed no change, but the qPCR results from the SN show downregulation[74]. In AD, the frontal cortex and hippocampus are downregulated according to qPCR results[75-77], whereas the cerebellum remains unchanged[76]. However, in some hippocampal and cerebellar cortex regions, downregulation was observed using both WES and qPCR [75]. Blood samples showed no changes in gene expression, whereas cerebral spinal fluid was downregulated[75,76]. These findings highlight the varied gene expression patterns across different regions and biological samples in both patients with PD and AD.

As an important modulator of mtDNAcn, PGC-1α has been implicated in the pathology of several neurodegenerative diseases. In a Drosophila model of synuclein-induced degeneration, PGC-1α exhibited protective effects by promoting mtDNA replication and mtDNAcn[78]. In human studies, PGC-1α was upregulated in the midbrain of sporadic PD patients[79]. However, it appears that some mutations in PGC-1α confer an increased vulnerability to nigral dopaminergic neuron death. In sporadic cases of AD, cerebrospinal fluid levels of the PGC-1α-targeted mitochondrial biogenesis driver sirtuin 1 negatively correlate with phosphorylated tau levels[78]. These studies indicate that a mechanism might promote an increase in mtDNAcn, whereas late disease mechanisms might promote its reduction. Importantly, these effectors interact with one another in several ways. Analysis of neurogenesis-enriched miRNAs revealed that the differential expression between AD subjects and controls could regulate many events.

mtDNAcn in metabolic disorders

In response to stresses or damage that compromise respiratory chain function, the cell can greatly amplify its mtDNAcn, which is intended to restore a normal ratio of mitochondrial and nuclear genomes, and is dependent on the transcriptional coactivator PGC-1α and its action on mitochondrial RNA POLγ. It is an attractive candidate for a biomarker because of the potential for it to point to early disease onset, the relative ease of obtaining tissue samples, and the feasibility of measuring mtDNAcn changes in archival collections, making it an attractive candidate.

Applications of mtDNAcn in the field of metabolism are largely directed toward mitochondrial disorders. Evidence suggests that disrupted mtDNA topology is disease dependent[80,81]. Studies that determined mtDNA deletion size and copy number in various tissues of subjects with different diseases indicated that mtDNA topology has the potential to serve as a disease signature at the genomic level[80]. Evaluation of mtDNA deletion size, which is readily detected by qPCR, has been employed as a clinical diagnostic tool for multiple mitochondrial disorders, including A3243G-induced mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)[15]. The feasibility of mtDNA deletion size evaluation in maternal plasma has been investigated as a noninvasive prenatal test for the prenatal diagnosis

Table 3 Clinical relevance of mitochondrial DNA copy number in diseases

Disease category	Role of mtDNAcn	Key observations	Diagnostic/prognostic value
Neurodegenerative disorders[64-70]	Biomarker for disease progression and severity	Reduced mtDNAcn in AD brains; increased mtDNAcn in peripheral blood of AD patients	Correlates with tau pathology in CSF; potential for non-invasive diagnosis using blood mtDNA levels
Cancer[15,62,100-102]	Indicator of tumor aggressiveness and treatment response	Elevated mtDNAcn associated with tumor proliferation; decreased mtDNAcn linked to poor prognosis	Distinguishes between cancerous and non-cancerous tissues; early-stage cancers show higher mtDNAcns, while advanced stages may show depletion
Metabolic disorders[4,15,80-83]	Reflects mitochondrial dysfunction	mtDNAcn dysregulated in diabetes and other metabolic syndromes, indicating stress or compensation mechanisms	Biomarker for mitochondrial stress in diabetes; changes in mtDNAcn can indicate early disease onset or progression
Aging[47-49,55,56]	Associated with age-related cellular dysfunction	Decline in mtDNAcn in various tissues (*e.g.*, blood, muscle) with age; some tissues exhibit increased mtDNA	Low mtDNAcn linked with poor health outcomes in aging populations, including cognitive and physical decline
Inherited mitochondrial disorders[82-93]	Indicates heteroplasmy levels and disease severity	Variations in mtDNAcn linked to phenotypes like MELAS, Pearson's syndrome, and Leber's hereditary optic neuropathy	High mtDNAcn linked to milder phenotypes; can guide prognosis and therapy for conditions like Kearns-Sayre syndrome and mitochondrial encephalopathy

mtDNAcn: Mitochondrial DNA copy number; AD: Alzheimer's disease; MELAS: Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; CSF: Cerebrospinal fluid.

of A3243G-induced MELAS. mtDNA depletion syndromes, a group of autosomal recessive disorders characterized by a significant tissue-specific reduction in mtDNA levels, arise from mutations in genes responsible for various aspects of mtDNA maintenance, including mitochondrial nucleotide metabolism, mtDNA replication, mitochondrial dynamics, and quality control mechanisms[15]. A few other examples of mtDNA mutations and their effects include Pearson's syndrome, in which the mtDNA mutation involves deletions. Blood samples were used for analysis. mtDNA levels were elevated ("up")[82], as quantified by qPCR. Similarly, in Kearns-Sayre syndrome (KSS), another deletion was identified in mtDNA, with blood and muscle samples used for analysis. The mtDNA levels were also elevated[82] using qPCR for quantification by qPCR. In MELAS, the mtDNA mutation, m.3243A>G, was detected in leukocytes. Studies have demonstrated that mtDNA levels can be elevated, unchanged, or decreased depending on the patient's age, as reported by[83], using qPCR for quantification. Similarly, in myoclonic epilepsy with ragged red fibers (MERRF), the m.8344A>G mutation in leukocytes was studied by Liu *et al*[84], and mtDNA levels were found to vary (up, unchanged, or down) depending on age.

For LHON, in the case of the m.11778G>A mutation, peripheral blood cells showed elevated mtDNA levels in a study using qPCR[85]. Other studies have reported the m.11778G>A mutation in blood samples, where mtDNA levels were found to be either elevated or unchanged, with qPCR used for quantification[84,86]. Additionally, in LHON with the m.3460G>A mutation, blood samples were analyzed using qPCR, and mtDNA levels were either elevated or unchanged, as shown in a few studies[84,86]. Interestingly, it was found that copy number tended to increase in younger patients, whereas it remained unchanged or even decreased in older patients. Other point mutations linked to mitochondrial disorders that can be detected at the nucleic acid level have also been evaluated for potential application in maternal plasma tests, including A1555G-induced non-syndromic hearing loss and mtDNA4977 deletion-induced Pearson syndrome[87].

As a biomarker, the mtDNAcn reflects the maintenance and modification of the mitochondrial genome. In surface cells, larger copy numbers are maintained by the replicative machinery involving mitochondrial POLγ. POLγ subunit A (*POLGA*), which encodes the catalytic subunit of mitochondrial POLγ, has been identified as one of several nuclear genes associated with mtDNA depletion syndrome. Over 300 mutations in the *POLGA* gene have been identified, contributing to a wide range of mtDNA defects that lead to varying disease severities, symptoms, and ages of onset. Interestingly, a mutation that severely impairs the proofreading activity of *POLGA* in mice resulted in numerous mtDNA mutations but had no effect on mtDNAcn[88].

Patients with *POLGA*-related disorders can present with either a significant reduction in mtDNAcn or an accumulation of mtDNA mutations and deletions without affecting overall mtDNA levels[89]. The crystal structure of POLγ has provided insights into how different mutations affect enzyme function and replication fidelity[90]. However, the relationship between genotype and phenotype remains complex and unpredictable, with significant phenotypic variability occurring within members of the same family[91-93]. Pathogenic mtDNA mutations, whether homoplasmic or heteroplasmic, are responsible for various mitochondrial syndromes, such as mitochondrial encephalopathy, MELAS, LHON, MERRF, Pearson's syndrome, and KSS. Studies have shown that mtDNAcn is elevated only in small cohorts of patients with either Pearson's or KSS, which is caused by large heteroplasmic mtDNA deletions, and that there is no correlation between mtDNAcn and the size or position of the deletion[15,46].

More extensive investigations have been conducted on mtDNAcn variations in patients with mtDNA point mutations. A study involving patients with MELAS carrying the heteroplasmic m.3243A>G mutation found that high mtDNAcn and low heteroplasmy levels were associated with less severe disease[93,94]. Other studies involving smaller cohorts reported that MELAS and MERRF patients with moderately elevated mtDNAcn s exhibited milder phenotypes[95]. In patients

with LHON, who mostly carry homoplasmic mutations, mtDNAcn has been implicated in disease penetrance, with asymptomatic carriers of the m.11778G>A or m.3460G>A mutations showing higher mtDNAcns than visually impaired patients[96,97].

mtDNAcn in cancer

The mtDNA polyploidy biomarker can classify different cancer types; changes in mtDNAcn have been reported in solid tumors and hematological malignancies, with higher levels reported in some cancer types and lower levels reported in others[15,62,80]. Cancer tissues normally display significant alterations in mtDNAcn (one hundredfold lower than normal tissue; mtDNA amplification up to twentyfold)[98]. An increase in mtDNA has been associated with mutations in the oncogenes Ras and Myc, and downregulation of Tfam expression (at mRNA and protein levels)[98,99].

Increased mtDNAcn has been proposed and observed as a potential mechanism to eliminate the effects of mitochondrial mutations in proliferative cancer cells. This association is explained by the selective growth advantage of tumor cells that have a higher mtDNA content than their non-cancerous neighbors[99,100]. Over time, this leads to gradual accumulation and propagation of mutant mitochondrial genomes in cellular and tissue mixtures[101]. Accordingly, early stage cancers with higher mtDNAcns are often accompanied by a lower mutation load.

Other cancer-related biomechanisms related to increased mtDNAcn include accelerated mitochondrial biogenesis, the Warburg effect, and increased bioenergetic demand, which are often proposed in parallel to maintain mitochondrial function and ATP production in cancer cells[101]. mtDNAcn levels can significantly distinguish cancerous tissues from non-cancerous tissues in matched samples[62,100,101]. This observation suggests the potential use of mtDNAcn level as a sensitive biomarker for early cancer screening. Decreased mtDNAcn has also been reported in breast cancer and is associated with poor prognosis[102]. Based on the relative detection thresholds of somatic mutations across different variant allele frequencies, some early-stage cancers with mtDNA deletions are difficult to detect.

mtDNAcn variation plays a significant role in cancer progression and varies across different cancer types, with both increases and decreases in mtDNA levels correlating with cancer risk and severity. In peripheral blood lymphocytes, studies have shown that an increase in mtDNA levels is associated with elevated cancer risk, as observed through a meta-analysis of the literature using qPCR[103]. In contrast, decreased mtDNA levels in bone tissue and peripheral blood lymphocytes are associated with a decreased risk of cancer[103].

In brain gliomas, elevated mtDNA levels in blood samples are associated with an increased risk of cancer[104], whereas in breast cancer, similar elevations in blood mtDNA levels have been reported to correlate with an increased cancer risk[105,106]. Blood samples from colon and rectal cancers exhibit increased mtDNA levels, further supporting the link between elevated mtDNA levels and cancer risk[107]. However, in the kidney, reduced mtDNA levels have been observed in peripheral blood lymphocytes from cancer patients[108], suggesting a complex role of mtDNA in different tissues. For lung cancer patients, increased mtDNA levels in blood samples are indicative of a higher cancer risk[109], and in pancreatic cancer cases, upregulation of mtDNA is similarly associated with cancer progression[110]. Patients with skin cancer also show increased mtDNA levels[106].

In matched tissue samples, changes in mtDNA levels correlated with disease severity. In bladder cancer, mtDNA levels are downregulated, contributing to an increase in disease severity[99]. Similar reductions have been observed in Ewing sarcoma[111], gliomas[112], and primary breast tumors[99,113], indicating a potential link between reduced mtDNA levels and more severe cancer stages. Conversely, increased mtDNA levels have been reported in colorectal carcinoma [114,115], colorectal adenoma[99,100], and lung cancer, where both small- and non-small-cell lung cancers show upregulated mtDNA, contributing to disease progression[116-118].

In kidney cancers, specifically chromophobe renal cell carcinoma, mtDNA levels are increased[119], whereas renal oncocytomas are decreased[120,121]. Hepatocellular carcinoma also shows a decline in mtDNA levels, which aligns with an increase in disease severity[99,122]. A notable decrease in mtDNA is observed in head and neck cancers, such as squamous cell carcinoma[99], as well as in certain prostate cancer types, particularly adenocarcinoma, where reduced mtDNA levels are linked to worse outcomes[122,123]. In the oral/digestive tract, increased mtDNA levels have been observed in esophageal cancer[99], whereas stomach cancer shows elevated levels[124]. Pancreatic endocrine tumors also display upregulation of mtDNA[123], and thyroid adenocarcinoma also exhibits an increase[123]. These variations in mtDNA levels across different cancers suggest that mtDNA could be a critical biomarker for both cancer risk assessment and monitoring disease progression.

These data reveal that mutations in mtDNA accumulate in virtually all types of cancer and have been associated with diagnostic or prognostic purposes. The pathophysiological relevance of these changes is still unknown; however, it is still unclear whether mtDNA mutations directly cause oncogenesis or whether they are merely the result of faster mtDNA replication in rapidly growing cancer cells. The proliferation of pre-existing heteroplasmic mutations or polymorphisms, which then undergo passive clonal expansion after several rounds of cell division during tumor formation, may be the cause of these mutations[125].

The notion that mtDNA mutations directly cause cancer has been refuted by strong evidence. Most tumor types, with the notable exception of kidney, colorectal, and thyroid cancers, counter-select for mutations that result in the truncation of mitochondrial proteins[123,126,127]. mtDNA levels have been found to be upregulated in these specific cancer types, indicating a compensatory mechanism meant to maintain mitochondrial function despite harmful mutations[123]. This observation underscores the complex relationship between mitochondrial dysfunction and tumor biology.

A comprehensive study that assessed the mutational landscape of over two-thousands cancer patients highlighted a strong strand bias in mtDNA mutations, indicating a replication-driven mechanism underlying their generation[123]. One likely scenario is that pre-existing heteroplasmic mutations, or mutations introduced by replication errors of DNA POLγ, may clonally expand to high frequencies in fast-dividing cancer cells, becoming fixed within certain tumor subpopulations over time. Oncocytomas, a type of tumor characterized by the accumulation of mtDNA mutations that

lead to severe OXPHOS dysfunction, provide a compelling example of this mechanism. In these tumors, mitochondrial dysfunction is accompanied by a compensatory increase in mitochondrial mass, which is likely to sustain bioenergetic capacity despite the OXPHOS defects[128,129]. Although oncocytomas are driven by high levels of mtDNA mutations, they typically present as benign lesions with low invasiveness and nonaggressive clinical phenotypes.

However, the extent to which mitochondrial dysfunction acts as a barrier for cancer progression remains unclear. Although some forms of mitochondrial impairment may limit the malignant potential of certain tumors, there is evidence to suggest that in some cancer types, the accumulation of mtDNA mutations can provide a selective advantage. This facilitates the survival and proliferation of transformed cells, directly contributing to cancer development. Supporting this hypothesis, a study demonstrated that metabolic alterations induced by mtDNA mutations promote tumorigenesis in colon cancer[130].

In addition to the mutational burden, the abundance of mtDNA within a cell has emerged as a critical biomarker of a cell's reliance on OXPHOS for energy production. Therefore, the regulation of mtDNAcn may be indicative of the bioenergetic adaptations that cancer cells undergo in response to mitochondrial dysfunction. The relationship between mtDNA mutations, their functional consequences, and their contribution to cancer progression continue to be the subject of intense investigation in the field of cancer biology.

Mounting evidence suggests that mtDNAcn may be dysregulated during carcinogenesis, aging, and neurodegenerative disease progression, among other disorders. Owing to its unique characteristics, such as maternal inheritance and absence of histones[2,6,15,48,63], mtDNA can be isolated not only from tissues but also from body fluids, such as plasma or saliva, among others. Moreover, the qPCR quantification of mtDNA is simple, inexpensive, and widely available. Hence, diagnostic applications of mtDNAcn in body fluids have gained significant attention. Nevertheless, there are important challenges that must be overcome to fully realize this potential. For example, a pre-analytical step must be conducted prior to any qPCR quantification of plasma to prevent potential contamination of qPCR reagents, calculation bias, and amplification of unreliably detected mtDNA. There is also the question of whether mtDNAcn response in tissues can be replicated in bodily fluids. Integration across the ranges of fluid and tissue types studied is necessary to understand the potential cross-application of tools and techniques and to identify species that cannot be integrated and require tailored approaches.

Further research is needed to elucidate these matters while ensuring that the use of mtDNAcn in fluid biopsies never compromises its application to tissues. There is also a substantial demand for computational tools that enable comparisons between different cohorts to harmonize nonlinear data. Moreover, sample pooling to reduce processing and qPCR costs can seriously confound the diagnostic strategies. Population-level cutoff norms for mtDNAcn must be established to ensure better comparability of the results. Finally, factors affected by sampling strategy and ethnic diversity must be explored further, as they can largely contribute to the inter-study variability already seen in the mtDNAcn literature. Addressing all these known hurdles will be fundamental to ensuring proper diagnostics using mtDNAcn in fluid biopsies in the future (Table 4).

THERAPEUTIC IMPLICATIONS OF MODULATING mtDNAcn

Abnormal mtDNAcn is involved in the pathogenesis of many human diseases, and numerous clinical studies have shown that alterations in mtDNAcn are correlated with disease conditions. Therefore, modulation of mtDNAcn has therapeutic implications for human health and disease. The development of a versatile genome editing tool is required for the examination of mtDNAcn and for a fundamental understanding of the pathogenic mechanisms of altered mtDNAcns. Platform technologies for the modulation of mtDNAcn and their application in human diseases are also desired.

The metabolic stress sensor 5′ adenosine monophosphate-activated protein kinase (AMPK) is activated by a decrease in the ATP/adenosine monophosphate ratio at the onset of various stress conditions, including energy deprivation, hypoxia, and nutrient starvation[131]. Once activated, AMPK maintains cellular energy homeostasis by enhancing ATP-generating pathways, such as fatty acid oxidation in the mitochondria, and the inhibition of energy-consuming biosynthetic pathways, such as lipid and protein synthesis[131,132]. Reports also have demonstrated that AMPK also upregulates mitochondrial biogenesis through the activation of the transcriptional coactivators PGC-1α and PGC-1β[133]. Furthermore, the activation of AMPK increases mtDNAcn in several human somatic cells, including endothelial and cancer cells. Depletion of mtDNA by chemical inhibition of mtDNA replication decreases the levels of mtDNA-encoded genes and OXPHOS complexes, resulting in the activation of stress response pathways, including the mitochondrial unfolded protein response[131-133]. Activation of unfolded protein response is known to enhance mitochondrial biogenesis and the clearance of damaged mitochondria, thus improving mitochondrial homeostasis[134].

Modulation of mtDNAcn has therapeutic implications in human diseases such as age-associated neurodegenerative diseases. Modification of the mtDNAcn is an attractive option for the development of therapeutic modalities against age-associated neurodegenerative diseases. Thus far, classes of small molecules, vitamins, and ionophores that increase mtDNAcn have been identified. Chemical screening using human cell models is a promising approach for the identification of additional small molecules that alter mtDNAcns. Two primary therapeutic strategies have been explored to enhance OXPHOS capacity and restore mitochondrial function. The first involves increasing the overall mitochondrial mass, while the second focuses on selectively altering mtDNA to modulate its copy number and/or heteroplasmy levels. These approaches predominantly target treatment of primary mitochondrial disorders. However, if human clinical trials yield positive outcomes, they may have broader applications in the management of age-related diseases.

One promising strategy to improve the balance in favor of functional mitochondria is to focus on selective elimination of damaged mitochondria[135]. This is accomplished by promoting autophagy, a cellular mechanism that breaks down

Table 4 Role of mtDNA copy number in various stages of cancer development and progression

Aspect	Role of mtDNAcn	Mechanism/impact	Examples
Cancer risk	Altered mtDNAcn (increase or decrease) may predispose individuals to cancer development	Imbalance in ROS production	Decreased mtDNAcn linked to breast cancer risk
		Compromised cellular energy metabolism	Increased mtDNAcn linked to lung cancer risk
Tumor initiation	Changes in mtDNAcn can affect mitochondrial biogenesis and metabolic reprogramming	Promotes a shift to aerobic glycolysis (Warburg effect)	Low mtDNAcn observed in colorectal cancer tissues
		Increases ROS, leading to genomic instability	
Tumor progression	Dynamic changes in mtDNAcn support adaptation to tumor microenvironment	High mtDNAcn enables oxidative metabolism in hypoxic conditions	Elevated mtDNAcn associated with metastatic breast cancer
		Supports invasive and metastatic properties	
Therapeutic resistance	Altered mtDNAcn contributes to drug resistance	High mtDNAcn enhances oxidative phosphorylation, reducing sensitivity to certain chemotherapies	Increased mtDNAcn linked to resistance in lung cancer treatments
Prognostic biomarker	mtDNAcn alterations can predict cancer outcomes	Low mtDNAcn correlates with poor prognosis in many cancers	Reduced mtDNAcn in gastric cancer linked to poor survival
		High mtDNAcn may predict aggressive tumor behavior	
Immune evasion	Changes in mtDNAcn influence immune responses within the tumor microenvironment	mtDNA release into the cytoplasm activates inflammatory pathways	mtDNA-derived DAMPs in melanoma
		Alters immune surveillance mechanisms	
Angiogenesis	mtDNAcn modulates energy demand and oxidative stress, indirectly affecting vascular growth	High mtDNAcn supports angiogenic signaling	Increased angiogenesis in glioblastoma with altered mtDNAcn
Metastasis	Altered mtDNAcn facilitates energy supply for metastatic spread	Provides metabolic flexibility for survival in secondary sites	Elevated mtDNAcn in metastatic colorectal cancer

mtDNAcn: Mitochondrial DNA copy number; ROS: Reactive oxygen species; DAMPs: Damage-associated molecular patterns.

and recycles a variety of components, including mitochondria, to increase mitochondrial turnover. Inhibition of mammalian target of rapamycin complex 1 (mTORC1), a crucial regulator of food sensing and metabolism, is a method of inducing autophagy[136,137]. The intricacy of the mTORC1 pathway, which controls a variety of cellular processes, is probably the reason why rapamycin-mediated mTORC1 suppression has been inconsistently beneficial in improving mitochondrial function in certain animal models[137]. However, in one study, rather than autophagy activation, the effect of rapamycin in a mouse model of leigh syndrome appeared to be due to a metabolic shift toward amino acid catabolism [138]. As a result, off-target effects may limit the therapeutic use of mTORC1 inhibitors such as rapamycin[139,140].

By increasing longevity in model organisms such as Caenorhabditis elegans and enhancing muscle function in elderly mice, more specialized autophagy enhancers, including urolithin A, have shown promise[140]. However, the regulatory processes controlling autophagy and mitochondrial quality are extremely intricate and poorly understood. Excessive mitochondrial clearance caused by the dysregulation of these mechanisms may result in negative long-term repercussions. Before embarking on therapeutic development based on these principles, caveats and concerns associated with manipulating mtDNAcns should be considered (Table 5).

Despite years of effort aimed at elucidating the regulation of mtDNAcn, our understanding remains rudimentary. There are several obstacles in mtDNAcn research. First, the rate-limiting steps of mtDNA replication remain elusive, forcing scientists to imagine rather than experiment with the mtDNA replication mechanism. Second, the expression of genes involved in the regulation of mtDNAcn is tissue-selective, and the regulatory mechanisms responsible for mtDNAcn alterations remain poorly defined. Additionally, discrepancies between the effects of mitochondrial respiration on mtDNAcn observed in different laboratory settings remain unclear. Unexpectedly, an increased mtDNAcn was observed under high glucose conditions in a cell type and in an experimental background-dependent manner. Considered as a simple internal control in qPCR, chloroplast DNA copy number was also reported to be altered in proud low mtDNAcn cells (ND7 and 964) but not in other cells. Third, it has been difficult to evaluate changes in mtDNAcn in human tissues owing to the complexity of the tissue architecture and biological variability. Controversies regarding the increase or decrease in mtDNAcn have been observed among comparable disorders across different laboratories. These confounding factors collectively hinder the understanding and comparison of mtDNAcn alterations in various diseases (Table 6).

Table 5 Comparison of techniques for modulating mtDNA copy number therapeutic strategy

Therapeutic strategy	Mechanism	Applications	Advantages	Challenges
AMPK activation	Enhances mitochondrial biogenesis *via* PGC-1α activation	Neurodegenerative diseases, aging	Promotes energy balance	Off-target effects, limited clinical trials
Genome editing tools	Targets mtDNA mutations or modulates copy number	Mitochondrial diseases, cancer therapy	Precision targeting	Ethical concerns, technical challenges
Autophagy induction	Removes damaged mitochondria	Improves mitochondrial quality	Enhances cellular health	Excessive clearance may have long-term side effects
Small molecules and vitamins	Increases mtDNAcn	Metabolic and neurodegenerative disorders	Cost-effective	Limited understanding of long-term effects

AMPK; Adenosine monophosphate-activated protein kinase; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; mtDNA: Mitochondrial DNA; mtDNAcn: Mitochondrial DNA copy number.

Table 6 Broad spectrum of therapeutic applications of mtDNA copy number

Application	Role of mtDNAcn assessment	Therapeutic implications	Examples
Cancer prognostication	mtDNAcn serves as a biomarker for predicting cancer outcomes	Guides risk stratification and treatment intensity Identifies patients with aggressive disease	Low mtDNAcn linked to poor prognosis in gastric and colorectal cancers
Therapeutic targeting	Abnormal mtDNAcn highlights mitochondrial vulnerabilities	Enables development of drugs targeting mitochondrial pathways (*e.g.*, OXPHOS inhibitors)	mtDNAcn modulation as a target in ovarian and pancreatic cancer therapies
Monitoring treatment response	Changes in mtDNAcn reflect tumor response to therapy	Serves as a real-time marker to monitor chemotherapy, radiotherapy, or immunotherapy efficacy	mtDNAcn alterations used to monitor cisplatin therapy in ovarian cancer
Personalized medicine	mtDNAcn variations help tailor therapies based on mitochondrial function	Facilitates selection of specific treatment modalities (*e.g.*, glycolysis inhibitors *vs* OXPHOS inhibitors)	mtDNAcn guiding metabolic therapy choices in lung and breast cancer
Radiotherapy sensitization	Altered mtDNAcn may increase sensitivity or resistance to radiotherapy	Identifies patients who might benefit from combined mitochondrial and radiotherapy interventions	Elevated mtDNAcn linked to radioresistance in glioblastoma
Metabolic modulation	mtDNAcn assessment reveals metabolic dependencies of tumors	Guides therapies targeting cancer metabolism (*e.g.*, ketogenic diets, mitochondrial uncouplers)	Low mtDNAcn tumors treated with glycolysis inhibitors
Early disease detection	mtDNAcn alterations in blood or tissue serve as a non-invasive biomarker for early cancer detection	Allows early initiation of treatment, potentially improving outcomes	Reduced mtDNAcn detected in circulating cell-free DNA in lung and breast cancers
Combination therapies	mtDNAcn dynamics predict synergy between mitochondrial-targeted drugs and conventional therapies	Combines metabolic modulators with standard chemotherapy or immunotherapy for enhanced efficacy	mtDNAcn-directed combination strategies in melanoma treatment
Toxicity management	mtDNAcn levels predict susceptibility to mitochondrial toxicity from certain drugs	Assists in preemptive dose adjustments or alternative drug selection to avoid adverse effects	Monitoring mtDNAcn to prevent cardiotoxicity from anthracyclines
Rare mitochondrial disorders	mtDNAcn assessment aids in the diagnosis and management of mitochondrial diseases with cancer overlap	Develops therapies that normalize mtDNAcn or enhance mitochondrial biogenesis	mtDNAcn restoration therapies in mitochondrial depletion syndromes

mtDNAcn: Mitochondrial DNA copy number; OXPHOS: Oxidative phosphorylation.

CONCLUSION

mtDNAcn is a crucial biomarker for understanding mitochondrial health, cellular energy metabolism, and disease pathogenesis. Its quantification using methods such as qPCR and NGS plays a vital role in the early diagnosis, prognosis, and monitoring of treatment responses in a wide range of diseases, including cancer, neurodegenerative disorders, and metabolic conditions. However, challenges, such as standardizing methodologies, addressing inter-study variability, and ensuring accurate and reproducible measurements, remain. Overcoming these obstacles is essential to effectively integrate mtDNAcn assessments into clinical diagnostics and therapeutic strategies.

FOOTNOTES

Author contributions: Parchwani D conceived the overall concept and design of the manuscript, contributed to the drafting of the manuscript and the critical revision of the intellectual content, and was also involved in the finalization of manuscript submissions; Singh R played a pivotal role in literature collection, assisted in the writing and revision of specific sections, provided valuable input for structuring the manuscript, and contributed to the manuscript's final editing; Patel D led the literature review process, ensuring that all relevant studies and articles were cited, assisting in drafting and revising manuscripts, and assisting in the preparation of tables; and all authors thoroughly reviewed and endorsed the final manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: India

ORCID number: Deepak Parchwani 0000-0001-5024-970X.

S-Editor: Bai Y
L-Editor: A
P-Editor: Zhang L

REFERENCES

1 **Gustafsson CM**, Falkenberg M, Larsson NG. Maintenance and Expression of Mammalian Mitochondrial DNA. *Annu Rev Biochem* 2016; **85**: 133-160 [PMID: 27023847 DOI: 10.1146/annurev-biochem-060815-014402]
2 **Bonekamp NA**, Larsson NG. SnapShot: Mitochondrial Nucleoid. *Cell* 2018; **172**: 388-388.e1 [PMID: 29328920 DOI: 10.1016/j.cell.2017.12.039]
3 **Tang JX**, Thompson K, Taylor RW, Oláhová M. Mitochondrial OXPHOS Biogenesis: Co-Regulation of Protein Synthesis, Import, and Assembly Pathways. *Int J Mol Sci* 2020; **21**: 3820 [PMID: 32481479 DOI: 10.3390/ijms21113820]
4 **Sturm G**, Karan KR, Monzel AS, Santhanam B, Taivassalo T, Bris C, Ware SA, Cross M, Towheed A, Higgins-Chen A, McManus MJ, Cardenas A, Lin J, Epel ES, Rahman S, Vissing J, Grassi B, Levine M, Horvath S, Haller RG, Lenaers G, Wallace DC, St-Onge MP, Tavazoie S, Procaccio V, Kaufman BA, Seifert EL, Hirano M, Picard M. OxPhos defects cause hypermetabolism and reduce lifespan in cells and in patients with mitochondrial diseases. *Commun Biol* 2023; **6**: 22 [PMID: 36635485 DOI: 10.1038/s42003-022-04303-x]
5 **Brüser C**, Keller-Findeisen J, Jakobs S. The TFAM-to-mtDNA ratio defines inner-cellular nucleoid populations with distinct activity levels. *Cell Rep* 2021; **37**: 110000 [PMID: 34818548 DOI: 10.1016/j.celrep.2021.110000]
6 **Villena JA**. New insights into PGC-1 coactivators: redefining their role in the regulation of mitochondrial function and beyond. *FEBS J* 2015; **282**: 647-672 [PMID: 25495651 DOI: 10.1111/febs.13175]
7 **Naini A**, Gilkerson R, Shanske S, Pang J. Detection of mitochondrial DNA (mtDNA) mutations. *Methods Cell Biol* 2020; **155**: 383-400 [PMID: 32183969 DOI: 10.1016/bs.mcb.2019.11.009]
8 **Roy A**, Kandettu A, Ray S, Chakrabarty S. Mitochondrial DNA replication and repair defects: Clinical phenotypes and therapeutic interventions. *Biochim Biophys Acta Bioenerg* 2022; **1863**: 148554 [PMID: 35341749 DOI: 10.1016/j.bbabio.2022.148554]
9 **Thompson K**, Collier JJ, Glasgow RIC, Robertson FM, Pyle A, Blakely EL, Alston CL, Oláhová M, McFarland R, Taylor RW. Recent advances in understanding the molecular genetic basis of mitochondrial disease. *J Inherit Metab Dis* 2020; **43**: 36-50 [PMID: 31021000 DOI: 10.1002/jimd.12104]
10 **Yu R**, Lendahl U, Nistér M, Zhao J. Regulation of Mammalian Mitochondrial Dynamics: Opportunities and Challenges. *Front Endocrinol (Lausanne)* 2020; **11**: 374 [PMID: 32595603 DOI: 10.3389/fendo.2020.00374]
11 **Yang J**, Guo Q, Feng X, Liu Y, Zhou Y. Mitochondrial Dysfunction in Cardiovascular Diseases: Potential Targets for Treatment. *Front Cell Dev Biol* 2022; **10**: 841523 [PMID: 35646910 DOI: 10.3389/fcell.2022.841523]
12 **Bustamante-Barrientos FA**, Luque-Campos N, Araya MJ, Lara-Barba E, de Solminihac J, Pradenas C, Molina L, Herrera-Luna Y, Utreras-Mendoza Y, Elizondo-Vega R, Vega-Letter AM, Luz-Crawford P. Mitochondrial dysfunction in neurodegenerative disorders: Potential therapeutic application of mitochondrial transfer to central nervous system-residing cells. *J Transl Med* 2023; **21**: 613 [PMID: 37689642 DOI: 10.1186/s12967-023-04493-w]
13 **Picca A**, Calvani R, Coelho-Junior HJ, Marzetti E. Cell Death and Inflammation: The Role of Mitochondria in Health and Disease. *Cells* 2021; **10**: 537 [PMID: 33802550 DOI: 10.3390/cells10030537]
14 **Huang C**, Chen L, Li J, Ma J, Luo J, Lv Q, Xiao J, Gao P, Chai W, Li X, Zhang M, Hu F, Hu D, Qin P. Mitochondrial DNA Copy Number and Risk of Diabetes Mellitus and Metabolic Syndrome. *J Clin Endocrinol Metab* 2023; **109**: e406-e417 [PMID: 37431585 DOI: 10.1210/clinem/dgad403]
15 **Filograna R**, Mennuni M, Alsina D, Larsson NG. Mitochondrial DNA copy number in human disease: the more the better? *FEBS Lett* 2021; **595**: 976-1002 [PMID: 33314045 DOI: 10.1002/1873-3468.14021]
16 **Ma Q**, Sun Y, Lei K, Luo W. Progress in diagnosis and treatment of Leber's hereditary optic neuropathy. *J Mol Med (Berl)* 2024; **102**: 1-10 [PMID: 37982904 DOI: 10.1007/s00109-023-02389-2]
17 **Pangou E**, Sumara I. The Multifaceted Regulation of Mitochondrial Dynamics During Mitosis. *Front Cell Dev Biol* 2021; **9**: 767221 [PMID: 34805174 DOI: 10.3389/fcell.2021.767221]
18 **Mertens J**, Regin M, De Munck N, Couvreu de Deckersberg E, Belva F, Sermon K, Tournaye H, Blockeel C, Van de Velde H, Spits C.

Mitochondrial DNA variants segregate during human preimplantation development into genetically different cell lineages that are maintained postnatally. *Hum Mol Genet* 2022; **31**: 3629-3642 [PMID: 35285472 DOI: 10.1093/hmg/ddac059]

19 **Leuthner TC**, Hartman JH, Ryde IT, Meyer JN. PCR-Based Determination of Mitochondrial DNA Copy Number in Multiple Species. *Methods Mol Biol* 2021; **2310**: 91-111 [PMID: 34096001 DOI: 10.1007/978-1-0716-1433-4_8]

20 **Shoop WK**, Gorsuch CL, Bacman SR, Moraes CT. Precise and simultaneous quantification of mitochondrial DNA heteroplasmy and copy number by digital PCR. *J Biol Chem* 2022; **298**: 102574 [PMID: 36209825 DOI: 10.1016/j.jbc.2022.102574]

21 **Dotolo S**, Esposito Abate R, Roma C, Guido D, Preziosi A, Tropea B, Palluzzi F, Giacò L, Normanno N. Bioinformatics: From NGS Data to Biological Complexity in Variant Detection and Oncological Clinical Practice. *Biomedicines* 2022; **10**: 2074 [PMID: 36140175 DOI: 10.3390/biomedicines10092074]

22 **Wheeler JH**, Young CKJ, Young MJ. Analysis of Human Mitochondrial DNA Content by Southern Blotting and Nonradioactive Probe Hybridization. *Curr Protoc Toxicol* 2019; **80**: e75 [PMID: 30982231 DOI: 10.1002/cptx.75]

23 **Prole DL**, Chinnery PF, Jones NS. Visualizing, quantifying, and manipulating mitochondrial DNA in vivo. *J Biol Chem* 2020; **295**: 17588-17601 [PMID: 33454000 DOI: 10.1074/jbc.REV120.015101]

24 **Ye W**, Tang X, Liu C, Wen C, Li W, Lyu J. Accurate quantitation of circulating cell-free mitochondrial DNA in plasma by droplet digital PCR. *Anal Bioanal Chem* 2017; **409**: 2727-2735 [PMID: 28154880 DOI: 10.1007/s00216-017-0217-x]

25 **Maggo S**, North LY, Ozuna A, Ostrow D, Grajeda YR, Hakimjavadi H, Cotter JA, Judkins AR, Levitt P, Gai X. A method for measuring mitochondrial DNA copy number in pediatric populations. *Front Pediatr* 2024; **12**: 1401737 [PMID: 38938506 DOI: 10.3389/fped.2024.1401737]

26 **Victor AR**, Brake AJ, Tyndall JC, Griffin DK, Zouves CG, Barnes FL, Viotti M. Accurate quantitation of mitochondrial DNA reveals uniform levels in human blastocysts irrespective of ploidy, age, or implantation potential. *Fertil Steril* 2017; **107**: 34-42.e3 [PMID: 27793366 DOI: 10.1016/j.fertnstert.2016.09.028]

27 **O'Hara R**, Tedone E, Ludlow A, Huang E, Arosio B, Mari D, Shay JW. Quantitative mitochondrial DNA copy number determination using droplet digital PCR with single-cell resolution. *Genome Res* 2019; **29**: 1878-1888 [PMID: 31548359 DOI: 10.1101/gr.250480.119]

28 **Longchamps RJ**, Castellani CA, Yang SY, Newcomb CE, Sumpter JA, Lane J, Grove ML, Guallar E, Pankratz N, Taylor KD, Rotter JI, Boerwinkle E, Arking DE. Evaluation of mitochondrial DNA copy number estimation techniques. *PLoS One* 2020; **15**: e0228166 [PMID: 32004343 DOI: 10.1371/journal.pone.0228166]

29 **Xue L**, Moreira JD, Smith KK, Fetterman JL. The Mighty NUMT: Mitochondrial DNA Flexing Its Code in the Nuclear Genome. *Biomolecules* 2023; **13**: 753 [PMID: 37238623 DOI: 10.3390/biom13050753]

30 **Refinetti P**, Warren D, Morgenthaler S, Ekstrøm PO. Quantifying mitochondrial DNA copy number using robust regression to interpret real time PCR results. *BMC Res Notes* 2017; **10**: 593 [PMID: 29132417 DOI: 10.1186/s13104-017-2913-1]

31 **de Menezes ECS**, Malik AN. Absolute Quantification of Cellular and Cell-Free Mitochondrial DNA Copy Number from Human Blood and Urinary Samples Using Real Time Quantitative PCR. *Methods Mol Biol* 2025; **2878**: 233-257 [PMID: 39546266 DOI: 10.1007/978-1-0716-4264-1_13]

32 **Ganel L**, Chen L, Christ R, Vangipurapu J, Young E, Das I, Kanchi K, Larson D, Regier A, Abel H, Kang CJ, Scott A, Havulinna A, Chiang CWK, Service S, Freimer N, Palotie A, Ripatti S, Kuusisto J, Boehnke M, Laakso M, Locke A, Stitziel NO, Hall IM. Mitochondrial genome copy number measured by DNA sequencing in human blood is strongly associated with metabolic traits *via* cell-type composition differences. *Hum Genomics* 2021; **15**: 34 [PMID: 34099068 DOI: 10.1186/s40246-021-00335-2]

33 **Liu Y**, Zhou K, Guo S, Wang Y, Ji X, Yuan Q, Su L, Guo X, Gu X, Xing J. NGS-based accurate and efficient detection of circulating cell-free mitochondrial DNA in cancer patients. *Mol Ther Nucleic Acids* 2021; **23**: 657-666 [PMID: 33575112 DOI: 10.1016/j.omtn.2020.12.017]

34 **Mahmud S**, Biswas S, Afrose S, Mita MA, Hasan MR, Shimu MSS, Paul GK, Chung S, Saleh MA, Alshehri S, Ghoneim MM, Alruwaily M, Kim B. Use of Next-Generation Sequencing for Identifying Mitochondrial Disorders. *Curr Issues Mol Biol* 2022; **44**: 1127-1148 [PMID: 35723297 DOI: 10.3390/cimb44030074]

35 **Wang D**, Xiang H, Ning C, Liu H, Liu JF, Zhao X. Mitochondrial DNA enrichment reduced NUMT contamination in porcine NGS analyses. *Brief Bioinform* 2020; **21**: 1368-1377 [PMID: 31204429 DOI: 10.1093/bib/bbz060]

36 **Bivins A**, Kaya D, Bibby K, Simpson SL, Bustin SA, Shanks OC, Ahmed W. Variability in RT-qPCR assay parameters indicates unreliable SARS-CoV-2 RNA quantification for wastewater surveillance. *Water Res* 2021; **203**: 117516 [PMID: 34412018 DOI: 10.1016/j.watres.2021.117516]

37 **Weinhouse C**. Mitochondrial-epigenetic crosstalk in environmental toxicology. *Toxicology* 2017; **391**: 5-17 [PMID: 28855114 DOI: 10.1016/j.tox.2017.08.008]

38 **Kauppila JHK**, Bonekamp NA, Mourier A, Isokallio MA, Just A, Kauppila TES, Stewart JB, Larsson NG. Base-excision repair deficiency alone or combined with increased oxidative stress does not increase mtDNA point mutations in mice. *Nucleic Acids Res* 2018; **46**: 6642-6669 [PMID: 29860357 DOI: 10.1093/nar/gky456]

39 **Sanchez-Contreras M**, Sweetwyne MT, Kohrn BF, Tsantilas KA, Hipp MJ, Schmidt EK, Fredrickson J, Whitson JA, Campbell MD, Rabinovitch PS, Marcinek DJ, Kennedy SR. A replication-linked mutational gradient drives somatic mutation accumulation and influences germline polymorphisms and genome composition in mitochondrial DNA. *Nucleic Acids Res* 2021; **49**: 11103-11118 [PMID: 34614167 DOI: 10.1093/nar/gkab901]

40 **Guo X**, Xu W, Zhang W, Pan C, Thalacker-Mercer AE, Zheng H, Gu Z. High-frequency and functional mitochondrial DNA mutations at the single-cell level. *Proc Natl Acad Sci U S A* 2023; **120**: e2201518120 [PMID: 36577067 DOI: 10.1073/pnas.2201518120]

41 **Koklesova L**, Mazurakova A, Samec M, Kudela E, Biringer K, Kubatka P, Golubnitschaja O. Mitochondrial health quality control: measurements and interpretation in the framework of predictive, preventive, and personalized medicine. *EPMA J* 2022; **13**: 177-193 [PMID: 35578648 DOI: 10.1007/s13167-022-00281-6]

42 **Li D**, Liang C, Zhang T, Marley JL, Zou W, Lian M, Ji D. Pathogenic mitochondrial DNA 3243A>G mutation: From genetics to phenotype. *Front Genet* 2022; **13**: 951185 [PMID: 36276941 DOI: 10.3389/fgene.2022.951185]

43 **Rahman S**, Poulton J, Marchington D, Suomalainen A. Decrease of 3243 A-->G mtDNA mutation from blood in MELAS syndrome: a longitudinal study. *Am J Hum Genet* 2001; **68**: 238-240 [PMID: 11085913 DOI: 10.1086/316930]

44 **Walker MA**, Lareau CA, Ludwig LS, Karaa A, Sankaran VG, Regev A, Mootha VK. Purifying Selection against Pathogenic Mitochondrial DNA in Human T Cells. *N Engl J Med* 2020; **383**: 1556-1563 [PMID: 32786181 DOI: 10.1056/NEJMoa2001265]

45 **Montano V**, Gruosso F, Simoncini C, Siciliano G, Mancuso M. Clinical features of mtDNA-related syndromes in adulthood. *Arch Biochem Biophys* 2021; **697**: 108689 [PMID: 33227288 DOI: 10.1016/j.abb.2020.108689]

46 **La Morgia C**, Maresca A, Caporali L, Valentino ML, Carelli V. Mitochondrial diseases in adults. *J Intern Med* 2020; **287**: 592-608 [PMID: 32463135 DOI: 10.1111/joim.13064]

47 **Rahman S**. Mitochondrial disease in children. *J Intern Med* 2020; **287**: 609-633 [PMID: 32176382 DOI: 10.1111/joim.13054]

48 **Kar B**, Castillo SR, Sabharwal A, Clark KJ, Ekker SC. Mitochondrial Base Editing: Recent Advances towards Therapeutic Opportunities. *Int J Mol Sci* 2023; **24**: 5798 [PMID: 36982871 DOI: 10.3390/ijms24065798]

49 **Frahm T**, Mohamed SA, Bruse P, Gemünd C, Oehmichen M, Meissner C. Lack of age-related increase of mitochondrial DNA amount in brain, skeletal muscle and human heart. *Mech Ageing Dev* 2005; **126**: 1192-1200 [PMID: 16099018 DOI: 10.1016/j.mad.2005.06.008]

50 **Arbeithuber B**, Hester J, Cremona MA, Stoler N, Zaidi A, Higgins B, Anthony K, Chiaromonte F, Diaz FJ, Makova KD. Age-related accumulation of de novo mitochondrial mutations in mammalian oocytes and somatic tissues. *PLoS Biol* 2020; **18**: e3000745 [PMID: 32667908 DOI: 10.1371/journal.pbio.3000745]

51 **Wachsmuth M**, Hübner A, Li M, Madea B, Stoneking M. Age-Related and Heteroplasmy-Related Variation in Human mtDNA Copy Number. *PLoS Genet* 2016; **12**: e1005939 [PMID: 26978189 DOI: 10.1371/journal.pgen.1005939]

52 **Nadalutti CA**, Ayala-Peña S, Santos JH. Mitochondrial DNA damage as driver of cellular outcomes. *Am J Physiol Cell Physiol* 2022; **322**: C136-C150 [PMID: 34936503 DOI: 10.1152/ajpcell.00389.2021]

53 **Wang Y**, Zhao G, Fang Z, Pan H, Zhao Y, Wang Y, Zhou X, Wang X, Luo T, Zhang Y, Wang Z, Chen Q, Dong L, Huang Y, Zhou Q, Xia L, Li B, Guo J, Xia K, Tang B, Li J. Genetic landscape of human mitochondrial genome using whole-genome sequencing. *Hum Mol Genet* 2022; **31**: 1747-1761 [PMID: 34897451 DOI: 10.1093/hmg/ddab358]

54 **Busnelli A**, Navarra A, Levi-Setti PE. Qualitative and Quantitative Ovarian and Peripheral Blood Mitochondrial DNA (mtDNA) Alterations: Mechanisms and Implications for Female Fertility. *Antioxidants (Basel)* 2021; **10**: 55 [PMID: 33466415 DOI: 10.3390/antiox10010055]

55 **Venkatesan D**, Iyer M, Narayanasamy A, Gopalakrishnan AV, Vellingiri B. Plausible Role of Mitochondrial DNA Copy Number in Neurodegeneration-a Need for Therapeutic Approach in Parkinson's Disease (PD). *Mol Neurobiol* 2023; **60**: 6992-7008 [PMID: 37523043 DOI: 10.1007/s12035-023-03500-x]

56 **Knez J**, Winckelmans E, Plusquin M, Thijs L, Cauwenberghs N, Gu Y, Staessen JA, Nawrot TS, Kuznetsova T. Correlates of Peripheral Blood Mitochondrial DNA Content in a General Population. *Am J Epidemiol* 2016; **183**: 138-146 [PMID: 26702630 DOI: 10.1093/aje/kwv175]

57 **Mengel-From J**, Thinggaard M, Dalgård C, Kyvik KO, Christensen K, Christiansen L. Mitochondrial DNA copy number in peripheral blood cells declines with age and is associated with general health among elderly. *Hum Genet* 2014; **133**: 1149-1159 [PMID: 24902542 DOI: 10.1007/s00439-014-1458-9]

58 **Picca A**, Guerra F, Calvani R, Coelho-Júnior HJ, Leeuwenburgh C, Bucci C, Marzetti E. The contribution of mitochondrial DNA alterations to aging, cancer, and neurodegeneration. *Exp Gerontol* 2023; **178**: 112203 [PMID: 37172915 DOI: 10.1016/j.exger.2023.112203]

59 **Kim SJ**, Miller B, Kumagai H, Silverstein AR, Flores M, Yen K. Mitochondrial-derived peptides in aging and age-related diseases. *Geroscience* 2021; **43**: 1113-1121 [PMID: 32910336 DOI: 10.1007/s11357-020-00262-5]

60 **Seo AY**, Joseph AM, Dutta D, Hwang JC, Aris JP, Leeuwenburgh C. New insights into the role of mitochondria in aging: mitochondrial dynamics and more. *J Cell Sci* 2010; **123**: 2533-2542 [PMID: 20940129 DOI: 10.1242/jcs.070490]

61 **Smith ALM**, Whitehall JC, Greaves LC. Mitochondrial DNA mutations in ageing and cancer. *Mol Oncol* 2022; **16**: 3276-3294 [PMID: 35842901 DOI: 10.1002/1878-0261.13291]

62 **Kobayashi H**, Imanaka S. Mitochondrial DNA Damage and Its Repair Mechanisms in Aging Oocytes. *Int J Mol Sci* 2024; **25**: 13144 [PMID: 39684855 DOI: 10.3390/ijms252313144]

63 **Fukunaga H**. Mitochondrial DNA Copy Number and Developmental Origins of Health and Disease (DOHaD). *Int J Mol Sci* 2021; **22**: 6634 [PMID: 34205712 DOI: 10.3390/ijms22126634]

64 **Peng F**, Wang S, Feng Z, Zhou K, Zhang H, Guo X, Xing J, Liu Y. Circulating cell-free mtDNA as a new biomarker for cancer detection and management. *Cancer Biol Med* 2023; **21**: 105-110 [PMID: 37823689 DOI: 10.20892/j.issn.2095-3941.2023.0280]

65 **Li L**, Hann HW, Wan S, Hann RS, Wang C, Lai Y, Ye X, Evans A, Myers RE, Ye Z, Li B, Xing J, Yang H. Cell-free circulating mitochondrial DNA content and risk of hepatocellular carcinoma in patients with chronic HBV infection. *Sci Rep* 2016; **6**: 23992 [PMID: 27063412 DOI: 10.1038/srep23992]

66 **Cerantonio A**, Citrigno L, Greco BM, De Benedittis S, Passarino G, Maletta R, Qualtieri A, Montesanto A, Spadafora P, Cavalcanti F. The Role of Mitochondrial Copy Number in Neurodegenerative Diseases: Present Insights and Future Directions. *Int J Mol Sci* 2024; **25**: 6062 [PMID: 38892250 DOI: 10.3390/ijms25116062]

67 **Yang SY**, Castellani CA, Longchamps RJ, Pillalamarri VK, O'Rourke B, Guallar E, Arking DE. Blood-derived mitochondrial DNA copy number is associated with gene expression across multiple tissues and is predictive for incident neurodegenerative disease. *Genome Res* 2021; **31**: 349-358 [PMID: 33441415 DOI: 10.1101/gr.269381.120]

68 **Gao R**, Ma SL. Is Mitochondria DNA Variation a Biomarker for AD? *Genes (Basel)* 2022; **13**: 1789 [PMID: 36292674 DOI: 10.3390/genes13101789]

69 **Rahman MA**, Rahman MDH, Rhim H, Kim B. Drug Target to Alleviate Mitochondrial Dysfunctions in Alzheimer's Disease: Recent Advances and Therapeutic Implications. *Curr Neuropharmacol* 2024; **22**: 1942-1959 [PMID: 39234772 DOI: 10.2174/1570159X22666240426091311]

70 **Wang W**, Zhao F, Ma X, Perry G, Zhu X. Mitochondria dysfunction in the pathogenesis of Alzheimer's disease: recent advances. *Mol Neurodegener* 2020; **15**: 30 [PMID: 32471464 DOI: 10.1186/s13024-020-00376-6]

71 **Gao XY**, Yang T, Gu Y, Sun XH. Mitochondrial Dysfunction in Parkinson's Disease: From Mechanistic Insights to Therapy. *Front Aging Neurosci* 2022; **14**: 885500 [PMID: 35795234 DOI: 10.3389/fnagi.2022.885500]

72 **Foo ASC**, Soong TW, Yeo TT, Lim KL. Mitochondrial Dysfunction and Parkinson's Disease-Near-Infrared Photobiomodulation as a Potential Therapeutic Strategy. *Front Aging Neurosci* 2020; **12**: 89 [PMID: 32308618 DOI: 10.3389/fnagi.2020.00089]

73 **Dölle C**, Flønes I, Nido GS, Miletic H, Osuagwu N, Kristoffersen S, Lilleng PK, Larsen JP, Tysnes OB, Haugarvoll K, Bindoff LA, Tzoulis C. Defective mitochondrial DNA homeostasis in the substantia nigra in Parkinson disease. *Nat Commun* 2016; **7**: 13548 [PMID: 27874000 DOI: 10.1038/ncomms13548]

74 **Müller-Nedebock AC**, van der Westhuizen FH, Kõks S, Bardien S. Nuclear Genes Associated with Mitochondrial DNA Processes as Contributors to Parkinson's Disease Risk. *Mov Disord* 2021; **36**: 815-831 [PMID: 33513296 DOI: 10.1002/mds.28475]

75 **Wei W**, Keogh MJ, Wilson I, Coxhead J, Ryan S, Rollinson S, Griffin H, Kurzawa-Akanbi M, Santibanez-Koref M, Talbot K, Turner MR, McKenzie CA, Troakes C, Attems J, Smith C, Al Sarraj S, Morris CM, Ansorge O, Pickering-Brown S, Ironside JW, Chinnery PF.

Mitochondrial DNA point mutations and relative copy number in 1363 disease and control human brains. *Acta Neuropathol Commun* 2017; **5**: 13 [PMID: 28153046 DOI: 10.1186/s40478-016-0404-6]

76 **Pyle A**, Anugrha H, Kurzawa-Akanbi M, Yarnall A, Burn D, Hudson G. Reduced mitochondrial DNA copy number is a biomarker of Parkinson's disease. *Neurobiol Aging* 2016; **38**: 216.e7-216.e10 [PMID: 26639155 DOI: 10.1016/j.neurobiolaging.2015.10.033]

77 **Ortega-Vázquez A**, Sánchez-Badajos S, Ramírez-García MÁ, Alvarez-Luquín D, López-López M, Adalid-Peralta LV, Monroy-Jaramillo N. Longitudinal Changes in Mitochondrial DNA Copy Number and Telomere Length in Patients with Parkinson's Disease. *Genes (Basel)* 2023; **14**: 1913 [PMID: 37895262 DOI: 10.3390/genes14101913]

78 **Ashleigh T**, Swerdlow RH, Beal MF. The role of mitochondrial dysfunction in Alzheimer's disease pathogenesis. *Alzheimers Dement* 2023; **19**: 333-342 [PMID: 35522844 DOI: 10.1002/alz.12683]

79 **MacMullen C**, Sharma N, Davis RL. Mitochondrial dynamics and bioenergetics in Alzheimer's induced pluripotent stem cell-derived neurons. *Brain* 2024 [PMID: 39513728 DOI: 10.1093/brain/awae364]

80 **Earls RH**, Menees KB, Chung J, Gutekunst CA, Lee HJ, Hazim MG, Rada B, Wood LB, Lee JK. NK cells clear α-synuclein and the depletion of NK cells exacerbates synuclein pathology in a mouse model of α-synucleinopathy. *Proc Natl Acad Sci U S A* 2020; **117**: 1762-1771 [PMID: 31900358 DOI: 10.1073/pnas.1909110117]

81 **Su X**, Chu Y, Kordower JH, Li B, Cao H, Huang L, Nishida M, Song L, Wang D, Federoff HJ. PGC-1α Promoter Methylation in Parkinson's Disease. *PLoS One* 2015; **10**: e0134087 [PMID: 26317511 DOI: 10.1371/journal.pone.0134087]

82 **Chinnery PF**, Samuels DC, Elson J, Turnbull DM. Accumulation of mitochondrial DNA mutations in ageing, cancer, and mitochondrial disease: is there a common mechanism? *Lancet* 2002; **360**: 1323-1325 [PMID: 12414225 DOI: 10.1016/S0140-6736(02)11310-9]

83 **Nissanka N**, Moraes CT. Mitochondrial DNA damage and reactive oxygen species in neurodegenerative disease. *FEBS Lett* 2018; **592**: 728-742 [PMID: 29281123 DOI: 10.1002/1873-3468.12956]

84 **Liu CS**, Cheng WL, Lee CF, Ma YS, Lin CY, Huang CC, Wei YH. Alteration in the copy number of mitochondrial DNA in leukocytes of patients with mitochondrial encephalomyopathies. *Acta Neurol Scand* 2006; **113**: 334-341 [PMID: 16629770 DOI: 10.1111/j.1600-0404.2006.00586.x]

85 **Fontana GA**, Gahlon HL. Mechanisms of replication and repair in mitochondrial DNA deletion formation. *Nucleic Acids Res* 2020; **48**: 11244-11258 [PMID: 33021629 DOI: 10.1093/nar/gkaa804]

86 **Giordano C**, Iommarini L, Giordano L, Maresca A, Pisano A, Valentino ML, Caporali L, Liguori R, Deceglie S, Roberti M, Fanelli F, Fracasso F, Ross-Cisneros FN, D'Adamo P, Hudson G, Pyle A, Yu-Wai-Man P, Chinnery PF, Zeviani M, Salomao SR, Berezovsky A, Belfort R Jr, Ventura DF, Moraes M, Moraes Filho M, Barboni P, Sadun F, De Negri A, Sadun AA, Tancredi A, Mancini M, d'Amati G, Loguercio Polosa P, Cantatore P, Carelli V. Efficient mitochondrial biogenesis drives incomplete penetrance in Leber's hereditary optic neuropathy. *Brain* 2014; **137**: 335-353 [PMID: 24369379 DOI: 10.1093/brain/awt343]

87 **Bianco A**, Bisceglia L, Russo L, Palese LL, D'Agruma L, Emperador S, Montoya J, Guerriero S, Petruzzella V. High Mitochondrial DNA Copy Number Is a Protective Factor From Vision Loss in Heteroplasmic Leber's Hereditary Optic Neuropathy (LHON). *Invest Ophthalmol Vis Sci* 2017; **58**: 2193-2197 [PMID: 28403426 DOI: 10.1167/iovs.16-20389]

88 **Bianco A**, Valletti A, Longo G, Bisceglia L, Montoya J, Emperador S, Guerriero S, Petruzzella V. Mitochondrial DNA copy number in affected and unaffected LHON mutation carriers. *BMC Res Notes* 2018; **11**: 911 [PMID: 30572950 DOI: 10.1186/s13104-018-4025-y]

89 **Fassad MR**, Desouky LM, Asal S, Abdalla EM. Screening for the mitochondrial A1555G mutation among Egyptian patients with non-syndromic, sensorineural hearing loss. *Int J Mol Epidemiol Genet* 2014; **5**: 200-204 [PMID: 25755848]

90 **Geurts J**, Nasi S, Distel P, Müller-Gerbl M, Prolla TA, Kujoth GC, Walker UA, Hügle T. Prematurely aging mitochondrial DNA mutator mice display subchondral osteopenia and chondrocyte hypertrophy without further osteoarthritis features. *Sci Rep* 2020; **10**: 1296 [PMID: 31992827 DOI: 10.1038/s41598-020-58385-w]

91 **Dimmock D**, Tang LY, Schmitt ES, Wong LJ. Quantitative evaluation of the mitochondrial DNA depletion syndrome. *Clin Chem* 2010; **56**: 1119-1127 [PMID: 20448188 DOI: 10.1373/clinchem.2009.141549]

92 **Pedersen ZO**, Holm-Yildiz S, Dysgaard T. Nutritional Interventions for Patients with Mitochondrial POLG-Related Diseases: A Systematic Review on Efficacy and Safety. *Int J Mol Sci* 2022; **23**: 10658 [PMID: 36142570 DOI: 10.3390/ijms231810658]

93 **Copeland WC**. The mitochondrial DNA polymerase in health and disease. *Subcell Biochem* 2010; **50**: 211-222 [PMID: 20012584 DOI: 10.1007/978-90-481-3471-7_11]

94 **Kaguni LS**. DNA polymerase gamma, the mitochondrial replicase. *Annu Rev Biochem* 2004; **73**: 293-320 [PMID: 15189144 DOI: 10.1146/annurev.biochem.72.121801.161455]

95 **Rahman S**, Copeland WC. POLG-related disorders and their neurological manifestations. *Nat Rev Neurol* 2019; **15**: 40-52 [PMID: 30451971 DOI: 10.1038/s41582-018-0101-0]

96 **Grady JP**, Pickett SJ, Ng YS, Alston CL, Blakely EL, Hardy SA, Feeney CL, Bright AA, Schaefer AM, Gorman GS, McNally RJ, Taylor RW, Turnbull DM, McFarland R. mtDNA heteroplasmy level and copy number indicate disease burden in m.3243A>G mitochondrial disease. *EMBO Mol Med* 2018; **10**: e8262 [PMID: 29735722 DOI: 10.15252/emmm.201708262]

97 **Nakamura M**, Yabe I, Sudo A, Hosoki K, Yaguchi H, Saitoh S, Sasaki H. MERRF/MELAS overlap syndrome: a double pathogenic mutation in mitochondrial tRNA genes. *J Med Genet* 2010; **47**: 659-664 [PMID: 20610441 DOI: 10.1136/jmg.2009.072058]

98 **Wang P**, Song M, Zeng ZL, Zhu CF, Lu WH, Yang J, Ma MZ, Huang AM, Hu Y, Huang P. Identification of NDUFAF1 in mediating K-Ras induced mitochondrial dysfunction by a proteomic screening approach. *Oncotarget* 2015; **6**: 3947-3962 [PMID: 25714130 DOI: 10.18632/oncotarget.2968]

99 **Mimaki M**, Wang X, McKenzie M, Thorburn DR, Ryan MT. Understanding mitochondrial complex I assembly in health and disease. *Biochim Biophys Acta* 2012; **1817**: 851-862 [PMID: 21924235 DOI: 10.1016/j.bbabio.2011.08.010]

100 **Moiseeva O**, Bourdeau V, Roux A, Deschênes-Simard X, Ferbeyre G. Mitochondrial dysfunction contributes to oncogene-induced senescence. *Mol Cell Biol* 2009; **29**: 4495-4507 [PMID: 19528227 DOI: 10.1128/MCB.01868-08]

101 **Kopinski PK**, Singh LN, Zhang S, Lott MT, Wallace DC. Mitochondrial DNA variation and cancer. *Nat Rev Cancer* 2021; **21**: 431-445 [PMID: 34045735 DOI: 10.1038/s41568-021-00358-w]

102 **Domínguez-de-la-Cruz E**, Muñoz ML, Pérez-Muñoz A, García-Hernández N, Moctezuma-Meza C, Hinojosa-Cruz JC. Reduced mitochondrial DNA copy number is associated with the haplogroup, and some clinical features of breast cancer in Mexican patients. *Gene* 2020; **761**: 145047 [PMID: 32783993 DOI: 10.1016/j.gene.2020.145047]

103 **Kim M**, Gorelick AN, Vàzquez-García I, Williams MJ, Salehi S, Shi H, Weiner AC, Ceglia N, Funnell T, Park T, Boscenco S, O'Flanagan CH,

Jiang H, Grewal D, Tang C, Rusk N, Gammage PA, McPherson A, Aparicio S, Shah SP, Reznik E. Single-cell mtDNA dynamics in tumors is driven by coregulation of nuclear and mitochondrial genomes. *Nat Genet* 2024; **56**: 889-899 [PMID: 38741018 DOI: 10.1038/s41588-024-01724-8]

104 **Thyagarajan B**, Wang R, Nelson H, Barcelo H, Koh WP, Yuan JM. Mitochondrial DNA copy number is associated with breast cancer risk. *PLoS One* 2013; **8**: e65968 [PMID: 23776581 DOI: 10.1371/journal.pone.0065968]

105 **Mi J**, Tian G, Liu S, Li X, Ni T, Zhang L, Wang B. The relationship between altered mitochondrial DNA copy number and cancer risk: a meta-analysis. *Sci Rep* 2015; **5**: 10039 [PMID: 25952580 DOI: 10.1038/srep10039]

106 **Shen J**, Song R, Lu Z, Zhao H. Mitochondrial DNA copy number in whole blood and glioma risk: A case control study. *Mol Carcinog* 2016; **55**: 2089-2094 [PMID: 26756431 DOI: 10.1002/mc.22453]

107 **Lemnrau A**, Brook MN, Fletcher O, Coulson P, Tomczyk K, Jones M, Ashworth A, Swerdlow A, Orr N, Garcia-Closas M. Mitochondrial DNA Copy Number in Peripheral Blood Cells and Risk of Developing Breast Cancer. *Cancer Res* 2015; **75**: 2844-2850 [PMID: 25977328 DOI: 10.1158/0008-5472.CAN-14-1692]

108 **Shen J**, Wan J, Song R, Zhao H. Peripheral blood mitochondrial DNA copy number, length heteroplasmy and breast cancer risk: a replication study. *Carcinogenesis* 2015; **36**: 1307-1313 [PMID: 26363030 DOI: 10.1093/carcin/bgv130]

109 **Huang B**, Gao YT, Shu XO, Wen W, Yang G, Li G, Courtney R, Ji BT, Li HL, Purdue MP, Zheng W, Cai Q. Association of leukocyte mitochondrial DNA copy number with colorectal cancer risk: Results from the Shanghai Women's Health Study. *Cancer Epidemiol Biomarkers Prev* 2014; **23**: 2357-2365 [PMID: 25139937 DOI: 10.1158/1055-9965.EPI-14-0297]

110 **Xing J**, Chen M, Wood CG, Lin J, Spitz MR, Ma J, Amos CI, Shields PG, Benowitz NL, Gu J, de Andrade M, Swan GE, Wu X. Mitochondrial DNA content: its genetic heritability and association with renal cell carcinoma. *J Natl Cancer Inst* 2008; **100**: 1104-1112 [PMID: 18664653 DOI: 10.1093/jnci/djn213]

111 **Lin YH**, Lim SN, Chen CY, Chi HC, Yeh CT, Lin WR. Functional Role of Mitochondrial DNA in Cancer Progression. *Int J Mol Sci* 2022; **23**: 1659 [PMID: 35163579 DOI: 10.3390/ijms23031659]

112 **Gentiluomo M**, Katzke VA, Kaaks R, Tjønneland A, Severi G, Perduca V, Boutron-Ruault MC, Weiderpass E, Ferrari P, Johnson T, Schulze MB, Bergmann M, Trichopoulou A, Karakatsani A, La Vecchia C, Palli D, Grioni S, Panico S, Tumino R, Sacerdote C, Bueno-de-Mesquita B, Vermeulen R, Sandanger TM, Quirós JR, Rodriguez-Barranco M, Amiano P, Colorado-Yohar S, Ardanaz E, Sund M, Khaw KT, Wareham NJ, Schmidt JA, Jakszyn P, Morelli L, Canzian F, Campa D. Mitochondrial DNA Copy-Number Variation and Pancreatic Cancer Risk in the Prospective EPIC Cohort. *Cancer Epidemiol Biomarkers Prev* 2020; **29**: 681-686 [PMID: 31932413 DOI: 10.1158/1055-9965.EPI-19-0868]

113 **Yu M**, Wan Y, Zou Q. Decreased copy number of mitochondrial DNA in Ewing's sarcoma. *Clin Chim Acta* 2010; **411**: 679-683 [PMID: 20123091 DOI: 10.1016/j.cca.2010.01.035]

114 **Hua L**, Juratli TA, Zhu H, Deng J, Wang D, Sun S, Xie Q, Wakimoto H, Gong Y. High Tumor Mitochondrial DNA Content Correlates With an Improved Patient's Outcome in WHO Grade III Meningioma. *Front Oncol* 2020; **10**: 542294 [PMID: 33072573 DOI: 10.3389/fonc.2020.542294]

115 **Li Y**, Sundquist K, Vats S, Hong MG, Wang X, Chen Y, Hedelius A, Saal LH, Sundquist J, Memon AA. Mitochondrial heteroplasmic shifts reveal a positive selection of breast cancer. *J Transl Med* 2023; **21**: 696 [PMID: 37798736 DOI: 10.1186/s12967-023-04534-4]

116 **Feng S**, Xiong L, Ji Z, Cheng W, Yang H. Correlation between increased copy number of mitochondrial DNA and clinicopathological stage in colorectal cancer. *Oncol Lett* 2011; **2**: 899-903 [PMID: 22866147 DOI: 10.3892/ol.2011.322]

117 **Wang Y**, He S, Zhu X, Qiao W, Zhang J. High copy number of mitochondrial DNA predicts poor prognosis in patients with advanced stage colon cancer. *Int J Biol Markers* 2016; **31**: e382-e388 [PMID: 27197581 DOI: 10.5301/jbm.5000211]

118 **Lin CS**, Wang LS, Tsai CM, Wei YH. Low copy number and low oxidative damage of mitochondrial DNA are associated with tumor progression in lung cancer tissues after neoadjuvant chemotherapy. *Interact Cardiovasc Thorac Surg* 2008; **7**: 954-958 [PMID: 18685121 DOI: 10.1510/icvts.2008.177006]

119 **Lee HC**, Yin PH, Lin JC, Wu CC, Chen CY, Wu CW, Chi CW, Tam TN, Wei YH. Mitochondrial genome instability and mtDNA depletion in human cancers. *Ann N Y Acad Sci* 2005; **1042**: 109-122 [PMID: 15965052 DOI: 10.1196/annals.1338.011]

120 **Dasgupta S**, Soudry E, Mukhopadhyay N, Shao C, Yee J, Lam S, Lam W, Zhang W, Gazdar AF, Fisher PB, Sidransky D. Mitochondrial DNA mutations in respiratory complex-I in never-smoker lung cancer patients contribute to lung cancer progression and associated with EGFR gene mutation. *J Cell Physiol* 2012; **227**: 2451-2460 [PMID: 21830212 DOI: 10.1002/jcp.22980]

121 **Zhang L**, Henske EP. Chromophobe renal cell carcinoma: New genetic and metabolic insights. *Urol Oncol* 2020; **38**: 678-681 [PMID: 32444178 DOI: 10.1016/j.urolonc.2020.04.035]

122 **Gopal RK**, Calvo SE, Shih AR, Chaves FL, McGuone D, Mick E, Pierce KA, Li Y, Garofalo A, Van Allen EM, Clish CB, Oliva E, Mootha VK. Early loss of mitochondrial complex I and rewiring of glutathione metabolism in renal oncocytoma. *Proc Natl Acad Sci U S A* 2018; **115**: E6283-E6290 [PMID: 29915083 DOI: 10.1073/pnas.1711888115]

123 **Chattopadhyay M**, Jenkins EC, Lechuga-Vieco AV, Nie K, Fiel MI, Rialdi A, Guccione E, Enriquez JA, Sia D, Lujambio A, Germain D. The portrait of liver cancer is shaped by mitochondrial genetics. *Cell Rep* 2022; **38**: 110254 [PMID: 35045282 DOI: 10.1016/j.celrep.2021.110254]

124 **Schöpf B**, Weissensteiner H, Schäfer G, Fazzini F, Charoentong P, Naschberger A, Rupp B, Fendt L, Bukur V, Giese I, Sorn P, Sant'Anna-Silva AC, Iglesias-Gonzalez J, Sahin U, Kronenberg F, Gnaiger E, Klocker H. OXPHOS remodeling in high-grade prostate cancer involves mtDNA mutations and increased succinate oxidation. *Nat Commun* 2020; **11**: 1487 [PMID: 32198407 DOI: 10.1038/s41467-020-15237-5]

125 **Yuan Y**, Ju YS, Kim Y, Li J, Wang Y, Yoon CJ, Yang Y, Martincorena I, Creighton CJ, Weinstein JN, Xu Y, Han L, Kim HL, Nakagawa H, Park K, Campbell PJ, Liang H; PCAWG Consortium. Comprehensive molecular characterization of mitochondrial genomes in human cancers. *Nat Genet* 2020; **52**: 342-352 [PMID: 32024997 DOI: 10.1038/s41588-019-0557-x]

126 **Toure S**, Mbaye F, Gueye MD, Fall M, Dem A, Lamy JB, Sembene M. Somatic Mitochondrial Mutations in Oral Cavity Cancers among Senegalese Patients. *Asian Pac J Cancer Prev* 2019; **20**: 2203-2208 [PMID: 31350985 DOI: 10.31557/APJCP.2019.20.7.2203]

127 **Pérez-Amado CJ**, Tovar H, Gómez-Romero L, Beltrán-Anaya FO, Bautista-Piña V, Dominguez-Reyes C, Villegas-Carlos F, Tenorio-Torres A, Alfaro-Ruíz LA, Hidalgo-Miranda A, Jiménez-Morales S. Mitochondrial DNA Mutation Analysis in Breast Cancer: Shifting From Germline Heteroplasmy Toward Homoplasmy in Tumors. *Front Oncol* 2020; **10**: 572954 [PMID: 33194675 DOI: 10.3389/fonc.2020.572954]

128 **Ju YS**, Alexandrov LB, Gerstung M, Martincorena I, Nik-Zainal S, Ramakrishna M, Davies HR, Papaemmanuil E, Gundem G, Shlien A, Bolli N, Behjati S, Tarpey PS, Nangalia J, Massie CE, Butler AP, Teague JW, Vassiliou GS, Green AR, Du MQ, Unnikrishnan A, Pimanda JE, Teh BT, Munshi N, Greaves M, Vyas P, El-Naggar AK, Santarius T, Collins VP, Grundy R, Taylor JA, Hayes DN, Malkin D; ICGC Breast Cancer Group; ICGC Chronic Myeloid Disorders Group; ICGC Prostate Cancer Group, Foster CS, Warren AY, Whitaker HC, Brewer D, Eeles R,

Cooper C, Neal D, Visakorpi T, Isaacs WB, Bova GS, Flanagan AM, Futreal PA, Lynch AG, Chinnery PF, McDermott U, Stratton MR, Campbell PJ. Origins and functional consequences of somatic mitochondrial DNA mutations in human cancer. *Elife* 2014; **3**: e02935 [PMID: 25271376 DOI: 10.7554/eLife.02935]

129 **Stewart JB**, Alaei-Mahabadi B, Sabarinathan R, Samuelsson T, Gorodkin J, Gustafsson CM, Larsson E. Simultaneous DNA and RNA Mapping of Somatic Mitochondrial Mutations across Diverse Human Cancers. *PLoS Genet* 2015; **11**: e1005333 [PMID: 26125550 DOI: 10.1371/journal.pgen.1005333]

130 **Sun X**, Zhan L, Chen Y, Wang G, He L, Wang Q, Zhou F, Yang F, Wu J, Wu Y, Xing J, He X, Huang Q. Increased mtDNA copy number promotes cancer progression by enhancing mitochondrial oxidative phosphorylation in microsatellite-stable colorectal cancer. *Signal Transduct Target Ther* 2018; **3**: 8 [PMID: 29610678 DOI: 10.1038/s41392-018-0011-z]

131 **Kürschner G**, Zhang Q, Clima R, Xiao Y, Busch JF, Kilic E, Jung K, Berndt N, Bulik S, Holzhütter HG, Gasparre G, Attimonelli M, Babu M, Meierhofer D. Renal oncocytoma characterized by the defective complex I of the respiratory chain boosts the synthesis of the ROS scavenger glutathione. *Oncotarget* 2017; **8**: 105882-105904 [PMID: 29285300 DOI: 10.18632/oncotarget.22413]

132 **Smith AL**, Whitehall JC, Bradshaw C, Gay D, Robertson F, Blain AP, Hudson G, Pyle A, Houghton D, Hunt M, Sampson JN, Stamp C, Mallett G, Amarnath S, Leslie J, Oakley F, Wilson L, Baker A, Russell OM, Johnson R, Richardson CA, Gupta B, McCallum I, McDonald SA, Kelly S, Mathers JC, Heer R, Taylor RW, Perkins ND, Turnbull DM, Sansom OJ, Greaves LC. Age-associated mitochondrial DNA mutations cause metabolic remodelling that contributes to accelerated intestinal tumorigenesis. *Nat Cancer* 2020; **1**: 976-989 [PMID: 33073241 DOI: 10.1038/s43018-020-00112-5]

133 **Herzig S**, Shaw RJ. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat Rev Mol Cell Biol* 2018; **19**: 121-135 [PMID: 28974774 DOI: 10.1038/nrm.2017.95]

134 **Kjøbsted R**, Hingst JR, Fentz J, Foretz M, Sanz MN, Pehmøller C, Shum M, Marette A, Mounier R, Treebak JT, Wojtaszewski JFP, Viollet B, Lantier L. AMPK in skeletal muscle function and metabolism. *FASEB J* 2018; **32**: 1741-1777 [PMID: 29242278 DOI: 10.1096/fj.201700442R]

135 **Halling JF**, Pilegaard H. PGC-1α-mediated regulation of mitochondrial function and physiological implications. *Appl Physiol Nutr Metab* 2020; **45**: 927-936 [PMID: 32516539 DOI: 10.1139/apnm-2020-0005]

136 **Suárez-Rivero JM**, Pastor-Maldonado CJ, Povea-Cabello S, Álvarez-Córdoba M, Villalón-García I, Talaverón-Rey M, Suárez-Carrillo A, Munuera-Cabeza M, Reche-López D, Cilleros-Holgado P, Piñero-Pérez R, Sánchez-Alcázar JA. Activation of the Mitochondrial Unfolded Protein Response: A New Therapeutic Target? *Biomedicines* 2022; **10**: 1611 [PMID: 35884915 DOI: 10.3390/biomedicines10071611]

137 **Dikic I**, Elazar Z. Mechanism and medical implications of mammalian autophagy. *Nat Rev Mol Cell Biol* 2018; **19**: 349-364 [PMID: 29618831 DOI: 10.1038/s41580-018-0003-4]

138 **Rabanal-Ruiz Y**, Otten EG, Korolchuk VI. mTORC1 as the main gateway to autophagy. *Essays Biochem* 2017; **61**: 565-584 [PMID: 29233869 DOI: 10.1042/EBC20170027]

139 **Barriocanal-Casado E**, Hidalgo-Gutiérrez A, Raimundo N, González-García P, Acuña-Castroviejo D, Escames G, López LC. Rapamycin administration is not a valid therapeutic strategy for every case of mitochondrial disease. *EBioMedicine* 2019; **42**: 511-523 [PMID: 30898651 DOI: 10.1016/j.ebiom.2019.03.025]

140 **van de Wal MAE**, Adjobo-Hermans MJW, Keijer J, Schirris TJJ, Homberg JR, Wieckowski MR, Grefte S, van Schothorst EM, van Karnebeek C, Quintana A, Koopman WJH. Ndufs4 knockout mouse models of Leigh syndrome: pathophysiology and intervention. *Brain* 2022; **145**: 45-63 [PMID: 34849584 DOI: 10.1093/brain/awab426]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 100598

DOI: 10.5662/wjm.v15.i3.100598

ISSN 2222-0682 (online)

MINIREVIEWS

Artificial intelligence in revolutionizing orthodontic practice

Paul Fawaz, Patrick El Sayegh, Bart Vande Vannet

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade D
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Maslova ZN

Received: August 20, 2024
Revised: October 7, 2024
Accepted: December 18, 2024
Published online: September 20, 2025
Processing time: 196 Days and 21.3 Hours

Paul Fawaz, Bart Vande Vannet, Faculty of Dentistry, Department of Orthodontics, University Lorraine, Nancy 54000, France

Patrick El Sayegh, Faculty of Dentistry, Saint Joseph University of Beirut, Beirouth 11042020, Lebanon

Bart Vande Vannet, Institut Jean Lamour, Campus Artem (403), University Lorraine, Nancy 54000, France

Co-first authors: Paul Fawaz and Patrick El Sayegh.

Corresponding author: Bart Vande Vannet, DDS, MSc, PhD, Professor, Department of Orthodontics, University Lorraine, Nancy 54000, France. bart.vande-vannet@univ-lorraine.fr

Abstract

This analytical research paper explores the transformative impact of artificial intelligence (AI) in orthodontics, with a focus on its objectives: Identifying current applications, evaluating benefits, addressing challenges, and projecting future developments. AI, a subset of computer science designed to simulate human intelligence, has seen rapid integration into orthodontic practice. The paper examines AI technologies such as machine learning, deep learning, natural language processing, computer vision, and robotics, which are increasingly used to analyze patient data, assist with diagnosis and treatment planning, automate routine tasks, and improve patient communication. AI systems offer precise malocclusion diagnoses, predict treatment outcomes, and customize treatment plans by leveraging dental imagery. They also streamline image analysis, improve diagnostic accuracy, and enhance patient engagement through personalized communication. The objectives include evaluating the benefits of AI in terms of efficiency, accuracy, and personalized care, while acknowledging the challenges like data quality, algorithm transparency, and practical implementation. Despite these hurdles, AI presents promising prospects in advanced imaging, predictive analytics, and clinical decision-making. In conclusion, AI holds the potential to revolutionize orthodontic practices by improving operational efficiency, diagnostic precision and patient outcomes. With collaborative efforts to overcome challenges, AI could play a pivotal role in advancing orthodontic care.

Key Words: Artificial intelligence; Orthodontics; Machine learning; Deep learning; Diagnosis; Treatment planning; Patient management; Efficiency; Accuracy; Personalized treatment; Challenges; Future directions

Core Tip: This paper explores the future of orthodontics, highlighting the integration of artificial intelligence (AI) and digital technologies. AI is becoming crucial in treatment planning, yet clinicians remain essential in decision-making. The growing role of 3D digital technologies in orthodontics reflects AI's increasing influence, but ethical and legal challenges persist. Emphasizing the need for clinical trials, the study calls for further exploration of AI's potential to transform traditional orthodontic practices.

Citation: Fawaz P, El Sayegh P, Vande Vannet B. Artificial intelligence in revolutionizing orthodontic practice. *World J Methodol* 2025; 15(3): 100598
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/100598.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.100598

INTRODUCTION

In recent years, the field of orthodontics has seen significant technological advancements, particularly in artificial intelligence (AI). AI, a branch of computer science that enables machines to simulate human intelligence, has become a powerful tool with the potential to transform various aspects of orthodontic practice. This emerging field allows computers to perform tasks previously done by humans. Though still in its early stages, AI is becoming integral to many sectors, including healthcare, where researchers are incorporating it to enhance patient care. Each dental specialty stands to benefit from AI through improved precision of care, more accurate diagnoses, and time savings in both clinical and administrative tasks. While the digital shift in the profession is well underway, it is crucial for clinicians to rigorously assess the reliability and accuracy of each AI system being introduced[1]. This article examines the current state of AI in orthodontics, its applications, benefits, challenges, and prospects.

OVERVIEW OF AI

AI encompasses a range of technologies and techniques that enable computers to perform tasks that traditionally require human intelligence[2]. These include machine learning (ML), deep learning, natural language processing, computer vision, and robotics. In orthodontics, AI systems leverage these technologies to analyze patient data, assist in diagnosis and treatment planning, automate tasks, and improve patient communication and management. Machine intelligence functions like machines. It adheres to the fundamental hierarchy of machines: Input, processing, and output. Input data can be voice data (handheld sounds), text data (medical or processing records, experimental parameters), or picture data (X-ray images, photos). The neural networks process this input data and provide an output. The result can be a prognosis, diagnosis, treatment, or disease prediction. In orthodontics, two of the previous options could be beneficial: Diagnosis and treatment planning (Figure 1).

APPLICATIONS OF AI IN ORTHODONTICS

ML in orthodontics

Various approaches can be employed to analyze different types of orthodontic records, leading to accurate and accelerated diagnoses. By directly engaging with patient records, these results can be used to develop effective treatment plans. ML and deep learning techniques have transformed medical diagnostics, including orthodontics. By utilizing vast datasets and sophisticated algorithms, these methods can swiftly and accurately analyze orthodontic records such as dental images, patient histories, and treatment outcomes. This leads to more precise diagnoses, enabling orthodontists to create treatment plans that are better tailored to each patient's needs.

Additionally, integrating ML into orthodontic practice can significantly speed up the diagnostic process. Traditional methods often involve manual examination of patient records and subjective interpretation by clinicians, which can be time-consuming and susceptible to human error. By contrast, ML algorithms can quickly process large volumes of data, identifying patterns and anomalies that may not be immediately noticeable to human observers. This expedited diagnostic process allows for faster treatment initiation, ultimately improving patient outcomes and satisfaction.

The synergy between ML systems and patient records supports a personalized approach to orthodontic treatment planning. By incorporating individual patient data, such as medical history, genetic factors, and treatment preferences, into the analysis, clinicians can develop customized treatment plans that address specific patient needs and goals[2].

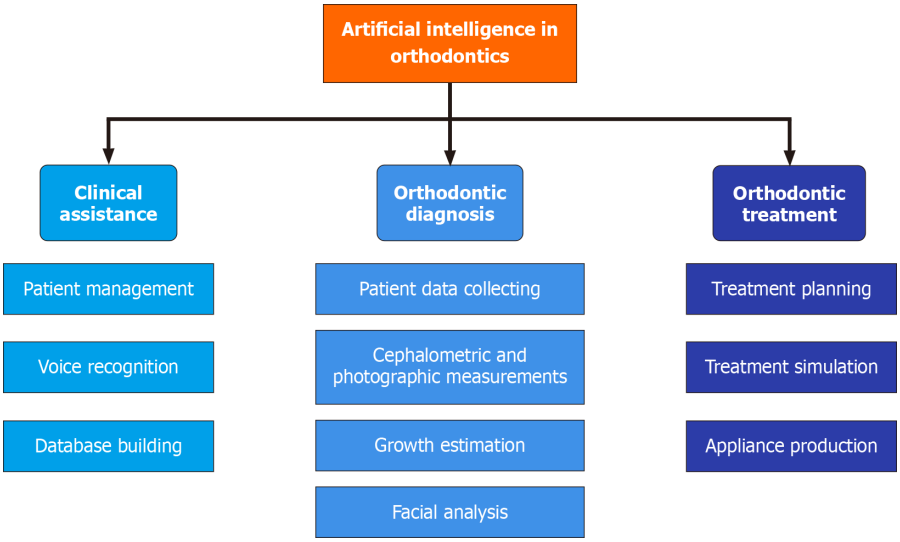


Figure 1 The role of artificial intelligence in orthodontics.

Diagnosis and treatment planning

AI algorithms can analyze patient records, including dental images such as X-rays, intraoral scans, and photographs, to assist orthodontists in diagnosing malocclusions, predicting treatment outcomes, and designing personalized treatment plans[3]. For instance, AI systems can accurately detect anomalies in dental images, measure tooth and bone structures, and identify risk factors for orthodontic issues.

Imaging and analysis

AI-powered software can automatically segment dental images, extract relevant features, and analyze them to detect abnormalities and track treatment progress[4]. This automation streamlines the diagnostic process, reduces errors, and allows orthodontists to focus more on patient care. Additionally, AI algorithms can enhance imaging techniques such as cone-beam computed tomography (CBCT) and magnetic resonance imaging (MRI), leading to improved visualization and analysis of dental structures.

Image analysis using AI can be divided into four categories: Image filtering and knowledge-based landmark search, model-based approaches, soft-computing and model-based approaches, and hybrid approaches. Many web-based applications are available for clinicians to perform image analysis *via* AI, including CephX (Herzliya, Israel), WebCeph (Gyeonggi-do, Republic of Korea), Dolphin Imaging (Los Angeles, CA, United States), and AudaxCeph (Ljubljana, Slovenia).

For example, WebCeph (Gyeonggi-do, Republic of Korea) is a fully automated web-based platform powered by AI that can perform nine different cephalometric analyses and provide interpretations based on the obtained cephalometric measurements. It can store and preserve digital cephalograms, orthopantomograms, and patient photographs. Its features, such as simulation and visual treatment overlays, are highly beneficial in daily orthodontic practice.

AI can perform various tasks, such as prediction and classification, using different algorithms. Hatice *et al*[5] aimed to determine cervical vertebral stages (CVS) for growth and development periods using six commonly used AI classifiers and compared their performances. The classifiers are k-nearest neighbors (k-NN), Naive Bayes (NB), decision tree (Tree), artificial neural networks (ANNs), support vector machine (SVM), random forest (RF), and logistic regression (LR). Among these algorithms, k-NN and LR had the lowest precision values, while SVM, RF, Tree, and NB had variable precision values. ANNs might be the preferred method for determining CVS.

Patient management and communication

The traditional method of acquiring orthodontic images, which involves manual classification, archiving, and monitoring, is time-consuming and prone to errors due to fatigue. With advancements in digital dentistry, imaging data is increasingly being indexed and stored in digital archives, allowing for easy retrieval for diagnostic, treatment, and subsequent monitoring purposes. Developing an efficient AI tool for the automated classification and monitoring of orthodontic images would be highly beneficial. AI-driven tools, such as chatbots and virtual assistants, can provide patients with personalized treatment information, appointment reminders, and post-treatment care instructions[6]. These digital tools enhance patient engagement, satisfaction, and adherence to treatment plans. Additionally, AI systems can analyze patient feedback and preferences to optimize treatment protocols and improve the overall patient experience.

Deep learning, a branch of ML, excels in analyzing high-dimensional data such as texts and images. In computer vision, deep learning has replaced many traditional ML tasks, including classification, segmentation, and detection. In orthodontics, studies have begun applying deep learning for diagnosis, screening, and decision-making[7,8].

The performance of the DeepID model was thoroughly evaluated through external tests and compared with orthodontists. The findings demonstrated that deep learning methods could automatically classify, archive, and monitor orthodontic images with greater accuracy and speed than manual methods[9] (Figure 2).

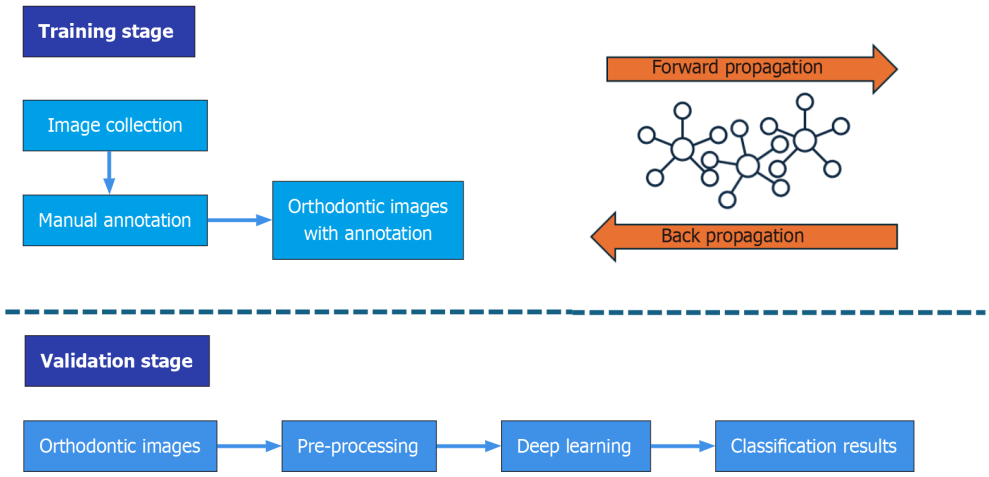


Figure 2 Deep learning workflow for orthodontic image analysis.

Dental monitoring (DM, Paris, France) is a new orthodontic application that combines teledentistry with AI using a knowledge-based algorithm, enabling precise semi-automatic treatment monitoring. It is the world's first Software as a Service (commonly referred to as SaaS) application designed for remote dental care monitoring, allowing orthodontists to remotely monitor patients' treatments using intraoral photos taken by patients with their smartphones and a special ScanBox. An AI-powered system in DM performs a preliminary assessment of data from intraoral images, partially automating communication between the doctor, staff, and patient. Unlike other dental applications, it provides validated real-time information through the knowledge-based algorithm.

During orthodontic treatment with aligners, remote monitoring should be performed at each aligner change. This allows clinicians to assess fit, ensure the adaptability of the aligners, check the presence or integrity of attachments, maintain elastic buttons, and monitor the integrity of the teeth and aligners. The DM system provides follow-up information to the patient based on a preliminary analysis conducted by AI. For aligner treatment, immediately after the intraoral scan, the DM application informs the patient whether they can proceed to switch to the next aligner and start using it (indicated by "GO") or if they should continue using the current one (indicated by "No Go"). If a "No Go" signal is given, a new intraoral scan may be required after a few days.

BENEFITS OF AI IN ORTHODONTICS

Improved efficiency

AI technologies automate repetitive tasks, such as image analysis and data processing, allowing orthodontists to work more efficiently and focus on complex cases[10]. This leads to faster diagnosis, treatment planning, and decision-making, ultimately reducing treatment times and enhancing patient outcomes.

Enhanced accuracy

AI algorithms can analyze large volumes of patient data with greater speed and accuracy than human professionals[11]. This allows orthodontists to make more informed decisions, avoid diagnostic errors, and achieve optimal treatment outcomes. Furthermore, AI systems can learn from past cases, continuously enhancing their diagnostic and predictive capabilities over time.

Personalized treatment

By analyzing patient-specific data, such as dental anatomy, facial characteristics, and treatment history, AI systems can tailor specific treatment plans to individual patient needs and preferences[12]. This individualized approach improves treatment outcomes, patient satisfaction, and long-term oral health.

Dental extractions

When considering dental extractions as part of orthodontic treatment, AI systems can analyze cases and guide orthodontists in selecting specific treatment plans. In orthodontic treatment planning, various factors affect the decision to extract teeth, such as systemic diseases, remaining growth, and patient's primary complaints. Space analysis results should not be the sole criterion for extraction but remain a priority when addressing crowding issues. For conditions such as bimaxillary protrusion, profile improvement, orthognathic surgery planning, and aesthetic considerations, extraction may be necessary based on established norms for dentomaxillary discrepancy. Orthodontists develop treatment plans based on clinical evidence, personal experience, and potential biases from past treatments. These plans are influenced by patient history, personal philosophy, and aesthetic standards. In some cases, treatment decisions can rely solely on intra-

oral photographs, which provide sufficient information for experienced clinicians. However, determining the need for extractions from clinical photos poses a limitation for AI, which relies on accumulated data for training (ML).

Two AI models have been developed using photographic input data for different purposes: Landmark detection models with crowding categorization capabilities and diagnostic models for orthodontic extractions. Factors beyond crowding may also influence extraction decisions, necessitating advanced AI algorithms to improve their generalizability and support clinical decision-making effectively[13].

Aligners and maxillary distalization

AI algorithms play a crucial role in the planning and staging of aligners. A key consideration in aligner preparation is dividing the desired dental movements into logical stages. Specialized AI algorithms typically handle this automatic staging by sequentially dividing the planned movements. Another important biomechanical consideration during aligner treatment is anchorage. For instance, when distalizing premolars and molars followed by retracting anterior teeth, maintaining anchorage and preventing lingual tipping of incisors are critical. This requires a sequential movement of teeth: First molars are distalized, followed by premolars, canines, and finally incisors. Some aligner treatment software incorporates AI algorithms that automatically manage this sequential dental movement as part of aligner treatment planning.

Clinical decision support systems

AI-powered clinical decision support systems aid orthodontists in making real-time decisions by providing evidence-based recommendations, treatment guidelines, and risk assessments[14]. These systems analyze patient data, scientific literature, and clinical guidelines to offer personalized treatment suggestions and enhance clinical decision-making.

To enhance orthodontic care, clinicians must utilize ML and AI tools to analyze relationships among dentition, craniofacial skeleton, and soft tissues. This knowledge can then be applied to advance orthodontic diagnosis, treatment planning, growth and development assessment, progress evaluation, treatment outcomes, and stability assessment.

Currently, companies manufacturing aligners utilize digital dental model data and AI algorithms to predict and plan tooth movement and perform tooth segmentation. However, clinicians should exercise caution when relying on predictions from these AI algorithms and when monitoring treatment outcomes. Moreover, these technological advancements require integrating multiple sources, including clinical information, CBCT, digital dental models, photographs, cephalograms, and panoramic images.

CHALLENGES AND LIMITATIONS OF AI IN ORTHODONTICS

Data quality and bias

AI algorithms rely on extensive and varied datasets for training to achieve reliable performance[15]. Nevertheless, the quality and representativeness of available data can vary, potentially introducing biases and inaccuracies into AI models. Moreover, ethical considerations such as data privacy and regulatory compliance are essential to ensure the responsible use of patient data in AI applications.

Interpretability and transparency

AI systems frequently function as "black boxes", which can pose challenges for orthodontists in comprehending how specific diagnoses or treatment recommendations are generated[16]. Improving the interpretability and transparency of AI algorithms is crucial to building trust and gaining acceptance among clinicians and patients.

Integration and adoption

Successfully integrating AI into orthodontic practice involves overcoming technical, organizational, and cultural hurdles [17]. Orthodontists need sufficient training to effectively utilize AI technologies, and practice workflows may require redesigning to incorporate AI-driven processes. Moreover, the initial high costs of implementing AI systems and the ongoing need for maintenance and updates can present adoption challenges, especially in smaller practices and resource-limited environments.

FUTURE DIRECTIONS AND OPPORTUNITIES

Despite the challenges, the future of AI in orthodontics is promising, with numerous opportunities for further research, development, and innovation. Some potential areas for future exploration include.

Advanced imaging techniques

AI algorithms have the potential to augment the capabilities of established imaging modalities like CBCT, MRI, and 3D scanning, offering comprehensive anatomical details and enhancing diagnostic precision[18]. Furthermore, AI-powered image reconstruction and enhancement algorithms can optimize image quality and minimize radiation exposure, thereby benefiting both clinicians and patients.

Predictive analytics

AI systems have the capability to analyze longitudinal patient data, enabling prediction of treatment outcomes, anticipation of complications, and optimization of treatment protocols[19]. With ML and predictive analytics techniques, orthodontists can discern patterns and trends in patient responses to treatment, facilitating the development of personalized intervention strategies aimed at enhancing outcomes.

CONCLUSION

The future of orthodontics will increasingly integrate digital technologies and AI, fostering a balance between technological advancements and the indispensable role of human intelligence and creativity. As AI continues to evolve, it will play a critical role in supporting digital treatment planning through the incorporation of computational geometry, biomechanics, 3D visualization, and enhanced human-machine interaction. AI algorithms are already becoming central to the field of digital orthodontics, influencing nearly every aspect of patient analysis, diagnosis, and treatment planning. However, while AI promises to significantly enhance the precision, efficiency, and customization of orthodontic practices, it will not replace the expertise and judgment of clinicians in the foreseeable future. The growing prominence of 3D digital technologies reflects AI's increasing role, but the responsibility for final health decisions will still rest with clinicians. As AI becomes more prevalent, the ethical and legal considerations surrounding its use in medical settings, including orthodontics, will demand greater attention, especially as medical responsibility and patient safety remain paramount. In addition, the legal recognition of AI in clinical practice will be crucial for its integration into mainstream orthodontics. To truly transform traditional orthodontic treatment approaches, more clinical trials focused on AI applications are essential. These trials will help ensure that AI's potential is fully realized while maintaining the highest standards of patient care.

FOOTNOTES

Author contributions: Fawaz P contributed to conceptualization, developing the ideas or research goals, writing-review and editing, creating visual representations of data or results, such as graphs or figures; Sayegh PE contributed to conceptualization, developing the ideas or research goals, designing the methodology or models used in the study, collecting and managing data, applying statistical, mathematical, or computational techniques to analyze data, writing-review and editing, performing experiments or gathering data; Vannet BV contributed to conceptualization, developing the ideas or research goals, writing-review and editing, designing the methodology or models used in the study, providing materials, funding, or other resources necessary for the research, overseeing the research process and team, managing the overall project, timelines, and logistics, confirming the accuracy or reproducibility of results.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: France

ORCID number: Bart Vande Vannet 0000-0002-5524-4630.

S-Editor: Liu H
L-Editor: A
P-Editor: Zhang YL

REFERENCES

1 **Fawaz P**, Sayegh PE, Vannet BV. What is the current state of artificial intelligence applications in dentistry and orthodontics? *J Stomatol Oral Maxillofac Surg* 2023; **124**: 101524 [PMID: 37270174 DOI: 10.1016/j.jormas.2023.101524]
2 **Abdul NS**, Shivakumar GC, Sangappa SB, Di Blasio M, Crimi S, Cicciù M, Minervini G. Applications of artificial intelligence in the field of oral and maxillofacial pathology: a systematic review and meta-analysis. *BMC Oral Health* 2024; **24**: 122 [PMID: 38263027 DOI: 10.1186/s12903-023-03533-7]
3 **Davenport T**, Kalakota R. The potential for artificial intelligence in healthcare. *Future Healthc J* 2019; **6**: 94-98 [PMID: 31363513 DOI: 10.7861/futurehosp.6-2-94]
4 **Krishnan G**, Singh S, Pathania M, Gosavi S, Abhishek S, Parchani A, Dhar M. Artificial intelligence in clinical medicine: catalyzing a sustainable global healthcare paradigm. *Front Artif Intell* 2023; **6**: 1227091 [PMID: 37705603 DOI: 10.3389/frai.2023.1227091]
5 **Strunga M**, Urban R, Surovková J, Thurzo A. Artificial Intelligence Systems Assisting in the Assessment of the Course and Retention of Orthodontic Treatment. *Healthcare (Basel)* 2023; **11** [PMID: 36900687 DOI: 10.3390/healthcare11050683]
6 **Kazimierczak N**, Kazimierczak W, Serafin Z, Nowicki P, Nożewski J, Janiszewska-Olszowska J. AI in Orthodontics: Revolutionizing

Diagnostics and Treatment Planning-A Comprehensive Review. *J Clin Med* 2024; **13** [PMID: 38256478 DOI: 10.3390/jcm13020344]

7 **Paudyal R**, Shah AD, Akin O, Do RKG, Konar AS, Hatzoglou V, Mahmood U, Lee N, Wong RJ, Banerjee S, Shin J, Veeraraghavan H, Shukla-Dave A. Artificial Intelligence in CT and MR Imaging for Oncological Applications. *Cancers (Basel)* 2023; **15**: 2573 [PMID: 37174039 DOI: 10.3390/cancers15092573]

8 **Dhopte A**, Bagde H. Smart Smile: Revolutionizing Dentistry With Artificial Intelligence. *Cureus* 2023; **15**: e41227 [PMID: 37529520 DOI: 10.7759/cureus.41227]

9 **Thorat V**, Rao P, Joshi N, Talreja P, Shetty AR. Role of Artificial Intelligence (AI) in Patient Education and Communication in Dentistry. *Cureus* 2024; **16**: e59799 [PMID: 38846249 DOI: 10.7759/cureus.59799]

10 **Ghaffari M**, Zhu Y, Shrestha A. A review of advancements of artificial intelligence in dentistry. *Dent Rev* 2024; **4**: 100081 [DOI: 10.1016/j.dentre.2024.100081]

11 **Aldoseri A**, Al-khalifa KN, Hamouda AM. Re-Thinking Data Strategy and Integration for Artificial Intelligence: Concepts, Opportunities, and Challenges. *Appl Sci* 2023; **13**: 7082 [DOI: 10.3390/app13127082]

12 **Soori M**, Arezoo B, Dastres R. Artificial intelligence, machine learning and deep learning in advanced robotics, a review. *Cogn Robot* 2023; **3**: 54-70 [DOI: 10.1016/j.cogr.2023.04.001]

13 **Auconi P**, Gili T, Capuani S, Saccucci M, Caldarelli G, Polimeni A, Di Carlo G. The Validity of Machine Learning Procedures in Orthodontics: What Is Still Missing? *J Pers Med* 2022; **12** [PMID: 35743742 DOI: 10.3390/jpm12060957]

14 **Dixon D**, Sattar H, Moros N, Kesireddy SR, Ahsan H, Lakkimsetti M, Fatima M, Doshi D, Sadhu K, Junaid Hassan M. Unveiling the Influence of AI Predictive Analytics on Patient Outcomes: A Comprehensive Narrative Review. *Cureus* 2024; **16**: e59954 [PMID: 38854327 DOI: 10.7759/cureus.59954]

15 **Mohammad-Rahimi H**, Nadimi M, Rohban MH, Shamsoddin E, Lee VY, Motamedian SR. Machine learning and orthodontics, current trends and the future opportunities: A scoping review. *Am J Orthod Dentofacial Orthop* 2021; **160**: 170-192.e4 [PMID: 34103190 DOI: 10.1016/j.ajodo.2021.02.013]

16 **Kök H**, Acilar AM, İzgi MS. Usage and comparison of artificial intelligence algorithms for determination of growth and development by cervical vertebrae stages in orthodontics. *Prog Orthod* 2019; **20**: 41 [PMID: 31728776 DOI: 10.1186/s40510-019-0295-8]

17 **Li S**, Guo Z, Lin J, Ying S. Artificial Intelligence for Classifying and Archiving Orthodontic Images. *Biomed Res Int* 2022; **2022**: 1473977 [PMID: 35127938 DOI: 10.1155/2022/1473977]

18 **Etemad L**, Wu TH, Heiner P, Liu J, Lee S, Chao WL, Zaytoun ML, Guez C, Lin FC, Jackson CB, Ko CC. Machine learning from clinical data sets of a contemporary decision for orthodontic tooth extraction. *Orthod Craniofac Res* 2021; **24** Suppl 2: 193-200 [PMID: 34031981 DOI: 10.1111/ocr.12502]

19 **Swinckels L**, Bennis FC, Ziesemer KA, Scheerman JFM, Bijwaard H, de Keijzer A, Bruers JJ. The Use of Deep Learning and Machine Learning on Longitudinal Electronic Health Records for the Early Detection and Prevention of Diseases: Scoping Review. *J Med Internet Res* 2024; **26**: e48320 [PMID: 39163096 DOI: 10.2196/48320]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 102688

DOI: 10.5662/wjm.v15.i3.102688

ISSN 2222-0682 (online)

MINIREVIEWS

Sotatercept: A novel therapeutic approach for pulmonary arterial hypertension through transforming growth factor-β signaling modulation

Jyoti Bajpai, Mehul Saxena, Akshyaya Pradhan, Surya Kant

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade B, Grade B, Grade C, Grade C

Novelty: Grade B, Grade B, Grade B, Grade B, Grade C

Creativity or Innovation: Grade A, Grade B, Grade B, Grade B, Grade C

Scientific Significance: Grade A, Grade A, Grade B, Grade B, Grade C

P-Reviewer: Ren J; Venkatesan N; Zhou SW

Received: October 28, 2024

Revised: December 31, 2024

Accepted: January 11, 2025

Published online: September 20, 2025

Processing time: 131 Days and 22.4 Hours

Jyoti Bajpai, Surya Kant, Department of Respiratory Medicine, King George's Medical University, Lucknow 226003, Uttar Pradesh, India

Mehul Saxena, Gandhi Memorial Hospital, King George's Medical University, Lucknow 226003, Uttar Pradesh, India

Akshyaya Pradhan, Department of Cardiology, King George's Medical University, Lucknow 226003, Uttar Pradesh, India

Corresponding author: Jyoti Bajpai, MD, Department of Respiratory Medicine, King George's Medical University, Shahmina Road, Chowk, Lucknow 226003, Uttar Pradesh, India. jyotibajpai33@gmail.com

Abstract

Pulmonary arterial hypertension (PAH) is a progressive disease marked by degeneration of the lung's blood vessels. As the disease progresses, the resistance to blood flow in the pulmonary arteries increases, putting a strain on the right side of the heart as it pumps blood through the lungs. PAH is characterized by changes in the structure of blood vessels and excessive cell growth. Untreated PAH leads to irreversible right-sided heart failure, often despite medical intervention. Patients experience a gradual decline in function until they are unable to perform daily activities. Advances in treatment have improved the prognosis for many PAH patients. Currently approved therapies target the prostacyclin, endothelin, nitric oxide, or phosphodiesterase pathways to slow the progression of the disease. To address the unmet need for effective PAH therapies, research efforts are focused on identifying new targets and developing therapies that specifically address the underlying disease mechanisms and restore vascular wall homeostasis. Among these, sotatercept, a fusion protein that targets the transforming growth factor-β superfamily signaling pathway, has emerged as a promising therapeutic option. In this review, we examine the available evidence from clinical trials to assess the potential of sotatercept as a treatment for PAH.

Key Words: Pulmonary artery; Drugs; Mean pulmonary artery pressure; Transforming growth factor-β pathway; Protein

Core Tip: Sotatercept is an activin receptor type IIA-Fc fusion protein that improve pulmonary artery pressure as well as cardiopulmonary function in pulmonary artery hypertension. It is administered as a subcutaneous injection every three weeks. There are three different pathways involve in pathogenesis of pulmonary arterial hypertension. Recently, a new pathway for pathogenesis of pulmonary arterial hypertension cellular proliferation has been discovered.

Citation: Bajpai J, Saxena M, Pradhan A, Kant S. Sotatercept: A novel therapeutic approach for pulmonary arterial hypertension through transforming growth factor-β signaling modulation. *World J Methodol* 2025; 15(3): 102688
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/102688.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.102688

INTRODUCTION

Pulmonary arterial hypertension (PAH) primarily stems from pulmonary vascular remodeling caused by an imbalance in pro- and anti-proliferative signaling pathways, leading to excessive vessel wall proliferation[1]. Impaired anti-proliferative bone morphogenetic protein receptor type II signaling in PAH allows uninhibited pro-proliferative activin signaling through activin receptor type 2A/B (ActRIIA/B)[2-4], resulting in excessive vessel wall (cell) proliferation. Existing treatment approaches for PAH primarily target three key pathways: The prostacyclin pathway using prostacyclin analogs, the endothelin pathway using endothelin receptor antagonists, and lastly the nitric oxide pathway using phosphodiesterase type 5 inhibitors or soluble guanylate cyclase stimulators[5-7]. While these therapies have improved symptoms and functional capacity, their ability to address the underlying vascular remodeling in PAH remains limited. Many patients fail to achieve low-risk status, and long-term survival remains poor, with a 50% 7-year survival rate post-diagnosis[7]. Moreover, these therapies primarily act as vasodilators and do not directly target the cellular and molecular mechanisms that cause disease progression. Sotatercept, a fusion protein, is believed to restore the balance between pro-proliferative and anti-proliferative signaling mediated by ActRIIA and bone morphogenetic protein receptor type II. It achieves this by binding to and sequestering specific ligands of the transforming growth factor β (TGF-β) superfamily. In preclinical models of PAH, statins have demonstrated the ability to reverse pulmonary artery and right ventricle (RV) remodeling. The safety and therapeutic efficacy of sotatercept as an adjunct to baseline PAH treatment is currently being evaluated in a comprehensive clinical trial program.

Preclinical studies have demonstrated that sotatercept's mechanism of action involves the modulation of pro- and anti-proliferative signaling pathways (Figure 1)[7]. Current PAH treatment recommendations emphasize a risk-adapted approach with combination medication therapies for the majority of patients[5,6]. With a poor 7-year survival rate after diagnosis, many patients fail to meet targeted treatment goals or achieve low-risk status, resulting in a poor long-term prognosis. Consequently, innovative therapies that directly address the underlying pathophysiology of PAH are essential to restore vascular wall homeostasis[7]. The TGF-β superfamily is central to the pathogenesis of PAH, regulating cellular proliferation and vascular remodeling. An imbalance in signaling in this superfamily contributes considerably to the vascular pathology observed in PAH. Specifically, excessive pro-proliferative drosophila mothers against decapentaplegic protein (Smad)2/3 signaling occurs alongside diminished antiproliferative Smad1/5/8 signaling, driving pathological vascular remodeling[8].

Activin-class ligands, including activin A, growth differentiation factor (GDF) 8, and GDF11, are key activators of the Smad2/3 signaling pathway. These ligands are considerably upregulated in the small pulmonary arteries of both experimental models and PAH patients[4]. Sotatercept, an Fc-fusion protein containing the extracellular domain of ActRIIA, sequesters activin-class ligands, thereby restoring the balance between Smad2/3 and Smad1/5/8 signaling. This rebalancing produces antiproliferative and anti-inflammatory effects on the pulmonary vasculature, reversing vascular remodeling and reducing pulmonary hypertension in preclinical models of PAH[9].

Moreover, studies have implicated activin receptor signaling in the abnormal remodeling of the RV, which is a critical indicator of prognosis in PAH[10]. In preclinical models of systemic pressure overload, ischemia, and aging, activin receptor signaling has been linked to detrimental RV remodeling[11]. By regulating this pathway, sotatercept could potentially mitigate RV dysfunction, as suggested by clinical trial observations of RV function improvements. Distinct from conventional vasodilator-based treatments that primarily focus on hemodynamic parameters, sotatercept's capacity to address the underlying mechanisms of vascular and RV remodeling classifies it as a disease-modifying agent. The therapeutic application of engineered fusion proteins such as sotatercept has been extensively investigated owing to their ability to modulate crucial signaling pathways in diseases such as PAH[12]. These mechanistic insights provide a robust foundation for the therapeutic efficacy observed with sotatercept in clinical studies, even in patients receiving multiple background therapies[1].

CLINICAL STUDIES

As shown in Table 1, several clinical trials have evaluated the efficacy of sotatercept in Pulmonary Hypertension.

Table 1 Clinical trials evaluating the role of sotatercept in pulmonary hypertension

Study	Type of study	No. of patients	Dose	Primary end point	Adverse events	Serious adverse events	Outcome	Type of PAH	Background regimen
Pulsar	Phase 2 randomized, double-blind	106 patients at 43 sites in 8 countries	0.3 or 0.7 mg/kg-1 mg/kg	To compare the efficacy and safety of sotatercept *vs* placebo when added to standard of care	Thrombocytopenia was the most common; Hemoglobin increase in 1 patient (3%) in the sotatercept 0.3-mg group and in 7 patients (17%) in sotatercept 0.7-mg group but in no in the placebo group	24% in 0.7 mg group	162.2 dyn. sec. cm (-5) in the sotatercept 0.3-mg group and a decrease of 255.9 dyn. sec. cm (-5) in the sotatercept 0.7-mg group, as compared with a decrease of 16.4 dyn. sec. cm (-5) in placebo	Idiopathic (58%), heritable (14%), connective tissue disorder (17%), and drug induced (7%)	Prostacyclin infusion (39%), triple therapy (57%), double therapy (34%)
Pulsar open	Phase 2, multicentre, randomised, placebo-controlled, double-blind study	106 participants	0.3 mg/kg-1 mg/kg or 0.7 mg/kg-1mg/kg	Change from baseline to months 18-24 in PVR, as measured by RHC. Change from baseline to months 18-24 in 6MWD, WHO-FC, and NT-proBNP were secondary endpoints. The study protocol was amended in July 2020 with the third RHC to be performed at month 18	TEAEs were reported in 102 (98.1%) participants, and 72 (69.2%) of these experienced TEAEs considered treatment-related	Serious TEAEs were reported in 32 (30.8%) participants. Five (4.8%) participants reported six serious TEAEs that were considered related to the study drug: Pyrexia, red blood cell increased, systemic lupus erythematosus, ischaemic stroke, pleural effusion and pulmonary hypertension	Statistically significant improvements occurred in all primary and secondary efficacy endpoints from baseline to months 18-24	Idiopathic (58%), heritable (14%), connective tissue disorder (17%), and drug induced (7%)	Triple (56.7%) or double (36.1%) background PAH therapy, and more than a third (36%) were receiving prostacyclin infusion therapy
Stellar	Phase 3 randomized, double-blind	323 underwent randomization (163 received sotatercept or 160 placebo at 91 sites in 21 countries	Starting dose of 0.3 mg/kg at visit 1 and was escalated to the target dose of 0.7 mg/kg at visit 2 (day 21, with a window of ± 3 days). Patients continued to receive a dose of 0.7 mg/kg for the duration of the trial	The change from baseline at week 24 in the 6MWD	10% of the patients in either group during the 24-week treatment period. Epistaxis, dizziness, telangiectasia, increased hemoglobin levels, thrombocytopenia, and increased blood pressure were common adverse events	Serious adverse events occurred in 23 patients (14.1%) in the sotatercept group and 36 patients (22.5%) in the placebo group	Sotatercept and placebo groups the change from baseline at week 24 in the 6-minute walk distance was 40.8 m ($0.001 < P$)	Idiopathic (50.9%), heritable (21.5%), drug-induced (4.3%), connective-tissue disease-associated (17.8%), or after shunt correction (5.5%)	Monotherapy (5.5%), double therapy (34.4%), or triple therapy (60.1%)
Spectra	Phase 2 open label	21	Sotatercept 0.3 mg/kg	Change from baseline to week 24 in peak oxygen uptake	16 (76%)	3 (14%). Three serious TEAEs were reported (hematochezia, complication associated with central line, and fluid overload)	There was a significant improvement from baseline in peak oxygen uptake, with a mean change of 102.74 mL/min (95% CIs: 27.72-177.76; $P = 0.0097$)	Idiopathic (6%), heritable (4%). Associated with connetive tissue disorder (14%)	Prostacyclin infusion therapy (57%), double therapy (57%), triple therapy (43%)

PVR: Pulmonary vascular resistance; RHC: Right heart catheterisation; 6MWD: 6-minute walk distance; WHO-FC: World Health Organization functional class; NT-proBNP: N-terminal pro-B-type natriuretic peptide; TEAEs: Treatment-emergent adverse events; PAH: Pulmonary arterial hypertension.

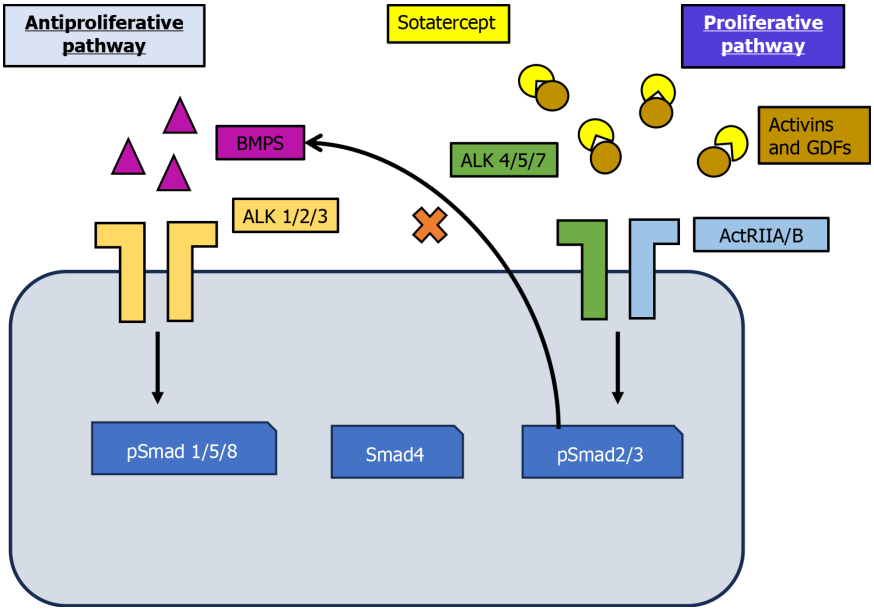


Figure 1 Schematic representation of the proposed mechanism of action of sotatercept. By binding and sequestering activin-class ligands - such as activin A, growth differentiation factor 8, and growth differentiation factor 11 - it reduces overactive drosophila mothers against decapentaplegic protein 2/3 signaling while restoring deficient drosophila mothers against decapentaplegic protein 1/5/8 signaling. This rebalancing exerts antiproliferative and anti-inflammatory effects on the pulmonary vasculature, reversing pathological remodeling of small pulmonary arteries. BMPS: Bone morphogenetic proteins; ALK: Anaplastic lymphoma kinase; Smad: Drosophila mothers against decapentaplegic protein; GDF: Growth differentiation factor; ActRIIA/B: Activin receptor type 2A/B.

Pulsar

The safety and efficacy of sotatercept in individuals with PAH who were receiving background medication for pulmonary hypertension were investigated in a multicenter, randomized, double-blind phase 2 trial[1]. This trial consisted of a 24-week placebo-controlled treatment phase followed by an 18-month active medication extension period. Patients were eligible for inclusion if they were classified as World Health Organization (WHO) functional class (FC) II or III and had confirmed PAH (group 1 of the latest WHO classification of pulmonary hypertension)[13]. The subtypes linked to portopulmonary disease, schistosomiasis, and human immunodeficiency virus infection were excluded. Three groups of eligible patients were randomly assigned to receive sotatercept at doses of 0.3, 0.7, or a placebo. Subcutaneous injections of sotatercept or placebo (saline) were administered every 21 days.

At week 24, the intention-to-treat analysis demonstrated a reduction in pulmonary vascular resistance from baseline in the 0.3 mg sotatercept group, with an even greater reduction in the 0.7 mg sotatercept group compared to the placebo group. The 0.7 mg sotatercept group experienced an approximately 35% reduction from baseline. The most common hematologic adverse events were thrombocytopenia and elevated hemoglobin levels. Sotatercept treatment has been shown to reduce pulmonary vascular resistance in patients receiving baseline monotherapy, double therapy, or triple therapy. The primary factor contributing to the reduction in pulmonary vascular resistance was the lower mean pulmonary artery pressure in the sotatercept groups compared to the placebo group. Additionally, the sotatercept group demonstrated concordant improvements from baseline in 6-minute walk distance (6MWD) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels.

Pulsar open label

The study aimed to assess the safety and effectiveness of sotatercept in treating PAH. A total of 106 individuals with WHO FC II-III and WHO group 1 PAH were included. Sotatercept (0.3 mg/kg or 0.7 mg/kg) was added to the standard of care during a 24-week double-blind, placebo-controlled trial[14]. In the extension phase, patients receiving sotatercept continued their current dosage, while those on placebo were rerandomized to either 0.3 mg/kg or 0.7 mg/kg of sotatercept. The improvements observed in 6MWD, NT-proBNP, and WHO FC at 24 weeks compared to baseline were maintained when sotatercept treatment was continued for up to 48 weeks. Individuals who were rerandomized from placebo to sotatercept at 48 weeks rather than 24 weeks also experienced improvements in 6MWD, NT-proBNP, and WHO FC.

Stellar

In phase 3 multicenter, double-blind, randomized, placebo-controlled trial, eligible patients with PAH who were receiving stable background therapy and classified as WHO FC II or III were randomly assigned in a 1:1 ratio to receive subcutaneous sotatercept every three weeks at a starting dose of 0.3 mg per kilogram of body weight (target dose: 0.7 mg per kilogram) or a placebo[15]. The primary outcome measure was the change in 6MWD from baseline at week 24. Patients with schistosomiasis, veno-occlusive disease, portopulmonary disease, and human immunodeficiency virus infection were excluded from the study. The primary outcome measure was the change in 6MWD from baseline at week

24. Secondary endpoints were ranked hierarchically. Multi-component improvement was calculated as the proportion of patients who met all three criteria at week 24 compared to baseline (*i.e.*, an increase of at least 30 minutes in 6MWD, a decrease of at least 30% in NT-proBNP level, or maintenance or achievement of an NT-proBNP level of less than 300 pg per milliliter, or an improvement in WHO FC from III to II or I, or II to I, or II).

Out of the 434 patients screened for eligibility, 323 were randomized to receive sotatercept or placebo at 91 locations in 21 countries. Eligible patients were randomized (1:1) to receive a placebo (160 patients) or sotatercept (163 patients) in addition to their ongoing background therapy. The study population was relatively young, with a mean age of 47.9 years and a mean time from diagnosis of 8.8 years. Among the 323 patients randomized, 198 (61.3%) were receiving triple therapy, and 129 (39.5%) were receiving prostacyclin infusion therapy. During the 24-week treatment period, at least 10% of patients in both groups reported experiencing adverse events. Epistaxis, telangiectasia, and dizziness were more common in the sotatercept group than in the placebo group.

In the sotatercept group, 23 patients (14.1%) experienced serious adverse events, compared to 36 patients (22.5%) in the placebo group. Mean hemoglobin levels increased by approximately 1.3 g/dL in the sotatercept group and decreased by approximately 0.1 g/dL in the placebo group at week 24. Addition of sotatercept to background therapy with currently available medications for 24 weeks was found to improve exercise capacity as measured by 6MWD. Improvements were also observed in pulmonary vascular resistance, WHO FC, NT-proBNP levels, risk of death, and the physical impacts and cardiopulmonary and symptoms domain scores of the PAH-symptoms and impact quality-of-life instrument. Sotatercept was associated with an 84% reduced risk of death or nonfatal clinical deterioration events compared to placebo. The safety profile of sotatercept was consistent with findings from the phase 2 pulsar study. The study had several limitations, including the underrepresentation of minority groups, patients from outside North America and Europe, and patients with PAH associated with drugs and toxins, congenital heart disease, or connective tissue disease. According to study results, patients with PAH may experience a nearly three-fold increase in life expectancy when sotatercept is added to stable background therapy. This benefit may be accompanied by a reduction in the need for lung/heart-lung transplantation, intravenous prostacyclin, and PAH-related hospitalizations.

Other trials

In the BELIEVE phase 2 trial, sotatercept was evaluated in patients with beta-thalassemia. Results showed that sotatercept considerably increased hemoglobin levels and reduced transfusion burden in patients with beta-thalassemia, suggesting a potential benefit in treating anemia in this patient population. Additionally, levastaruzent is being studied in a phase 3 trial called medalist (NCT02631070) in patients with very low-, low-, and intermediate-risk myelodysplastic syndromes (MDS). Despite the discontinuation of sotatercept's trials in β-thalassemia owing to its binding to activin A, sotatercept is the first-in-class agent to target TGF-β superfamily inhibition for the treatment of ineffective erythropoiesis. For patients with β-thalassemia, TGF-β superfamily inhibition may provide an alternative or adjunctive therapeutic approach[16]. In this study, sotatercept demonstrated safety and efficacy in patients with β-thalassemia. In a phase 2 study, sotatercept treatment was also found to increase hemoglobin levels and reduce transfusion requirements in anemic patients with lower-risk MDS[17]. These findings suggest that sotatercept may have a common underlying mechanism of action that ameliorates ineffective erythropoiesis across multiple disease states.

Sotatercept has been investigated in numerous clinical trials across various therapeutic areas, with a focus on its potential to treat conditions such as MDS, PAH, and others. In the commands trial, sotatercept was found to improve anemia and reduce the need for red blood cell transfusions in patients with lower-risk MDS. These findings suggest that sotatercept may be a promising therapeutic option for patients with MDS. The cherish trial demonstrated that sotatercept could improve iron overload markers and increase hemoglobin levels in patients with non-transfusion-dependent beta-thalassemia, suggesting its potential as a therapeutic option for this patient population. Collectively, these studies indicate that sotatercept holds promise for the treatment of various conditions characterized by anemia, impaired red blood cell production, and related complications.

The ongoing, open-label extension study, SOTERIA, is evaluating the long-term safety, tolerability, and efficacy of sotatercept when added to background therapy for the treatment of PAH in patients who have completed prior sotatercept studies without premature discontinuation[18]. SPECTRA is a phase 2, open-label trial evaluating the change in peak oxygen consumption during exercise in adult PAH patients[19]. Similarly, MOONBEAM and CADENCE are ongoing phase 2 trials investigating the pharmacodynamics and pharmacokinetics of sotatercept, as well as its role in reducing pulmonary vascular resistance, respectively[20-22]. The hyperion and zenith trials are randomized, controlled phase 3 studies evaluating the effect of sotatercept on time to clinical worsening or first morbidity or mortality events[20].

CHALLENGES AND LIMITATIONS

The clinical translation of sotatercept for PAH faces several challenges that need to be addressed to ensure its effective implementation. One considerable hurdle lies in the high cost of biological therapies. Sotatercept, as a complex biologic, may carry a substantial price tag, particularly in resource-constrained settings. This could limit access to the drug for patients in low- and middle-income countries where healthcare resources are often limited. Overcoming this challenge will require exploring cost-effective manufacturing methods and advocating for policy changes that can improve affordability and accessibility, thus ensuring broader availability for those in need. Another challenge lies in managing the potential side effects associated with sotatercept. Despite its promising effects in reversing vascular remodeling, clinical trials have reported adverse events, including thrombocytopenia and elevated hemoglobin levels. These side effects require careful monitoring and effective management strategies to ensure patient safety. Establishing standardized

protocols for patient monitoring, encompassing strategies for early detection and intervention, will be crucial to minimizing risks and optimizing therapeutic outcomes.

Ultimately, the successful clinical implementation of sotatercept depends on its integration into existing healthcare frameworks. This entails timely and accurate diagnosis of PAH, providing adequate training for clinicians on its use, and incorporating sotatercept into established treatment protocols. Ensuring that healthcare systems possess the necessary infrastructure to effectively manage these novel treatment options is paramount to maximizing their benefits and improving patient outcomes. Addressing these barriers will be essential to fully realize the transformative potential of sotatercept in the treatment of PAH.

A noteworthy limitation of this study lies in the exclusion of metabolites with insufficient single nucleotide polymorphisms, an approach adopted to maintain statistical rigor and minimize the risk of false-positive associations. However, this approach may have inadvertently limited the ability to identify novel metabolite-gene associations, especially for metabolites that are understudied or lack sufficient genetic data. Future studies should consider alternative approaches, such as imputation-based methods or integrating multi-omics datasets, to improve the analysis of these metabolites. Because sotatercept, an activin signaling inhibitor, is the first medication to target a completely new pathway in nearly two decades, its inclusion in the therapeutic arsenal has considerably raised expectations. Ongoing long-term research will be critical to further understanding the drug's overall benefits and safety profile.

CONCLUSION

Sotatercept, a novel therapeutic approach, targets the underlying pathophysiology of PAH by addressing the imbalance between pro- and anti-proliferative signaling pathways. This innovative mechanism of action sets it apart from conventional therapies, offering a new avenue for the management of PAH. Clinical trials, including pulsar, stellar, and others, have substantiated sotatercept's efficacy in considerably reducing pulmonary vascular resistance, enhancing exercise capacity, and positively influencing key biomarkers such as NT-proBNP. Notably, its safety profile has remained consistent across studies; however, ongoing monitoring of long-term effects is warranted.

Sotatercept shows promise in enhancing patient outcomes when added to background PAH therapy. Ongoing trials, such as SOTERIA, MOONBEAM, and CADENCE, in the open-label long-term follow-up study to evaluate the effects of sotatercept in PAH treatment will offer further insights into its long-term efficacy and safety. Sotatercept's unique mechanism of action offers a promising new direction in PAH treatment, potentially extending survival, reducing morbidity, and improving the quality of life for patients who continue to face poor prognoses despite current treatment options. However, it is important to note that much of the genetic and mechanistic data informing these advances primarily come from genome-wide association studies performed in European populations. This limits the generalizability of findings to other populations and underscores the need for more diverse and representative datasets in PAH research. Addressing these gaps is crucial to ensuring that novel therapeutics such as sotatercept are broadly applicable and effective across diverse patient populations. Future research should focus on elucidating the sotatercept's role in various PAH subgroups and exploring the potential of combination therapies to optimize outcomes. In essence, sotatercept is poised to become a key component of PAH management, addressing unmet medical needs and offering hope for patients battling this challenging condition.

FOOTNOTES

Author contributions: Bajpai J and Pradhan A conceptualized the idea and prepared the first draft; Saxena M and Kant S perform the literature search; Kant S and Bajpai J critically reviewed the draft and prose the changes; Bajpai J prepared the final manuscript and submitted; and all authors thoroughly reviewed and endorsed the final manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: India

ORCID number: Jyoti Bajpai 0000-0001-6337-856X; Akshyaya Pradhan 0000-0002-2360-7580; Surya Kant 0000-0001-7520-5404.

S-Editor: Bai Y
L-Editor: A
P-Editor: Guo X

REFERENCES

1 **Humbert M**, McLaughlin V, Gibbs JSR, Gomberg-Maitland M, Hoeper MM, Preston IR, Souza R, Waxman A, Escribano Subias P, Feldman J, Meyer G, Montani D, Olsson KM, Manimaran S, Barnes J, Linde PG, de Oliveira Pena J, Badesch DB; PULSAR Trial Investigators. Sotatercept for the Treatment of Pulmonary Arterial Hypertension. *N Engl J Med* 2021; **384**: 1204-1215 [PMID: 33789009 DOI: 10.1056/NEJMoa2024277]

2 **Tielemans B**, Delcroix M, Belge C, Quarck R. TGFβ and BMPRII signalling pathways in the pathogenesis of pulmonary arterial hypertension. *Drug Discov Today* 2019; **24**: 703-716 [PMID: 30529762 DOI: 10.1016/j.drudis.2018.12.001]

3 **Morrell NW**, Aldred MA, Chung WK, Elliott CG, Nichols WC, Soubrier F, Trembath RC, Loyd JE. Genetics and genomics of pulmonary arterial hypertension. *Eur Respir J* 2019; **53**: 1801899 [PMID: 30545973 DOI: 10.1183/13993003.01899-2018]

4 **Yung LM**, Yang P, Joshi S, Augur ZM, Kim SSJ, Bocobo GA, Dinter T, Troncone L, Chen PS, McNeil ME, Southwood M, Poli de Frias S, Knopf J, Rosas IO, Sako D, Pearsall RS, Quisel JD, Li G, Kumar R, Yu PB. ACTRIIA-Fc rebalances activin/GDF versus BMP signaling in pulmonary hypertension. *Sci Transl Med* 2020; **12**: eaaz5660 [PMID: 32404506 DOI: 10.1126/scitranslmed.aaz5660]

5 **Bisserier M**, Pradhan N, Hadri L. Current and emerging therapeutic approaches to pulmonary hypertension. *Rev Cardiovasc Med* 2020; **21**: 163-179 [PMID: 32706206 DOI: 10.31083/j.rcm.2020.02.597]

6 **Mayeux JD**, Pan IZ, Dechand J, Jacobs JA, Jones TL, McKellar SH, Beck E, Hatton ND, Ryan JJ. Management of Pulmonary Arterial Hypertension. *Curr Cardiovasc Risk Rep* 2021; **15**: 2 [PMID: 33224405 DOI: 10.1007/s12170-020-00663-3]

7 **7 Sitbon O**, Gomberg-Maitland M, Granton J, Lewis MI, Mathai SC, Rainisio M, Stockbridge NL, Wilkins MR, Zamanian RT, Rubin LJ. Clinical trial design and new therapies for pulmonary arterial hypertension. *Eur Respir J* 2019; **53**: 1801908 [PMID: 30545975 DOI: 10.1183/13993003.01908-2018]

8 **Andre P**, Joshi SR, Briscoe SD, Alexander MJ, Li G, Kumar R. Therapeutic Approaches for Treating Pulmonary Arterial Hypertension by Correcting Imbalanced TGF-β Superfamily Signaling. *Front Med (Lausanne)* 2021; **8**: 814222 [PMID: 35141256 DOI: 10.3389/fmed.2021.814222]

9 **9 Joshi SR**, Liu J, Bloom T, Karaca Atabay E, Kuo TH, Lee M, Belcheva E, Spaits M, Grenha R, Maguire MC, Frost JL, Wang K, Briscoe SD, Alexander MJ, Herrin BR, Castonguay R, Pearsall RS, Andre P, Yu PB, Kumar R, Li G. Sotatercept analog suppresses inflammation to reverse experimental pulmonary arterial hypertension. *Sci Rep* 2022; **12**: 7803 [PMID: 35551212 DOI: 10.1038/s41598-022-11435-x]

10 **Prisco SZ**, Thenappan T, Prins KW. Treatment Targets for Right Ventricular Dysfunction in Pulmonary Arterial Hypertension. *JACC Basic Transl Sci* 2020; **5**: 1244-1260 [PMID: 33426379 DOI: 10.1016/j.jacbts.2020.07.011]

11 **Magga J**, Vainio L, Kilpiö T, Hulmi JJ, Taponen S, Lin R, Räsänen M, Szabó Z, Gao E, Rahtu-Korpela L, Alakoski T, Ulvila J, Laitinen M, Pasternack A, Koch WJ, Alitalo K, Kivelä R, Ritvos O, Kerkelä R. Systemic Blockade of ACVR2B Ligands Protects Myocardium from Acute Ischemia-Reperfusion Injury. *Mol Ther* 2019; **27**: 600-610 [PMID: 30765322 DOI: 10.1016/j.ymthe.2019.01.013]

12 **Chen Y**, Feng H, Chen L, Zhou W, Zhou S. Construction of homologous branched oligomer megamolecules based on linker-directed protein assembly. *Soft Matter* 2024; **20**: 6889-6893 [PMID: 39177042 DOI: 10.1039/d4sm00673a]

13 **13 Pitre T**, Su J, Cui S, Scanlan R, Chiang C, Husnudinov R, Khalid MF, Khan N, Leung G, Mikhail D, Saadat P, Shahid S, Mah J, Mielniczuk L, Zeraatkar D, Mehta S. Medications for the treatment of pulmonary arterial hypertension: a systematic review and network meta-analysis. *Eur Respir Rev* 2022; **31**: 220036 [PMID: 35948391 DOI: 10.1183/16000617.0036-2022]

14 **Humbert M**, McLaughlin V, Gibbs JSR, Gomberg-Maitland M, Hoeper MM, Preston IR, Souza R, Waxman AB, Ghofrani HA, Escribano Subias P, Feldman J, Meyer G, Montani D, Olsson KM, Manimaran S, de Oliveira Pena J, Badesch DB. Sotatercept for the treatment of pulmonary arterial hypertension: PULSAR open-label extension. *Eur Respir J* 2023; **61**: 2201347 [PMID: 36041750 DOI: 10.1183/13993003.01347-2022]

15 **Hoeper MM**, Badesch DB, Ghofrani HA, Gibbs JSR, Gomberg-Maitland M, McLaughlin VV, Preston IR, Souza R, Waxman AB, Grünig E, Kopeć G, Meyer G, Olsson KM, Rosenkranz S, Xu Y, Miller B, Fowler M, Butler J, Koglin J, de Oliveira Pena J, Humbert M; STELLAR Trial Investigators. Phase 3 Trial of Sotatercept for Treatment of Pulmonary Arterial Hypertension. *N Engl J Med* 2023; **388**: 1478-1490 [PMID: 36877098 DOI: 10.1056/NEJMoa2213558]

16 **Cappellini MD**, Porter J, Origa R, Forni GL, Voskaridou E, Galactéros F, Taher AT, Arlet JB, Ribeil JA, Garbowski M, Graziadei G, Brouzes C, Semeraro M, Laadem A, Miteva D, Zou J, Sung V, Zinger T, Attie KM, Hermine O. Sotatercept, a novel transforming growth factor β ligand trap, improves anemia in β-thalassemia: a phase II, open-label, dose-finding study. *Haematologica* 2019; **104**: 477-484 [PMID: 30337358 DOI: 10.3324/haematol.2018.198887]

17 **Komrokji R**, Garcia-Manero G, Ades L, Prebet T, Steensma DP, Jurcic JG, Sekeres MA, Berdeja J, Savona MR, Beyne-Rauzy O, Stamatoullas A, DeZern AE, Delaunay J, Borthakur G, Rifkin R, Boyd TE, Laadem A, Vo B, Zhang J, Puccio-Pick M, Attie KM, Fenaux P, List AF. Sotatercept with long-term extension for the treatment of anaemia in patients with lower-risk myelodysplastic syndromes: a phase 2, dose-ranging trial. *Lancet Haematol* 2018; **5**: e63-e72 [PMID: 29331635 DOI: 10.1016/S2352-3026(18)30002-4]

18 **Acceleron Pharma, Inc**. A Long-term Follow-up Study of Sotatercept for PAH Treatment (MK-7962-004/A011-12). (SOTERIA). [accessed 2024 Nov 27]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available online from https://clinicaltrials.gov/study/NCT04796337 ClinicalTrials.gov Identifier: NCT04796337

19 **Waxman AB**, Systrom DM, Manimaran S, de Oliveira Pena J, Lu J, Rischard FP. SPECTRA Phase 2b Study: Impact of Sotatercept on Exercise Tolerance and Right Ventricular Function in Pulmonary Arterial Hypertension. *Circ Heart Fail* 2024; **17**: e011227 [PMID: 38572639 DOI: 10.1161/CIRCHEARTFAILURE.123.011227]

20 **Torbic H**, Tonelli AR. Sotatercept for Pulmonary Arterial Hypertension in the Inpatient Setting. *J Cardiovasc Pharmacol Ther* 2024; **29**: 10742484231225310 [PMID: 38361351 DOI: 10.1177/10742484231225310]

21 **Auth R**, Klinger JR. Emerging pharmacotherapies for the treatment of pulmonary arterial hypertension. *Expert Opin Investig Drugs* 2023; **32**: 1025-1042 [PMID: 37881882 DOI: 10.1080/13543784.2023.2274439]

22 **Pradhan A**, Tyagi R, Sharma P, Bajpai J, Kant S. Shifting Paradigms in the Management of Pulmonary Hypertension. *Eur Cardiol* 2024; **19**: e25 [DOI: 10.15420/ecr.2024.11]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 102703

DOI: 10.5662/wjm.v15.i3.102703

ISSN 2222-0682 (online)

MINIREVIEWS

Third space endoscopy pulmonary complications and chylothorax post peroral endoscopic myotomy

Ahmed Tawheed, Alaa Ismail, Ahmed El-Tawansy, Karim Maurice, Ahmed Ali, Amr El-Fouly, Ahmad Madkour

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade A, Grade C, Grade C, Grade C

Novelty: Grade A, Grade B, Grade B, Grade B

Creativity or Innovation: Grade A, Grade B, Grade B, Grade C

Scientific Significance: Grade A, Grade B, Grade B, Grade B

P-Reviewer: Bonk MN; Li J; Vyshka G

Received: October 28, 2024

Revised: January 17, 2025

Accepted: January 23, 2025

Published online: September 20, 2025

Processing time: 131 Days and 13.2 Hours

Ahmed Tawheed, Amr El-Fouly, Ahmad Madkour, Department of Endemic Medicine, Faculty of Medicine, Helwan University, Cairo 11795, Egypt

Alaa Ismail, Faculty of Medicine, Helwan University, Cairo 11795, Egypt

Ahmed El-Tawansy, Department of Anesthesia and Surgical Intensive Care, Helwan University, Cairo 11795, Egypt

Karim Maurice, Department of General and Laparoscopic Surgery, Cairo University, Cairo 12613, Egypt

Ahmed Ali, Department of Gastroenterology, Mansoura International Hospital, Al Mansurah 35516, Ad Daqahlīyah, Egypt

Co-first authors: Ahmed Tawheed and Alaa Ismail.

Corresponding author: Ahmed Tawheed, MD, PhD, Department of Endemic Medicine, Faculty of Medicine, Helwan University, Ain Helwan, Cairo 11795, Egypt.
ahmed.tawhid@med.helwan.edu.eg

Abstract

Third-space endoscopy (TSE) has emerged as an effective treatment modality for various gastrointestinal motility diseases and gastrointestinal tumors. TSE is based on the concept of working in the submucosa using a mucosal flap valve technique, which is the underlying premise for all TSE procedures; thus, some complications are shared across the spectrum of TSE procedures. Despite the high safety profiles of most TSE procedures, studies have reported various adverse events, including insufflation-related complications, bleeding, perforation, and infection. Although the occurrence rate of those complications is not very high, they sometimes result in critical conditions. No reports of chylous effusion following TSE procedures, particularly per-oral endoscopic myotomy, have been documented previously. We are presenting the first reported case of chylous pleural effusion after per-oral endoscopic myotomy. Additionally, we aim to present a comprehensive overview, discuss the existing data, and provide insights into pulmonary post-endoscopic complications in light of recent advancements in endoscopic procedures, especially TSE.

Key Words: Endoscopy; Third-space endoscopy; Submucosal endoscopy; Per oral endoscopic myotomy; Endoscopic submucosal dissection; Pulmonary complications;

Chylothorax; Pleural effusion

Core Tip: In this review, we discuss a case of post-per oral endoscopic myotomy chylothorax, focusing on its management, which was carried out with the assistance of other specialties through a multidisciplinary team including a cardiothoracic surgeon, nutritionist, anesthesiologist, and, of course, gastroenterologists. We also provide a brief review of the current known data in the literature regarding post-endoscopic pulmonary complications, especially in relation to third-space endoscopic procedures such as per oral endoscopic myotomy.

Citation: Tawheed A, Ismail A, El-Tawansy A, Maurice K, Ali A, El-Fouly A, Madkour A. Third space endoscopy pulmonary complications and chylothorax post peroral endoscopic myotomy. *World J Methodol* 2025; 15(3): 102703
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/102703.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.102703

INTRODUCTION

Third-space endoscopy (TSE), or submucosal endoscopy, is an interventional endoscopic technique that reaches the submucosa and beyond under direct visualization accomplished *via* fluid injection and expansion of the submucosal space. As shown by Sumiyama *et al*[1], the peritoneal cavity and mediastinum could be accessed safely *via* a submucosal tunnel secured by a mucosal flap valve, thus minimizing the risk of contamination and leakage after secure mucosal incision closure[1,2]. TSE uses the submucosal space as the default working space to treat various gastrointestinal (GI) diseases or to endoscopically resect early GI tumors as an alternative to surgery.

TSE is now the primary treatment for several GI disorders. For example, peroral endoscopic myotomy has been suggested as an alternative to surgery for achalasia. It has since become the preferred method of managing achalasia after proving to be effective and safe in long-term follow-up studies. Multiple subsidiary procedures have been introduced using the same concept, such as gastric-per oral endoscopic myotomy (POEM)[3] to treat gastroparesis, diverticular-POEM for epiphrenic diverticulum (pulsion diverticulum)[4], Zenker-POEM for Zenker's (hypopharyngeal) diverticulum [5]. Recently, peroral endoscopic fundoplication has been introduced to treat post-POEM gastroesophageal reflux, considered a pure natural orifice transluminal endoscopic surgery procedure[6]. In terms of managing early GI tumors, this began with advances in techniques such as endoscopic mucosal resection and endoscopic submucosal dissection (ESD), submucosal endoscopic tumoral resection and now includes more invasive techniques such as endoscopic full-thickness resection.

The typical steps of POEM involve submucosal injection of fluid, allowing the endoscopist to make a tunnel in the submucosa, followed by myotomy and, lastly, the closure of the mucosa, which stands as the only barrier between the esophageal lumen and the mediastinum[7]. ESD shares with POEM the concept of working in the third space/submucosa, but instead of myotomy, mucosectomy will be done to resect the tumor[8]. Due to the quite invasive nature of those procedures compared to luminal endoscopy, there are expected to be several complications not reported during and after luminal endoscopy[9]. This review will focus on pulmonary complications of these procedures, which most commonly are aspiration pneumonia, pulmonary embolism, and gas-related complications like retroperitoneal air, pneumoperitoneum, pneumothorax, and pneumomediastinum. Although studies have recognized pleural effusion as a possible post-POEM complication, no reports of post-POEM chylothorax have been published so far[10,11]. Therefore, we are also reporting a rare case of chylothorax as an adverse event after POEM and presenting the outlines of management of such cases using the help of similar cases after cardiothoracic surgeries.

OVERVIEW OF THE COMPLICATIONS

In most luminal endoscopic procedures (first space endoscopy), the type of anesthesia is usually conscious sedation using midazolam and/or propofol. Generally, cardiopulmonary complications represent nearly 60% of the total adverse events that happen during or after all types of endoscopic procedures. The most common type of complication that could happen is hypoxia, which could happen in as low as 1.5% and up to 70% of patients, especially in patients undergoing endoscopic variceal ligation[12]. However, this is usually reversible within a few minutes after proper management and usually has no future morbidity. Prevention of such complications could be done by proper assessment, patient selection, tailored anesthesia according to each case, and continuous monitoring of vital signs during the procedure. Management requires early detection of the adverse event, reporting, and a multidisciplinary approach[13]. Advanced endoscopic techniques such as TSE are usually associated with a higher incidence of complications, which tend to be more severe pulmonary complications.

In a prospective post-POEM computed tomography (CT) esophagram cohort that included 84 patients who underwent POEM for achalasia. The CT revealed that nearly 86% of patients had pneumomediastinum, 67% had pneumoperitoneum, 52% had subcutaneous emphysema, and 46% had pleural effusion[14]. Other findings that had lower frequencies in their cohort included retroperitoneal air in 38% of patients, pneumothorax in 19%, atelectasis in 14%, intramural air in 13%, pericardial effusion in 2%, and pneumopericardium in 2%. Despite the high incidence rates of post-POEM pulmonary events, only 6% (5 patients, 4 with pneumonia and 1 with leak) of patients in the total cohort required an intervention based on that imaging. The authors concluded that an early post-POEM CT could help detect complications before symptoms appear. Chartier *et al*[15] also studied this, where the early CT post-POEM detected adverse events that required intervention in 14% of their cohort. However, the authors concluded that follow-up using CT without symptoms remains questionable. Multiple grading systems have been introduced to assess the severity of surgical complications, for example the Clavien-Dindo classification, originally used for surgical adverse events but then adapted to include endoscopic adverse events[16]. New grading systems have been originally designated to evaluate the adverse events of endoscopic procedures. Nass *et al*[17] introduced the adverse events in GI endoscopy classification (Table 1). Another proposed classification was proposed by Chavan *et al*[18], divided into 4 classes (not an adverse event, mild, moderate, and severe) (Table 2).

COMPLICATIONS HIGHLY ASSOCIATED WITH TSE

Insufflation-related complications

The definition of insufflation-related complications needs a consensus from experts worldwide before it can be validated. However, it was suggested that it should only be defined as a complication if the insufflation-related events required an intervention[19]. The rate of insufflation-related events usually depends on multiple factors, including the definition of complication, the type of gas used (air or CO_2), the nature and duration of the procedure, and the method of diagnosis [15]. Most pulmonary complications are reported after POEM compared to other endoscopic procedures; this could be explained by the anatomy of the esophagus, where after myotomy, the mucosa will be the only barrier with the mediastinum[19]. The most common respiratory complications with POEM are insufflation-related complications. These include subcutaneous emphysema (7.5%) and pneumoperitoneum (6.8%). pneumothorax (1.2%), and pneumomediastinum (1.1%)[20].

The use of CO_2 instead of air has been associated with low insufflation-related complications; this is because CO_2 has a higher diffusion capacity and quicker absorption than air[21,22]. Cai *et al*[22] reported a study where room air was used in 52% of patients, and insufflation-related complications included pneumomediastinum (48%), pneumoperitoneum (37%), subcutaneous emphysema (28%), and pneumothorax (17%). In contrast, Zhang *et al*[23] reported a decrease in the incidence rate of insufflation-related complications from 3.3% to 1.9% after switching to CO_2. The previous results highlight the most important preventive measure to decrease the incidence of insufflation-related complications: Using CO_2 instead of room air. Other measures that could help include positive pressure ventilation, using the low-flow gas tube, and dissecting a wider submucosal tunnel[24-27]. However, all these measures cannot completely prevent complications related to insufflation.

The diagnosis of insufflation-related complications should be done as early as possible to prevent irreversible damage. This could be done by clinical examination and other investigations, including monitoring the end-tidal CO_2 and peak airway pressure. Fluoroscopy could help differentiate between capnoperitoneum and retroperitoneal CO_2[28].

In the Nabi *et al*[19] review article, where the authors discussed the insufflation-related complications of POEM, they suggested a 4-pronged approach to be applied to manage such complications. The approach includes: (1) Gastric decompression through suctioning; (2) Temporary pausing of the procedure to allow CO_2 absorption; (3) Subtle changes in ventilator settings to increase minute ventilation and augment CO_2 washout; and (4) Lastly, needle decompression[19, 29]. However, we would like to add a fifth point to this approach that we believe should be number one: Good communication between the endoscopist and the anesthesiologist before, during, and after the procedure. Since POEM is a procedure that causes an increase in the peak inspiratory pressure and means arterial pressure with a lot of CO_2 insufflation leading to systemic absorption[30], this will not be possible to be controlled only by an anesthesiologist. Thus, endoscopists should also be involved in the management of such situations.

Subcutaneous emphysema could happen in TSE procedures as an insufflation-related complication with a potential risk of airway obstruction. It has been found that 60% of the instances of raised end-tidal CO_2 > 50 mmHg are associated with subcutaneous emphysema[31]. Therefore, general anesthesia with intubation is necessary to allow the regulation of ventilation volume. The uptake of CO_2 produced by the insufflation could be resolved through hyperventilation. While mild cases can be treated with hyperventilation, this may not be sufficient to maintain normocapnia in more severe cases where subcutaneous emphysema has occurred and requires needle drainage[31]. A meta-analysis study reported that the incidence rate of pneumothorax during or after POEM is 1.2%[20]. In most cases, pneumothorax can be treated conservatively or by needle decompression. On the other hand, post-POEM tension pneumothorax, as reported by Kang *et al*[32] and Rajmohan *et al*[33] requires a chest tube insertion and could be a life-threatening condition.

Although pneumoperitoneum is not a direct pulmonary complication, its incidence could result in severe lung injury. This happens due to either gastric distention or pneumoperitoneum due to continuous insufflation, eventually leading to an increase in the peak inspiratory pressure and lung injury. To decrease the peak inspiratory pressure, the anesthesiologist usually decreases the tidal volume and increases the respiratory rate to keep ventilation. If these measures fail, the anesthesiologist could ask the endoscopist to start gastric decompression, decrease the amount of CO_2 insufflation, and percutaneous abdominal needle decompression (the 5-pronged approach)[30]. However, Inoue *et al*[7] recommended

Table 1 The adverse events in gastrointestinal endoscopy classification

Grading	Definition
No adverse event	A telephone contact with the general practitioner, outpatient clinic, or endoscopy service without any intervention or extended observation of the patient after the procedure, < 3 hours, without any intervention
Grade I	Adverse events with any deviation of the standard postprocedural course, without the need for pharmacologic treatment or endoscopic, radiologic, or surgical interventions
	Presentation at the emergency ward, without any intervention
	Hospital admission (< 24 hours), without any intervention
	Allowed therapeutic regimens are drugs as antiemetics, antipyretics, analgesics, and electrolytes
	Allowed diagnostic tests: Radiology and laboratory tests
Grade II	Adverse events requiring pharmacologic treatment with drugs other than those allowed for grade I adverse events (*i.e.*, antibiotics, antithrombotics, *etc.*)
	Blood or blood product transfusions
	Hospital admission for more than 24 hours
Grade III	Adverse events requiring endoscopic, radiologic, or surgical intervention
Grade IIIa	Endoscopic or radiologic intervention
Grade IIIb	Surgical intervention
Grade IV	Adverse events requiring intensive care unit/critical care unit admission
Grade IVa	Single-organ dysfunction (including dialysis)
Grade IVb	Multiorgan dysfunction
Grade V	Death

Definition of adverse event: All negative outcomes for a patient that prevent completion of the planned procedure or cause any deviation from the standard postprocedural course.

keeping the positive pressure ventilation above the levels produced by the CO_2 insufflation by the endoscopist, believing this could be a protective measure against emphysema and embolism. The disagreement regarding the limitation of peak inspiratory pressure during POEM has persisted. However, we recommend keeping peak inspiratory pressure under 30 cmH_2O is best.

Empyema, pneumonia, and mediastinitis

Since the peritoneal and mediastinal cavities are exposed in POEM, it is postulated that contamination of these spaces could lead to infection-related complications[34,35]. Contamination and sepsis are frequently seen following dehiscence of mucosal entry or delayed mucosal perforations, leading to mucosal barrier failure[10,23]. Zhang *et al*[23] reported 13 cases of delayed mucosal barrier failure leading to infection. To prevent such complications, prophylactic antibiotics are routinely administered before POEM and for a few days postoperatively[36]. Post-operative infections can be managed by antibiotics only in mild cases. Significant infections usually require drainage or surgery along with a prolonged course of antibiotics[10]. Delayed mucosal barrier failure requires nil per os, nasogastric/naso-jejunal feeds, and intravenous antibiotics.

Aspiration pneumonia

The incidence of aspiration pneumonia in POEM is very low > 0.1%[36-38]. As most patients lie in a left lateral decubitus position during endoscopy, when aspiration pneumonia happens, it is usually in the left lung. Aspiration pneumonia is mainly diagnosed clinically with fever, cough, and sputum symptoms. A chest X-ray or a CT scan can also detect the radiographic signs of pneumonia. White blood cell count, C-reactive protein level, and body temperature are reported to be significantly higher in patients with aspiration pneumonia than in those without aspiration pneumonia[39]. Preventive measures include a clear liquid diet for at least 24 hours preoperative, adequate suction of the oral cavity to remove saliva, esophageal content aspiration, if present, before induction of anesthesia, and rapid sequence intubation with cricoid pressure in a semi-reclining position[37,40,41]. If the patient develops post-POEM aspiration pneumonia, antibiotics should be administered to avoid prolonged hospital stays[39].

Pulmonary embolism

Pulmonary embolism is a rare complication; however, it could be fatal if not discovered early. Khashab *et al*[42] reported an event of pulmonary embolism managed with as a possible complication of POEM. The source of the embolism could be either a venous thromboembolism (VTE) or an air embolism. The risk of VTE is increased because of prolonged

Table 2 Classification of adverse events of third space endoscopy[18]

Category	Type of adverse event
Severe	Any events requiring prolongation of hospital for > 10 days and ICU admission > 1 night
	Insufflation related events
	Causing hemodynamic instability
	Requiring premature termination of procedure ± drainage
	Mucosal injury (during or after)
	Requiring special closure techniques (stenting/sponge/surgery/drainage)
	Bleeding (during or after)
	Requiring blood transfusion
	Causing hemodynamic instability
	Endoscopic reintervention or surgery
	Post procedure leak
	Requiring endoscopic reintervention, drainage or surgery
	Cardiopulmonary events
	Causing hemodynamic instability
	Requiring premature termination of procedure
	Infection
	Causing hemodynamic instability requiring antibiotics ± drainage or surgery
Moderate	Any events requiring prolongation of hospital for 4-10 days and ICU admission for 1 day
	Insufflation related events with high probability of hemodynamic compromise requiring prolonged withholding of procedure (15 minutes) ± immediate drainage
	Capno-pericardium
	Mediastinal emphysema
	Tension pneumothorax
	Pleural effusion (during or after)
	Requiring drainage ± antibiotics
Mild	Insufflation related events requiring temporary withholding of procedure ± drainage
	Retroperitoneum
	Pneumothora
	Capno-peritoneum
	Mucosal injury (during or after) which can closed endoscopically
	Bleeding (during or after) not requiring blood transfusion or additional endoscopic intervention
	Infection requiring prolonged antibiotics
Not an adverse event	Insufflation related events not requiring any measures and accidently detected during fluoroscopy
	Small pneumothorax
	Small pneumoperitoneum
	Retroperitoneum
	Mild mediastinal emphysema
	Subcutaneous emphysema

Citation: Chavan R, Nabi Z, Reddy DN. Adverse events associated with third space endoscopy: Diagnosis and management. *Int J Gastrointest Interv* 2020; 9: 86-97. Copyright © *International Journal of Gastrointestinal Intervention*. Published by The Society of Gastrointestinal Intervention. Powered by INFOrang Co., Ltd. ICU: Intensive care unit.

recumbency during TSE and after the procedure because of the intravenous sedation. Kusunoki *et al*[43] reported an overall frequency of asymptomatic VTE after ESD of 10%. To prevent VTE, it is recommended to use mechanical prophylactic measures such as changing the posture right after the procedure, massaging the lower limbs, and wearing elastic stockings. Early detection of VTE is important to minimize the risk of pulmonary embolism; thus, the D-dimer level on the day after ESD, particularly, could be valuable. It is reported to be potentially associated with the risk for VTE in ESD patients[43]. Air embolism is a very rare insufflation-related complication. The main problem is that it can be difficult to notice because it mimics sedation-related problems. Therefore, it should be considered with prolonged altered mental status, anisocoria, tachycardia, tachypnea, dyspnea, or ST-T changes in the electrocardiogram. The only proven method to reduce the incidence of air embolism is by using CO_2 instead of room air[44].

Hemothorax

Hemothorax is another post-POEM adverse event where earlier detection is important. Werner *et al*[10] reported a delayed bleeding event causing hemothorax. The event required surgery for definitive treatment.

Pleural effusion

Pleural effusion that requires drainage is a rare complication of POEM. According to some reports, it only occurs in 0.2% of patients who undergo POEM[10]. Several studies examined the rate of post-POEM pleural effusion. Werner *et al*[10] reported that, up to 2017, data from literature studying post-POEM complications recruited 4117 patients; of those who underwent POEM, 11.7% developed pleural effusion, and only 0.2% of patients required drainage. Another multicentric study recruited 1826 patients and reported that only 0.2% of patients developed pleural effusion post-POEM. However, in most cases, the pleural effusion spontaneously resolved[35]. In a recent Korean cohort, the data of 328 patients were collected retrospectively, showing that the frequency of pleural effusion post-POEM was 0.9%[11]. Based on the above-mentioned studies, the frequency of developing pleural effusion after POEM ranged from 0.2% to 11.7%, with a small number of reported cases of pulmonary embolism.

PREVENTION OF PULMONARY COMPLICATIONS

To reduce pulmonary complications in TSE and POEM it is recommended to follow the following recommendations.

Preoperative preparation

Preoperative prevention strategies include keeping the patient on clear liquids for at least 24 hours before the procedure, aspirating the esophageal contents before induction of anesthesia and rapid sequence intubation with cricoid pressure, starting induction/intubation with the patient in a semi-reclining position, and giving antibiotic prophylaxis to reduce postoperative infections.

Intraoperative technical optimization

Intraoperative prevention strategies include the use of CO_2 instead of room air, avoidance of full-thickness myotomy, and avoidance of over-insufflation when full-thickness myotomies have been recognized. These strategies will reduce insufflation-related complications and pneumothorax. Frequent abdominal palpation could help in the early detection of tense pneumoperitoneum. Inadvertent entry in the mediastinum should be promptly recognized as it could lead to pneumoperitoneum and pneumopericardium. In cases where clinical instability is thought to be due to pneumopericardium or pneumothorax, it is recommended to briefly interrupt the procedure without insufflation to reestablish clinical stability and continue the procedure. In cases with cardiac arrest, bilateral chest tubes and subxiphoid pericardial windows can be lifesaving.

Postoperative monitoring strategies

Thorough postoperative monitoring is recommended to early detect and manage postoperative complications.

FUTURE RESEARCH DIRECTIONS

Investigating the underlying mechanisms of pulmonary complications associated with TSE, particularly the impact of insufflation techniques, will enrich our understanding of how these complications develop and how they could be further prevented. Moreover, exploring advancements in surgical techniques aimed at minimizing the risk of such complications would be valuable.

CASE REPORT

To our knowledge, no cases of chylothorax have been reported post-POEM. However, we report a post-POEM chylothorax in a 58-year-old female patient without notable medical history apart from suffering from type II achalasia for which she underwent an open Heller's myotomy 20 years ago. With an Eckardt score of 11, the patient presented with

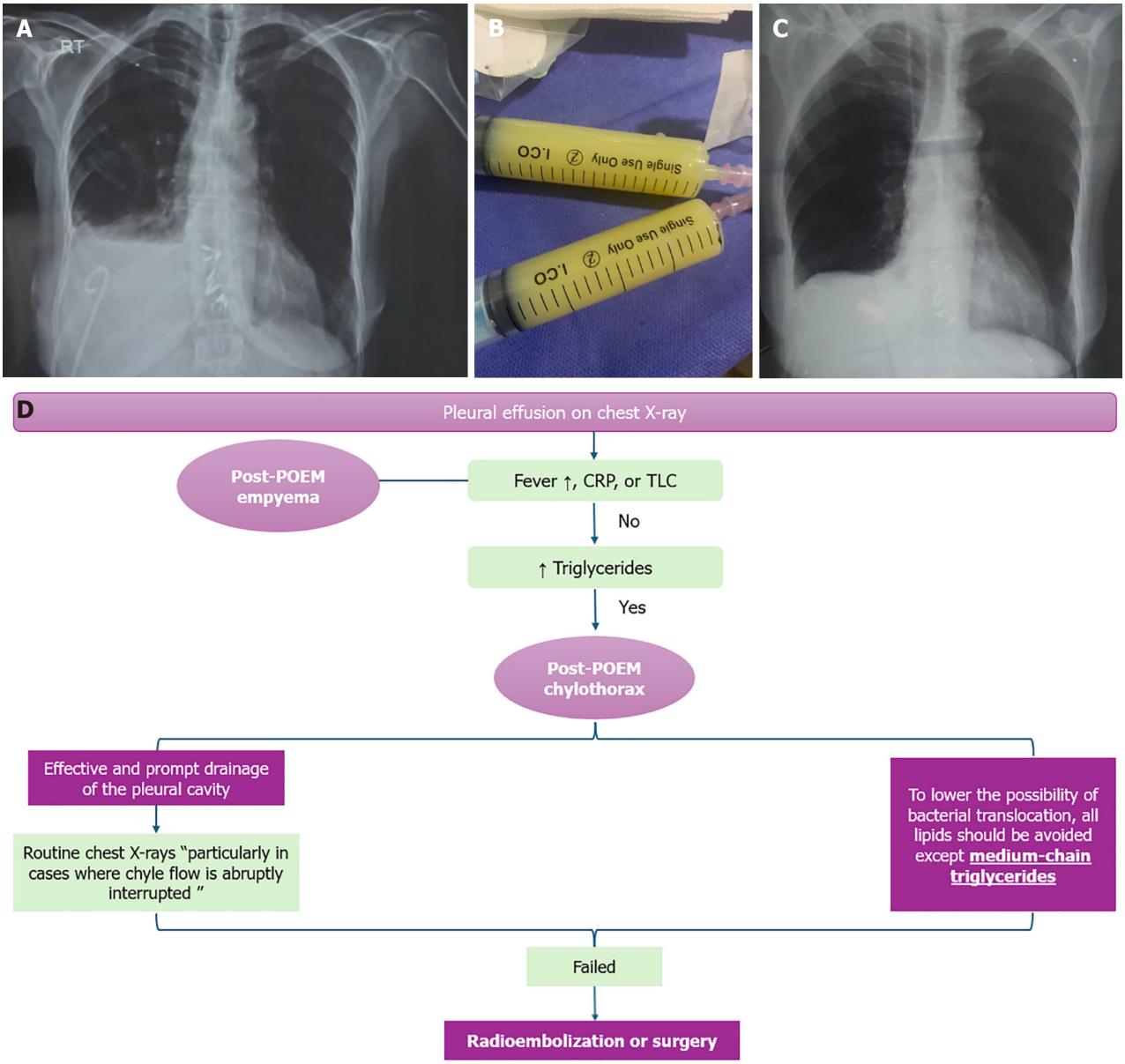


Figure 1 Case report. A: Chylous pleural effusion with pigtail inserted; B: Syringes showing the aspirated white pleural fluid "chylous effusion"; C: X-ray showing the total resolution of the previous chylothorax after our management plan; D: Flowchart showing the management of post-peroral endoscopic myotomy chylothorax. POEM: Peroral endoscopic myotomy; CRP: C-reactive protein; TLC: Total leukocyte count.

recurring dysphagia, vomiting, and significant weight loss. Esophagoscopy revealed a dilated esophagus, and esophageal manometry confirmed the recurrence of type II achalasia. An 8-cm tunnel was started above the cardia and extended for 3 cm on the gastric side. We were driven to use a higher current due to the severe submucosal fibrosis (forced coagulation effect 2 at 60 watts). Myotomy was performed successfully without intraprocedural complications.

36 hours later, the patient started a clear fluid diet without developing complications and was discharged a day later. On post-procedural day 4, the patient presented with severe dyspnea. Chest examination revealed absent breath sounds on the right side. The patient was readmitted to the hospital, and a chest X-ray revealed a massive right pleural effusion with a left mediastinal shift. A pigtail catheter was inserted (Figure 1A), which drained a white milky effusion (Figure 1B), and the patient was kept nil per os. The diagnosis of empyema was excluded based on the absence of fever, normal C-reactive protein, and total leucocytic count. Fluid analysis revealed high triglycerides; otherwise, the patient had normal biochemical parameters in her effusion sample. After three days of drainage, the catheter evacuated most of the fluid, leaving a scant amount of drained serous fluid with a normal triglycerides level on fluid analysis.

A CT of the chest with oral and intravenous contrast confirmed the diagnosis of pulmonary embolism and also confirmed the absence of any esophageal leaks or esophageal-pleural fistulas. The patient was kept on anticoagulation for pulmonary embolism. After a trial to restart the clear fluid diet, the patient developed chylothorax again despite the absence of an esophageal leak. A thoracic duct leak was excluded using lymphoscintigraphy. The high morbidity and mortality of thoracic duct ligation pushed us to apply the algorithmic approach described by Chalret du Rieu *et al*[45] in 2011 in order to manage post-operative chylothorax based on when the chylothorax formed and how the drainage flowed. Upon applying the algorithm, the decision was for a conservative treatment. The patient was kept on exclusive

parenteral nutrition, and based on our nutritionist's recommendations, total elimination of fats was applied. Two weeks later, the patient started an oral diet with the elimination of all fats except for medium-chain triglycerides to reduce the risk of bacterial translocation, which was of beneficial value. During the following week, the patient became symptom-free, and the effusion resolved, which was confirmed using imaging (Figure 1C), leading to the removal of the pigtail catheter. The patient was discharged safely and was advised to continue a fat-free diet supplemented with medium-chain triglycerides for another 3 months.

During the subsequent follow-up visits, there was a significant improvement in pre-POEM symptoms, especially dysphagia. In addition, the patient gained nearly 25 kg, and her Eckardt score dropped to 2. Regarding the effusion, the patient remained asymptomatic without any evidence of chylothorax recurrence to date (1 year). The management of such a post-POEM chylothorax was challenging, especially after the exclusion of a thoracic duct injury and the presence of any esophageal leaks or fistulas. Based on our experience and after reviewing the literature, we suggest the following algorithm for the management of post-POEM chylothorax (Figure 1D)[45,46]. This includes conservative treatment in the form of diminution of chyle flow, efficient drainage of the pleural cavity, nutritional support for the prevention of chronic adverse events, and the avoidance of septic consequences; regular chest X-rays; total cessation of oral or enteral fat consumption at the time of chylothorax diagnosis which can reduce chyle flow by a factor of 10[46]. Surgery and radiographic embolization should be considered if conservative measures fail to treat chylothorax.

CONCLUSION

TSE has transformed the field of GI endoscopy, making it possible to replace surgery with minimally invasive procedures either in some diseases such as achalasia, gastroparesis, or to resect early GI tumors. Under the umbrella of TSE, many procedures have been developed, including POEM, submucosal tunnelling endoscopic resection, Zenker-POEM, gastric-POEM, diverticular-POEM, and peroral endoscopic fundoplication. Although TSE is technically challenging, major complications are rarely reported; however, once they occur, they require careful observation and prompt management. In this review, we aimed to spotlight the pulmonary complications of TSE, especially POEM. We could summarize the management of these complications in the sentence, "working as a team". The management could never be done by an anesthesiologist or the endoscopist alone; instead, it requires a team effort. However, the main steps are the 4-pronged approach that includes gastric decompression, temporary holding of the procedure, increasing the minute ventilation, and needle decompression.

Also, we are reporting the first case of chylothorax after POEM. We faced challenges in managing such cases because of the lack of similarly reported cases and, thus, the absence of guidelines for managing this complication. We overcame this obstacle by consulting our colleagues in the cardiothoracic surgery specialties, which allowed us to successfully manage this case and develop an algorithm of management for similar cases after POEM. However, further studies and research are needed to validate the use of this algorithm on a larger number of patients.

FOOTNOTES

Author contributions: Tawheed A designed the overall concept and outline of the manuscript; Tawheed A and Ismail A wrote the manuscript, they contributed equally to this article, they are the co-first authors of this manuscript; El-Tawansy A revised manuscript and provided critical points from an anesthesia point of view; Ali A followed up on the patient's medical plan; Maurice K provided the management plan for the case; El-Fouly A and Madkour A performed the per-oral endoscopic myotomy procedure for the patient; and all authors contributed to this article and approved the final version of the manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: Egypt

ORCID number: Ahmed Tawheed 0000-0002-9382-8733; Alaa Ismail 0000-0002-7314-9311; Ahmad Madkour 0000-0001-8416-6013.

S-Editor: Bai Y
L-Editor: A
P-Editor: Yu HG

REFERENCES

1 **Sumiyama K**, Gostout CJ, Rajan E, Bakken TA, Knipschield MA. Transesophageal mediastinoscopy by submucosal endoscopy with mucosal flap safety valve technique. *Gastrointest Endosc* 2007; **65**: 679-683 [PMID: 17383463 DOI: 10.1016/j.gie.2006.10.017]

2 **Sumiyama K**, Gostout CJ, Rajan E, Bakken TA, Knipschield MA, Marler RJ. Submucosal endoscopy with mucosal flap safety valve. *Gastrointest Endosc* 2007; **65**: 688-694 [PMID: 17324411 DOI: 10.1016/j.gie.2006.07.030]

3 **McCurdy GA**, Gooden T, Weis F, Mubashir M, Rashid S, Raza SM, Morris J, Cai Q. Gastric peroral endoscopic pyloromyotomy (G-POEM) in patients with refractory gastroparesis: a review. *Therap Adv Gastroenterol* 2023; **16**: 17562848231151289 [PMID: 37007216 DOI: 10.1177/17562848231151289]

4 **Ren L**, Ye H, Zhu Y, Xie W, Liang Y, Liu Y, Dong J, Chen W, Chen X, Wang B, Pan L, Shi R. Diverticular peroral endoscopic myotomy (D-POEM) for symptomatic oesophageal diverticulum: a multicentre cohort study with a minimum follow-up of 3 years. *Surg Endosc* 2024; **38**: 253-259 [PMID: 37985492 DOI: 10.1007/s00464-023-10471-6]

5 **Kaminski MF**, Budnicka A, Przybysz A, Pilonis ND. Traditional septotomy or Z-POEM for Zenker's diverticulum. *Best Pract Res Clin Gastroenterol* 2024; **71**: 101943 [PMID: 39209416 DOI: 10.1016/j.bpg.2024.101943]

6 **Tawheed A**, Bahcecioglu IH, Yalniz M, El-Kassas M. Gastroesophageal reflux after per-oral endoscopic myotomy: Management literature. *World J Gastroenterol* 2024; **30**: 2947-2953 [PMID: 38946871 DOI: 10.3748/wjg.v30.i23.2947]

7 **Inoue H**, Minami H, Kobayashi Y, Sato Y, Kaga M, Suzuki M, Satodate H, Odaka N, Itoh H, Kudo S. Peroral endoscopic myotomy (POEM) for esophageal achalasia. *Endoscopy* 2010; **42**: 265-271 [PMID: 20354937 DOI: 10.1055/s-0029-1244080]

8 **Draganov PV**, Aihara H, Karasik MS, Ngamruengphong S, Aadam AA, Othman MO, Sharma N, Grimm IS, Rostom A, Elmunzer BJ, Jawaid SA, Westerveld D, Perbtani YB, Hoffman BJ, Schlachterman A, Siegel A, Coman RM, Wang AY, Yang D. Endoscopic Submucosal Dissection in North America: A Large Prospective Multicenter Study. *Gastroenterology* 2021; **160**: 2317-2327.e2 [PMID: 33610532 DOI: 10.1053/j.gastro.2021.02.036]

9 **Inoue H**, Shiwaku H, Iwakiri K, Onimaru M, Kobayashi Y, Minami H, Sato H, Kitano S, Iwakiri R, Omura N, Murakami K, Fukami N, Fujimoto K, Tajiri H. Clinical practice guidelines for peroral endoscopic myotomy. *Dig Endosc* 2018; **30**: 563-579 [PMID: 30022514 DOI: 10.1111/den.13239]

10 **Werner YB**, von Renteln D, Noder T, Schachschal G, Denzer UW, Groth S, Nast JF, Kersten JF, Petzoldt M, Adam G, Mann O, Repici A, Hassan C, Rösch T. Early adverse events of per-oral endoscopic myotomy. *Gastrointest Endosc* 2017; **85**: 708-718.e2 [PMID: 27609778 DOI: 10.1016/j.gie.2016.08.033]

11 **Lee JY**, Lim CH, Kim DH, Jung HY, Youn YH, Jung DH, Park JC, Moon HS, Hong SJ; Therapeutic Endoscopy and Instrument for Functional Gastrointestinal Disorders Study Group Under the Korean Society of Neurogastroenterology and Motility. Adverse Events Associated With Peroral Endoscopic Myotomy Affecting Extended Hospital Stay: A Multi-center Retrospective Study in South Korea. *J Neurogastroenterol Motil* 2022; **28**: 247-254 [PMID: 35362451 DOI: 10.5056/jnm21081]

12 **Lotfy A**, Elgazzar AE, Awad M, Yusuf A, Talaat Fathy. Thoracic complications of upper gastrointestinal endoscopy in Zagazig University Hospitals. A cross-sectional single center study. *Egy J Chest Dis Tu* 2017; **66**: 729-734 [DOI: 10.1016/j.ejcdt.2017.09.002]

13 **Waddingham W**, Kamran U, Kumar B, Trudgill NJ, Tsiamoulos ZP, Banks M. Complications of diagnostic upper Gastrointestinal endoscopy: common and rare - recognition, assessment and management. *BMJ Open Gastroenterol* 2022; **9**: e000688 [PMID: 36572454 DOI: 10.1136/bmjgast-2021-000688]

14 **Pannu D**, Yang D, Abbitt PL, Draganov PV. Prospective evaluation of CT esophagram findings after peroral endoscopic myotomy. *Gastrointest Endosc* 2016; **84**: 408-415 [PMID: 26907745 DOI: 10.1016/j.gie.2016.02.022]

15 **Chartier M**, Barat M, Dohan A, Belle A, Oudjit A, Abou Ali E, Hallit R, Leandri C, Scialom S, Coriat R, Chaussade S, Soyer P, Barret M. Clinical impact of routine CT esophagogram after peroral endoscopic myotomy (POEM) for esophageal motility disorders. *Endosc Int Open* 2021; **9**: E1355-E1360 [PMID: 34466359 DOI: 10.1055/a-1512-9638]

16 **El Abiad R**, Ashat M, Khashab M. Complications related to third space endoscopic procedures. *Best Pract Res Clin Gastroenterol* 2024; **71**: 101908 [PMID: 39209411 DOI: 10.1016/j.bpg.2024.101908]

17 **Nass KJ**, Zwager LW, van der Vlugt M, Dekker E, Bossuyt PMM, Ravindran S, Thomas-Gibson S, Fockens P. Novel classification for adverse events in GI endoscopy: the AGREE classification. *Gastrointest Endosc* 2022; **95**: 1078-1085.e8 [PMID: 34890695 DOI: 10.1016/j.gie.2021.11.038]

18 **Chavan R**, Nabi Z, Reddy DN. Adverse events associated with third space endoscopy: Diagnosis and management. *Int J Gastrointest Interv* 2020; **9**: 86-97 [DOI: 10.18528/ijgii200010]

19 **Nabi Z**, Reddy DN, Ramchandani M. Adverse events during and after per-oral endoscopic myotomy: prevention, diagnosis, and management. *Gastrointest Endosc* 2018; **87**: 4-17 [PMID: 28987545 DOI: 10.1016/j.gie.2017.09.029]

20 **Akintoye E**, Kumar N, Obaitan I, Alayo QA, Thompson CC. Peroral endoscopic myotomy: a meta-analysis. *Endoscopy* 2016; **48**: 1059-1068 [PMID: 27617421 DOI: 10.1055/s-0042-114426]

21 **Ren Z**, Zhong Y, Zhou P, Xu M, Cai M, Li L, Shi Q, Yao L. Perioperative management and treatment for complications during and after peroral endoscopic myotomy (POEM) for esophageal achalasia (EA) (data from 119 cases). *Surg Endosc* 2012; **26**: 3267-3272 [PMID: 22609984 DOI: 10.1007/s00464-012-2336-y]

22 **Cai MY**, Zhou PH, Yao LQ, Zhu BQ, Liang L, Li QL. Thoracic CT after peroral endoscopic myotomy for the treatment of achalasia. *Gastrointest Endosc* 2014; **80**: 1046-1055 [PMID: 24998467 DOI: 10.1016/j.gie.2014.05.004]

23 **Zhang XC**, Li QL, Xu MD, Chen SY, Zhong YS, Zhang YQ, Chen WF, Ma LL, Qin WZ, Hu JW, Cai MY, Yao LQ, Zhou PH. Major perioperative adverse events of peroral endoscopic myotomy: a systematic 5-year analysis. *Endoscopy* 2016; **48**: 967-978 [PMID: 27448052 DOI: 10.1055/s-0042-110397]

24 **Wang X**, Tan Y, Zhang J, Liu D. Risk factors for gas-related complications of peroral endoscopic myotomy in achalasia. *Neth J Med* 2015; **73**: 76-81 [PMID: 25753072]

25 **Bechara R**, Onimaru M, Ikeda H, Inoue H. Per-oral endoscopic myotomy, 1000 cases later: pearls, pitfalls, and practical considerations. *Gastrointest Endosc* 2016; **84**: 330-338 [PMID: 27020899 DOI: 10.1016/j.gie.2016.03.1469]

26 **Ramchandani M**, Nageshwar Reddy D, Darisetty S, Kotla R, Chavan R, Kalpala R, Galasso D, Lakhtakia S, Rao GV. Peroral endoscopic myotomy for achalasia cardia: Treatment analysis and follow up of over 200 consecutive patients at a single center. *Dig Endosc* 2016; **28**: 19-26 [PMID: 26018637 DOI: 10.1111/den.12495]

27 **Familiari P**, Gigante G, Marchese M, Boskoski I, Tringali A, Perri V, Costamagna G. Peroral Endoscopic Myotomy for Esophageal Achalasia: Outcomes of the First 100 Patients With Short-term Follow-up. *Ann Surg* 2016; **263**: 82-87 [PMID: 25361224 DOI: 10.1097/SLA.0000000000000992]

28 **Nabi Z**, Ramchandani M, Chavan R, Kalapala R, Darisetty S, Rao GV, Reddy N. Per-oral endoscopic myotomy for achalasia cardia: outcomes in over 400 consecutive patients. *Endosc Int Open* 2017; **5**: E331-E339 [PMID: 28484733 DOI: 10.1055/s-0043-105517]

29 **Darisetty S**, Nabi Z, Ramchandani M, Chavan R, Kotla R, Nageshwar Reddy D. Anesthesia in per-oral endoscopic myotomy: A large tertiary care centre experience. *Indian J Gastroenterol* 2017; **36**: 305-312 [PMID: 28840505 DOI: 10.1007/s12664-017-0782-0]

30 **Bang YS**, Park C. Anesthetic Consideration for Peroral Endoscopic Myotomy. *Clin Endosc* 2019; **52**: 549-555 [PMID: 31288505 DOI: 10.5946/ce.2019.033]

31 **Murata H**, Ichinomiya T, Hara T. Anesthesia for peroral endoscopic myotomy in Japan. *Curr Opin Anaesthesiol* 2019; **32**: 511-516 [PMID: 30994477 DOI: 10.1097/ACO.0000000000000742]

32 **Kang S**, Kim Y, Kim DH. A rare complication: Tension pneumothorax after peroral endoscopic myotomy. *Int J Gastrointest Interv* 2022; **11**: 139-142 [DOI: 10.18528/ijgii210057]

33 **Rajmohan N**, Sadath A, Nelson F, Vamadevan BT. Problems in beginning a "POEM". *Indian J Anaesth* 2019; **63**: 508-510 [PMID: 31263311 DOI: 10.4103/ija.IJA_29_19]

34 **Shiwaku H**, Inoue H, Yamashita K, Ohmiya T, Beppu R, Nakashima R, Takeno S, Sasaki T, Nimura S, Yamashita Y. Peroral endoscopic myotomy for esophageal achalasia: outcomes of the first over 100 patients with short-term follow-up. *Surg Endosc* 2016; **30**: 4817-4826 [PMID: 26932548 DOI: 10.1007/s00464-016-4813-1]

35 **Haito-Chavez Y**, Inoue H, Beard KW, Draganov PV, Ujiki M, Rahden BHA, Desai PN, Pioche M, Hayee B, Haji A, Saxena P, Reavis K, Onimaru M, Balassone V, Nakamura J, Hata Y, Yang D, Pannu D, Abbas A, Perbtani YB, Patel LY, Filser J, Roman S, Rivory J, Mion F, Ponchon T, Perretta S, Wong V, Maselli R, Ngamruengphong S, Chen YI, Bukhari M, Hajiyeva G, Ismail A, Pieratti R, Kumbhari V, Galdos-Cardenas G, Repici A, Khashab MA. Comprehensive Analysis of Adverse Events Associated With Per Oral Endoscopic Myotomy in 1826 Patients: An International Multicenter Study. *Am J Gastroenterol* 2017; **112**: 1267-1276 [PMID: 28534521 DOI: 10.1038/ajg.2017.139]

36 **Stavropoulos SN**, Modayil RJ, Friedel D, Savides T. The International Per Oral Endoscopic Myotomy Survey (IPOEMS): a snapshot of the global POEM experience. *Surg Endosc* 2013; **27**: 3322-3338 [PMID: 23549760 DOI: 10.1007/s00464-013-2913-8]

37 **Goudra B**, Singh PM, Gouda G, Sinha AC. Peroral endoscopic myotomy-initial experience with anesthetic management of 24 procedures and systematic review. *Anesth Essays Res* 2016; **10**: 297-300 [PMID: 27212764 DOI: 10.4103/0259-1162.171462]

38 **Tantau M**, Crisan D. Peroral endoscopic myotomy: Time to change our opinion regarding the treatment of achalasia? *World J Gastrointest Endosc* 2015; **7**: 237-246 [PMID: 25789094 DOI: 10.4253/wjge.v7.i3.237]

39 **Watari J**, Tomita T, Toyoshima F, Sakurai J, Kondo T, Asano H, Yamasaki T, Okugawa T, Tanaka J, Daimon T, Oshima T, Fukui H, Hori K, Matsumoto T, Miwa H. The incidence of "silent" free air and aspiration pneumonia detected by CT after gastric endoscopic submucosal dissection. *Gastrointest Endosc* 2012; **76**: 1116-1123 [PMID: 23164512 DOI: 10.1016/j.gie.2012.07.043]

40 **Yang D**, Pannu D, Zhang Q, White JD, Draganov PV. Evaluation of anesthesia management, feasibility and efficacy of peroral endoscopic myotomy (POEM) for achalasia performed in the endoscopy unit. *Endosc Int Open* 2015; **3**: E289-E295 [PMID: 26357672 DOI: 10.1055/s-0034-1391965]

41 **Tanaka E**, Murata H, Minami H, Sumikawa K. Anesthetic management of peroral endoscopic myotomy for esophageal achalasia: a retrospective case series. *J Anesth* 2014; **28**: 456-459 [PMID: 24185834 DOI: 10.1007/s00540-013-1735-0]

42 **Khashab MA**, El Zein M, Kumbhari V, Besharati S, Ngamruengphong S, Messallam A, Abdelgalil A, Saxena P, Tieu AH, Raja S, Stein E, Dhalla S, Garcia P, Singh VK, Pasricha PJ, Kalloo AN, Clarke JO. Comprehensive analysis of efficacy and safety of peroral endoscopic myotomy performed by a gastroenterologist in the endoscopy unit: a single-center experience. *Gastrointest Endosc* 2016; **83**: 117-125 [PMID: 26212369 DOI: 10.1016/j.gie.2015.06.013]

43 **Kusunoki M**, Miyake K, Shindo T, Ueki N, Kawagoe T, Gudis K, Futagami S, Tsukui T, Takagi I, Hosaka J, Sakamoto C. The incidence of deep vein thrombosis in Japanese patients undergoing endoscopic submucosal dissection. *Gastrointest Endosc* 2011; **74**: 798-804 [PMID: 21855867 DOI: 10.1016/j.gie.2011.06.015]

44 **Nonaka S**, Saito Y, Takisawa H, Kim Y, Kikuchi T, Oda I. Safety of carbon dioxide insufflation for upper gastrointestinal tract endoscopic treatment of patients under deep sedation. *Surg Endosc* 2010; **24**: 1638-1645 [PMID: 20108154 DOI: 10.1007/s00464-009-0824-5]

45 **Chalret du Rieu M**, Baulieux J, Rode A, Mabrut JY. Management of postoperative chylothorax. *J Visc Surg* 2011; **148**: e346-e352 [PMID: 22033151 DOI: 10.1016/j.jviscsurg.2011.09.006]

46 **Schild HH**, Strassburg CP, Welz A, Kalff J. Treatment options in patients with chylothorax. *Dtsch Arztebl Int* 2013; **110**: 819-826 [PMID: 24333368 DOI: 10.3238/arztebl.2013.0819]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 100903

DOI: 10.5662/wjm.v15.i3.100903

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Retrospective Study

"Electronic Pediatrician", a non-machine learning prototype artificial intelligence software for pediatric computer-assisted pathophysiologic diagnosis — general presentation

Andrei-Lucian Drăgoi, Roxana-Maria Nemeș

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B

Novelty: Grade A

Creativity or Innovation: Grade A

Scientific Significance: Grade B

P-Reviewer: Ling Y

Received: September 6, 2024

Revised: October 24, 2024

Accepted: November 25, 2024

Published online: September 20, 2025

Processing time: 181 Days and 13.5 Hours

Andrei-Lucian Drăgoi, Roxana-Maria Nemeș, Medical Doctoral School of University "Titu Maiorescu", Bucharest 040051, Romania

Andrei-Lucian Drăgoi, The Emergency County Hospital Târgoviște (SJUT), Dambovita 130095, Târgoviște, Romania

Corresponding author: Andrei-Lucian Drăgoi, MD, Doctor, Researcher, Medical Doctoral School of University "Titu Maiorescu", Str. General I. E. Florescu nr. 9, Bl. D11, Sc. B, Ap. 9, Bucharest 040051, Romania. dr.dragoi@yahoo.com

Abstract

BACKGROUND

Knowledge-based systems (KBS) are software applications based on a knowledge database and an inference engine. Various experimental KBS for computer-assisted medical diagnosis and treatment were started to be used since 70s (VisualDx, GIDEON, DXPlain, CADUCEUS, Internist-I, Mycin *etc.*).

AIM

To present in detail the "Electronic Pediatrician (EPed)", a medical non-machine learning artificial intelligence (nml-AI) KBS in its prototype version created by the corresponding author (with database written in Romanian) that offers a physio-pathology-based differential and positive diagnosis and treatment of ill children.

METHODS

EPed specifically focuses on the physiopathological reasoning of pediatric clinical cases. EPed has currently reached its prototype version 2.0, being able to diagnose 302 physiopathological macro-links (briefly named "clusters") and 269 pediatric diseases: Some examples of diagnosis and a previous testing of EPed on a group of 34 patients are also presented in this paper.

RESULTS

The prototype EPed can currently diagnose 269 pediatric infectious and non-infectious diseases (based on 302 clusters), including the most frequent respiratory/digestive/renal/central nervous system infections, but also many other non-infectious pediatric diseases like autoimmune, oncological, genetical diseases and even intoxications, plus some important surgical pathologies.

CONCLUSION
EPed is the first and only physiopathology-based nml-AI KBS focused on general pediatrics and is the first and only pediatric Romanian KBS addressed to medical professionals. Furthermore, EPed is the first and only nml-AI KBS that offers not only both a physiopathology-based differential and positive disease diagnosis, but also identifies possible physiopathological "clusters" that may explain the signs and symptoms of any child-patient and may help treating that patient physiopathologically (until a final diagnosis is found), thus encouraging and developing the physiopathological reasoning of any clinician.

Key Words: Knowledge-based systems; Computer-assisted medical diagnosis; Non-machine learning artificial intelligence; DXPlain; General pediatrics; "Electronic Pediatrician" software

Core Tip: Electronic Pediatrician (EPed) is the first and only physiopathology-based non-machine learning artificial intelligence (nml-AI) knowledge-based system (KBS) focused on general pediatrics and is the first and only Romanian pediatric KBS addressed to medical professionals. Furthermore, EPed is the first and only nml-AI KBS that offers not only both a physiopathology-based differential and positive disease diagnosis, but also identifies possible physiopathological "clusters" that may explain the signs and symptoms of any child-patient and may help treating that patient physiopathologically (until a final diagnosis is found), thus encouraging and developing the physiopathological reasoning of any clinician.

Citation: Drăgoi AL, Nemeș RM. "Electronic Pediatrician", a non-machine learning prototype artificial intelligence software for pediatric computer-assisted pathophysiologic diagnosis — general presentation. *World J Methodol* 2025; 15(3): 100903
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/100903.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.100903

INTRODUCTION

Knowledge-based systems (KBS) are software with 2 pivotal components: (1) A knowledge (data-)base (KB); and (2) An inference engine (which deduces new information based on that KB)[1-3].

Medical expert systems (MES)[4] are artificial intelligence (AI)-based KBS[5-7] which emulate human experts and can be used as important adjuvants in clinical decision making[8].

However, because MES are mainly based on if–then rules, they are hard to build and maintain, thus quite expensive. Some examples of MES are: VisualDx (focused on dermatology), GIDEON & Mycin (both focused on infectious diseases), DXplain[9-12], CADUCEUS, Internist-I (all focused on internal medicine) *etc*[13,14].

The here-proposed software application "Electronic Pediatrician (EPed)" (built as a prototype by the corresponding author of this article) is a non-machine learning AI (nml-AI) KBS, a much cheaper alternative for MES, with other two main advantages: (1) EPed is the first nml-AI KBS written in Romanian and focused on general pediatrics; and (2) EPed is the first and only physiopathology-based nml-AI KBS that offers both a list of possible diseases for any proposed child-patient and a list of possible pathophysiological "clusters" that may explain the set of clinical and paraclinical signs of that child-patient (and thus help treating the patients physiopathologically until a specific disease diagnosis is obtained) encouraging and developing the physiopathological reasoning of any clinician (thus having potential pedagogical importance in the future, including a helpful resource in orientating the anamnesis and the clinical exam). This EPed prototype was also tested on a group of selected patients treated in the Children's Infectious Diseases Ward of The Emergency County Hospital Târgoviște (Romania).

MATERIALS AND METHODS

The "cluster" concept used by EPed

The "cluster" concept (used by EPed) is defined as any morphological and/or functional anomaly (macroscopic or microscopic) that produces at least one non-trivial clinical or paraclinical sign (or symptom) and can be a physiopathological macro-link in the physiopathogenic chain of one or more diseases or syndromes. This "cluster" concept is much wider and flexible than a "syndrome" because it contains all the clinical and paraclinical signs of a specific physiopathological process: For example, the "pharyngitis" cluster is defined as "an acute or chronic infectious/non-infectious inflammation of the pharynx" plus all the non-trivial clinical and paraclinical signs generated by this inflammation; the same for laryngitis, epiglottitis, sinusitis *etc*.

The 7 types of clusters used by EPed

EPed uses 7 major types of clusters [each having a relatively specific clinico-paraclinical picture (CPP)], which can be regarded as modules of "healthy" physiopathological clinical reasoning of any medical professional: (1) Syndromes that can also have the role of physiopathological macro-links in various diseases, for example: Respiratory functional syndrome, central neuron syndrome, peripheral neuron syndrome, *etc.*; (2) Inflammation of various tissues, anatomical structures, organs, *etc.*, for example: "pharyngitis" (defined as acute/chronic infectious/non-infectious inflammation of the pharynx), "laryngitis" (defined as acute/chronic infectious/non-infectious inflammation of the larynx), *etc.*; (3) Deformities (including cracks/ruptures) of tissues, anatomical structures and organs, *etc.*, for example: Left ventricular hypertrophy, right ventricular hypertrophy, *etc.*; (4) Various functional imbalances, for example: Left systolic ventricular failure, left diastolic ventricular failure, *etc.*; (5) Various metabolic/homeostatic imbalances, for example: Metabolic acidosis, respiratory acidosis, hypoxia, hypoxemia, *etc.*; (6) Changes in lab parameters (*e.g.* hypernatremia, hyperkalemia, direct/total hyperbilirubinemia) that also produce at least one non-trivial sign/symptom (different from the name of the cluster): For example, hypernatremia also produces non-trivial clinical signs (*e.g.* convulsions), *etc.*; and (7) Various genetic abnormalities (each with a certain CPP), for example: Trisomy 21 (Down syndrome), trisomy 13 (Patau syndrome), mutations of the dystrophin gene (muscular dystrophies Duchenne, Becker) *etc.*

Example of a cluster-based description of a disease

For example, acute viral hepatitis A (VHA) is described in EPed by a specific set of clusters: (1) Viremia (predefined by EPed as the presence of the Hepatovirus A in the bloodstream); (2) Hepatitis (predefined by EPed as the non-specific global inflammation of the liver); (3) Hepatocytolysis (predefined by EPed as the necrosis of hepatocytes); (4) Intrahepatic cholestasis; (5) Extrahepatic cholestasis (that may sometimes complicate VHA); (6) Biological/systemic inflammatory syndrome (predefined by EPed as the increase in serum levels of the main inflammatory markers); (7) Cholecystitis (predefined by EPed as the nespecific inflammation of the biliary vesicle that may sometimes appear in VHA); (8) Cholangitis (that may sometimes complicate VHA); and (9) Gastritis (predefined by EPed as the inflammation of the gastric mucosa that may sometimes appear in VHA). Note: (1) The "cluster" concept can be regarded as a "vertical synapse" between various biochemical (microscopic) sub-processes of any disease (the pathophysiological micro-links of that disease) and the (macroscopic) clinical signs and symptoms of a patient. EPed essentially tries to create a common and unifying physiopathological language between several pediatric subspecialties (and not only!) by using this "cluster" concept as a unifying binder with a central role in the general algorithm of diagnosis and treatment proposed by EPed; (2) EPed primarily uses clusters to ultra-concisely describe diseases and to generate a physiopathology-based complex/combined differential diagnosis on N clinical/paraclinical signs (CPS)/symptoms simultaneously, an advantage impossible to be accomplished by any medical/pediatric manual: Furthermore, the physiopathological foundation of this complex differential diagnosis is not found in any other software of computer-assisted medical diagnosis; and (3) Moreover, EPed also has a promising performance in establishing a pediatric positive diagnosis, as we have already shown in a recent article in which we've tested EPed on 34 child-patients with 24 distinct real diagnoses established by the medical doctor[15] (Table 1; Figure 1).

The real positive-diagnoses (established by the medical doctor for each tested child-patient in part) were found to occupy an average list-index of 12 (varying from 1 to 57) in the list of possible disease-diagnoses proposed by EPed for each input case in part, which is an acceptable performance of medical positive diagnostic performance for a prototype version (Figure 2).

Furthermore, in 2025, we plan to retrospectively test the positive-diagnosis performance of EPed on at least 1000 Romanian child-patients from at least one major Romanian hospital, with a total of at least 200-250 distinct diagnoses.

The 2-steps diagnosis accomplished by EPed: EPed uses an original 2-steps physiopathology-based differential diagnostic algorithm: (1) Finding all possible clusters that may explain the set of clinical and paraclinical signs of a child-patient; and (2) Finding all possible diseases containing those previously found possible clusters. Important note. EPed uses a newly-proposed universal diagnostic algorithm based on *Occam's razor* (the parsimony principle) also combined with the relative incidence (RI) of diseases.

The structure of EPed: As a nml-AI KBS, EPed has 2 major components: (1) A medical knowledge database (composed of text files describing clusters and diseases respectively) and (2) A processing engine (which analyzes any child-patient and offers lists of possible clusters and diseases for that child-patient).

The interface of EPed: EPed has an interface window (IW) with 2 main sections: (1) (The lower-half of IW) by which the user can rapidly gather (by using predefined lists of terms) all the clinical and paraclinical signs of a child-patient; and (2) (The upper-half of IW) containing the lists of possible clusters and diseases proposed by EPed for that child-patient (Figure 3).

The steps of using EPed are listed next: (1) The user adds any CPS to a distinct list dedicated to the CPP of a child-patient. See the next figure in which the user has added the "cough" symptom ("tuse" in Romanian) to the list dedicated to CPP (Figure 4A); and (2) After all CPS (gathered from a patient) are added in the CPP-list, EPed automatically analyzes that CPP-list and finds all possible clusters (Figure 4B) and diseases (diseases that contain at least one of the possible clusters). This 2nd step has two sub-steps: (1) For each possible cluster, EPed calculates a coverage score (CS) which measures the percent in which that CPP-list is explainable by that possible cluster found; and (2) EPed then generates a list of possible diseases (LPD) that may explain at least one cluster from the list of possible clusters (LPC). For each disease (from LPD), 3 types of scores are calculated and assigned to that disease: (1) The RI of that specific disease (1 = very rare; 2 = rare; 3 = medium incidence; 4 = frequent; 5 = very frequent); (2) A cluster CS (CCS) measuring the percent

Table 1 The 24 distinct real diagnoses of the tested group (as established by the medical doctor)

The real diagnoses of the tested group (as established by the medical doctor)	The number of tested cases per each real diagnosis (also in percents from the *n* = 34 patients in total) (%)	The indexed cases tested for each diagnosis in part
Viral diarrhea (rotavirus, norovirus, SARS-CoV2, adenovirus)	7 (21)	Cases No. 4, 5, 6, 14, 17, 19, 25
Bacterial diarrhea (Clostridium, Salmonella, Campylobacter, Shigella)	5 (15)	Cases No. 1, 5, 7, 14, 15, 18
Infectious mononucleosis (Epstein-Barr virus and/or citomegalovirus)	4 (12)	Cases No. 2, 23, 27, 34
Pyelonephritis (ESBL-neg./pos. *E. coli*)	4 (12)	Cases No. 4, 24, 29, 30
Viral URTI (adenovirus)	3 (9)	Cases No. 8, 25, 30
Iron-deficiency anemia	3 (9)	Cases No. 8, 15, 29
COVID-19 (both respiratory and digestive forms of COVID-19)	3 (9%)	Cases No. 10, 16, 19
Streptococcal pharyngitis/tonsillitis	2 (6)	Cases No. 3, 33
Viral encephalitis (varicella-zoster virus)	2 (6)	Cases No. 12, 22
Minor poststreptococcal inflammatory syndrome	1 (3)	Case No. 4
Viral pneumonia	1 (3)	Case No. 6
Hand-foot-and-mouth disease	1 (3)	Case No. 8
Type-A influenza	1 (3)	Case No. 9
Viral tracheobronchitis	1 (3)	Case No. 11
Bacterial acute otitis media	1 (3)	Case No. 11
Varicella	1 (3)	Case No. 12
Giardiasis (Giardia lamblia)	1 (3)	Case No. 13
Hepatitis A	1 (3)	Case No. 20
Floor-of-mouth abscess (sublingual gland abscess)	1 (3)	Case No. 21
Scarlet fever	1 (3)	Case No. 26
Acute lymphoblastic leukemia (ALL)	1 (3)	Case No. 28
Vulvovaginal (Vv.) candidiasis	1 (3)	Case No. 30
Marshall syndrome (PFAPA)	1 (3)	Case No. 31
Mediterranean spotted fever (Rickettsia conorii) (confirmed by pos. IgM anti-Rickettsia conorri)	1 (3)	Case No. 32

COVID-19: Coronavirus disease 2019.

of LPC that can be explained by each possible disease in part; and (3) A combined score of any (possible) disease CSD = RI*CCS (Figure 4C).

RESULTS

This section of the paper offers an example of a simulation based on a processed respiratory CPP: EPed generates both an LPC and LPD for this given respiratory CPP (Table 2).

DISCUSSION

EPed is the first and only nml-AI KBS for "hybrid" (pediatric) physiopathological and disease medical diagnosis in Romania and worldwide. Although not a MES, EPed is very versatile.

Table 2 A simulation applied on a respiratory clinical and paraclinical picture example

A respiratory CPP example	The possible clusters that may partially or integrally explain the given respiratory CPP (%)	The possible diseases that may explain the possible clusters found by EPed
This CPP example contains these 7 listed signs (which are simultaneously processed as a single CPP	EPed has found many possible clusters that may partially explain the given respiratory CPP (the percent in parentheses represents the degree by which that specific cluster theoretically/potentially explains the given CPP	EPed has found many possible diseases that may explain the possible clusters found by EPed
Cough ("tuse")	Systemic (biological) inflammatory syndrome (57%)	Tuberculosis (CSD = 1.1)
Fever ("febra")	Systemic dissemination of Mycobacterium tuberculosis (42%)	Viral pneumonia (CSD = 1.1)
Wheezing ("wheezing")	Broncho-esophageal fistula (42%)	Bacterial pneumonia (CSD = 1)
Increased erythrocyte sedimentation rate (ESR) ("VSH crescut")	Tracheo-esophageal fistula (42%)	COVID-19 (CSD = 0.9)
Thrombocytopenia ("trombocitopenie")	Bronchiolitis (a cluster defined by EPed as an inflammation of bronchioli) (28%)	Scarlet fever (CSD = 0.9)
Neutrophilia ("neutrofilie")	Bronchiolitis obliterans (28%)	Acute viral diarrhea (CSD = 0.8)
Monocytosis ("monocitoza")	Respiratory failure (28%)	Viral URTI (CSD = 0.8)
	Pericarditis (28%)	Bronchiolitis (CSD = 0.6)
	Functional respiratory syndrome of obstructive type (28%)	Measles (CSD = 0.5)
	Dehydration (28%)	Influenza (CSD = 0.5)
	Pleurisy (28%)	Congenital broncho-esophageal fistula (disease) (CSD = 0.5)
	Alveolar pneumonia (28%)	Congenital tracheo-esophageal fistula (disease) (CSD = 0.5)
	Interstitial pneumonia (28%)	Bacterial URTI (CSD = 0.4)
	Bronchitis (14%)	Infectious mononucleosis with EBV or/and CMV (CSD = 0.4)
	Carditis (pancarditis) (14%)	Cystic fibrosis (CSD = 0.4)
	Gastroesophageal reflux disease (14%)	Bacterial epiglottitis (CSD = 0.3)
	Rhinitis (a cluster defined by EPed as an inflammation of the nasal mucosa) (14%)	Viral laryngeal tracheitis (CSD = 0.2)
	Adenoiditis (a cluster defined by EPed as an inflammation of the nasal adenoids) (14%)	Periamygdalian abscess (phlegmon) (CSD = 0.2)
	Bacteremia (14%)	Cervical adenoflegmon (CSD = 0.2)
	Cholangitis (14%)	Asthma (CSD = 0.2)
	Endocarditis (14%)	Gastroesophageal reflux disease (CSD = 0.1)
	[…]	Hiatal hernia (CSD = 0.1)
	Pyelonephritis (14%)	*etc.*
	Sepsis (14%)	
	Toxic bacterial syndrome (14%)	
	SIRS (14%)	
	CNS tuberculoma (14%)	
	Viremia (14%)	
	Hypersplenism (14%)	
	Vasculitis (14%)	
	etc.	

CPP: Clinico-paraclinical picture; EPed: Electronic Pediatrician.

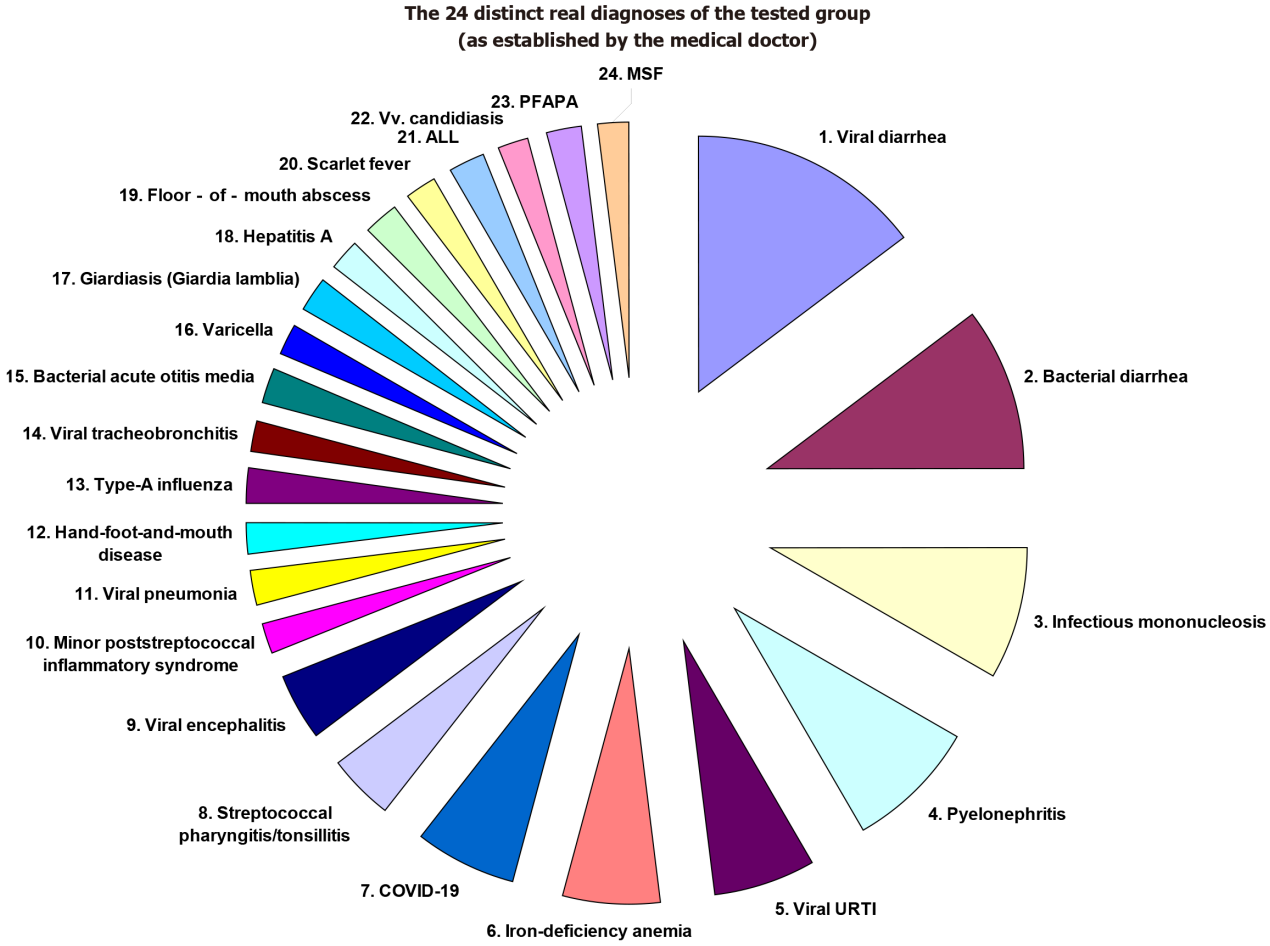


Figure 1 The 24 distinct real diagnoses of the tested group (as established by the medical doctor).

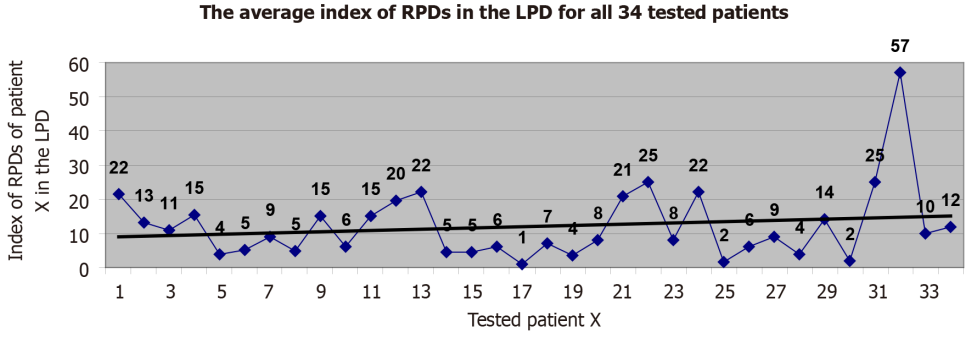


Figure 2 The average list-index of real positive-diagnoses in the list of Electronic Pediatrician-predicted diagnoses for each of the 34 tested child-patients. RPDs: Real positive-diagnoses; LPD: List of Electronic Pediatrician-predicted diagnoses.

The entire EPed prototype (including its unpacked database containing 302 clusters and 269 diseases) takes less than 3Mb on any USB memory stick or hard-disk, which makes it very practical, portable and RAM&ROM-memory efficient.

EPed is very efficient in its speed of input, because any CPS can be rapidly searched (even by using a word fragment!) in a pre-generated list of all CPSs from the database of EPed: In takes maximum 5 seconds to find any sign in the database, so that it takes about 30 seconds to input a set of 6 CPSs gathered from the patient, even in the emergency room.

EPed is very efficient in its speed of listing all the possible clusters and diseases, because it pre-loads its entire database in the RAM memory of any computer (with minimal RAM and ROM resources): In 2-3 seconds EPed identifies all possible clusters and diseases plus all the pseudo-probabilistic scores that serve in its 3 types of listings (by various scores or combination of scores).

The speed of input and computing of EPed is comparable to any online medical symptom checker. However, its focus on physiopathological clusters (by using a simple and robust nml-AI architecture), makes EPed much more versatile than any other symptom checker, because it intelligently and causally links CPSs to diseases not directly, but *via* clusters (which are *causal medical concepts* much more general and flexible than classical syndromes). The costs of periodically

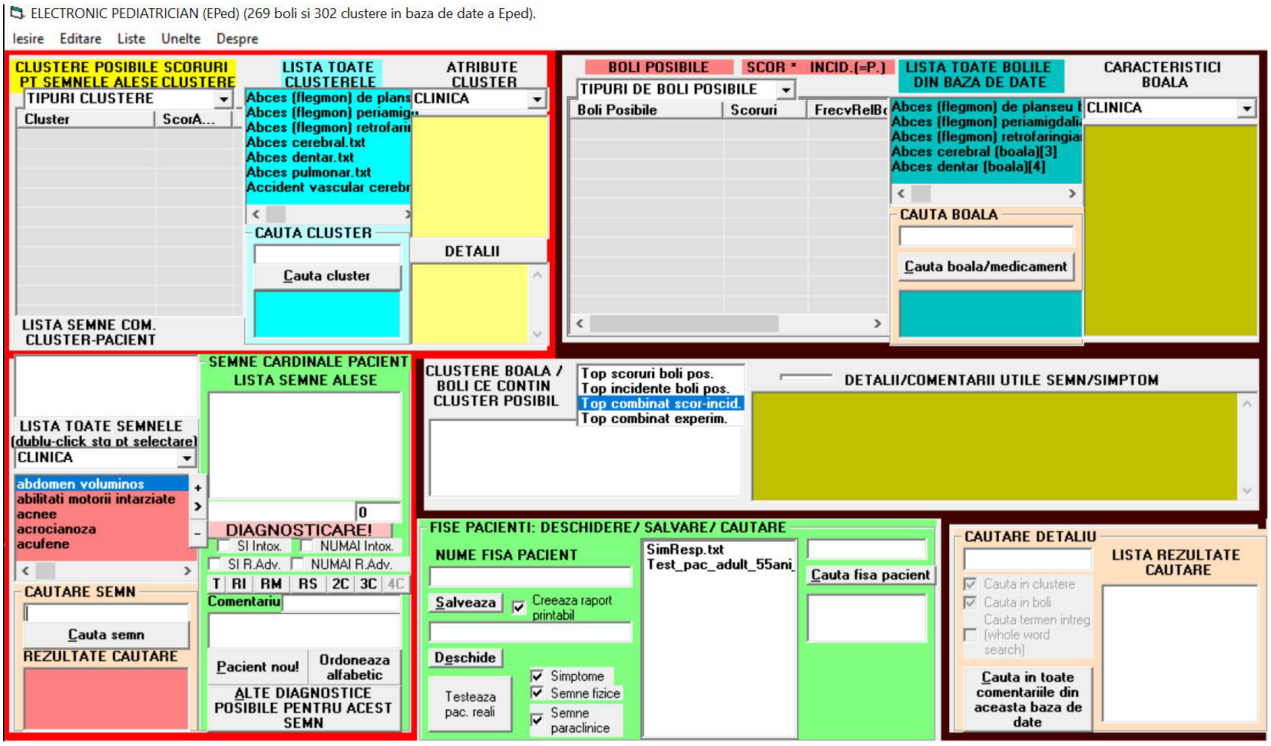


Figure 3 The interface of Electronic Pediatrician.

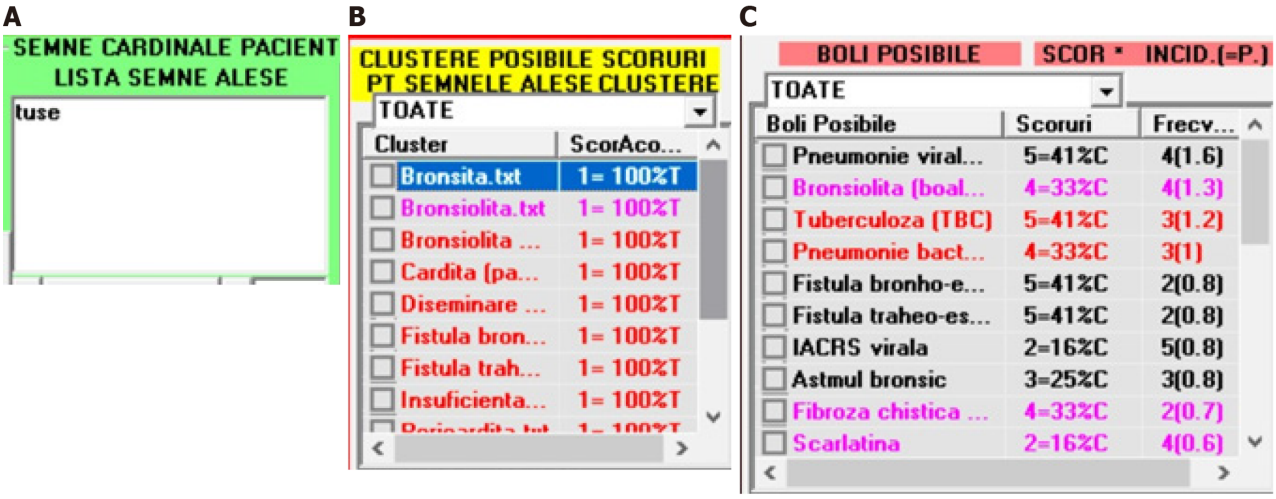


Figure 4 A simple test in which Electronic Pediatrician provides two distinct lists of all possible clusters and all possible diseases respectively for one single symptom: "cough". A: The sign "cough" ("tuse" in Romanian) inserted in the list dedicated to the clinico-paraclinical picture (CPP); B: The possible clusters identified by Electronic Pediatrician (EPed) to explain "cough" listed in the list of (all) possible clusters (LPC) ("100%T" means that all those clusters cover the CPP by 100%, because the "cough" is the only inserted clinical sign in this example): (1) Bronchitis (a cluster defined by EPed as an inflammation of bronchi); (2) Bronchiolitis (a cluster defined by EPed as an inflammation of bronchioli); (3) Bronchiolitis obliterans; (4) Carditis (pancarditis); (5) Systemic dissemination of Mycobacterium tuberculosis; (6) Broncho-esophageal fistula; (7) Tracheo-esophageal fistula; (8) Respiratory failure; (9) Pericarditis; (10) Gastroesophageal reflux disease; (11) Rhinitis; and (12) Functional respiratory syndrome of obstructive type; C: The possible diseases identified by EPed to explain all the clusters that may imply "cough" listed in the list of (all) possible disease (LPD) (listed in the descending order of CSD): (1) Viral pneumonia (CSD = 1.6); (2) Bronchiolitis (CSD = 1.3); (3) Tuberculosis (CSD = 1.2); (4) Bacterial pneumonia (CSD = 1); (5) Congenital broncho-esophageal fistula (CSD = 0.8); (6) Congenital tracheo-esophageal fistula (CSD = 0.8); (7) Viral URTI (CSD = 0.8); (8) Asthma (CSD = 0.8); (9) Cystic fibrosis (CSD = 0.7); (10) Scarlet fever (CSD = 0.6); (11) COVID-19 (CSD = 0.6); (12) Measles (CSD = 0.5); (13) Bacterial epiglottitis (CSD = 0.5); (14) Acute viral diarrhea (CSD = 0.4); (15) Influenza (CSD = 0.3); (16) Bacterial URTI (0.3); (17) Viral laryngeal tracheitis (croup) (CSD = 0.3); (18) Chickenpox (CSD = 0.3); (19) Infectious mononucleosis with EBV or/and CMV (CSD = 0.3); (20) Peptic ulcer (CSD = 0.2); (21) Gastroesophageal reflux disease (CSD = 0.2); (22) Hiatal hernia (CSD = 0.2); (23) Roseola infantum (6th childhood disease) (CSD = 0.2); and (24) Acute rheumatoid arthritis (CSD = 0.2) *etc*.

expanding and updating EPed (including its database) are exponentially lower than any medical AI diagnostic system, with EPed being much more energy-efficient than AI systems which are quite expensive and notorious for their huge energy expenditure and need for large human resources in their development. Furthermore, EPed doesn't "hallucinate" as AI systems do. EPed can be also easily adapted in the future for Android phones/tablets and also for online use. EPed can be easily adapted and improved over time by using minimal human resource.

This prototype EPed was also preliminarily tested on real child-patients by comparing the EPed-proposed list of possible diseases with the list of all diagnoses of the child-patient at discharge (as established by a real MD)[15].

CONCLUSION

EPed may be used not only by students, interns and MD pediatricians but also by any other medical professional from any other (medical) specialty close to pediatrics (or interested in it): General practitioners, infectionists *etc.* EPed can also be regarded as a potential pedagogical tool usable in training future medical students and resident physicians by developing their physiopathological reasoning and differential diagnosis skills.

ACKNOWLEDGEMENTS

Many thanks (for all the encouragements and critical views!) to my Ph.D. supervisor, prof. dr. Roxana-Maria Nemeș, a well-known and very appreciated Romanian pneumologist, pediatrician and researcher.

FOOTNOTES

Author contributions: Drăgoi AL designed, analyzed, interpreted, and prepared this paper; Nemeș RM has initially reviewed this paper and suggested important improvements in its form.

Institutional review board statement: It is not the case for this paper.

Informed consent statement: Patients were not additionally required to give informed consent to this study of the EPed software, because the analysis accomplished by EPed used anonymous data that were obtained after each parent of each admitted child-patient (diagnosed and treated in the Pediatric Infectious Diseases Ward of our hospital) agreed to all labs, imagistic and treatment interventions on his/her child by written consent (which consent was part of each medical folder in part).

Conflict-of-interest statement: The authors of this paper declare no existing competing interests.

Data sharing statement: No additional data.

Country of origin: Romania

ORCID number: Andrei-Lucian Drăgoi 0000-0002-2261-1350.

S-Editor: Qu XL
L-Editor: A
P-Editor: Guo X

REFERENCES

1 **Sowa JF**. Knowledge Representation: Logical, Philosophical, and Computational Foundations (1st ed.). Available from: https://books.google.ro/books/about/Knowledge_Representation.html?id=dohQAAAAMAAJ&redir_esc=y
2 **Lenert MC**, Walsh CG, Miller RA. Discovering hidden knowledge through auditing clinical diagnostic knowledge bases. *J Biomed Inform* 2018; **84**: 75-81 [PMID: 29940263 DOI: 10.1016/j.jbi.2018.06.014]
3 **O'Shea JS**. Computer-assisted pediatric diagnosis. *Am J Dis Child* 1975; **129**: 199-202 [PMID: 1091139 DOI: 10.1001/archpedi.1975.02120390033007]
4 **Johnson KB**, Feldman MJ. Medical informatics and pediatrics. Decision-support systems. *Arch Pediatr Adolesc Med* 1995; **149**: 1371-1380 [PMID: 7489077 DOI: 10.1001/archpedi.1995.02170250077014]
5 **Liang H**, Tsui BY, Ni H, Valentim CCS, Baxter SL, Liu G, Cai W, Kermany DS, Sun X, Chen J, He L, Zhu J, Tian P, Shao H, Zheng L, Hou R, Hewett S, Li G, Liang P, Zang X, Zhang Z, Pan L, Cai H, Ling R, Li S, Cui Y, Tang S, Ye H, Huang X, He W, Liang W, Zhang Q, Jiang J,

Yu W, Gao J, Ou W, Deng Y, Hou Q, Wang B, Yao C, Liang Y, Zhang S, Duan Y, Zhang R, Gibson S, Zhang CL, Li O, Zhang ED, Karin G, Nguyen N, Wu X, Wen C, Xu J, Xu W, Wang B, Wang W, Li J, Pizzato B, Bao C, Xiang D, He W, He S, Zhou Y, Haw W, Goldbaum M, Tremoulet A, Hsu CN, Carter H, Zhu L, Zhang K, Xia H. Evaluation and accurate diagnoses of pediatric diseases using artificial intelligence. *Nat Med* 2019; **25**: 433-438 [PMID: 30742121 DOI: 10.1038/s41591-018-0335-9]

6 **Li YW**, Liu F, Zhang TN, Xu F, Gao YC, Wu T. Artificial intelligence in pediatrics. *Chin Med J (Engl)* 2020; **133**: 358-360 [PMID: 31929357 DOI: 10.1097/CM9.0000000000000563]

7 **Li Y**, Zhang T, Yang Y, Gao Y. Artificial intelligence-aided decision support in paediatrics clinical diagnosis: development and future prospects. *J Int Med Res* 2020; **48**: 300060520945141 [PMID: 32924683 DOI: 10.1177/0300060520945141]

8 **Jackson P**. Introduction to Expert Systems (book, 3rd ed, 1998). Addison Wesley. Available from: https://books.google.ro/books/about/Introduction_to_Expert_Systems.html?id=9rJQAAAAMAAJ&redir_esc=y

9 **Barnett GO**. DXplain. *JAMA* 1987; **258**: 67 [DOI: 10.1001/jama.1987.03400010071030]

10 **London S**. DXplain: a Web-based diagnostic decision support system for medical students. *Med Ref Serv Q* 1998; **17**: 17-28 [PMID: 10557826 DOI: 10.1300/J115v17n02_02]

11 **Elkin PL**, Liebow M, Bauer BA, Chaliki S, Wahner-Roedler D, Bundrick J, Lee M, Brown SH, Froehling D, Bailey K, Famiglietti K, Kim R, Hoffer E, Feldman M, Barnett GO. The introduction of a diagnostic decision support system (DXplain™) into the workflow of a teaching hospital service can decrease the cost of service for diagnostically challenging Diagnostic Related Groups (DRGs). *Int J Med Inform* 2010; **79**: 772-777 [PMID: 20951080 DOI: 10.1016/j.ijmedinf.2010.09.004]

12 **Martinez-Franco AI**, Sanchez-Mendiola M, Mazon-Ramirez JJ, Hernandez-Torres I, Rivero-Lopez C, Spicer T, Martinez-Gonzalez A. Diagnostic accuracy in Family Medicine residents using a clinical decision support system (DXplain): a randomized-controlled trial. *Diagnosis (Berl)* 2018; **5**: 71-76 [PMID: 29730649 DOI: 10.1515/dx-2017-0045]

13 **Bond WF**, Schwartz LM, Weaver KR, Levick D, Giuliano M, Graber ML. Differential diagnosis generators: an evaluation of currently available computer programs. *J Gen Intern Med* 2012; **27**: 213-219 [PMID: 21789717 DOI: 10.1007/s11606-011-1804-8]

14 **Berner ES**, Jackson JR, Algina J. Relationships among performance scores of four diagnostic decision support systems. *J Am Med Inform Assoc* 1996; **3**: 208-215 [PMID: 8723611 DOI: 10.1136/jamia.1996.96310634]

15 **Drăgoi AL**, Nemeş RM. The "Electronic Pediatrician (EPed®)" - A clinically tested prototype software for computer-assisted pathophysiologic diagnosis and treatment of ill children. *Int J Med Inform* 2023; **178**: 105169 [PMID: 37562316 DOI: 10.1016/j.ijmedinf.2023.105169]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 101837

DOI: 10.5662/wjm.v15.i3.101837

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Retrospective Study

Machine learning-based models for prediction of in-hospital mortality in patients with dengue shock syndrome

Luan Thanh Vo, Thien Vu, Thach Ngoc Pham, Tung Huu Trinh, Thanh Tat Nguyen

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade B

Novelty: Grade B, Grade B

Creativity or Innovation: Grade B, Grade B

Scientific Significance: Grade B, Grade B

P-Reviewer: Kong MW

Received: September 29, 2024

Revised: November 3, 2024

Accepted: November 19, 2024

Published online: September 20, 2025

Processing time: 159 Days and 6.3 Hours

Luan Thanh Vo, Thach Ngoc Pham, Tung Huu Trinh, Thanh Tat Nguyen, Department of Infectious Diseases, Children's Hospital No. 2, Ho Chi Minh City 700000, Viet Nam

Thien Vu, AI Nutrition Project, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), Osaka 5670001, Japan

Thien Vu, NCD Epidemiology Research Center, Shiga University of Medical Science, Otsu, Shiga 5200003, Japan

Thanh Tat Nguyen, Department of Tuberculosis and Epidemiology, Woolcock Institute of Medical Research, Ho Chi Minh City 700000, Viet Nam

Co-corresponding authors: Thien Vu and Thanh Tat Nguyen.

Corresponding author: Thanh Tat Nguyen, MD, PhD, Senior Researcher, Department of Tuberculosis and Epidemiology, Woolcock Institute of Medical Research, Pham Ngoc Thach Street, Ho Chi Minh City 700000, Viet Nam. thanhhonor@gmail.com

Abstract

BACKGROUND

Severe dengue children with critical complications have been attributed to high mortality rates, varying from approximately 1% to over 20%. To date, there is a lack of data on machine-learning-based algorithms for predicting the risk of in-hospital mortality in children with dengue shock syndrome (DSS).

AIM

To develop machine-learning models to estimate the risk of death in hospitalized children with DSS.

METHODS

This single-center retrospective study was conducted at tertiary Children's Hospital No. 2 in Viet Nam, between 2013 and 2022. The primary outcome was the in-hospital mortality rate in children with DSS admitted to the pediatric intensive care unit (PICU). Nine significant features were predetermined for further analysis using machine learning models. An oversampling method was used to enhance the model performance. Supervised models, including logistic regression, Naïve Bayes, Random Forest (RF), K-nearest neighbors, Decision Tree and Extreme Gradient Boosting (XGBoost), were employed to develop predictive

models. The Shapley Additive Explanation was used to determine the degree of contribution of the features.

RESULTS
In total, 1278 PICU-admitted children with complete data were included in the analysis. The median patient age was 8.1 years (interquartile range: 5.4-10.7). Thirty-nine patients (3%) died. The RF and XGboost models demonstrated the highest performance. The Shapley Addictive Explanations model revealed that the most important predictive features included younger age, female patients, presence of underlying diseases, severe transaminitis, severe bleeding, low platelet counts requiring platelet transfusion, elevated levels of international normalized ratio, blood lactate and serum creatinine, large volume of resuscitation fluid and a high vasoactive inotropic score (> 30).

CONCLUSION
We developed robust machine learning-based models to estimate the risk of death in hospitalized children with DSS. The study findings are applicable to the design of management schemes to enhance survival outcomes of patients with DSS.

Key Words: Dengue shock syndrome; Dengue mortality; Machine learning; Supervised models; Logistic regression; Random forest; K-nearest neighbors; Support vector machine; Extreme Gradient Boost; Shapley addictive explanations

Core Tip: The in-hospital mortality rate of children with dengue shock syndrome (DSS) at a large tertiary pediatric hospital in Viet Nam was 3%. The supervised models showed good predictive value. In particular, the Random Forest and Extreme Gradient Boost models demonstrated the highest model performance. The supervised machine learning model showed that the nine most important predictive variables included younger age, presence of underlying diseases, severe transaminitis, critical bleeding, platelet transfusion requirement, elevated international normalized ratio and blood lactate levels, and high vasoactive inotropic score (> 30). Identification of mortality predictors in patients with DSS will help optimize management protocols to enhance survival outcomes.

Citation: Vo LT, Vu T, Pham TN, Trinh TH, Nguyen TT. Machine learning-based models for prediction of in-hospital mortality in patients with dengue shock syndrome. *World J Methodol* 2025; 15(3): 101837
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/101837.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.101837

INTRODUCTION

Dengue has posed a huge disease burden in tropical and subtropical countries, particularly Southeast Asia, South Asia, and South America, with its incidence increasing by 85.5% from 1990 to 2019 worldwide[1]. Dengue-associated severe complications have been attributed to increasing mortality rates, varying from 1% to > 20%[2,3]. Common severe dengue-related complications among hospitalized children include dengue shock syndrome (DSS), severe bleeding, acute liver failure, huge plasma leakage, and respiratory failure[4-7]. To date, many prognostic models for dengue-related death have been reported in adult cohorts; however, there are insufficient data on the pediatric population in this regard[8-12]. In addition, several published prognostic models to estimate the risk of death among hospitalized dengue children are restricted by the relatively limited sample size and the lack of robust statistical methods to determine the predictive model effect[3,4]. Notably, we recently reported a cohort of 492 hospitalized children with DSS to predict fatality; however, this study was restricted by the limited number of fatal outcomes (n = 26 deaths, 5.3%)[3]. Another prospective study cohort showed more important predictors of death in children with severe dengue; however, the study was limited by the small sample size of 78 pediatric patients included in the final analysis[4]. Recently, machine learning methods, with their high performance in prediction and classification, have achieved significant advances and applicability in a multitude of medical fields, particularly tropical diseases[13-15]. We aimed to use machine learning to identify the strong predictors of mortality among hospitalized children with DSS. Determining the predictors of death will aid in optimizing the prognosis and management protocols of hospitalized children with DSS to enhance survival outcomes.

MATERIALS AND METHODS

Study design, setting, population and data source

This single-center retrospective cohort study, the Viet Nam Dengue-Infected Study, was conducted at Children's Hospital No. 2 in Ho Chi Minh City, Viet Nam. This study included all pediatric patients admitted with DSS between 2013 and 2022. Data were collected from patient records to ensure the comprehensive coverage of clinical and laboratory variables

relevant to patient outcomes. The inclusion criteria were age < 18 years, laboratory-confirmed dengue infection, and the presence of DSS[2]. The exclusion criteria were a lack of serological confirmation of dengue infection and missing data for covariables of interest (≥ 50%).

Study outcome and candidate predictors

The main outcome was the in-hospital mortality rate in pediatric intensive care unit (PICU)-admitted children with DSS. Candidate predictors were predetermined based on clinical knowledge, disease pathogenesis, and medical literature, including patient age, underlying diseases on admission, systolic shock index, critical bleeding, severe transaminitis, international normalized ratio (INR), peak hematocrit (%), platelet cell count, platelet transfusion requirement, blood lactate, serum creatinine, cumulative fluid infused from referral hospitals and within 24 h PICU admission, colloid to crystalloid fluid infusion ratio, and vasoactive inotropic score (VIS).

Data collection of variables

The dataset was gathered from hospital medical records, originally comprising 1278 observations and 81 variables, and was classified into the following major categories:

Demographic data: Patient's age, gender, and accompanied underlying diseases.

Clinical data: Day of onset of dengue shock, severity of dengue shock, severe bleeding, platelet transfusion, respiratory rate, systolic shock index, VIS, cumulative amount of fluid infused from the referral hospital and during 24 h of PICU admission, and colloid-to-crystalloid fluid infusion ratio.

Laboratory data: Hematological parameters, liver enzyme levels, and biomarkers indicating organ dysfunction.

Data preprocessing and feature selection

Handling missing data: Missing data patterns were examined using the naniar, rms, VIM, and dlookr packages in R. Visualizations, such as missing data patterns, Pareto charts, and hierarchical clustering, were utilized to manipulate missing values. Variables with more than 50% missing data were excluded from the analysis. Missing data imputation was performed using Multivariate Imputation by Chained Equations (MICE) method. The MICE algorithm was performed with predictive mean matching, generating five imputed datasets with 50 iterations each.

Data cleaning and transformation: Categorical variables were converted into factors. Binary variables were converted into numeric (0/1) formats. Outliers and irrelevant categories were removed where applicable.

Feature selection: A predefined set of clinical and laboratory covariables was preselected using clinical knowledge, the Least Absolute Shrinkage and Selection Operator (LASSO), and stepwise selection using the Akaike information criterion (AIC) method. To prevent overfitting by selecting a combination of predetermined features, we prudently manipulated the penalizing coefficient (lambda) from the LASSO regression in balance with the AIC method.

Statistical analyses

All steps in the predictive model development are described in Figure 1. The original dataset was first oversampled using the synthetic minority oversampling technique (SMOTE) to address the mortality outcome imbalance[16]. After oversampling, the dataset was split into training (70%) and testing (30%) sets.

Machine learning models

Supervised models were implemented, including logistic regression, Random Forest, Naive Bayes, Decision Tree, K-nearest neighbors (KNN) and Extreme Gradient Boost (XGBoost). The models were trained using 5-fold cross-validation to optimize the performance metrics. The hyperparameters were tuned using grid search to identify the optimal settings for each model. Performance metrics, including accuracy, sensitivity, specificity, precision, area under the curve (AUC), and F1-score, were calculated with 95% confidence intervals for the test set using bootstrapping techniques.

Model explanation

Shapley additive explanations (SHAP) analysis was used to interpret the contribution of each feature to model predictions. The SHAP values provide insights into the contribution of each feature to the model's predictions. SHAP summary and dependence plots were generated to visualize the importance of individual features and their interactions with the outcome.

All study analyses were conducted using R (version 4.3.2) with packages including tidyverse, compare Groups, rms, naniar, VIM, dlookr, missRanger, glmnet, caret, xgboost, SHAP forxgboost, ggplot2, and cutpointr. Statistical significance was set at $P < 0.05$.

RESULTS

Baseline characteristics of study participants upon PICU admission

Between 2013 and 2022, approximately 2000 children with DSS were admitted to the PICU, of whom 1278 met the

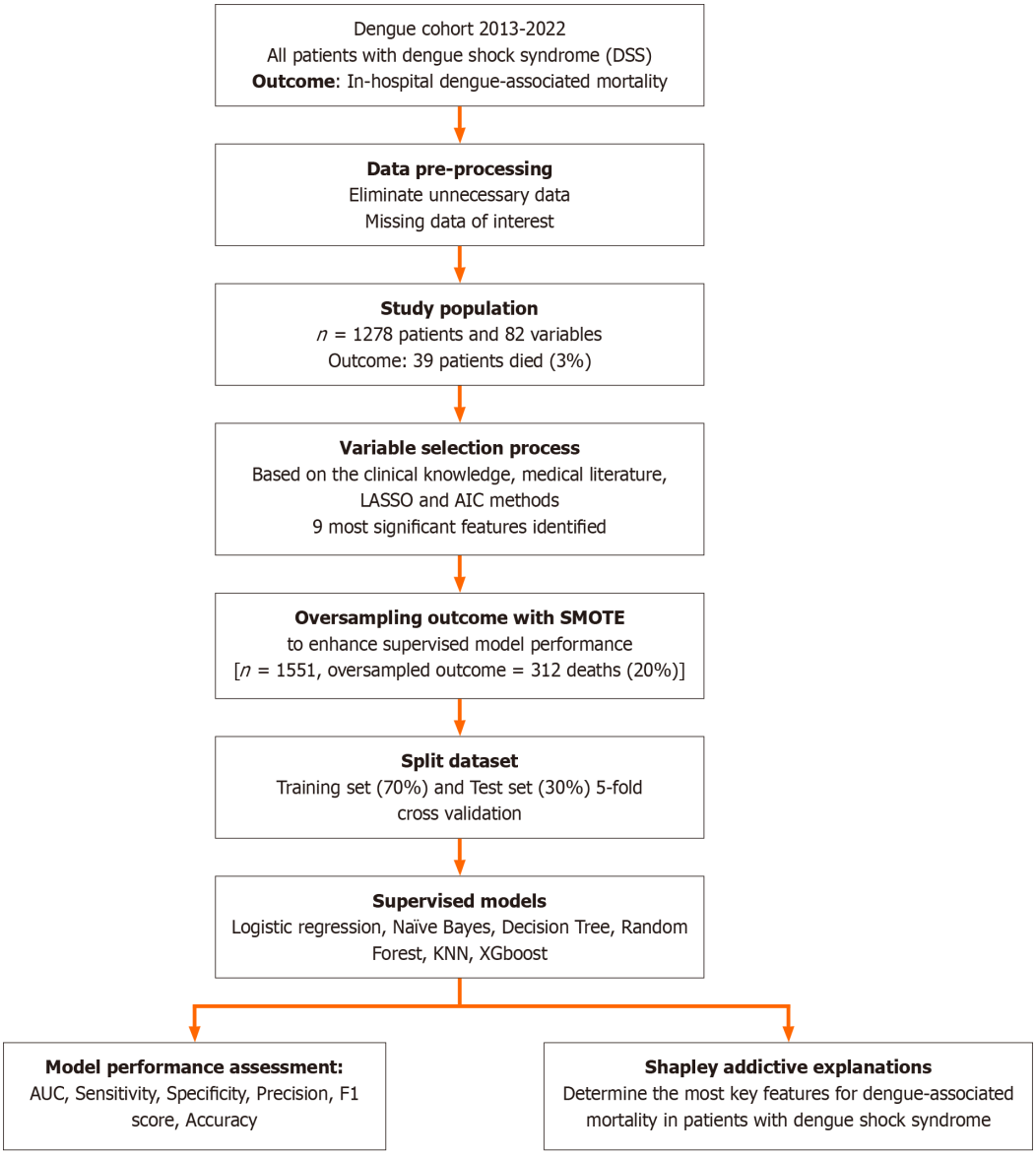


Figure 1 The study flow-chart. LASSO: Least Absolute Shrinkage and Selection Operator; DSS: Dengue shock syndrome; AUC: Area under the curve; AIC: Akaike information criterion; SMOTE: Synthetic minority oversampling technique; KNN: K-nearest neighbors.

eligibility criteria and were included in the analysis. Of these, 39 patients (3%) died in-hospital, whereas the remaining 1239 patients (97%) survived. Baseline clinical and laboratory characteristics of the participants are presented in Table 1. The non-survivors were generally younger and had a higher prevalence of underlying diseases. The occurrence of DSS was observed earlier in non-survivors, with a median duration of four days after the onset of fever. Patients who did not survive experienced greater disease severity, with a significantly higher proportion of patients developing decompensated DSS. Critical bleeding complications were more prevalent among non-survivors and were accompanied by a markedly higher systolic shock index. These patients also exhibited more critical hepatic injury, as indicated by dramatically elevated levels of aspartate aminotransferase and alanine aminotransferase along with severe hepatic transaminases. Additionally, non-survivors had higher blood lactate levels, reflecting more severe metabolic acidosis. Regarding coagulation, non-survivors had a significantly elevated INR and lower hemoglobin and nadir hematocrit levels than the survivors. Platelet counts were lower in the non-survivors than in the survivors. In terms of respiratory function, non-survivors had a higher respiratory rate, indicating more respiratory distress. Fluid resuscitation was more pronounced in non-survivors, with greater cumulative intravenous fluid administration and higher colloid-to-crystalloid ratio. Furthermore, non-survivors required significantly more vasoactive inotropic support, with a larger proportion of patients requiring a VIS > 30, underscoring the severity of hemodynamic instability. Overall, non-survivors with severe dengue exhibited a more severe clinical profile and required more intensive treatment, which correlated with the fatal outcomes observed in this group.

Clinical predictors of in-hospital mortality of patients with DSS upon PICU admission

As presented in Table 2, in the multivariable logistic regression, the full predefined model showed significant clinical predictors of in-hospital mortality in patients with DSS, including accompanying underlying diseases, severe bleeding,

Table 1 Baseline clinical and laboratory characteristics of study participants on pediatric intensive care unit admission (*n* = 1278), *n* (%)/median (interquartile range)

Characteristics	Non-survivor (*n* = 39)	Survivors (*n* = 1239)	*P* value
Age (years)	7 (5-10)	8.1 (5.4-10.7)	0.16
Female sex	18 (46)	601 (49)	0.77
Underlying diseases	7 (18)	73 (6)	< 0.01
Day of occurrence of dengue shock since onset of fever (days)	4 (4-5)	5 (4-5)	0.09
Grading of DSS			
Compensated DSS	11 (28)	1143 (92)	< 0.001
Decompensated DSS	28 (72)	96 (8)	
Severe bleeding	29 (74)	66 (5.3)	< 0.001
Respiratory rate (breaths/min)	30 (24-40)	25 (22-30)	< 0.001
Systolic shock index (bpm/mmHg)	1.47 (1.33-1.88)	1.3 (1.11-1.5)	< 0.01
White blood cell count (× 10^9/L)	7.15 (3.86-12.9)	4.74 (3.36-6.7)	0.93
Hemoglobin (g/dL)	13.8 (11.5-15.1)	14.9 (13.5-16.1)	0.01
Peak hematocrit (%)	46 (42-52)	48 (45-51)	0.07
Nadir hematocrit (%)	33 (29-34)	38 (35-41)	0.001
Platelet cell count (× 10^9/L)	25 (14-42.4)	36 (23-55)	0.21
Platelet transfusion	34 (87)	129 (10.4)	< 0.001
Aspartate aminotransferase (IU/L)	2219 (572-4458)	148 (86-314)	< 0.001
Alanine aminotransferase (IU/L)	724 (324-1670)	63 (34-149)	< 0.001
Severe transaminitis	25 (64)	105 (9)	< 0.001
International normalized ratio	2.52 (1.83-3.57)	1.21 (1.1-1.47)	< 0.001
Blood lactate (mmol/L)	7.2 (3.1-13)	2.3 (1.6-3.1)	< 0.001
Serum creatinine (mmol/L)	65 (56-111)	51 (44-59)	0.001
Cumulative fluid infused from referral hospitals and 24 h of PICU admission (mL/kg)	301 (220-393)	133 (104-176)	< 0.001
Ratio of colloid-to-crystalloid infusion	6.0 (1.7-9.1)	0.7 (0-2.0)	< 0.001
Vasoactive inotropic score during first 24 h	60 (40-165)	0 (0-15)	< 0.001
Vasoactive inotropic score > 30	30 (79)	17 (13)	< 0.001

DSS: Dengue shock syndrome; PICU: Pediatric intensive care unit.

high INR, platelet transfusion requirement, elevated blood lactate and serum creatinine levels, larger volume of fluid infusion from referral hospitals and 24 h of PICU admission, and a high VIS (> 30). No significant interactions were found between the covariates. Multivariable analyses from the LASSO and AIC methods generated results similar to those from the full predefined model. However, the LASSO penalized underlying diseases as insignificant variables in the final model, whereas the AIC preserved this covariate in the final model. Although both LASSO and AIC models disregarded age variables, we retained this covariate in the final prognostic models based on its clinical importance in disease pathogenesis and the medical literature.

Performance of supervised models for in-hospital mortality in DSS patients without SMOTE

Table 3 summarizes the performance parameters of various supervised machine learning models employed to estimate the risk of in-hospital mortality in patients with DSS. The logistic regression model achieved an AUC of 0.92 (95%CI: 0.90-0.94), with a sensitivity of 0.99, low specificity of 0.50, precision of 0.98, F1 score of 0.61, and an accuracy of 0.97. The Naïve Bayes model further revealed an AUC of 0.86 (95%CI: 0.70-1), sensitivity 0.91, specificity 0.82, precision 0.83, F1 score 0.87, and an accuracy of 0.86. The Random Forest model demonstrated high performance with an AUC of 0.86 (95%CI: 0.69-1), sensitivity 0.82, specificity 0.91, precision 0.90, F1 score and accuracy of 0.86. In addition, the KNN model yielded almost similar results to the Random Forest model. The Decision Tree model exhibited an AUC of 0.77 (95%CI:

Table 2 Multivariable models for in-hospital mortality of patients with dengue shock syndrome upon pediatric intensive care unit admission

Candidate predictors	Full model[1]		Reduced model 1[2]		Reduced model 2[3]	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age (years)	0.85 (0.71-1.02)	0.09	0.93 (0.8-1.08)	0.32	0.94 (0.81-1.09)	0.39
Underlying diseases	5.14 (1.22-21.7)	0.03			4.1 (1.02-16.3)	0.04
Systolic shock index (mmHg/bpm)	0.46 (0.08-2.48)	0.36				
Severe bleeding	4.28 (1.37-13.4)	0.01	3.38 (1.19-9.57)	0.02	3.38 (1.2-9.58)	0.02
Severe transaminitis	1.16 (0.4-3.38)	0.78				
International normalized ratio	1.56 (1.12-2.17)	< 0.01	1.45 (1.05-1.99)	0.02	1.47 (1.07-2.01)	0.01
Peak hematocrit (%)	1.08 (1.0-1.16)	0.05				
Platelet counts (< 20 × 10^9/L)	0.95 (0.32-2.85)	0.92				
Platelet transfusion	4.48 (1.11-18.1)	0.03	4.1 (1.07-15.7)	0.03	4.89 (1.27-18.9)	0.02
Blood lactate (mmol/L)	1.2 (1.02-1.41)	0.03	1.2 (1.04-1.39)	0.01	1.19 (1.03-1.37)	0.02
Serum creatinine (μmol/L)	1.02 (1.0-1.03)	0.01	1.01 (1-1.03)	0.09	1.01 (1.0-1.03)	0.06
Log-2 Cumulative fluid infused from referral hospitals and within 24 h-PICU admission (mL/kg)[4]	2.76 (1.25-6.1)	0.01	3.69 (1.95-7.0)	< 0.001	3.61 (1.88-6.91)	< 0.001
Colloid to crystalloid fluid infusion ratio	1.07 (0.98-1.17)	0.15				
Vasoactive inotropic score (> 30)	9.58 (2.36-39)	< 0.01	4.78 (1.49-15.4)	< 0.01	4.59 (1.42-14.8)	0.01

[1]Full model based on predefined covariates with multiple imputation data analysis.
[2]Reduced model 1 based on Least Absolute Shrinkage and Selection Operator method.
[3]Reduced model 2 based on stepwise selection with the lowest value of Akaike information criterion.
[4]Standardization of covariates by Log-2 transformation.
DSS: Dengue shock syndrome; OR: Odds ratio; PICU: Pediatric intensive care unit.

Table 3 Performance of supervised models to estimate the risk of mortality in patients with dengue shock syndrome without the synthetic minority oversampling technique

Models	AUC (95%CI)	Sensitivity	Specificity	Precision	F1 score	Accuracy
Logistic regression	0.92 (0.90-0.94)	0.99	0.50	0.98	0.61	0.97
Naïve Bayes	0.86 (0.70-1)	0.91	0.82	0.83	0.87	0.86
Random forest	0.86 (0.69-1)	0.82	0.91	0.90	0.86	0.86
KNN	0.86 (0.71-1)	0.82	0.91	0.90	0.86	0.86
Decision tree	0.77 (0.59-0.94)	0.82	0.73	0.75	0.78	0.77
XGBoost	0.86 (0.71-1)	0.82	0.91	0.90	0.86	0.86

AUC: Area under the curve; KNN: K-nearest neighbors; SMOTE: Synthetic minority oversampling technique; XGboost: Extreme gradient boosting.

0.59-0.94), sensitivity 0.82, specificity 0.73, precision 0.75, F1 score 0.78, and an accuracy of 0.77. Finally, the XGBoost model also performed well, with an AUC of 0.86 (95%CI: 0.71-1), sensitivity 0.82, specificity 0.91, precision 0.90, F1 score and an accuracy of 0.86. Considering the performance of all these models, the Random Forest, KNN, and XGBoost models demonstrated the highest performance across multiple metrics, indicating their strong predictive capabilities.

Performance of supervised models for in-hospital mortality in DSS patients from oversampling with the SMOTE method

Table 4 summarizes the performance of the supervised machine learning models in predicting the in-hospital mortality in DSS patients when oversampling with SMOTE was employed. Overall, all supervised models using the SMOTE method showed superior performance compared to the models without SMOTE applicability. The XGBoost model demonstrated excellent performance compared with the other models.

Table 4 Performance of supervised models to estimate the risk of mortality in patients with dengue shock syndrome from oversampling data with the synthetic minority oversampling technique

Models	AUC (95%CI)	Sensitivity	Specificity	Precision	F1 score	Accuracy
Logistic regression	0.93 (0.89-0.97)	0.94	0.93	0.77	0.85	0.93
Naïve Bayes	0.94 (0.91-0.97)	0.95	0.94	0.80	0.87	0.94
Random forest	0.97 (0.95-0.99)	0.98	0.97	0.88	0.93	0.97
KNN	0.91 (0.87-0.95)	0.92	0.89	0.69	0.78	0.90
Decision tree	0.95 (0.92-0.98)	0.95	0.96	0.85	0.90	0.96
XGBoost	0.97 (0.95-0.99)	0.98	0.96	0.86	0.92	0.96

AUC: Area under the curve; KNN: K-nearest neighbors; SMOTE: Synthetic minority oversampling technique; XGboost: Extreme gradient boosting.

Features of importance from the Random-forest model with oversampling method

As presented in Figure 2, the oversampled random-forest model showed the most significant features (ranked by the Gini score), including the cumulative amount of fluid infused from the referral hospital and during the 24 h of admission, blood lactate, INR, and serum creatinine. Other important features included younger age, female patients, severe bleeding, low platelet count requiring platelet transfusion, a high VIS score, and underlying diseases.

The SHAP model

The SHAP model identified key variables contributing to the risk of fatality in children with severe dengue who were admitted to the PICU. The SHAP plot highlights the most important predictors contributing to the risk of death in hospitalized children with DSS, as presented in Figure 3. Among predetermined covariates, platelet transfusion had the highest SHAP value, making it the most important predictor of mortality. The cumulative amount of fluid infusion was also a major contributing factor. A larger volume of intravenous infusion and more colloids than crystalloid fluid administration were highly associated with an increased risk of mortality. Elevated serum creatinine and lactate levels were significant predictors of mortality. Critical hepatic injury, high INR, and severe bleeding highly predicted mortality in patients with DSS. A high systolic shock index and VIS (> 30) were substantial contributors to the mortality risk, reflecting the hemodynamic instability of patients. Peak hematocrit levels were highlighted as a relevant factor, while demographic variables, such as age and sex also played a role in predicting mortality, with older age surprisingly showing a protective effect in contrast to the younger population. The presence of underlying diseases, decompensated DSS, and platelet counts (< 20 × 10^9/L) had lower SHAP values, indicating that they were less influential than other variables.

DISCUSSION

Severe DSS significantly increases the risk of mortality among hospitalized children by up to 20%. Therefore, identifying prognostic factors associated with mortality in patients with DSS is imperative to optimize the allocation of medical resources, enhance monitoring capabilities, and prioritize treatment strategies to improve patient survival rates.

This study aimed to construct and validate predictive models for assessing the mortality risk in pediatric patients diagnosed with DSS. By analyzing data from 1278 patients, we identified 39 fatalities, corresponding to a mortality rate of 3%. Key variables incorporated into the final predictive model included severe bleeding, INR, platelet transfusion requirement, blood lactate levels, serum creatinine, cumulative intravenous fluid infusion from referral hospitals and within the first 24 h of PICU admission, and a VIS exceeding 30. These predictors exhibited robust predictive power for mortality risk, thereby offering valuable insights into the clinical management and prognosis improvement in patients with DSS. Our findings are consistent with the existing literature on the mortality risk factors for severe DSS. Each variable was carefully predetermined based on the pathophysiological relevance of the disease and corroborated with previous studies. Notably, our study distinguished itself by employing advanced machine learning techniques, such as Random Forest and XGBoost, to validate the reliability of the model. In this study, severe bleeding was demonstrated to be a critical prognostic factor, which is consistent with previous studies[17,18]. The INR, reflecting coagulation dysfunction, was also significantly associated with elevated mortality. This may have resulted from potential liver dysfunction or consumption of clotting factors in patients with severe DSS. Notably, coagulopathy combined with thrombocytopenia can lead to uncontrolled bleeding, further escalating mortality risk. Platelet transfusion requirement is a strong predictor in the prognostic model. In clinical practice, platelet transfusions are administered to DSS patients with severe thrombocytopenia and active hemorrhage or those requiring invasive procedures, such as endotracheal intubation, central venous catheterization, or abdominal decompression. Thus, platelet transfusion serves as an indicator of ongoing critical hemorrhage and is necessary in patients undergoing invasive interventions. Elevated blood lactate levels, commonly observed in patients with prolonged DSS or multi-organ failure, were significantly associated with mortality. This finding corroborates previous studies that established blood lactate as an independent predictor of mortality in

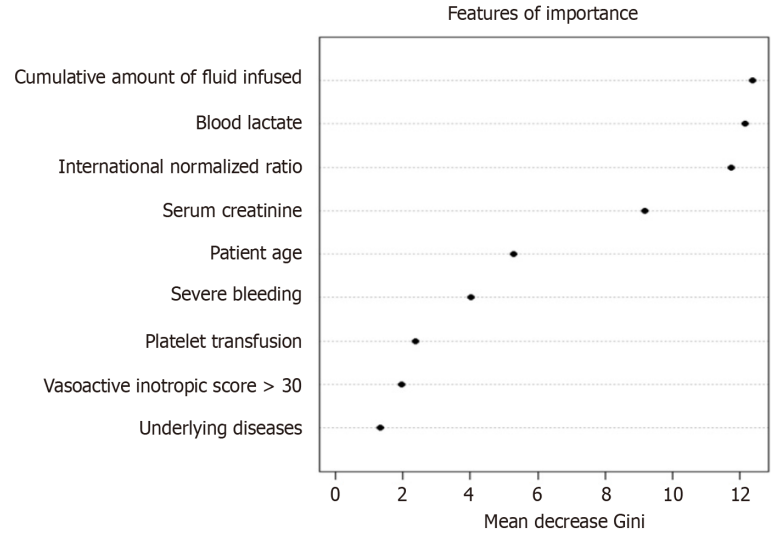


Figure 2 Features of importance from the Random Forest model.

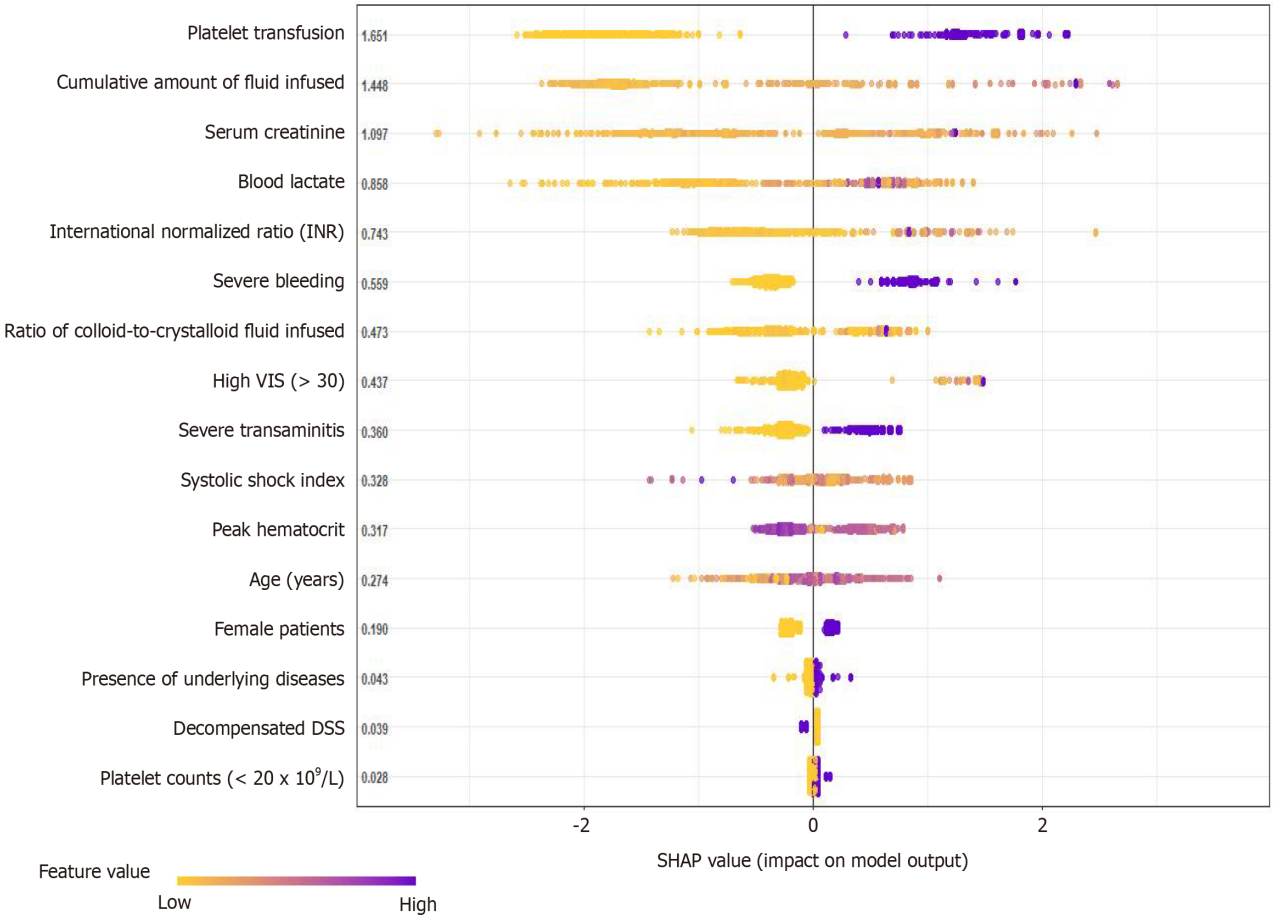


Figure 3 The SHAP analysis shows the impact of predefined variables on the model's prediction of mortality in patients with dengue shock syndrome.

patients with DSS[3,4,19]. Additionally, increased serum creatinine levels indicate acute kidney injury, which is frequently observed in patients with prolonged shock or reduced perfusion due to increased intra-abdominal pressure from large-volume fluid resuscitation[5]. This variable is also correlated with higher mortality rates[5]. Our model showed that a larger volume of intravenous fluid infusion was associated with increased mortality risk. Effective fluid management is crucial in treating patients with DSS in accordance with the 2009 World Health Organization (WHO) dengue guidelines[2]. The 2009 WHO dengue guidelines have recommended that tissue perfusion should be maintained through the administration of a minimal amount of intravenous fluid[2]. Numerous studies have demonstrated that fluid

overload is associated with poor outcomes, not only in patients with DSS but also in extensive critical care scenarios[3,4,19]. A high cumulative volume of intravenous fluid intake indicated several critical adverse conditions, including prolonged huge plasma leakage, delayed hospitalization accompanied by severe shock, and inappropriate large-volume intravenous fluid administration. These factors can lead to progressive respiratory failure, increased intra-abdominal and thoracic pressures, and the development of a combination of hypovolemic and obstructive shock. Furthermore, an increased VIS indicates the need for high-dose vasopressors to sustain blood pressure and oxygen delivery to organs, signifying severe cardiac failure or critical shock with a poor prognosis unless aggressive interventions are performed[3]. In this study, a high VIS (> 30) was a significant predictor of mortality risk in patients with DSS, which is consistent with previous studies[3,6].

To date, there are scant data on the prognostic value of estimating the risk of in-hospital death among pediatric patients. A small prospective cohort (n = 78 children with severe dengue) revealed significant clinical predictors of mortality among hospitalized children, including increased blood lactate levels, VIS, and positive fluid balance[4]. In addition, we recently developed a conventional statistical model to predict the in-hospital mortality in 492 PICU-admitted children with DSS. We also showed prognosticators for mortality in DSS children, including critical bleeding, high volume of fluid infusion, elevated blood lactate and increased VIS (> 30) during the first 24 h of PICU admission[3]. However, the study was restricted by its retrospective design, high rate of missing data for biochemistry tests, and small number of fatal outcomes (n = 26 deaths, 5.3%). The limitations of the inadequate sample size in the aforementioned studies cannot be manipulated by the conventional statistical analysis. A similar issue was observed in the current study, as there were 39 deaths (3%) among the sample size of 1278 children with DSS. To address this issue, we implemented the SMOTE. This approach effectively mitigated data imbalance and enhanced the model performance beyond that achieved by conventional statistical methods. After adjustment with the SMOTE, all supervised machine learning models showed a dramatic improvement in the performance metrics, and the XGBoost model demonstrated the best performance in predicting the risk of mortality.

Considering its simplicity, conventional statistical modelling has gained wide popularity among researchers. However, it requires a priori assumptions, a refined set of clinically important variables consistent with the underlying biological mechanisms, an adequate sample size to ensure study power, and an appropriate number of outcomes compared to the total predictors in the predictive models[20]. However, machine learning models are abstract to interpret, with or without a priori assumptions and oversampling techniques for limited outcome events. Notably, machine learning modelling has a weakness in that it is prone to overfitting. Hence, we integrated the two methods of conventional and machine learning analyses to maximize the strength of both techniques in our study. We based the AIC and LASSO methods on a predetermined set of features, and these conventional methods helped minimize overfitting, which is commonly associated with machine learning. Oversampling with the SMOTE significantly enhanced the study power and model performance metrics.

The XGBoost-based SHAP model was developed to evaluate the importance of SHAP values and the magnitude of the impact of each clinical predictor. Notably, the most significant predictors of in-hospital mortality among DSS patients were platelet transfusion requirement, critical bleeding, severe coagulation disorder and transaminitis, high VIS (> 30), large volume of fluid infused with more colloids than crystalloids, kidney failure, and high serum lactate levels. These prognostic factors had high-ranking SHAP values, reflecting their greater significance in the mortality prediction model for DSS. Furthermore, the SHAP model also revealed that other covariables, including demographic factors (age and sex), underlying diseases, and severity of dengue shock, were less important in the final prognostic model, as indicated by the small and narrow magnitudes of the SHAP values. Therefore, the SHAP model provides a transparent interpretation of how each variable affects the overall model's decisions, offering a comprehensive understanding of the interplay between the predictors and mortality risk. Interventions targeting the clinical predictors of high weight, as mentioned above, can significantly improve the survival outcomes of children with DSS.

This study has several significant implications for clinical practice. First, these machine learning-based predictive models can potentially transform the prognosis and management of hospitalized children with DSS. In particular, the XGBoost model had the highest predictive accuracy, identifying critical factors including younger age, female sex, underlying diseases, severe transaminitis, critical bleeding, low platelet counts necessitating transfusion, high INR, elevated blood lactate and serum creatinine levels, a large volume of resuscitation fluid, and a VIS > 30. Second, this result can be manipulated to design therapeutic schemes based on modifiable factors such as platelet transfusion, judicious administration of infused fluid and vasopressors, and management of critical transaminases. Third, a risk-scoring system can be developed based on the identified predictors from supervised and SHAP models to classify high- and low-risk mortality groups. This will aid clinicians in early recognition and assessment of the disease severity among hospitalized children with DSS. Therefore, interventions targeting these predictors can significantly improve the survival outcomes of children with DSS.

However, this study had certain limitations. First, as this was a single-center retrospective study, the generalizability of the findings to other treatment centers or diverse patient populations may be limited. Second, despite employing sophisticated techniques to process data and mitigate imbalances, missing or incomplete data might have had an impact on the analysis. Finally, there was variability in treatment standards during the study period from 2013 to 2022. In our study, the study population included children presenting with DSS, which has been well reported as the most common complication of severe dengue and is attributed to a high mortality rate (up to 20%)[2]. Thus, the study findings can be externally validated in WHO-reported regions with the highest dengue-related burdens, particularly South America, Africa, and Southeast Asia[1,2]. External validation across different dengue-endemic regions will increase the validity and generalizability of the model developed in this study.

CONCLUSION

We developed robust machine-learning-based models to estimate the risk of mortality in hospitalized children with DSS. The study findings can be manipulated to optimize the prognosis and management of children with severe dengue and enhance patient survival outcomes.

ACKNOWLEDGEMENTS

We are grateful to the patients and administrative staff, for their support with this study.

FOOTNOTES

Author contributions: Nguyen TT, Vo LT, Pham TN, Trinh TH contributed to conceptualization, funding acquisition; Nguyen TT, Vo LT contributed to data curation, investigation; Nguyen TT, Vu T contributed to formal analysis; Nguyen TT, Vo LT, Pham NT contributed to methodology; Vo LT, Vu T, Nguyen TT contributed to writing-original draft; Vo LT, Vu T, Pham NT, Trinh TH, Nguyen TT contributed to revision of the final manuscript. All authors have contributed to and approved the final manuscript.

Institutional review board statement: This sub-study stemmed from the primary published study, "Prognostic values of serum lactate-to-bicarbonate ratio and lactate for predicting 28-day in-hospital mortality in children with dengue shock syndrome". The primary study was approved by the Scientific Committee and Institutional Review Board (IRB) of the Children's Hospital No. 2, Ho Chi Minh City, Viet Nam (IRB. No. 893/QD-BVND2, signed on 06-June-2022).

Informed consent statement: We used a secondary dataset from primary research, which was considered to cause less than minimal risk to the participants. Therefore, the need for ethical approval was waived. This study was performed in accordance with the principles of Good Clinical Practice and ethical guidelines of the Declaration of Helsinki.

Conflict-of-interest statement: All authors declare that there is no conflict of interest.

Data sharing statement: The original contributions of this study are included in the article and Supplementary material. Further requests can be directed to the corresponding authors.

Country of origin: Viet Nam

ORCID number: Luan Thanh Vo 0000-0003-3188-5967; Thien Vu 0000-0002-6956-0191; Thanh Tat Nguyen 0000-0001-7600-6757.

S-Editor: Liu H
L-Editor: Webster JR
P-Editor: Guo X

REFERENCES

1 **Yang X**, Quam MBM, Zhang T, Sang S. Global burden for dengue and the evolving pattern in the past 30 years. *J Travel Med* 2021; **28** [PMID: 34510205 DOI: 10.1093/jtm/taab146]
2 Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control: New Edition. Geneva: World Health Organization; 2009– [PMID: 23762963]
3 **Nguyen Tat T**, Vo Hoang-Thien N, Nguyen Tat D, Nguyen PH, Ho LT, Doan DH, Phan DT, Duong YN, Nguyen TH, Nguyen TK, Dinh HT, Dinh TT, Pham AT, Do Chau V, Trinh TH, Vo Thanh L. Prognostic values of serum lactate-to-bicarbonate ratio and lactate for predicting 28-day in-hospital mortality in children with dengue shock syndrome. *Medicine (Baltimore)* 2024; **103**: e38000 [PMID: 38669370 DOI: 10.1097/MD.0000000000038000]
4 **Sachdev A**, Pathak D, Gupta N, Simalti A, Gupta D, Gupta S, Chugh P. Early Predictors of Mortality in Children with Severe Dengue Fever: A Prospective Study. *Pediatr Infect Dis J* 2021; **40**: 797-801 [PMID: 34321449 DOI: 10.1097/INF.0000000000003179]
5 **Laoprasopwattana K**, Khantee P, Saelim K, Geater A. Mortality Rates of Severe Dengue Viral Infection Before and After Implementation of a Revised Guideline for Severe Dengue. *Pediatr Infect Dis J* 2022; **41**: 211-216 [PMID: 34840312 DOI: 10.1097/INF.0000000000003411]
6 **Vo LT**, Do VC, Trinh TH, Vu T, Nguyen TT. Combined Therapeutic Plasma Exchange and Continuous Renal Replacement Therapy in Children With Dengue-Associated Acute Liver Failure and Shock Syndrome: Single-Center Cohort From Vietnam. *Pediatr Crit Care Med* 2023; **24**: 818-828 [PMID: 37310173 DOI: 10.1097/PCC.0000000000003304]
7 **Preeprem N**, Phumeetham S. Paediatric dengue shock syndrome and acute respiratory failure: a single-centre retrospective study. *BMJ*

Paediatr Open 2022; **6** [PMID: 36645744 DOI: 10.1136/bmjpo-2022-001578]

8 **Amâncio FF**, Heringer TP, de Oliveira Cda C, Fassy LB, de Carvalho FB, Oliveira DP, de Oliveira CD, Botoni FO, Magalhães Fdo C, Lambertucci JR, Carneiro M. Clinical Profiles and Factors Associated with Death in Adults with Dengue Admitted to Intensive Care Units, Minas Gerais, Brazil. *PLoS One* 2015; **10**: e0129046 [PMID: 26090676 DOI: 10.1371/journal.pone.0129046]

9 **Pinto RC**, Castro DB, Albuquerque BC, Sampaio Vde S, Passos RA, Costa CF, Sadahiro M, Braga JU. Mortality Predictors in Patients with Severe Dengue in the State of Amazonas, Brazil. *PLoS One* 2016; **11**: e0161884 [PMID: 27564084 DOI: 10.1371/journal.pone.0161884]

10 **Mallhi TH**, Khan AH, Sarriff A, Adnan AS, Khan YH. Determinants of mortality and prolonged hospital stay among dengue patients attending tertiary care hospital: a cross-sectional retrospective analysis. *BMJ Open* 2017; **7**: e016805 [PMID: 28698348 DOI: 10.1136/bmjopen-2017-016805]

11 **Huy BV**, Hoa LNM, Thuy DT, Van Kinh N, Ngan TTD, Duyet LV, Hung NT, Minh NNQ, Truong NT, Chau NVV. Epidemiological and Clinical Features of Dengue Infection in Adults in the 2017 Outbreak in Vietnam. *Biomed Res Int* 2019; **2019**: 3085827 [PMID: 31815129 DOI: 10.1155/2019/3085827]

12 **Kaur G**, Kumar V, Puri S, Tyagi R, Singh A, Kaur H. Predictors of dengue-related mortality in young adults in a tertiary care centre in North India. *J Family Med Prim Care* 2020; **9**: 694-697 [PMID: 32318404 DOI: 10.4103/jfmpc.jfmpc_605_19]

13 **Sarker IH**. Machine Learning: Algorithms, Real-World Applications and Research Directions. *SN Comput Sci* 2021; **2**: 160 [PMID: 33778771 DOI: 10.1007/s42979-021-00592-x]

14 **Sippy R**, Farrell DF, Lichtenstein DA, Nightingale R, Harris MA, Toth J, Hantztidiamantis P, Usher N, Cueva Aponte C, Barzallo Aguilar J, Puthumana A, Lupone CD, Endy T, Ryan SJ, Stewart Ibarra AM. Severity Index for Suspected Arbovirus (SISA): Machine learning for accurate prediction of hospitalization in subjects suspected of arboviral infection. *PLoS Negl Trop Dis* 2020; **14**: e0007969 [PMID: 32059026 DOI: 10.1371/journal.pntd.0007969]

15 **Huang SW**, Tsai HP, Hung SJ, Ko WC, Wang JR. Assessing the risk of dengue severity using demographic information and laboratory test results with machine learning. *PLoS Negl Trop Dis* 2020; **14**: e0008960 [PMID: 33362244 DOI: 10.1371/journal.pntd.0008960]

16 **Dablain D**, Krawczyk B, Chawla NV. DeepSMOTE: Fusing Deep Learning and SMOTE for Imbalanced Data. *IEEE Trans Neural Netw Learn Syst* 2023; **34**: 6390-6404 [PMID: 35085094 DOI: 10.1109/TNNLS.2021.3136503]

17 **Yuan K**, Chen Y, Zhong M, Lin Y, Liu L. Risk and predictive factors for severe dengue infection: A systematic review and meta-analysis. *PLoS One* 2022; **17**: e0267186 [PMID: 35427400 DOI: 10.1371/journal.pone.0267186]

18 **Sangkaew S**, Ming D, Boonyasiri A, Honeyford K, Kalayanarooj S, Yacoub S, Dorigatti I, Holmes A. Risk predictors of progression to severe disease during the febrile phase of dengue: a systematic review and meta-analysis. *Lancet Infect Dis* 2021; **21**: 1014-1026 [PMID: 33640077 DOI: 10.1016/S1473-3099(20)30601-0]

19 **Md-Sani SS**, Md-Noor J, Han WH, Gan SP, Rani NS, Tan HL, Rathakrishnan K, A-Shariffuddin MA, Abd-Rahman M. Prediction of mortality in severe dengue cases. *BMC Infect Dis* 2018; **18**: 232 [PMID: 29783955 DOI: 10.1186/s12879-018-3141-6]

20 **Rajula HSR**, Verlato G, Manchia M, Antonucci N, Fanos V. Comparison of Conventional Statistical Methods with Machine Learning in Medicine: Diagnosis, Drug Development, and Treatment. *Medicina (Kaunas)* 2020; **56** [PMID: 32911665 DOI: 10.3390/medicina56090455]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 100950

DOI: 10.5662/wjm.v15.i3.100950

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Observational Study

Evaluation of macular and peripapillary structure and microvasculature with optical coherence tomography angiography in migraine in the Indian population

Pankti Shah, Virna M Shah, Veerappan Rathinasabapathy Saravanan, Karthik Kumar, Siddharth Narendran

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade C
Novelty: Grade C
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Vengateswaran HT

Received: September 2, 2024
Revised: November 21, 2024
Accepted: December 9, 2024
Published online: September 20, 2025
Processing time: 187 Days and 5.3 Hours

Pankti Shah, Virna M Shah, Karthik Kumar, Department of Neuro Ophtalmology, Aravind Eye Hospital, Coimbatore 641014, Tamil Nādu, India

Veerappan Rathinasabapathy Saravanan, Department of Vitreoretina, Aravind Eye Hospital, Coimbatore 641014, Tamil Nādu, India

Siddharth Narendran, Division of Microbiology, Department of Cataract Services, Aravind Medical Research Foundation Regional Centre, Aravind Eye Hospital, Coimbatore 641014, Tamil Nādu, India

Corresponding author: Virna M Shah, DO, Chief Physician, Department of Neuro Ophthalmology, Aravind Eye Hospital, Tamilnadu, Coimbatore 641014, Tamil Nādu, India. virna@aravind.org

Abstract

BACKGROUND

Migraine has been proposed as a potential contributing factor to ischemic complications involving the retina and optic nerve. Ophthalmic disorders connected with migraine encompass occlusions of the branch and central retinal arteries and veins, alongside anterior and posterior ischemic optic neuropathy. With the advent of optical coherence tomography angiography (OCTA), it is easy to identify these macular subclinical microvascular and structural changes.

AIM

To evaluate macular and peripapillary structural and microvasculature changes in patients with migraine with aura (MA), migraine without aura (MW), and healthy control (HC) participants using OCTA.

METHODS

In this observational cross-sectional study, we studied a total of 100 eyes: (1) 32 eyes of 16 patients with MA; (2) 36 eyes of 18 patients with MW, recruited based on the International Classification of Headache Disorders; and (3) 32 eyes of 16 age and sex-matched healthy participants. Foveal flux, foveal avascular zone (FAZ), peripapillary flux obtained from OCTA, and foveal and peripapillary ganglion cell layer (GCL) thickness calculated *via* optical coherence tomography were compared among the groups.

RESULTS
The mean FAZ area measured in patients with MA and MW was significantly larger than that in the control participants (P = 0.002). However, there was no significant difference between the FAZ of the MA and MW groups. Macular perfusion in the superficial capillary plexus in patients with MA was significantly lower compared to MW (P = 0.0018) and HCs (P = 0.002). There was also significant thinning of the GCL in patients with MA and MW (P = 0.001) compared to HCs. However, there was no significant difference in temporal GCL thickness between the MA and MW groups.

CONCLUSION
Significant changes have been found in structural and microvascular parameters in patients with migraines compared with HCs. OCTA can serve as a valuable non-invasive imaging technique for identifying microcirculatory disturbances, aiding in better understanding the pathogenesis of different types of migraine and establishing their link with other ischemic retinal and systemic pathologies.

Key Words: Migraine; Aura; Optical coherence tomography angiography; Foveal microvasculature; Macular perfusion

Core Tip: Our observational study shows that there are microvascular and structural changes seen on optical coherence tomography angiography in migraine, and these changes could serve as a valuable non-invasive biomarker in its diagnosis.

Citation: Shah P, Shah VM, Saravanan VR, Kumar K, Narendran S. Evaluation of macular and peripapillary structure and microvasculature with optical coherence tomography angiography in migraine in the Indian population. *World J Methodol* 2025; 15(3): 100950
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/100950.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.100950

INTRODUCTION

Migraine, ranked as the third most prevalent disease worldwide, has an overall prevalence of 14.7% (18.8% in females and 10.7% in males)[1]. The term 'migraine' originates from the Latin word 'hemicrania,' signifying 'half skull.' This designation was initially used by the Greek physician Galenus of Pergamon[2]. Migraine auras refer to sensory symptoms (neurologic, gastrointestinal, and autonomic) that may manifest before or during a migraine attack. These symptoms include phenomena such as light flashes, blind spots, or tingling in the hands or face[2]. Their usual duration spans from 4 hours to 72 hours, and often cause significant incapacitation. These manifestations can occur episodically (fewer than 15 days per month) or chronically (more than 15 days per month). Among migraine headache sufferers, 70% experience migraine without aura (MW)[2]. While the complete pathophysiology of migraine remains elusive, there appears to be a complex interplay between neural and vascular factors that play a pivotal role[3].

For years, migraine has been proposed as a potential contributing factor to ischemic complications involving the retina and optic nerve[4]. Ophthalmic disorders associated with migraine include occlusions of the branch and central retinal arteries and veins, as well as anterior and posterior ischemic optic neuropathy[5]. Furthermore, there is evidence suggesting that migraine could increase the risk of onset or progression of normal tension glaucoma[6,7]. These disorders have been observed during both acute migraine attacks and the periods between attacks[8]. Consequently, both immediate and sustained alterations in ocular perfusion among patients with migraine might make them more susceptible to the aforementioned ocular complications[8,9].

There have been studies from Turkey and the United States describing optical coherence tomography (OCT) and OCT angiography (OCTA) parameters among patients with migraine[10], revealing numerous vascular differences compared to the healthy population[11-15]. Nonetheless, there is little literature available on the structural and microvascular parameters of the macula and peripapillary area among the Indian population.

In this study, we investigated the following parameters: (1) Foveal flux as a measure of foveal vasculature (flux is a novel metric that approximates the number of red blood cells moving through vessel segments per unit area); and (2) Foveal avascular zone (FAZ), peripapillary flux in the superior, inferior, nasal, and temporal quadrants through OCTA, and ganglion cell layer thickness (GCL) at the macula and in the superior, inferior, nasal, and temporal peripapillary quadrants as structural parameters through OCT. This study aimed to better understand the differences among three groups: patients with migraine with aura (MA), without aura (MW), and healthy controls (HCs).

MATERIALS AND METHODS

After approval from the Institutional Ethics Committee of our institute (Project No. RES2023067CLI), we prospectively

studied a total of 100 eyes: (1) 32 eyes from 16 patients with MA; (2) 36 eyes from 18 patients with MW; and (3) 32 eyes from 16 HCs. We assessed distant and near visual acuity, refractive error, slit-lamp anterior segment evaluation, posterior segment evaluation by indirect ophthalmoscopy, intraocular pressure by applanation tonometry, and OCT and OCTA parameters, as previously discussed. Patients were selected from the outpatient clinic of the neuro-ophthalmology department of our institute and those referred by neurologists, with inclusion based on the International Classification of Headache Disorders, 3rd edition[14]. Exclusion criteria for all groups included any neurologic disorder other than migraine (including neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease); any optic nerve disorders such as glaucoma, ischemic optic neuropathy, or retinal diseases; a history of intraocular surgery other than cataract extraction; systemic conditions affecting the microvasculature such as diabetes mellitus, hypertension, vasculitis, or renal disease; and ocular media opacity precluding high-quality imaging. HCs were also excluded if taking vasoactive medications, such as calcium channel blockers. The demographic parameters of participants are described in Table 1.

OCTA images were acquired using the AngioPlex matrix software on the CIRRUS HD-OCT Model 6000© 2021 (Carl Zeiss Meditec, Inc., Dublin, CA, United States). The scanning area captured consisted of a 6 mm × 6 mm section centered on the fovea, a 4.5 mm × 4.5 mm section centered on the optic nerve head (ONH), and a 200 µm × 200 µm optic disc cube. The superficial retinal capillary plexus (SCP) was segmented with an inner boundary 3 µm posterior to the internal limiting membrane (ILM) and an outer boundary 15 µm posterior to the outer aspect of the inner plexiform layer. Optic nerve scans were segmented into ONH and radial peripapillary capillary (RPC) slabs. The ONH slab was segmented with an inner boundary at the anterior border of the ILM and an outer boundary 150 µm posterior to the ILM. The software automatically assigned two concentric circles centered at the fovea and ONH. The radii of the inner and outer circles were 1 mm and 2 mm, respectively, providing a ring width of 1 mm. The flux, as a measure of vascularity, of the macular and RPC was evaluated between these rings in the peripapillary region and in four sectors (superior, inferior, nasal, and temporal) around the ONH. The segmentation was between the ILM and the posterior limit of the retinal nerve fiber layer (RNFL). Color-encoded slabs representing different layers were generated. The FAZ area was directly measured with AngioPlex software using a slab from the ILM to 75 µm above the retinal pigment epithelium. CIRRUS 6000 automatically centers and optimizes B-scan settings. The device also automatically corrects for the patient's refractive error and balances fundus brightness and contrast. Eye tracking was done using FastTrac™.

The macular and peripapillary GCL thickness was also evaluated in the peripapillary region and in four sectors using the same ONH analysis software. OCTA scans with low-quality or inadequate signal strength index (less than 6 on a 10-point scale) were excluded. Scans with blink artifacts, motion artifacts, media opacities interfering with the vessel signals, or segmentation errors were not included in the study.

Statistical analyses

Age, sex, intraocular pressure, refractive errors, and OCT and OCTA parameters were compared among the three groups: (1) MA; (2) MW; and (3) HCs. An independent *t*-test was used to test the significant difference between the means of two independent groups. Analysis of variance was used to determine whether the difference between group means (more than two groups) was statistically significant. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 32 eyes from 16 patients in the MA group, 36 eyes from 18 patients in the MW group, and 32 eyes from 16 healthy participants were enrolled. The mean ages for the three groups were as follows. (1) The MA group had a mean age of 28.18 years ± 7.23 years; (2) The MW group had a mean age of 28.94 years ± 5.95 years; and (3) The HC group had a mean age of 27.87 years ± 6.97 years. Across the three groups, the distribution of sexes was similar. In the MA group, 37.5% were male and 62.5% were female; in the MW group, 44.44% were male and 55.56% were female; and in the HC group, 43.75% were male and 56.25% were female. The age ($P = 0.89$) and sex ($P = 0.92$) differences were not significant among the groups. Additionally, the intraocular pressure ($P = 0.92$) and mean refractive errors (0.55) were not significantly different among the groups, as described in Table 1.

All patients enrolled in the study had a best-corrected visual acuity of 6/6 by distance Snellen's chart and near acuity of N6 by the Jaeger chart. Representative OCTA scans of the MA, MW, and HC participants are shown in Figure 1A (macula) and Figure 1B (optic nerve). Quantitative analysis of macular OCTA findings in the patients with MA and MW, and HCs is shown in Table 2. The mean FAZ area measured 0.34 mm² ± 0.12 mm² in the patients with MA, which was significantly larger than that in the control participants (0.201 mm² ± 0.007 mm²; $P = 0.002$). The FAZ of patients with MW (0.29 mm² ± 0.14 mm²) was also significantly larger than that in the HCs ($P = 0.007$). However, there was no significant difference between the FAZ areas of the MA and MW groups.

Macular perfusion in the SCP was significantly lower in patients with MA (4.16 ± 1.47) compared to those with MW (6.21 ± 3.10; $P = 0.0018$) and HCs (6.18 ± 2.12; $P = 0.002$). There was no difference in foveal perfusion between the MW and HC groups. There were no significant differences in peripapillary perfusion in the superior, inferior, nasal, and temporal quadrants among the MA, MW, and HC groups, as shown in Table 2.

When comparing structural parameters obtained from OCT, no statistical differences were found in GCL thickness among the MA, MW, and HC groups in the foveal, superior, inferior, and nasal peripapillary regions. However, there was significant thinning of the GCL in patients with MA (29.43 ± 4.01; $P = 0.001$) compared to HC participants (30.59 ± 3.44). Additionally, the temporal GCL thickness in patients with MW (27.38 ± 3.76; $P = 0.009$) was significantly reduced compared to HC participants. However, there was no significant difference in temporal GCL thickness between the MA and MW groups.

Table 1 Demographic parameters of participants, *n* (%)

Group	Mean age in years	Male	Female	Mean refraction (spherical)	Mean refraction (cylinder)	Mean intraocular pressure
Migraine with aura (*n* = 16)	28.18 ± 7.23	6 (37.5)	10 (62.5)	0.71 ± 0.67	0.31 ± 0.36	15.25 ± 2.87
Migraine without aura (*n* = 18)	28.94 ± 5.95	8 (44.44)	10 (55.55)	0.47 ± 0.48	0.20 ± 0.24	15.18 ± 2.58
Healthy controls (*n* = 16)	27.875 ± 6.97	7 (43.75)	9 (56.25)	0.27 ± 0.36	0.18 ± 0.26	15.82 ± 2.88

"Male" and "female" show the count and percentage of participants in each sex category for each group.

Table 2 Average ± standard deviation values of optical coherence tomography angiography parameters

Optical coherence tomography angiography parameters	Migraine with aura	Migraine without aura	Healthy controls
Foveal flux	4.16 ± 1.47	6.21 ± 3.10	6.18 ± 2.12
Foveal avascular zone	0.34 ± 0.12	0.29 ± 0.14	0.20 ± 0.07
Superior peripapillary flux	18.57 ± 4.70	19.03 ± 0.93	18.69 ± 3.46
Inferior peripapillary flux	18.22 ± 1.84	18.49 ± 1.12	18.65 ± 1.25
Nasal peripapillary flux	17.88 ± 1.69	17.14 ± 3.96	17.76 ± 2.08
Temporal peripapillary flux	17.55 ± 1.81	22.51 ± 1.83	18.71 ± 1.42

Table 3 Optical coherence tomography parameters of migraine with aura, migraine without aura, and healthy controls

Optical coherence tomography parameters	Migraine with aura	Migraine without aura	Healthy controls
Macular GCL thickness	9.46 ± 2.25	9.25 ± 2.27	10.15 ± 3.44
Superior GCL	29.43 ± 3.76	28.58 ± 3.78	30.68 ± 3.16
Inferior GCL	29.40 ± 4.22	29.30 ± 4.02	29.06 ± 3.59
Nasal GCL	29.43 ± 4.01	28.11 ± 3.59	28.75 ± 3.75
Temporal GCL	29.43 ± 4.01	27.38 ± 3.76	30.59 ± 3.44

GCL: Ganglion cell layer.

DISCUSSION

After analyzing a total of 100 eyes divided into three groups (MA, MW, and HCs), we concluded that the FAZ was enlarged in patients with migraine, both with and without aura, compared to HCs. Microvascular ischemic events or capillary remodeling of near-normal FAZ may be the cause of FAZ enlargement. The FAZ area is reportedly associated with the severity of systemic diseases, such as diabetes, and some studies have indicated that screening for FAZ might help detect early microvascular abnormalities. OCT parameters of MA, MW, and HC are shown in Table 3.

Enlarged FAZ has also been observed in several studies conducted in Turkey and the United States[13,15]. Our finding of an enlarged FAZ in patients with MW compared to HCs was supported by the results of Hamurcu *et al*[16] and Taşlı and Ersoy[17].

Another important conclusion of our study was the reduced foveal vascularity in the SCP in patients with MA compared to patients with MW and HCs. Similar findings have been reported by various studies[13,15,18,19]. The decreased foveal vessel density in the SCP in patients with MA is supportive of retinal microvascular disease. The chronic retinovascular alterations demonstrated by OCTA in patients with MA may be related to the increased risk of ocular complications in patients with migraine, even in the absence of an acute attack[13]. Since the advent of modern techniques, many studies have used various parameters to assess retinal vasculature to establish a link between migraine and other ischemic ocular and systemic diseases. Acer *et al*[20] reported that ocular pulse amplitude did not significantly differ between patients with migraine without aura and control cases. We employed foveal flux, a novel metric that approximates the number of red blood cells moving through vessel segments per unit area. *In vitro* and *in vivo* studies demonstrated that foveal flux is not only a complementary parameter to conventional vessel density but also a potentially

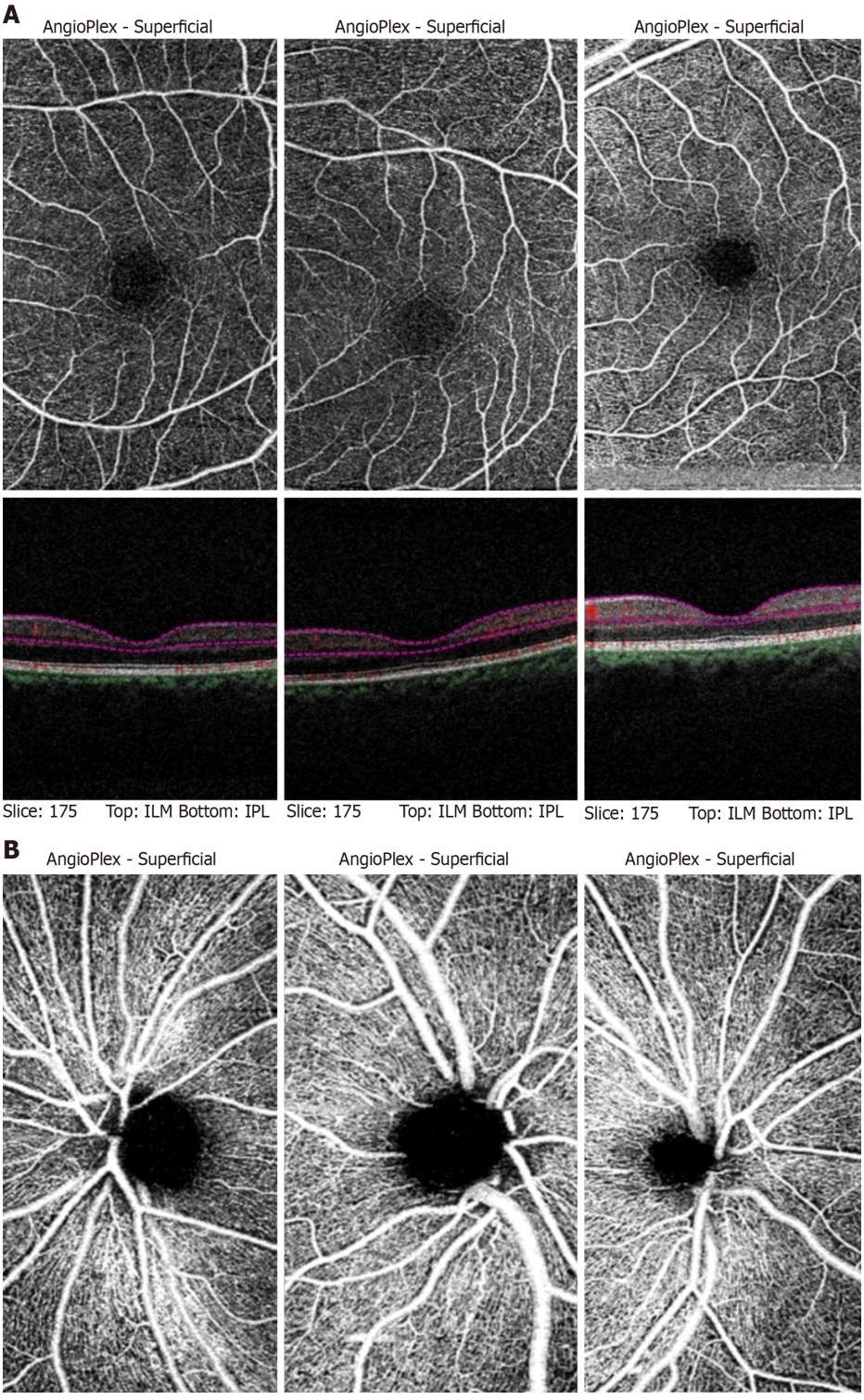


Figure 1 Representative optical coherence tomography angiography scans of migraine with aura, migraine without aura, and healthy control participants. A: Optical coherence tomography angiography (OCTA) images at superficial retinal capillary plexus of fovea of migraine with aura (MA), migraine without aura (MW) and healthy controls (HCs) from left to right with their corresponding B scan below; B: OCTA scans at optic nerve head in the MA, MW, HC groups from left to right, respectively. ILM: Internal limiting membrane; IPL: Inner plexiform layer.

useful measure of retinal perfusion, in addition to other OCTA-derived parameters.

We did not find any significant difference in peripapillary vasculature among the three groups. However, the temporal GCL thickness in patients with MA and MW was found to be significantly reduced compared to the HC group. Similar thinning of the GCC and RNFL has been documented in various studies[11,12]. Compromised choroidal blood flow can lead to focal ischemic damage in the optic disc[21]. These structural changes might provide insights into the association of migraine with optic nerve diseases such as anterior and posterior ischemic optic neuropathy, normal tension glaucoma, and others[5,6]. Amaurosis fugax in migraineurs has been proposed as a sign of affected choroidal blood flow[22].

Although most studies have shown more structural and microvascular changes in patients with aura compared to those with MW, we found in our study that FAZ enlargement and temporal GCL thinning were equally significant in patients with MW. Thus, we conclude that all patients with migraine, including those with and without aura, exhibit vascular and structural abnormalities compared to healthy eyes. Although the exact pathogenesis of migraine is still unknown, it is believed to be triggered by specific vessels and nerves in the brain. As the eye is an extension of the brain, these features could be reflected in the retinal blood vessels and nerves as observed by OCTA. Enlargement of the FAZ and loss of GCL could be early changes in migraine cases and may serve as potential biomarkers in the future.

To the best of our knowledge, our study is the first to employ OCT and OCTA parameters in three groups—MA, MW, and HC—to study structural and vascular changes in migraine within the Indian population.

This study had several limitations. First, the sample size was small, which might have contributed to the lack of significant results for some parameters. Additionally, most participants were in the interictal period, so vascular findings during an acute migraine attack could not be assessed. We also failed to include vascularity measures in the deep capillary plexus at the fovea and ONH, thus limiting access to important information. A possible confounding factor could have been the duration of migraine symptoms, as structural changes are chronic phenomena that we did not consider. Finally, migraine is a heterogeneous disorder, making it difficult to categorize patients into distinct groups.

CONCLUSION

In conclusion, we found significant FAZ enlargement in patients with migraine, both with and without aura, compared to HCs. Additionally, we observed reduced foveal vascularity in patients with aura compared to patients without aura and HCs. These microvascular changes can help establish a link between migraines and other retinal and choroidal ischemic diseases, providing a better understanding of migraine's lesser-established pathophysiology. We also found temporal GCL thinning in both groups of patients with migraine compared to HC. Repeated insults due to reduced blood supply during attacks may explain these structural changes even during attack-free periods.

Having conducted a study involving 100 eyes across three groups—MA, MW, and HC—we can now consider the future prospects of our research. Building on this pilot study, there is potential to conduct extensive longitudinal investigations. This broader research could encompass factors such as the duration of migraine episodes, acquiring scans during the attack phase, and incorporating a more comprehensive array of parameters.

FOOTNOTES

Author contributions: Shah P and Kumar K conducted the study; Shah P and Narendran S analyzed the data and wrote the paper; Shah VM supervised the study; Shah VM and Saravanan VR designed the study; All authors read and approved the final version of the manuscript to be published.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of Aravind Eye Hospital, Madurai, India.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to use of anonymous patient data for research at the time of registration in the outpatient department. We applied the opt-out method to obtain consent for this study by using a poster. The poster was approved by the Institutional Review Board.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at virna@aravind.org.

STROBE statement: The authors have read the STROBE Statement-checklist of items-and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

Country of origin: India

ORCID number: Virna M Shah 0000-0001-6899-6690.

S-Editor: Luo ML
L-Editor: Filipodia
P-Editor: Guo X

REFERENCES

1 **Vos T**, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY, Ali MK, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Bahalim AN, Barker-Collo S, Barrero LH, Bartels DH, Basáñez MG, Baxter A, Bell ML, Benjamin EJ, Bennett D, Bernabé E, Bhalla K, Bhandari B, Bikbov B, Bin Abdulhak A, Birbeck G, Black JA, Blencowe H, Blore JD, Blyth F, Bolliger I, Bonaventure A, Boufous S, Bourne R, Boussinesq M, Braithwaite T, Brayne C, Bridgett L, Brooker S, Brooks P, Brugha TS, Bryan-Hancock C, Bucello C, Buchbinder R, Buckle G, Budke CM, Burch M, Burney P, Burstein R, Calabria B, Campbell B, Canter CE, Carabin H, Carapetis J, Carmona L, Cella C, Charlson F, Chen H, Cheng AT, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahiya M, Dahodwala N, Damsere-Derry J, Danaei G, Davis A, De Leo D, Degenhardt L, Dellavalle R, Delossantos A, Denenberg J, Derrett S, Des Jarlais DC, Dharmaratne SD, Dherani M, Diaz-Torne C, Dolk H, Dorsey ER, Driscoll T, Duber H, Ebel B, Edmond K, Elbaz A, Ali SE, Erskine H, Erwin PJ, Espindola P, Ewoigbokhan SE, Farzadfar F, Feigin V, Felson DT, Ferrari A, Ferri CP, Fèvre EM, Finucane MM, Flaxman S, Flood L, Foreman K, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabbe BJ, Gabriel SE, Gakidou E, Ganatra HA, Garcia B, Gaspari F, Gillum RF, Gmel G, Gosselin R, Grainger R, Groeger J, Guillemin F, Gunnell D, Gupta R, Haagsma J, Hagan H, Halasa YA, Hall W, Haring D, Haro JM, Harrison JE, Havmoeller R, Hay RJ, Higashi H, Hill C, Hoen B, Hoffman H, Hotez PJ, Hoy D, Huang JJ, Ibeanusi SE, Jacobsen KH, James SL, Jarvis D, Jasrasaria R, Jayaraman S, Johns N, Jonas JB, Karthikeyan G, Kassebaum N, Kawakami N, Keren A, Khoo JP, King CH, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lalloo R, Laslett LL, Lathlean T, Leasher JL, Lee YY, Leigh J, Lim SS, Limb E, Lin JK, Lipnick M, Lipshultz SE, Liu W, Loane M, Ohno SL, Lyons R, Ma J, Mabweijano J, MacIntyre MF, Malekzadeh R, Mallinger L, Manivannan S, Marcenes W, March L, Margolis DJ, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGill N, McGrath J, Medina-Mora ME, Meltzer M, Mensah GA, Merriman TR, Meyer AC, Miglioli V, Miller M, Miller TR, Mitchell PB, Mocumbi AO, Moffitt TE, Mokdad AA, Monasta L, Montico M, Moradi-Lakeh M, Moran A, Morawska L, Mori R, Murdoch ME, Mwaniki MK, Naidoo K, Nair MN, Naldi L, Narayan KM, Nelson PK, Nelson RG, Nevitt MC, Newton CR, Nolte S, Norman P, Norman R, O'Donnell M, O'Hanlon S, Olives C, Omer SB, Ortblad K, Osborne R, Ozgediz D, Page A, Pahari B, Pandian JD, Rivero AP, Patten SB, Pearce N, Padilla RP, Perez-Ruiz F, Perico N, Pesudovs K, Phillips D, Phillips MR, Pierce K, Pion S, Polanczyk GV, Polinder S, Pope CA 3rd, Popova S, Porrini E, Pourmalek F, Prince M, Pullan RL, Ramaiah KD, Ranganathan D, Razavi H, Regan M, Rehm JT, Rein DB, Remuzzi G, Richardson K, Rivara FP, Roberts T, Robinson C, De Leòn FR, Ronfani L, Room R, Rosenfeld LC, Rushton L, Sacco RL, Saha S, Sampson U, Sanchez-Riera L, Sanman E, Schwebel DC, Scott JG, Segui-Gomez M, Shahraz S, Shepard DS, Shin H, Shivakoti R, Singh D, Singh GM, Singh JA, Singleton J, Sleet DA, Sliwa K, Smith E, Smith JL, Stapelberg NJ, Steer A, Steiner T, Stolk WA, Stovner LJ, Sudfeld C, Syed S, Tamburlini G, Tavakkoli M, Taylor HR, Taylor JA, Taylor WJ, Thomas B, Thomson WM, Thurston GD, Tleyjeh IM, Tonelli M, Towbin JA, Truelsen T, Tsilimbaris MK, Ubeda C, Undurraga EA, van der Werf MJ, van Os J, Vavilala MS, Venketasubramanian N, Wang M, Wang W, Watt K, Weatherall DJ, Weinstock MA, Weintraub R, Weisskopf MG, Weissman MM, White RA, Whiteford H, Wiersma ST, Wilkinson JD, Williams HC, Williams SR, Witt E, Wolfe F, Woolf AD, Wulf S, Yeh PH, Zaidi AK, Zheng ZJ, Zonies D, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**: 2163-2196 [PMID: 23245607 DOI: 10.1016/S0140-6736(12)61729-2]

2 **Shankar Kikkeri N**, Nagalli S. Migraine With Aura. 2024 Feb 29. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [PMID: 32119498]

3 **Jacobs B**, Dussor G. Neurovascular contributions to migraine: Moving beyond vasodilation. *Neuroscience* 2016; **338**: 130-144 [PMID: 27312704 DOI: 10.1016/j.neuroscience.2016.06.012]

4 **Beversdorf D**, Stommel E, Allen C, Stevens R, Lessell S. Recurrent branch retinal infarcts in association with migraine. *Headache* 1997; **37**: 396-399 [PMID: 9237415 DOI: 10.1046/j.1526-4610.1997.3706396.x]

5 **Lee AG**, Brazis PW, Miller NR. Posterior ischemic optic neuropathy associated with migraine. *Headache* 1996; **36**: 506-510 [PMID: 8824007 DOI: 10.1046/j.1526-4610.1996.3608506.x]

6 **Drance S**, Anderson DR, Schulzer M; Collaborative Normal-Tension Glaucoma Study Group. Risk factors for progression of visual field abnormalities in normal-tension glaucoma. *Am J Ophthalmol* 2001; **131**: 699-708 [PMID: 11384564 DOI: 10.1016/s0002-9394(01)00964-3]

7 **Katsarava Z**, Buse DC, Manack AN, Lipton RB. Defining the differences between episodic migraine and chronic migraine. *Curr Pain Headache Rep* 2012; **16**: 86-92 [PMID: 22083262 DOI: 10.1007/s11916-011-0233-z]

8 **Coppeto JR**, Lessell S, Sciarra R, Bear L. Vascular retinopathy in migraine. *Neurology* 1986; **36**: 267-270 [PMID: 3945397 DOI: 10.1212/wnl.36.2.267]

9 **Greven CM**, Slusher MM, Weaver RG. Retinal arterial occlusions in young adults. *Am J Ophthalmol* 1995; **120**: 776-783 [PMID: 8540551 DOI: 10.1016/s0002-9394(14)72731-x]

10 **Chaliha DR**, Vaccarezza M, Charng J, Chen FK, Lim A, Drummond P, Takechi R, Lam V, Dhaliwal SS, Mamo JCL. Using optical coherence tomography and optical coherence tomography angiography to delineate neurovascular homeostasis in migraine: a review. *Front Neurosci* 2024; **18**: 1376282 [PMID: 38686331 DOI: 10.3389/fnins.2024.1376282]

11 **Ekinci M**, Ceylan E, Cağatay HH, Keleş S, Hüseyinoğlu N, Tanyildiz B, Cakici O, Kartal B. Retinal nerve fibre layer, ganglion cell layer and choroid thinning in migraine with aura. *BMC Ophthalmol* 2014; **14**: 75 [PMID: 24885597 DOI: 10.1186/1471-2415-14-75]

12 **Demircan S**, Ataş M, Arık Yüksel S, Ulusoy MD, Yuvacı İ, Arifoğlu HB, Başkan B, Zararsız G. The impact of migraine on posterior ocular structures. *J Ophthalmol* 2015; **2015**: 868967 [PMID: 25767720 DOI: 10.1155/2015/868967]

13 **Chang MY**, Phasukkijwatana N, Garrity S, Pineles SL, Rahimi M, Sarraf D, Johnston M, Charles A, Arnold AC. Foveal and Peripapillary Vascular Decrement in Migraine With Aura Demonstrated by Optical Coherence Tomography Angiography. *Invest Ophthalmol Vis Sci* 2017; **58**: 5477-5484 [PMID: 29059314 DOI: 10.1167/iovs.17-22477]

14 **Headache Classification Committee of the International Headache Society (IHS)**. The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia* 2013; **33**: 629-808 [PMID: 23771276 DOI: 10.1177/0333102413485658]

15 **Liu Z**, Jie C, Wang J, Hou X, Zhang W, Wang J, Deng Y, Li Y. Retina and microvascular alterations in migraine: a systemic review and meta-analysis. *Front Neurol* 2023; **14**: 1241778 [PMID: 37840933 DOI: 10.3389/fneur.2023.1241778]

16 **Hamurcu MS**, Gultekin BP, Koca S, Ece SD. Evaluation of migraine patients with optical coherence tomography angiography. *Int Ophthalmol* 2021; **41**: 3929-3933 [PMID: 34291402 DOI: 10.1007/s10792-021-01962-3]

17 **Taşlı NG**, Ersoy A. Altered Macular Vasculature in Migraine Patients without Aura: Is It Associated with Ocular Vasculature and White Matter Hyperintensities? *J Ophthalmol* 2020; **2020**: 3412490 [PMID: 32351718 DOI: 10.1155/2020/3412490]

18 **Freiberg FJ**, Pfau M, Wons J, Wirth MA, Becker MD, Michels S. Optical coherence tomography angiography of the foveal avascular zone in diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol* 2016; **254**: 1051-1058 [PMID: 26338819 DOI: 10.1007/s00417-015-3148-2]

19 **Ulusoy MO**, Horasanlı B, Kal A. Retinal vascular density evaluation of migraine patients with and without aura and association with white matter hyperintensities. *Acta Neurol Belg* 2019; **119**: 411-417 [PMID: 30762208 DOI: 10.1007/s13760-019-01094-7]

20 **Acer S**, Oğuzhanoğlu A, Çetin EN, Ongun N, Pekel G, Kaşıkçı A, Yağcı R. Ocular pulse amplitude and retina nerve fiber layer thickness in migraine patients without aura. *BMC Ophthalmol* 2016; **16**: 1 [PMID: 26728474 DOI: 10.1186/s12886-015-0180-2]

21 **Flammer J**, Pache M, Resink T. Vasospasm, its role in the pathogenesis of diseases with particular reference to the eye. *Prog Retin Eye Res* 2001; **20**: 319-349 [PMID: 11286896 DOI: 10.1016/s1350-9462(00)00028-8]

22 **Madill SA**. Transient Visual Loss in Young Females with Crowded Optic Discs: A Proposed Aetiology. *Neuroophthalmology* 2021; **45**: 372-379 [PMID: 34720267 DOI: 10.1080/01658107.2021.1937231]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 98201

DOI: 10.5662/wjm.v15.i3.98201

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Basic Study

Selective procedure for the instant identification of cellular apoptosis induced by natural products

Ying-Yu Cui

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade C
Novelty: Grade C
Creativity or Innovation: Grade C
Scientific Significance: Grade C

P-Reviewer: Guo D

Received: June 20, 2024
Revised: November 19, 2024
Accepted: December 23, 2024
Published online: September 20, 2025
Processing time: 259 Days and 1.4 Hours

Ying-Yu Cui, Department of Cell Biology, Institute of Medical Genetics, State Key Laboratory of Cardiology, Tongji University School of Medicine, Shanghai 200331, China

Corresponding author: Ying-Yu Cui, PhD, Associate Professor, Teacher, Department of Cell Biology, Institute of Medical Genetics, State Key Laboratory of Cardiology, Tongji University School of Medicine, No. 500 Zhennan Road, Putuo District, Shanghai 200331, China. yycui@tongji.edu.cn

Abstract

BACKGROUND

Recently, the identification of cell apoptosis induced by natural products has become research hotspot and frontier in the biopharmaceutical and food industries under the umbrella of global green development worldwide. Traditionally, cell apoptosis is identified using morphological, biochemical, and cell cycle experiments, which is time consuming, and experimental materials are not from the same group, and it is very hard to ensure the identity and veracity of results of former and latter experiments.

AIM

To establish a selective, instant, and practical protocol to identify cell apoptosis induced by natural products.

METHODS

A one transient cell processing procedure (OTCPP) was used to detect human colorectal cancer LoVo cell apoptosis after treatment with *Pinus massoniana* bark extract (PMBE) at the morphological, biochemical, and cell cycle levels. The methods used included treatment with DNA gel electrophoresis, fluorescence microscopy, and flow cytometry.

RESULTS

In PMBE-treated LoVo cells, we observed a DNA ladder on gel electrophoresis and fluorescence microscopy revealed "nuclear shrinkage, chromatin condensation or fragmentation". In addition, flow cytometry showed an "obvious apoptosis curve". Thus OTCPP achieved synchronous detection of the morphology, biochemistry, cell cycle, and the DNA content of the cells.

CONCLUSION

OTCPP can quickly identify apoptosis and measure the apoptosis rate, thereby

unifying qualitative and quantitative analysis.

Key Words: Apoptosis identification; Fluorescence microscopy; DNA gel electrophoresis; Flow cytometry; *Pinus massoniana* bark extract; One transient cell processing procedure

Core Tip: According to the global green development hypothesis, the identification of cell apoptosis induced by natural products is a research hotspot. However, routine laboratory identification of apoptosis suffers from experimental errors and poor repeatability. Herein, we explored the simultaneous morphological, biochemical and molecular identification of apoptosis, while reducing experimental errors. We established a protocol to instantly identify apoptosis following natural product treatment, termed one transient cell processing procedure (OTCPP), comprising eleven steps. It could identify apoptosis of cultured LoVo cells in a little as 4 days. The OTCPP procedure is easy, rapid and high efficient, especially for researchers in developing countries.

Citation: Cui YY. Selective procedure for the instant identification of cellular apoptosis induced by natural products. *World J Methodol* 2025; 15(3): 98201
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/98201.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.98201

INTRODUCTION

Apoptosis is a normal physiological phenomenon[1,2], widely involved in development, tissue homeostasis, and some pathological processes, further becomes a frontier of bio-medical research. Classic methods for apoptosis identification include fluorescence microscopy, gel electrophoresis, and flow cytometry, among which DNA ladder resulting from gel electrophoresis is regarded as gold standard. However, these methods have different protocols and are performed with different cells cultured in different dishes, respectively, consuming more time. Apoptotic cells, whose degraded smaller DNA leaked out, fixed with ethanol and then stained with fluorescein, will become sub-diploids because of their DNA contents less than those of G1 phase[3,4]. The extracted DNA can be effectively controlled by phosphate-citric acid buffer (0.2 M, pH7.8), and the leaked DNA can form DNA ladder under gel electrophoresis, while the residual cells' cell cycle distribution can be analyzed with flow cytometry[5,6].

Currently, the global green development hypothesis is promoting research into natural products, thus they have become hotspot in the biopharmaceutical and food industries. Certain natural products can promote or inhibit cell growth, or even induce their apoptosis[7]. The identification of natural product-induced apoptosis is thus an important subject. Consequently, the present study aimed to identify the effect of *Pinus massoniana* bark extract (PMBE) on human colorectal cancer LoVo cells to demonstrate the integrative procedure termed one transient cell processing procedure (OTCPP). OTCPP produces uses different instruments to achieve apoptosis detection, with relatively satisfactory results. We believe that OTCPP will play an important role in accelerating the progress of basic research, clinical diagnosis, and screening candidate natural drugs.

MATERIALS AND METHODS

Reagents

The Institute of Songzhen nutritional resource (Guangzhou, China) supplied the PMBE. The other reagents comprised HEPES (Amresco, Solon, OH, United States), Roswell Park Memorial Institute (RPMI) 1640 (HyClone, Logan, UT, United States), Penicillin-Streptomycin (Penicillin 10000 units/mL, Streptomycin 10000 μg/mL) (Invitrogen, Waltham, MA, United States), Fetal bovine serum (FBS; Sijiqing Co. Hangzhou, China), Trypsin (HyClone), propidium iodide (PI) (Sigma, St. Louis, MO, United States), RNase A (Sino-American Biotechnology Co., Beijing, China) and Proteinase K (Merck, Darmstadt).

Cell line

This study used the human colorectal cancer cell line LoVo (ATCC, Manassas, VA, United States; number CCL-229).

Equipment

The OTCPP process used an incubator, fluorescence microscope (BH-2, Olympus, Tokyo, Japan), electrophoresis apparatus (Bio-Rad, Hercules, CA, United States), a flow cytometer (ELITE, Beckman Coulter, Indianapolis, IN, United States), and MULTICYCLE analysis software (Phoenix Flow Systems, San Diego, CA, United States).

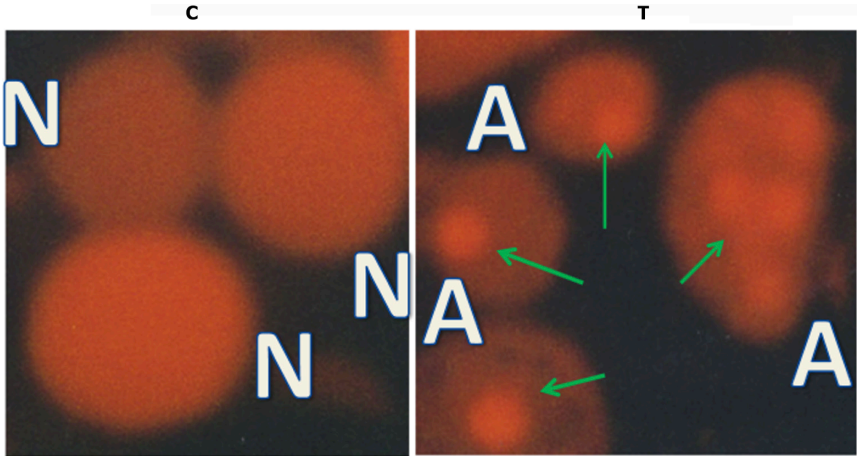


Figure 1 Fluorescence microscopy observation of LoVo cell apoptosis. Propidium iodide staining of LoVo cells untreated and treated with 140 μg/mL *Pinus massoniana* bark extract for 24 hours under fluorescence microscope with G.B. filter. Normal cells depicted in the panel show light and homogeneous staining of their nuclei. In contrast, apoptotic cells represented in the panel show thick and irregular staining of their nuclei as a result of chromatin condensation and nuclear fragmentation. Magnification, 400 ×. N: Normal cells; A: Apoptotic cells; C: Control group; T: Treatment group.

OTCPP Protocol

(1) Cell Culture and Treatment: Two wells of LoVo cells were cultured in complete RPMI 1640 media (10% FBS, 10 mmol/L HEPES, 100 IU/mL penicillin, 100 μg/mL streptomycin) in a flat-bottom plate (Costar, Corning, NY, United States), respectively, until to the logarithmic growth period (about 10^{4-6} cells/mL), then labeled as experimental group and control group, further adding 140 μg/mL PMBE to the culture media or not, respectively, all incubating for 24 hours; (2) Cell Collection and fixation: 1 mL of 0.25% trypsin was used to trypsinize the LoVo cells (1-2 × 10^7) at 37°C for appropriate time. Then, add 0.5% FBSRPMI 1640 media to stop trypsinization and gentle pipetting the cell clumps into single cells. The cells were transferred into an Eppendorf tube, centrifuged at 100 *g* for 5 minutes, and then discarded the supernatant. The cell pellet was washed twice using 1 × PBS with centrifugation at 100 *g* for 5 minutes each time. The cells were resuspended into single cell status using 1 × PBS (20 μL), added with 2 mL 70% ethanol, and placed at -20°C overnight to fix the cells; (3) Centrifuged 100 *g*, 10 minutes, discarded supernatant to remove ethanol; (4) Resuspended in 0.5 mL 1 × PBS, transferred to a new micro-tube, centrifuged 150 *g*, 10 minutes, discarded supernatant; (5) Added 40 μL of 0.2 M phosphate-citric acid buffer (pH7.8), RT 30 minutes, intermittent shaking; (6) Centrifuged 100 *g*, 10 minutes, and then transferred the supernatant to a new micro-tube, incubated on ice for fluorescence observation and flow cytometry analysis; (7) Added 3 μL of 0.25% NP40, 3 μL of RNase A solution (1 mg/mL) to the supernatant, vortexed, further incubated at 37°C, 30 minutes; (8) Added 3 μL of Proteinase K (1 mg/mL) to the mixture, vortexed thoroughly, and then incubated at 37°C for 30 minutes; (9) Then, 16 μL of the mixture was mixed thoroughly with 2 μL of 6 × DNA loading buffer, resolved on agarose gel at 220V, 2 hours, and then stained with EB. The gel was visualized under UV light; (10) Cell pellets of step (6) were resuspended in 0.5 mL 1 × PBS. Then, added 10 μL of Proteinase K (1 mg/mL), vortexed gently, and left RT, 30 minutes, then washed the cells two times using 1 × PBS with centrifugation 100 *g*, 5 minutes each time, and discarded supernatant, resuspended again in 20 μL 1 × PBS into single cells. Then, added 300 μL of DNA staining solution (containing 150 μg/mL PI and 20 U/mL RNase A) and incubated RT, 30 minutes; and (11) Placed a drop of the single cell suspension on a air-drying glass slide, covered with a coverslip, and observed under a fluorescence microscope (G.B filter, 400 ×). The remaining cell suspension for flow cytometry, analyzing the ratio of apoptotic cells.

Duration of use

4 days (2 ON incubation, 1 ON fixation, and 1 day with 2-3 hours of operation time).

Key notes

(1) Trypsinize the cells just well; (2) Rotation speed no more than 150 *g*; (3) Resuspend the cells into a single cell suspension using a small amount of 1 × PBS, then fix them with ethanol; and (4) Recommend removing large multi-cellular aggregates with a nylon monofilament mesh screen before analysis on the flow cytometer, ensuring that the cells flow through the cytometer in a single line.

RESULTS

Using a one-station operation for one-time cell culture and OTCPP, obtained enough experimental materials to identify cell apoptosis with different instruments, letting synchronous identification of LoVo cell apoptosis at morphological, biochemical, cell cycle, and DNA content level, respectively, come true. Further, reach the perfect combination of qualitative and quantitative analysis, which not only shortened the experimental time, but also completed the task in four working days rather than the original 9 working days. The results were quite satisfactory (Figure 1, Figure 2, and

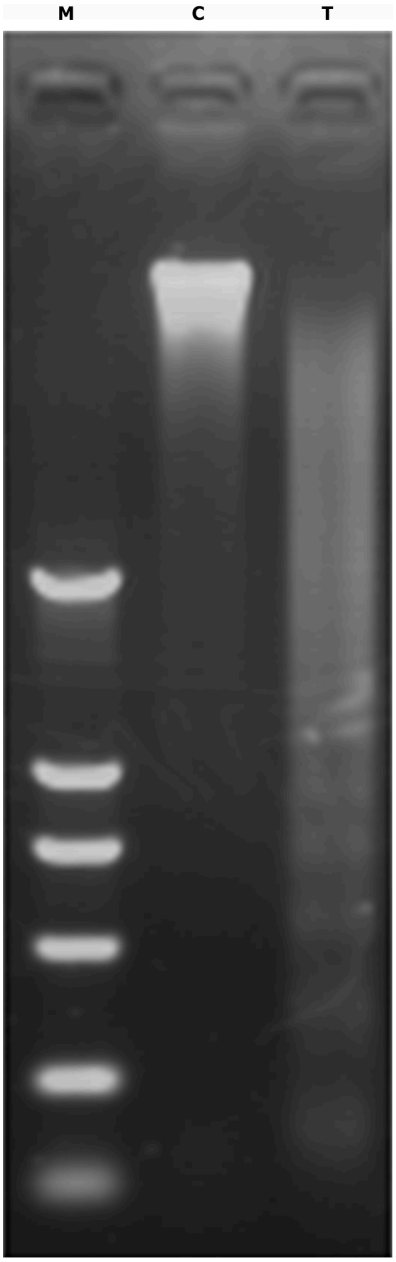


Figure 2 DNA gel electrophoresis of LoVo cell apoptosis. DNA ladder of apoptotic LoVo cells treated with 140 μg/mL *Pinus massoniana* bark extract for 24 hours. Results of gel electrophoresis of relative low-molecular-weight DNA oozed out of apoptotic LoVo cells. M: DNA marker DL2000; C: Control group; T: Treatment group.

Figure 3). Briefly, an integrative protocol for apoptosis identification and measurement was established.

PI staining of control LoVo cells and cells treated with 140 μg/mL PMBE for 24 hours under a fluorescence microscope with a G.B. filter. Normal cells (N) depicted in the panel show light and homogeneous staining of their nuclei. In contrast, apoptotic cells (A) in the panel show thick and irregular staining of their nuclei. Magnification, 400 ×.

DNA ladder from apoptotic LoVo cells treated with 140 μg/mL PMBE for 24 hours. Results of gel electrophoresis showing that relatively low molecular-weight DNA had extruded out of apoptotic LoVo cells. M DNA marker DL2000; 1 Control; 2 PMBE Treatment

Results of flow cytometry analysis of control LoVo cells and LoVo cells treated with 140 μg/mL PMBE for 24 hours: (1) Shows a normal cell cycle distribution of untreated LoVo cells stained with PI, showing no obvious apoptotic-sub-G1 curve, but with 10.9% of the cells showing spontaneous apoptosis; (2) Shows the cell cycle distribution of LoVo cell treated with PMBE and stained with PI. There is an obvious apoptotic-sub-G1 curve before the G1 curve, with 31.4% of the cells showing apoptosis; and (3) Shows the statistical histogram of LoVo cell apoptosis induced by PMBE treatment compared with Control. $^{a}P < 0.05$ *vs* Control, $n = 3$, mean ± SD.

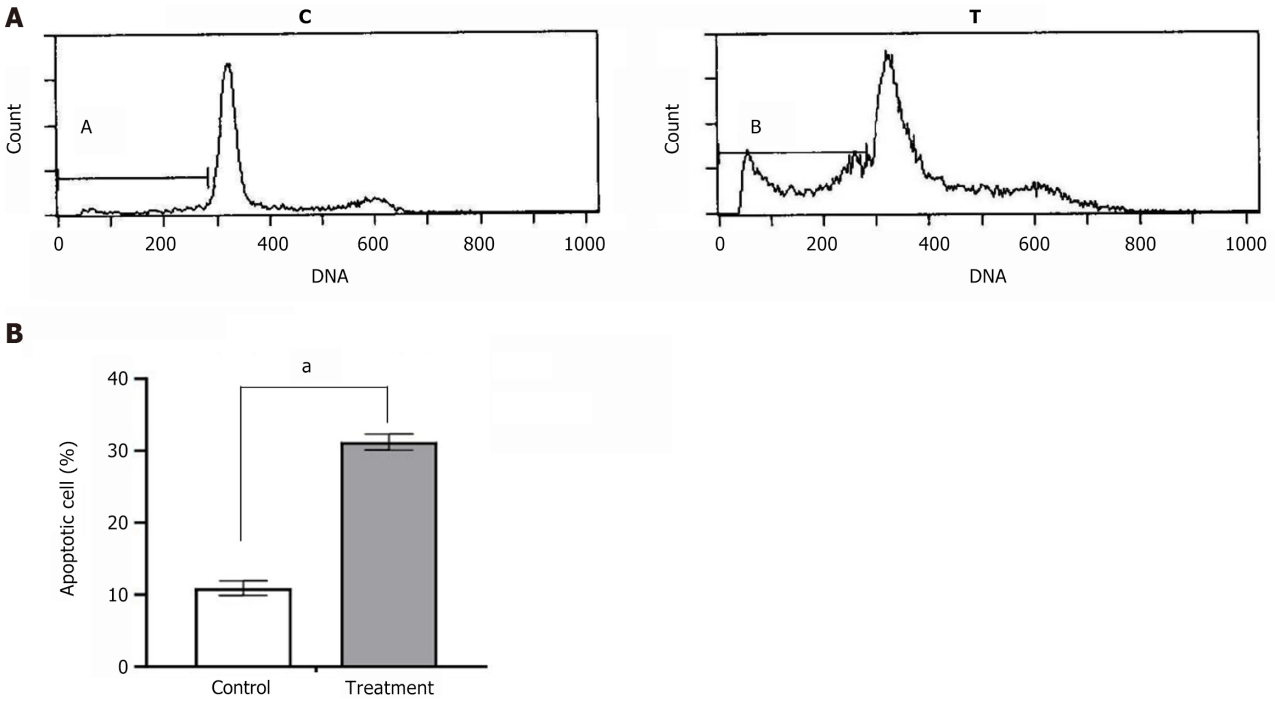


Figure 3 Pathological changes of LoVo cells after treatment with 140 μg/mL *Pinus massoniana* bark extract for 24 hours or not by flow cytometry analysis. A: Control group, a normal cell cycle distribution of an untreated LoVo cell line stained with propidium iodide (PI), there is no obvious apoptotic curve- sub-G1 curve but with a percentage of 10.9% spontaneous apoptosis. Treatment group, altered cell cycle distribution of a treated LoVo cell line stained with PI, there is an obvious apoptotic curve-sub- G1 curve before G1 curve with a gross percentage of 31.4% induced apoptosis; B: Statistical histogram of LoVo cell apoptosis induced by PMBE treatment compared with Control. $^{a}P < 0.05$ *vs* Control, $n = 3$, mean ± SD. C: Control group; T: Treatment group.

DISCUSSION

Through culturing cells once and subsequent OCTPP, obtain enough experimental materials to detect the target cell apoptosis with different instruments, reaching the simultaneous identification of apoptosis at morphological, biochemical, cell cycle, and DNA content level, respectively, further realize the unity of quantitative and qualitative analyses, shortening the experimental time obviously, while producing relatively good results.

Current measures for apoptosis identification involve detecting morphological, biochemical, and DNA content changes. At morphological level, fluorescence microscopy requires culturing cells on coverslips until monolayers form, which involves in the complicated steps and the inferior picture quality due to cell-cell junctions. What's more, it is qualitative other than quantitative. At molecular level, DNA for gel electrophoresis was not extracted classically after cells are lysed with buffer, not only time-consuming, but also potentially toxic, and possibly shearing DNA as well, further decreasing the reproducibility of experiments. Although pulse alternative field gel electrophoresis can avoid shearing DNA *via* pre-embedding cells in low-melting-point agarose, which will increase the time taken and the cost of the experiments. Moreover, these DNA detection methods are qualitative, or at most semi-quantitative. By contrast, flow cytometry is simple, rapid, quantitative, and multi-parametric.

Performing these three independent experiment results analyses (morphological, biochemical, and DNA content analyses) is time consuming, and the experimental materials are frequently not from the same cell culture batch, making it hard to ensure the consistency and veracity of the results. Herein, prefixed the cells in 70% cold ethanol, then 0.2 M phosphate-citrate buffer at pH7.8 was used to extract DNA, and then spin 100 *g*, 5-10 minutes to make the smaller DNA leaked out thoroughly. The supernatant was then sequentially incubated with RNase A and proteinase K, further for direct electrophoresis. The cell pellets were stained with PI, a proportion of which was for slide preparation and fluorescence microscope observation, the remaining for flow cytometry. OTCPP allows the rapid identification of the classical hallmarks of apoptosis, which owns merits as follows: (1) 70% cold ethanol fixation makes cells stored for relatively longer time before analysis, without any significant DNA degradation from pathogenic infection and cell autolysis; (2) Multiple targets (nuclear shrinkage, DNA ladder and sub-G1 curve) can be detected almost simultaneously, shortening the experimental time sharply; (3) Culturing cells on coverslips for nuclear morphology observation can be omitted completely; (4) DNA extraction is simple and rapid, with no phenol, chloroform, or other toxic reagents, and the inconvenience of pulse field gel electrophoresis; (5) 0.2 M phosphatecitrate buffer (pH 7.8) helps the smaller DNA fragments completely extruded out of apoptotic cells[8], increasing the sensitivity and specificity of flow cytometry; and (6) The same experimental samples can be ensured, enhancing the consistency during experimentation. Briefly and vividly, OTCPP successfully achieved three birds of cell apoptosis in one stone. Thus, OTCPP is an improvement on classical methods of apoptosis identification, and researchers can select it according to their different purposes and own experimental conditions. However, OTCPP might have potential limitations, unsuitable for long-lived cells lacking or just weak proliferative ability, *e.g.* neural cells, myocardial cells, and stem cells with relatively small numbers in different

tissues in organisms.

CONCLUSION

The present study presents a novel selective procedure for the rapid identification of human colon cancer LoVo cell apoptosis induced by the natural product, PMBE, using the OTCPP protocol. The use of OTCPP might pave the way for future basic and clinical research by optimizing experimental conditions (*e.g.* single-molecular augmented capture and imaging) to improve detection sensitivity in other cell types and/or disease models, *e.g.* neurons, cardiomyocytes and stem cells in critical diseases.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my laboratory mates for their invaluable assistance which significantly improved this work.

FOOTNOTES

Author contributions: Cui YY designed and performed the research, and analyzed the data and wrote the manuscript.

Supported by 2023 Key R&D Projects of Tongji University, No. 150029607160-24323; and Industrial Key Program Foundation of Guangdong Province: R&D of Natural Novel Anticancer Drugs, No. 2004B10401033.

Institutional review board statement: No human and/or animal subjects was involved in the present study.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: No additional data are available.

Country of origin: China

ORCID number: Ying-Yu Cui 0000-0002-6324-0795.

S-Editor: Qu XL
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Kerr JF**, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 1972; **26**: 239-257 [PMID: 4561027 DOI: 10.1038/bjc.1972.33]
2 **Nössing C**, Ryan KM. 50 years on and still very much alive: 'Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics'. *Br J Cancer* 2023; **128**: 426-431 [PMID: 36369364 DOI: 10.1038/s41416-022-02020-0]
3 **Darzynkiewicz Z**, Bruno S, Del Bino G, Gorczyca W, Hotz MA, Lassota P, Traganos F. Features of apoptotic cells measured by flow cytometry. *Cytometry* 1992; **13**: 795-808 [PMID: 1333943 DOI: 10.1002/cyto.990130802]
4 **Costigan A**, Hollville E, Martin SJ. Discriminating Between Apoptosis, Necrosis, Necroptosis, and Ferroptosis by Microscopy and Flow Cytometry. *Curr Protoc* 2023; **3**: e951 [PMID: 38112058 DOI: 10.1002/cpz1.951]
5 **Hotz MA**, Gong J, Traganos F, Darzynkiewicz Z. Flow cytometric detection of apoptosis: comparison of the assays of in situ DNA degradation and chromatin changes. *Cytometry* 1994; **15**: 237-244 [PMID: 8187583 DOI: 10.1002/cyto.990150309]
6 **Gong J**, Traganos F, Darzynkiewicz Z. A selective procedure for DNA extraction from apoptotic cells applicable for gel electrophoresis and flow cytometry. *Anal Biochem* 1994; **218**: 314-319 [PMID: 8074286 DOI: 10.1006/abio.1994.1184]
7 **Cui YY**, Xie H, Wang JF. [Transient detection of apoptosis of human liver cancer cells induced by Pinus massoniana bark extract (PMBE) *in vitro*]. *Zhongguo Zuzhihuaxue Yu Xibaohuaxue Zazhi* 2005; **14**: 80-83
8 **Zhu XQ**, Wang GS, Zhang XJ, Zhao Q. [Effects of sodium ferculate on the viability and apoptosis of human hepatoma BEL-7404 cells]. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 715-716

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 101057

DOI: 10.5662/wjm.v15.i3.101057

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Basic Study

Comparative evaluation of retentive capacity of three different attachment systems for implant retained overdentures: An *in vitro* study

Radha Chauhan, Narendra Padiyar, Pragati Kaurani, Ajay Gupta, Sachin Chauhan

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade C

Novelty: Grade B, Grade B

Creativity or Innovation: Grade B, Grade C

Scientific Significance: Grade B, Grade C

P-Reviewer: Hussain S

Received: September 3, 2024

Revised: November 19, 2024

Accepted: December 18, 2024

Published online: September 20, 2025

Processing time: 184 Days and 2.3 Hours

Radha Chauhan, Narendra Padiyar, Pragati Kaurani, Ajay Gupta, Department of Prosthodontics and Crown & Bridge, Mahatma Gandhi Dental College and Hospital, Jaipur 302022, Rājasthān, India

Sachin Chauhan, Department of Conservative Dentistry and Endodontics, Sudha Rustagi College of Dental Sciences and Research, Faridabad 121002, Haryāna, India

Corresponding author: Sachin Chauhan, Department of Conservative Dentistry and Endodontics, Sudha Rustagi College of Dental Sciences and Research, 1519 Sector 28, Faridabad 121002, Haryāna, India. drsachinchauhan13@gmail.com

Abstract

BACKGROUND

The primary issue in managing edentulous patients is the severely resorbed mandibular ridge, particularly in older individuals with diminished adaptive capacities. This compromised situation leads to the fabrication of inadequate dentures that lack retention and stability, potentially causing psychosocial issues.

AIM

To determine the difference in retentive capacity between three attachment systems in implant-retained overdentures.

METHODS

Three edentulous mandibular models were fabricated using heat-cured polymethacrylate resin, with two implant replicas placed in the intra-foraminal region of each model. 30 acrylic resin mandibular overdentures were fabricated with provisions for three different overdenture attachment systems: A prefabricated ball/O-ring attachment, a locator attachment system, and an equator attachment system. Each model was subjected to 15000 pulls using a universal testing machine to remove the overdenture from the acrylic model and the force data were recorded.

RESULTS

The ball/O-ring attachment system demonstrated superior retentive capacity for 15 years, while the locator and equator attachment systems maintained excellent retentive capacity for 5 years.

CONCLUSION
The ball/O-ring attachment system outperformed better than the other two attachment systems regarding retentive capacity. The locator and equator attachment systems presented sufficient retentive abilities until 15000 cycles. After 7500 cycles, significant differences in retentive force between the systems evolved.

Key Words: Dentures; Overdenture attachment systems; Equator; Dislodging cycles; Retentive capacity

Core Tip: The extensively resorbed mandibular ridge is the most common issue when treating edentulous patients, particularly as they age and lose some of their adaptive abilities. This often leads to the fabrication of unsatisfactory dentures with low retention and stability, potentially exacerbating psychological issues. In contrast, patients with maladaptive dentures showed excellent clinical outcomes with implant-supported overdentures. For edentulous patients, the mandibular 2-implant overdenture is the preferred treatment approach.

Citation: Chauhan R, Padiyar N, Kaurani P, Gupta A, Chauhan S. Comparative evaluation of retentive capacity of three different attachment systems for implant retained overdentures: An *in vitro* study. *World J Methodol* 2025; 15(3): 101057
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/101057.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.101057

INTRODUCTION

Reduced adaptive capabilities are the most common problem with severely resorbed mandibular ridges in complete dentures, resulting in poor retention and stability[1-3]. With high success rates, edentulous patients have received various prosthetic treatment options using osseointegrated implants[4-6]. An implant-supported overdenture improves aesthetics and oral hygiene, simplifies fabrication, and is more cost-effective[7,8]. The long-term functionality of implant-supported overdentures relies significantly on the attachment system's retentiveness[9]. Nowadays, implant-supported overdentures use a variety of attachment systems to improve their functionality[10,11]. Clinical applications with tooth-supported or implant-supported overdentures use ball attachments, and locator attachments are the most accessible types of attachments[12-16]. When prosthetic space is limited, a novel attachment method called the "OT Equator" with the smallest vertical height and diameter was designed[17]. An implant-retained overdenture attachment should have appropriate retentive characteristics that improve prosthesis retention while allowing for the patient's simple placement and removal[18].

Physical nature and retention, such as frictional contacts, mechanical interlocking, or magnetic forces, may influence the degree of retentive force[19-21]. Therefore, consuming food and liquids while chewing, wearing, and removing the prosthesis is likely to affect the retentive capacity of the attachment systems. During mastication and insertion, micro and macro movement happens between the retentive surfaces of an attachment system. Over time, taking out the overdenture causes wear and lowers the retentive pressure[22]. The purpose of this *in vitro* study was to compare and evaluate the retentive capacity of three different attachment systems for implant-supported overdentures, *i.e.*, the ball, the locator, and the equator system. The retentive capacity of each system was evaluated after multiple simulated insertion-removal cycles. The retentive values remained constant after many simulated insertion and removal cycles.

The objectives include: (1) Measure the retentive capacity of three different implant-retained overdenture attachment systems at the 1st, 7500th, and 15000th levels; (2) Compare the retentive capacity of the three implant-retained overdenture attachment systems at the 1st, 7500th, and 15000th; (3) Measure the retentive strength of three implant-retained overdenture attachment systems changes after being put in and taken out several times in a simulated manner at the 1st, 7500th, and 15000th stages; and (4) Compare the changes in the retentive capacity of three different attachment systems after multiple simulated insertion-removal cycles at the 1st, 7500th, and 15000th stages.

MATERIALS AND METHODS

The mandibular edentulous acrylic resin models were made with heat-polymerized polymethyl methacrylate resin (DPI Heat Cure, DPI, Mumbai, Maharashtra, India). The two implant replicas (Collagen Meniscus Implant) have a diameter of 3.75 mm and a length of 10 mm were placed. The acrylic resin mandibular overdentures were fabricated using heat-polymerized polymethyl methacrylate resin (DPI Heat Cure, DPI, Mumbai, Maharashtra, India). A prefabricated ball/O-ring attachment (Bioline dental implant series), OT Equator® attachment (Rhein 83, Bologna, Italy), Locator® attachment (Zest Anchor, Escondido, CA, United States), resin cement (RelyxTM, 3M ESPE, United States), universal testing machine (Instron 5567), manual thermocycling unit (two S-U-Polytub, Schuler Dental, Germany), and surveyor table and metallic clips were used in this study.

This study, which adhered strictly to all pertinent ethical principles, received approval from the ethical scientific committee of the local institution (Mahatma Gandhi Dental College and Hospital, Jaipur, India). The study model was fabricated using three wax patterns of standard mandibular edentulous models, which were made using modeling wax (Figure 1). Each wax model inserted two implant replicas in a parallel direction within the osteotomy site in the mandible at sites B and D. According to Misc *et al*[23], the two implants were separate, running alongside each other, positioned at the same horizontal level, perpendicular to the occlusal plane, and equidistant from the midline. We assessed their parallelism at the implant site by employing paralleled pins. Acrylic wax models were subsequently created using the compression molding technique (Figure 2).

Group 1 used the pre-fabricated ball/O-ring attachment (Bioline dental implant series). In pre-fabricated ball/O-ring attachment, a diameter of 2 mm, a metallic housing with a rubber O-ring component was used. Group 2 used the OT Equator® attachment (Rhein 83, Bologna, Italy). In pre-fabricated OT equator attachments, a diameter of 2 mm and a metallic housing with a nylon insert were used. It has a castable Hader bar with a length of 22 mm, a diameter of 1.8 mm, and a gauge of 13. The nylon rider measures 5 mm in length and 2.6 mm in width, with a moderate retention rate. The nylon rider measures 5 mm in length and 2.6 mm in width, with a moderate retention rate. Group 3 used the Locator® attachment (Zest Anchor, Escondido, CA, United States). The implant replicas (Collagen Meniscus Implant, 3.75 mm × 10 mm) were put into the acrylic models using a physio dispenser, similar to an implant that would be put into an osteotomy site in the mandible. Locator® attachment (Zest Anchor, Escondido, CA, United States) has Tissue cuff length = 1.0 mm, diameter = 3.86 mm. The retention force for the male blue locator inserts is 6.7 N, and maximum convergence = 20°. The models were sealed with resin cement (RelyxTM, 3M ESPE, United States). Each attachment system was secured into the implant replicas on the acrylic resin model, placed the overdentures with the corresponding housing on it, and tightened to 35 N/cm.

Experimental setup

Acrylic-made edentulous mandibles hold acrylic overdentures with their respective attachment systems (Figure 3). Some metallic clips were attached to the dentures and secured with clear auto-polymerized acrylic resin (Figure 4). The edentulous acrylic models were fixed in place using a surveyor table (Figure 5 and Figure 6).

Retention force testing

The Instron 8874 universal testing machine was set to perform 15000 insertion and de-insertion cycles on each denture specimen. There were cycles of a 2 mm upward movement at a crosshead speed of 50 mm/minute, followed by a downward movement with the same characteristics created in universal testing machine. The test machine was programmed for frequency and recorded retention strength data for each specimen over 15000 cycles. The readings were recorded from the start of the test, along with the retention force data for each cycle, ranging from 1 to 15000. The means of thirty values from the 1st, 500th, 1500th, 3000th, 4500th, 6000th, 7500th, and 9000th cycles were calculated for statistical analysis, using a significant level *P* of 0.05.

RESULTS

Table 1 and Figure 7 display descriptive statistics for changing retention strengths in the three study groups throughout the cycle sequence outlined below. In group 3, average retention decreased from 22.460 ± 2.2 N at baseline to 11.79 ± 1.4 N after 15000 cycles. The equator system reduced retention from 19.2 ± 2.7 N to 11.2 ± 1.7 N, while the ball attachment system decreased from 22.62 ± 2.1 N to 13.78 ± 3.1 N at the end of the cycle series. The graph depicts the distribution of retention values across the three groups. All three groups showed statistically significant differences in the retentive value at the 7500th and 15000th cycles. Three systems demonstrated enhanced retention strength after the initial 1500 cycles. Until the 4500-cycle, the locator and equator systems showed similar retention levels. However, there was more fluctuation in the ball attachment group. Up to the 4500th cycle, the locator and equator groups had higher median values, with the most significant disparity observed in the ball attachment system.

The Kruskal-Wallis test was performed. Once again, there were notable disparities in retention values across the whole cycle, except the 500th cycle. Compared to the baseline value, the locator group experienced a mean percentage change of 36.3% and 90.5% at the 7500th and 15000th cycles, respectively. In the equator group, 31.1% and 46.1% had a change in value from the baseline at the 7500th and 15000th cycles, respectively. The percentage change in the ball attachment group was 23.1% and 39.1% at the same cycles of the baseline value, respectively. A nonparametric statistical method known as the Kruskal-Wallis test compares the medians of two or more groups of data. This test evaluates whether the medians of the groups are identical. We randomly select the samples to ensure the independence of the observations and to maintain a minimum ordinal measurement scale for the dependent variable. When we violate the assumptions of normality and homogeneity of variance, this method becomes useful. This method proves advantageous when analyzing data that deviates from the normal distribution, such as microbiome data in health research. The test establishes and ranks the data from smallest to largest, sums the ranks in each subgroup, and determines the statistic *H* value. The null hypothesis states that the medians of both groups are equal. We reject the null hypothesis if the H statistic value demonstrates significance [20]. We used the Kruskal-Wallis test (Table 2, Figure 8) to compare the initial and end retention, revealing substantial disparities in median values. This suggests that the ball attachment method experienced reduced retention loss during the fatigue testing cycles. The mean retentive force for the ball attachment system was 22.620 N, 17.390 N, and 13.88 N from the baseline at the 7500th and 15000th cycles, respectively. The Locator® attachment system came in second with 22.46 N (base), 14.3 N (cycle 7500), and 11.79 N (cycle 15000). And the OT Equator® attachment system came in third with 19.2 N

Table 1 Evolution of retention strength in all groups according to number of insertion/de-insertion cycle, ball attachment system, equator attachment system, and locator attachment system

Cycles	Min	Max	Mean	Standard deviation	Mean differences	Change, %	*P* value	*P* value *vs* 1 cycle
Ball attachment system								
1	21.8	23.9	22.620	0.7899			0.001 (S)	
7500	12.0	15.1	17.390	0.7430	5.23	23.1		0.001 (S)
15000	15.9	18.5	13.78	0.79	8.84	39.1		0.001 (S)
Equator attachment system								
1	17.4	20.1	19.200	0.7318			0.001 (S)	
7500	12.5	14.2	13.22	0.48	5.98	31.1		0.001 (S)
15000	10.3	12	11.2	0.53	8	41.6		0.001 (S)
Locator attachment system								
1	21.4	23.6	22.460	0.7230			0.001 (S)	
7500	13.5	14.8	14.3	0.46	8.16	36.3		0.001 (S)
15000	11.3	12.7	11.79	0.37	10.67	90.5		0.001 (S)

Table 2 Kruskal-Wallis test results ($P < 0.05$) and mean values

Circles	*P* value
Initial	0.001
500	0.2
1500	0.001
3000	0.001
4500	0.001
6000	0.001
7500	0.001
9000	0.001
15000	0.001

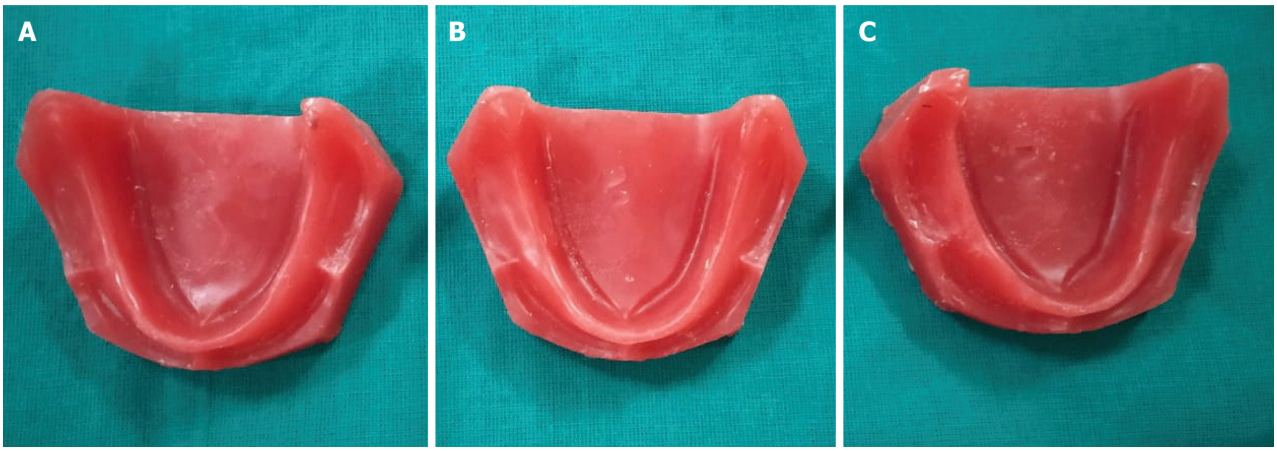


Figure 1 Wax models. A: Ball/O-ring attachment; B: Locator attachment system; C: Equator attachment system.

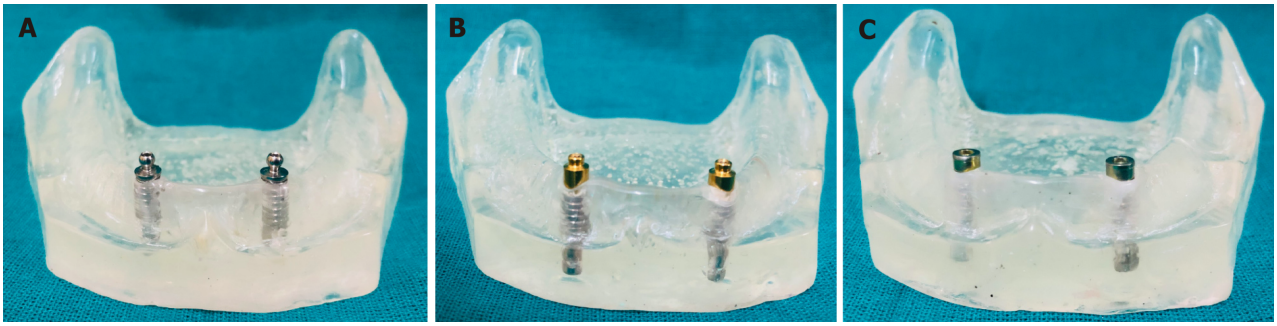


Figure 2 Attachments. A: Ball/O-ring attachment; B: Locator attachment system; C: Equator attachment system.

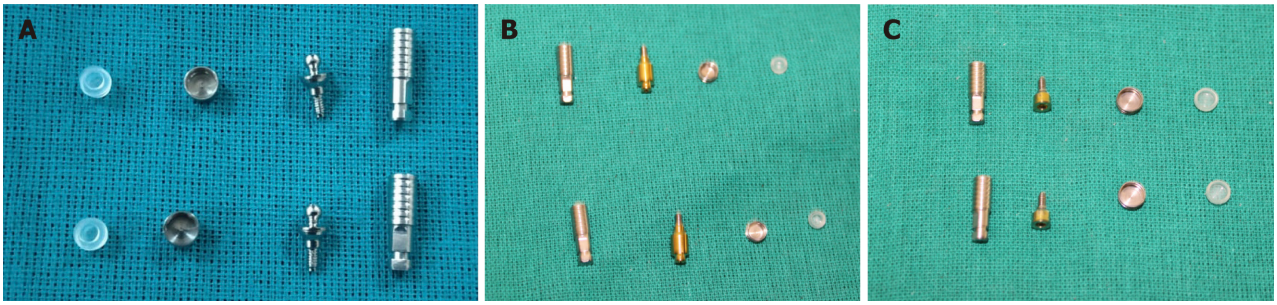


Figure 3 Attachment assembly. A: Ball/O-ring attachment; B: Locator attachment system; C: Equator attachment system.

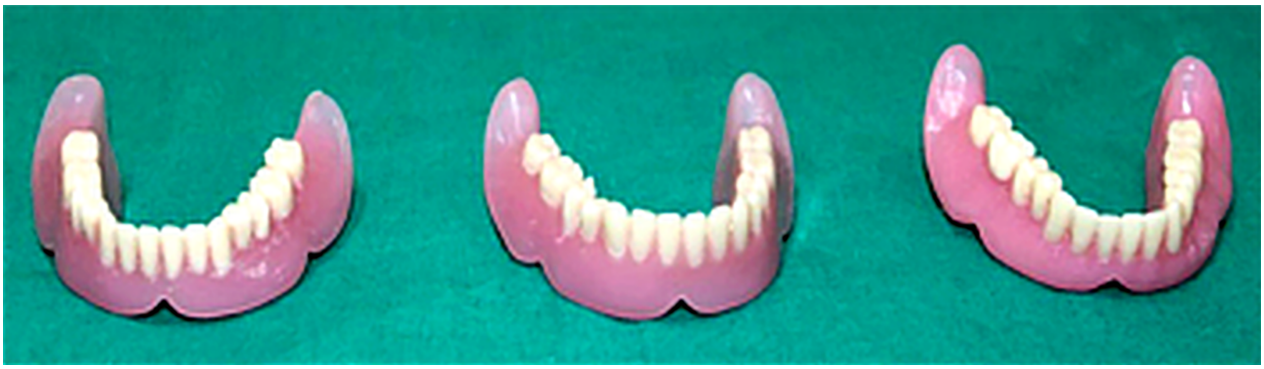


Figure 4 Mandibular dentures fabricated in a conventional manner using heat-cured acrylic resin.

(base), 13.22 N (cycle 7500), and 11.2 N (cycle 15000). All three groups showed statistically significant differences in the retentive value at the 7500th and 15000th cycles.

DISCUSSION

Complete dentures pose a challenge for retention because of the complex oral musculature and its attachments. In most edentulous patients, decreased retention in prostheses due to bone resorption causes mastication discomfort and dissatisfaction with conventional complete dentures. Implant placement in a completely edentulous mandibular arch is one of the treatment options for retaining or supporting long-term restorations[24]. Mandibular implant-retained prostheses outperform conventional dentures in each aspect, potentially overshadowing the challenge of excellent retention[2]. The purpose of this *in vitro* study was to compare and rate how well the ball, equator, and locator attachment systems held their shape during fatigue testing, which involved putting and taking out the attachments up to 15000 times. This *in vitro* study simulated two implant-retained overdentures by placing two implant analogs 23 mm apart at the canine eminence. For completely edentulous patients, this is considered the minimum standard of care. The procedure involved vertically separating the denture from the base. Fatigue or failure of overdenture attachments adversely affect their function, maintenance, and patient satisfaction. We generated 15000 pulls of separation for each specimen to assess the retentive capacity of the attachment systems. In their study, Al-Ghafli *et al*[25] found that if someone used the prosthesis every day for ten years, it would separate 15000 times, assuming they took out and put back in the overdenture four times a day.

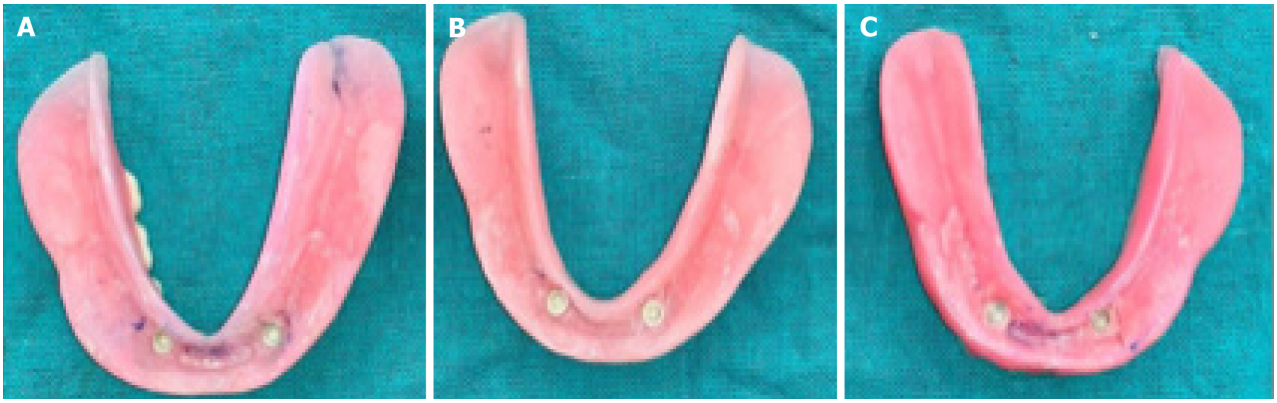


Figure 5 Acrylic resin overdenture. A: O-ring housing for ball attachment; B: Equator metallic housing and nylon insert; C: Locator metallic housing and male insert.

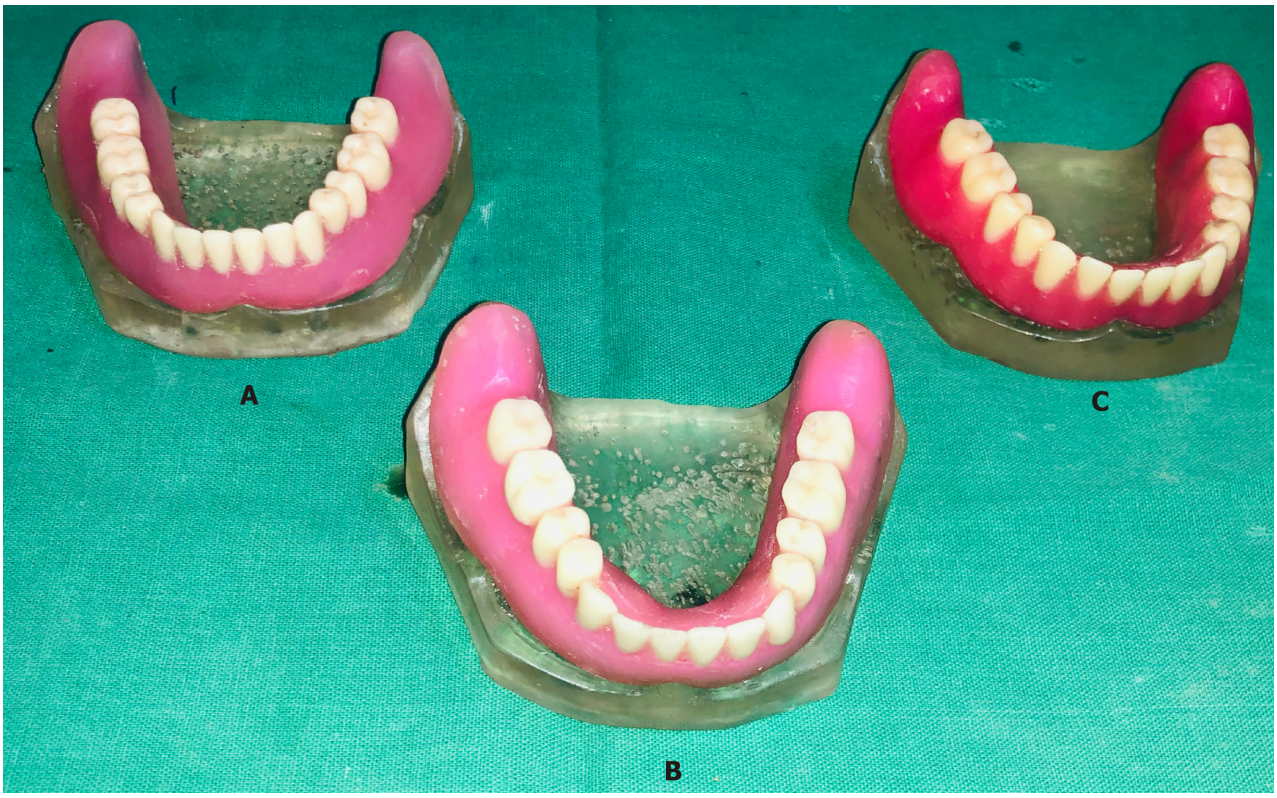


Figure 6 Mandibular overdenture models. A: O-ring housing for ball attachments; B: Equator metallic housing and nylon insert; C: Locator metallic housing and male insert.

The most common instrument for vertical separation and peak load force testing in *in vitro* studies is the Instron universal testing machine. We used an upward movement of 2 mm at a crosshead speed of 50 mm/minute, which approximates the rate at which patients remove the implant overdenture[9]. Although the speed of over-denture removal remains unproven, Sarnat *et al*[26] proposed that it could approximate the speed at which a real overdenture detaches from its holding elements under vertical force. We subjected the obtained results to statistical analysis and conducted comparisons to assess the retentive ability during different cycles corresponding to previous years of denture usage. The results indicated differences in peak load-to-dislocation between the three types of attachments. The ball attachment (28.4 ± 5.86 N) showed the maximum amount of retention, followed by the equator attachment (26.9 ± 7.75 N) and the locator attachment (26.6 ± 4.14 N).

Further data was analyzed to determine the percentage changes in the three attachment systems. The ball and locator systems showed similar characteristics at baseline, with a mean retention capacity of 22.6 N and 22.4 N, respectively, and the Equator system showed a retention capacity of 19.2 N. However, at the 7500th cycle, there was a reduction of 23.1% for the ball attachment system, 31.1% for the equator, and 36.3% for the locator attachment. After that, the decline in retentive values continued for all three attachment systems until the 15000th cycle, when the ball attachment system showed a mean retention of 13.7 ± 8.84 N (reduction by 39.1%) and the locator system demonstrated a mean retention of 11.79 ± 10.67 N

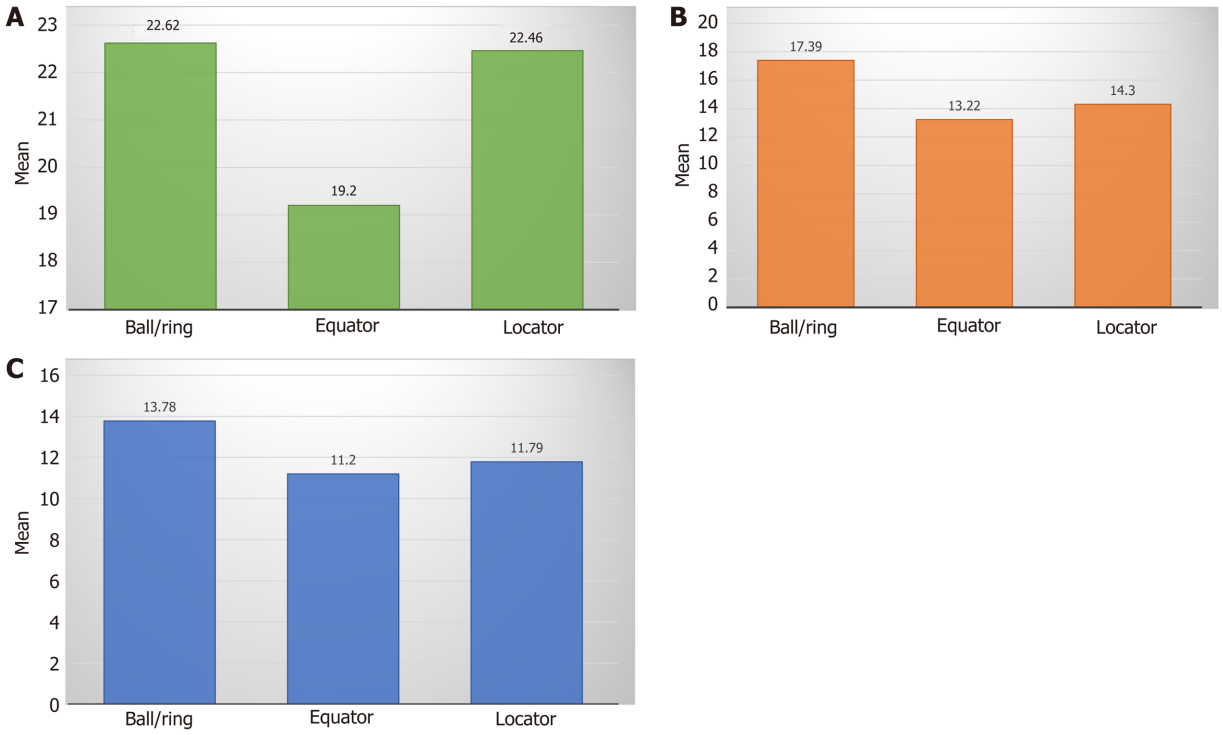


Figure 7 Mean values. A: Mean values of the retentive capacity of three attachment systems at cycle 1; B: Mean values of the retentive capacity of three attachment systems at cycle 7500; C: Mean values of the retentive capacity of three attachment systems at cycle 15000.

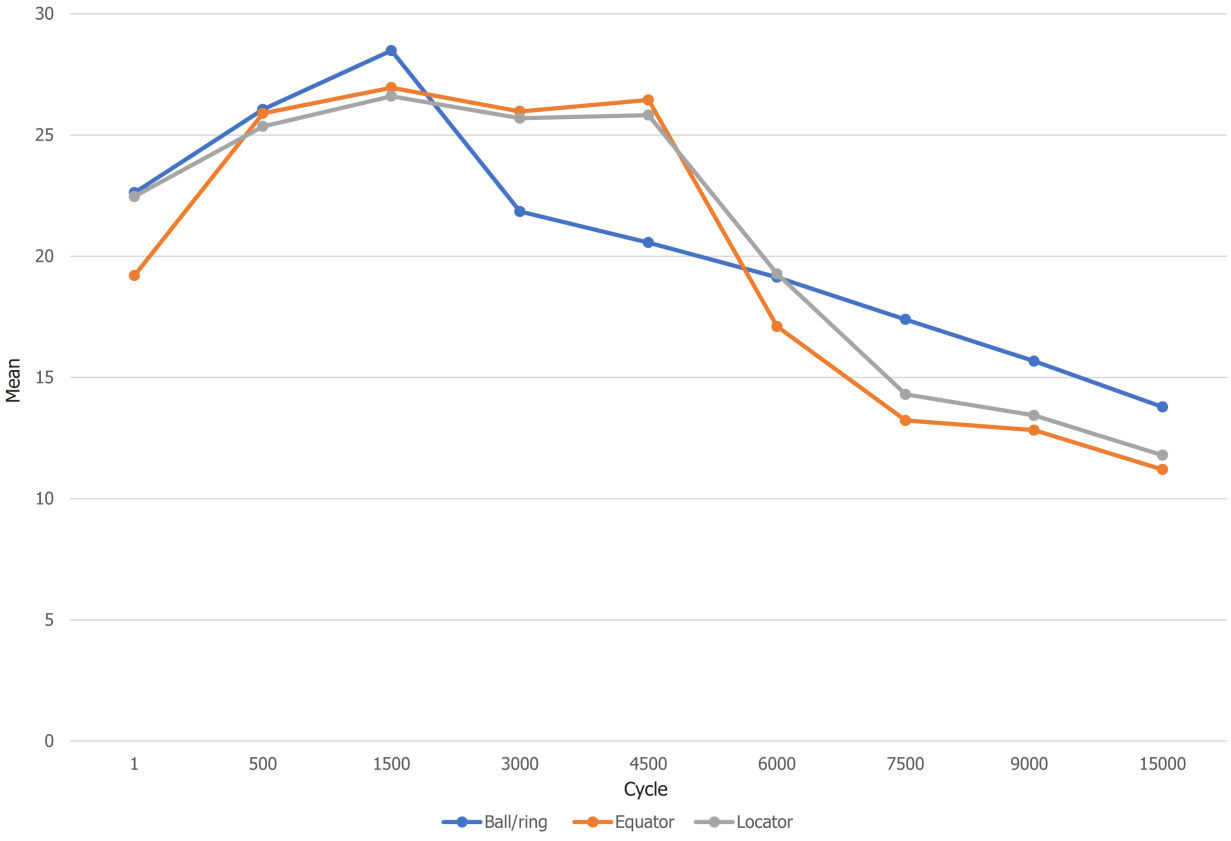


Figure 8 Kruskal-Wallis test results ($P < 0.05$) and mean values.

(reduction by 90.5%). In contrast, the OT equator had a mean value of 11.2 ± 8 N (a decrease of 41.6%).

The results indicated that the three attachment systems maintained or increased retention until around three years of usage. Ball attachment systems showed the highest retention until one year, when the patient inserts an overdenture four times daily. Equator and locator showed their highest retention for 3 years. There is a direct association between adequate retention, improved patient satisfaction, and increased quality of life. Various studies[27-30] have shown that forces varying between 10 and 20 N are sufficient for minimum retention in mandibular ODs, ensuring stability. Mínguez-Tomás *et al*[31] in their study evaluated retentive capacity in different attachment systems and found sufficient retention capacities after 14600 cycles and provided more precise data on attachment wear and retention loss.

Al-Ghafli *et al*[25] suggested some of the factors influencing retention loss, like the number and position of implants, the type of material used to fabricate the attachments, prosthetic design, and forces of different magnitudes in different directions. Conversely, Rutkunas *et al*[9] proposed that friction between the male and female retention elements, resulting from deformation or dimensional changes in the inner diameter of attachment nylon inserts, causes wear during the simple daily use of prosthesis. Different groups of researchers did an *in vitro* study that measured retention force in several different areas. These included fatigue (after 100, 200, 500, 1000, and 5000 dislodgement cycles), thermal undulation (10000 cycles at 5 and 55 degrees), implant angulation (0, 5, and 10 degrees), and disinfectants (three different agents). Repeated dislodging and thermal undulation did not alter the retention forces. Locator attachments showed a notable reduction in retention force of up to 58%. Implant angulation did not induce any significant changes in retention forces[24-27,31].

This study's result suggests that the ball attachment system in OD-1 maintains its superior retentive capacity until it begins to decline one year later. Still, the equator and locator maintain excellent retention for 3-4 years and then show rapid decline thereon. After 5 years, all three maintain good retention - even up to 10 years in the testing scenario. This could be due to the ball attachment system, which consists of a titanium male unit and a rubber-ring female unit. This system transfers stress to the abutments and provides an excellent shock-resorbing effect during operation. This can be due to the design of the ball attachment, which provides a bigger mechanical undercut. We performed this study in a controlled experimental setting to evaluate the retentive capacity of three different systems used in implant-supported overdentures, but we only applied mono-directional forces during the evaluation. This approach does not represent a realistic model for a clinical situation with overdentures. Therefore, the first molars generate the main forces, which in turn generate rotational forces on the attachments through leverage[32-34].

CONCLUSION

Given the limitations of this *in vitro* study, the following conclusion can be drawn: (1) The ball/O-ring attachment system showed superior retentive capacity among all three attachment systems; (2) Locator, OT equator, and ball attachment systems maintain clinically acceptable retention after 10 years; (3) Retention increases from baseline values until around the 1500th cycle mark in the ball attachment system; (4) Retention values were similar for the locator and equator attachment systems until the 4500th cycle; and (5) All three groups showed statistically significant differences in the retentive value at 7500th and 15000th. The ball attachment and locator attachment systems maintain their retentive capacity longer than the OT equator attachment systems. All three attachment systems showed a statistically significant decrease in the retention force. Further research is required to understand the loss in the retention force of various overdenture attachment systems.

FOOTNOTES

Author contributions: Padiyar UN was responsible for conception and supervision; Kaurani P designed the study and reviewed literature; Chauhan R collected data and wrote the manuscript; Chauhan R, Chauhan S, and Gupta A analyzed and interpreted data.

Institutional review board statement: This study received approval from the ethical scientific committee of the local institution (Mahatma Gandhi Dental College and Hospital, Jaipur, India).

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The dataset is available from the corresponding author atdrsachinchauhan13@gmail.com.

Country of origin: India

ORCID number: Sachin Chauhan 0000-0003-4800-3959.

S-Editor: Wei YF
L-Editor: A

P-Editor: Zhang YL

REFERENCES

1 **Gunne HS**, Bergman B, Enbom L, Högström J. Masticatory efficiency of complete denture patients. A clinical examination of potential changes at the transition from old to new denture. *Acta Odontol Scand* 1982; **40**: 289-297 [PMID: 6960628 DOI: 10.3109/00016358209024072]

2 **Bergman B**, Carlsson GE. Clinical long-term study of complete denture wearers. *J Prosthet Dent* 1985; **53**: 56-61 [PMID: 3882949 DOI: 10.1016/0022-3913(85)90066-6]

3 **van Waas MA**. The influence of clinical variables on patients' satisfaction with complete dentures. *J Prosthet Dent* 1990; **63**: 307-310 [PMID: 2308091 DOI: 10.1016/0022-3913(90)90202-n]

4 **Zitzmann NU**, Marinello CP. A review of clinical and technical considerations for fixed and removable implant prostheses in the edentulous mandible. *Int J Prosthodont* 2002; **15**: 65-72 [PMID: 11887602]

5 **Adell R**, Eriksson B, Lekholm U, Brånemark PI, Jemt T. Long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. *Int J Oral Maxillofac Implants* 1990; **5**: 347-359 [PMID: 2094653]

6 **Nogawa T**, Takayama Y, Ishikawa M, Yokoyama A. The impact of an additional implant under the saddle of removable partial dentures in Kennedy Class II edentulism on oral health-related quality of life and oral function: a case series report. *Int J Implant Dent* 2022; **8**: 60 [PMID: 36454445 DOI: 10.1186/s40729-022-00463-x]

7 **Kim SM**, Choi JW, Jeon YC, Jeong CM, Yun MJ, Lee SH, Huh JB. Comparison of changes in retentive force of three stud attachments for implant overdentures. *J Adv Prosthodont* 2015; **7**: 303-311 [PMID: 26330977 DOI: 10.4047/jap.2015.7.4.303]

8 **Feine JS**, Carlsson GE, Awad MA, Chehade A, Duncan WJ, Gizani S, Head T, Lund JP, MacEntee M, Mericske-Stern R, Mojon P, Morais J, Naert I, Payne AG, Penrod J, Stoker GT, Tawse-Smith A, Taylor TD, Thomason JM, Thomson WM, Wismeijer D. The McGill consensus statement on overdentures. Mandibular two-implant overdentures as first choice standard of care for edentulous patients. Montreal, Quebec, May 24-25, 2002. *Int J Oral Maxillofac Implants* 2002; **17**: 601-602 [PMID: 12182304]

9 **Rutkunas V**, Mizutani H, Takahashi H, Iwasaki N. Wear simulation effects on overdenture stud attachments. *Dent Mater J* 2011; **30**: 845-853 [PMID: 22123008 DOI: 10.4012/dmj.2011-057]

10 **Carlsson GE**. Implant and root supported overdentures - a literature review and some data on bone loss in edentulous jaws. *J Adv Prosthodont* 2014; **6**: 245-252 [PMID: 25177466 DOI: 10.4047/jap.2014.6.4.245]

11 **Marin DOM**, Leite ARP, Oliveira Junior NM, Paleari AG, Pero AC, Compagnoni MA. Retention Force and Wear Characteristics of three Attachment Systems after Dislodging Cycles. *Braz Dent J* 2018; **29**: 576-582 [PMID: 30517481 DOI: 10.1590/0103-6440201802074]

12 **Sadowsky SJ**. Mandibular implant-retained overdentures: a literature review. *J Prosthet Dent* 2001; **86**: 468-473 [PMID: 11725274 DOI: 10.1067/mpr.2001.119921]

13 **Payne AG**, Solomons YF. Mandibular implant-supported overdentures: a prospective evaluation of the burden of prosthodontic maintenance with 3 different attachment systems. *Int J Prosthodont* 2000; **13**: 246-253 [PMID: 11203640]

14 **Gotfredsen K**, Holm B. Implant-supported mandibular overdentures retained with ball or bar attachments: a randomized prospective 5-year study. *Int J Prosthodont* 2000; **13**: 125-130 [PMID: 11203620]

15 **Kuroshima S**, Ohta Y, Uto Y, Al-Omari FA, Sasaki M, Sawase T. Implant-assisted removable partial dentures: Part I. a scoping review of clinical applications. *J Prosthodont Res* 2024; **68**: 20-39 [PMID: 37164658 DOI: 10.2186/jpr.JPR_D_22_00252]

16 **Zhang H**, Ramos V Jr, Bratos M, Liu PP, He W. Effect of the attachments on clinical outcomes of mandibular distal extension implant-supported removable partial dentures: A systematic review. *J Prosthet Dent* 2022; **128**: 1211-1220 [PMID: 34301416 DOI: 10.1016/j.prosdent.2021.04.008]

17 **See WL**, Khoo TL, Mohan M, Nimbalkar S, Patil PG. Effect of surgical and prosthodontic protocols of distal extension implant-supported removable partial dentures on clinical and patient-reported outcomes: A systematic review. *J Prosthet Dent* 2024 [PMID: 38653688 DOI: 10.1016/j.prosdent.2024.03.021]

18 **Trakas T**, Michalakis K, Kang K, Hirayama H. Attachment systems for implant retained overdentures: a literature review. *Implant Dent* 2006; **15**: 24-34 [PMID: 16569958 DOI: 10.1097/01.id.0000202419.21665.36]

19 **Nogawa T**, Saito M, Murashima N, Takayama Y, Yokoyama A. Influence of rigidity of retainers on dynamic behavior of implant-supported removable partial dentures. *Int J Implant Dent* 2020; **6**: 60 [PMID: 33089410 DOI: 10.1186/s40729-020-00260-4]

20 **Besimo CE**, Guarneri A. In vitro retention force changes of prefabricated attachments for overdentures. *J Oral Rehabil* 2003; **30**: 671-678 [PMID: 12791150 DOI: 10.1046/j.1365-2842.2003.01140.x]

21 **Ichikawa H**, Yoda N, Ogawa T, Iwamoto M, Kawata T, Egusa H, Sasaki K. Impact of implant location on load distribution of implant-assisted removable partial dentures: a review of in vitro model and finite-element analysis studies. *Int J Implant Dent* 2023; **9**: 31 [PMID: 37725286 DOI: 10.1186/s40729-023-00500-3]

22 **Rutkunas V**, Mizutani H, Takahashi H. Evaluation of stable retentive properties of overdenture attachments. *Stomatologija* 2005; **7**: 115-120 [PMID: 16501313]

23 **Misch CE**, Perel ML, Wang HL, Sammartino G, Galindo-Moreno P, Trisi P, Steigmann M, Rebaudi A, Palti A, Pikos MA, Schwartz-Arad D, Choukroun J, Gutierrez-Perez JL, Marenzi G, Valavanis DK. Implant success, survival, and failure: the International Congress of Oral Implantologists (ICOI) Pisa Consensus Conference. *Implant Dent* 2008; **17**: 5-15 [PMID: 18332753 DOI: 10.1097/ID.0b013e3181676059]

24 **van Waas MA**. [Neutral zone. An anatomic space which is often neglected while fabricating removable complete dentures]. *Ned Tijdschr Tandheelkd* 2011; **118**: 563-567 [PMID: 22235519 DOI: 10.5177/ntvt.2011.11.11126]

25 **Al-Ghafli SA**, Michalakis KX, Hirayama H, Kang K. The in vitro effect of different implant angulations and cyclic dislodgement on the retentive properties of an overdenture attachment system. *J Prosthet Dent* 2009; **102**: 140-147 [PMID: 19703620 DOI: 10.1016/S0022-3913(09)60134-7]

26 **Sarnat AE**. The efficiency of cobalt samarium (Co5Sm) magnets as retention units for overdentures. *J Dent* 1983; **11**: 324-333 [PMID: 6365997 DOI: 10.1016/0300-5712(83)90117-3]

27 **Bandiaky ON**, Lokossou DL, Soueidan A, Le Bars P, Gueye M, Mbodj EB, Le Guéhennec L. Implant-supported removable partial dentures

compared to conventional dentures: A systematic review and meta-analysis of quality of life, patient satisfaction, and biomechanical complications. *Clin Exp Dent Res* 2022; **8**: 294-312 [PMID: 35014207 DOI: 10.1002/cre2.521]

28 **Alsabeeha NH**, Payne AG, Swain MV. Attachment systems for mandibular two-implant overdentures: a review of in vitro investigations on retention and wear features. *Int J Prosthodont* 2009; **22**: 429-440 [PMID: 20095190]

29 **Setz I**, Lee SH, Engel E. Retention of prefabricated attachments for implant stabilized overdentures in the edentulous mandible: an in vitro study. *J Prosthet Dent* 1998; **80**: 323-329 [PMID: 9760365 DOI: 10.1016/s0022-3913(98)70133-7]

30 **Pigozzo MN**, Mesquita MF, Henriques GE, Vaz LG. The service life of implant-retained overdenture attachment systems. *J Prosthet Dent* 2009; **102**: 74-80 [PMID: 19643220 DOI: 10.1016/S0022-3913(09)60112-8]

31 **Mínguez-Tomás N**, Alonso-Pérez-Barquero J, Fernández-Estevan L, Vicente-Escuder Á, Selva-Otaolaurruchi EJ. In vitro retention capacity of two overdenture attachment systems: Locator® and Equator®. *J Clin Exp Dent* 2018; **10**: e681-e686 [PMID: 30057711 DOI: 10.4317/jced.54834]

32 **Shastry T**, Anupama NM, Shetty S, Nalinakshamma M. An in vitro comparative study to evaluate the retention of different attachment systems used in implant-retained overdentures. *J Indian Prosthodont Soc* 2016; **16**: 159-166 [PMID: 27141166 DOI: 10.4103/0972-4052.176520]

33 **Gallucci GO**, Doughtie CB, Hwang JW, Fiorellini JP, Weber HP. Five-year results of fixed implant-supported rehabilitations with distal cantilevers for the edentulous mandible. *Clin Oral Implants Res* 2009; **20**: 601-607 [PMID: 19302389 DOI: 10.1111/j.1600-0501.2008.01699.x]

34 **Yang TC**, Maeda Y, Gonda T, Kotecha S. Attachment systems for implant overdenture: influence of implant inclination on retentive and lateral forces. *Clin Oral Implants Res* 2011; **22**: 1315-1319 [PMID: 21426402 DOI: 10.1111/j.1600-0501.2010.02137.x]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 95559

DOI: 10.5662/wjm.v15.i3.95559

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Primary author contact for systematic reviews of randomized controlled trials: A systematic review

Vasiliki Sinopoulou, Eshan Shah, Morris Gordon, Tonia E Tony-Jimmy

Specialty type: Methodology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade C
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Xie Y

Received: April 19, 2024
Revised: November 1, 2024
Accepted: December 3, 2024
Published online: September 20, 2025
Processing time: 321 Days and 0.1 Hours

Vasiliki Sinopoulou, Morris Gordon, Biomedical Evidence Synthesis and Translation to Practice Unit, School of Medicine, Preston PR1 7BH, United Kingdom

Eshan Shah, Tonia E Tony-Jimmy, School of Medicine and Dentistry, University of Central Lancashire, Preston PR1 2HE, United Kingdom

Co-first authors: Vasiliki Sinopoulou and Eshan Shah.

Corresponding author: Morris Gordon, MBChB, PhD, Professor, Biomedical Evidence Synthesis and Translation to Practice Unit, School of Medicine, HA340, Harrington Building, 135A Adelphi St, Preston PR1 7BH, United Kingdom. mgordon@uclan.ac.uk

Abstract

BACKGROUND

Systematic reviews (SRs) synthesize and evaluate data, mainly from randomized trials, which then guides the development of clinical recommendations in evidence-based medicine. However, the data and methodological information in the included papers can often be lacking or unclear, and reviewers usually need to contact the authors of included studies for clarifications. Contacting authors is recommended, but it is unclear how often SR teams do it, or what the level of response is.

AIM

To investigate how often reviewers undertake contact with the authors of included randomized controlled trials (RCTs) for clarification on data and risk of bias concerns, to explore the factors that influence whether SR authors contact or do not contact the authors, and the content and level of responses.

METHODS

We conducted a systematic electronic database search in MEDLINE using the search string "(systematic review)" AND "(RCT OR randomized OR trial)" for articles published between 1 January 2024 and 19 February 2024, without language restrictions. Screening and data extraction was done independently by two reviewers, and conflicts resolved by a senior author. Contact authors of included SRs were contacted for clarifications.

RESULTS

Of the 329 included SRs, 38% (n = 125) explicitly mentioned contact with the authors of included studies. The remaining 62% (n = 204) did not. We attempted

contact with all SR teams for clarifications and received 90 responses (19.4%). Of the 50 respondents who did not explicitly mention contact in their SRs, 25 (50%) replied that they did make contact. We received a total of 64 responses on the level and content of information sought. The mean ± SD contacts SR teams made were 10 (10), replies received 5 (6.7), and response waiting time 10.1 (28.3) weeks. Resources, time, poor previous experience, perceived likelihood of poor response and bias concerns were reported as barriers to attempting contact.

CONCLUSION

The majority of SRs published in 2024 did not confirm seeking clarifying or missing information from primary study authors. However, SR teams reported that 50% of contacted primary authors respond. Additional research can clarify this rate of response and establish methods to increase the integration of this core methodological element in SRs.

Key Words: Systematic review; Methodology; Contacting authors; Interventions; Randomized control trials

Core Tip: We found that a majority of systematic review teams do not seek clarifying or missing information from primary study authors. Time and resources are seen as a barrier, however, we found that almost 50% of contacted primary authors were reported to respond. Contacting authors should be seen as a core methodological requirement for systematic reviewers, and further steps should be taken to investigate and promote it.

Citation: Sinopoulou V, Shah E, Gordon M, Tony-Jimmy TE. Primary author contact for systematic reviews of randomized controlled trials: A systematic review. *World J Methodol* 2025; 15(3): 95559
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/95559.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.95559

INTRODUCTION

In clinical medicine, evidence-based medicine has been the cornerstone of decision making for more than three decades, underpinned by systematic reviews (SRs) to develop clinical guideline recommendations[1]. Most SRs primarily include randomized controlled trials (RCTs) in their search and evidence synthesis process, as RCTs are the gold standard design for assessing the efficacy of a medical intervention[2]. This is derived from the reduced risk of bias inherent in the methods of an RCT.

There is substantial evidence that completeness and quality of methods information is still sporadically reported, even in high impact journals[3]. There is also evidence that authors often release different forms of data over time meaning that SR authors may have questions that need clarifying or data that needs confirming[4]. Missing data, due to inadequate reporting of summary statistics or overall findings when results are unfavourable or null, is also a challenge and represents a significant source of bias. This can be observed in situations where the authors may hold the notion that certain results do not add value to their publication or limit impact, or there are pressures to deliver positive results due to commercial demands[5]. Incomplete data that do not make it into SRs can result into healthcare decision makers making less-than-ideal choices for treatment protocols, resource allocation, and overall care, potentially resulting in poorer patient outcomes[6].

When conducting a SR, contacting the authors of eligible and included RCTs to clarify material or provide additional recorded data that may be missing is suggested[7]. This can be beneficial for SRs, as they are able to more clearly discern the risk of bias in each RCT and include RCTs that they would otherwise have had to discount. Their results would then be of a more representative sample and draw wider conclusions in their meta-analysis. Nonetheless, reviewers applying this guidance face challenges in identifying the authors of RCTs, frequently experience RCT authors failing to reply, and struggle with summarizing the process in their own SR. Missing info about quality appraisal can also be common.

The aim of this study was to investigate how often systematic reviewers undertake contact with the authors of included papers for clarification on data and risk of bias concerns. We explored the factors that influence whether SR authors contact or do not contact the authors of the included studies, and the content and level of response to the communication. We also explored variables including impact factor of journal and available funding on whether author contact was initiated or not.

MATERIALS AND METHODS

A protocol for this review was deposited in the online repository for the University of Central Lancashire prior to the initiation of this review[8].

This work was exempt from ethical approval as there were no ethics issues related to it. Only corresponding authors who have made their contact information publicly available for the purpose of being contacted about their publications were contacted, solely with questions about their publications.

The report of this review followed the preferred reporting items for SRs and meta-analyses reporting guidelines.

Literature search

We performed a systematic electronic database search of all the RCTs published between 1 January 2024 and 19 February 2024 from the single database MEDLINE on 19 February 2024. The search strategy was "systematic review" (Title/Abstract) AND ["RCT" (Title/Abstract) OR "randomized" (Title/Abstract) OR "trial" (Title/Abstract)]. All retrieved citations from this search were imported into Covidence and results de-duplicated.

Study selection

Two authors (Shah E and Tony-Jimmy TE) independently screened all titles and abstracts, discarding those not meeting inclusion criteria. Disagreements were resolved by discussion and consensus in the presence of a third and fourth author (Sinopoulou V and Gordon M). All SRs selected for full-text screening were downloaded and independently reviewed by two authors (Shah E and Tony-Jimmy TE) to confirm whether papers met the inclusion criteria. All differences were resolved by Gordon M and Sinopoulou V, in a similar manner.

Inclusion criteria for full-text SR manuscripts were: (1) Full-text SR manuscripts of RCTs involving human participants; (2) Full-text SR manuscripts of RCT with interventions for the management of symptoms, involving any pharmacological or non-pharmacological intervention compared to any other intervention, placebo, no treatment, or usual care; and (3) Full-text SR manuscripts with outcome measures that directly impact patient health or risk to health. SRs that included any phase RCTs were eligible. There were no limitations on language or region.

Exclusion criteria were: (1) Manuscripts which reported outcome data of non-randomized or quasi-randomized trials; (2) Manuscripts that reported on non-medical interventions such as service evaluation, delivery, safety, and education trials; (3) Manuscripts on in-vitro interventions; and (4) Manuscripts without outcome results (*e.g.* protocols, trial registrations).

Outcomes

The outcomes for this review were: (1) Whether contact with the included studies' authors was initiated or not; (2) Number of contacts initiated, and number of responses received when contact was initiated; (3) Time given to primary authors to respond; (4) Type of information requested when author contact was initiated; (5) Factors that prevented reviewers from contacting authors; and (6) Whether impact factor (higher or lower than 5) and funding (industry, publicly funded or not reported) are correlated with whether author contact was initiated or not.

Data extraction

Two authors (Shah E and Tony-Jimmy TE) independently extracted data using a predesigned extraction form and disagreements were resolved by a third and fourth reviewer (Sinopoulou V and Gordon M).

The extracted data were key characteristics such as year of publication, contact author name and email, journal source, impact factor of SR publication journal, funding sources, DOI; including quotations or any comments made regarding author contact and data for the above outcomes.

Missing information

SR correspondence authors were contacted *via* email at their listed correspondence email when outcome information was lacking. Authors were given two weeks to reply at which point a reminder was sent if no response was received, and an additional two weeks were given for response. Emails were sent and responses received in March 2024.

Statistical analysis

We calculated descriptive statistics as absolute numbers and percentages for all binary outcomes, and mean ± SD for continuous outcomes. We conducted χ^2 tests to test for differences in relation to impact factor and funding source.

RESULTS

Our search identified 866 unique papers. After abstract screening, 484 SR were excluded with reasoning, leaving 382 SRs that met our inclusion criteria for full text screening. Of those included, 3 SRs were not accessible for full text screening. Thus, 379 SRs were included for full text screening, of which 329 met the inclusion criteria and were included in the review (Figure 1).

No included SRs provided enough information for our data extraction in their text, and we attempted contact with the corresponding authors for all included SRs. The questions we asked were structured on the outcomes of this review as outlined in the methods section.

Was contact with the included studies' authors initiated or not

Of the 329 full-text SRs examined, 38% (n = 125) explicitly mention contact with the primary authors of included studies as part of their planned protocol and methods. The remaining 62% (n = 204) of the examined SRs did not explicitly

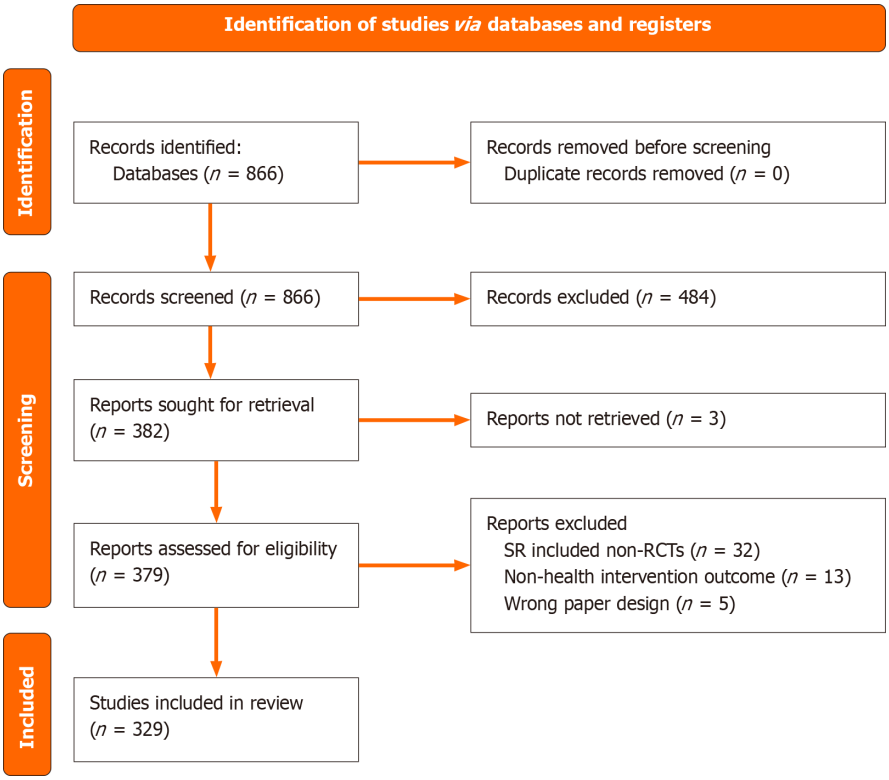


Figure 1 Prisma diagram. SR: Systematic review; RCTs: Randomized controlled trials.

mention contact with the primary study authors or that this was a part of their protocol.

Our team contacted the corresponding authors of included SRs *via* email for further information on the nature of their primary author contact. 7% (*n* = 23) of the 329 included SRs could not be contacted *via* email, due to inaccurate, outdated or lack of contact information, or being on leave. Of the 125 SRs that mentioned primary author contact in their papers, 116 could be contacted. From the 116 emails that were successfully sent, 40 replies were received, with 1 reply deemed unclear, yielding a 34% (39/116) response rate from SRs that explicitly mention author contact with primary studies' authors.

Of the 204 SRs that did not explicitly mention contact with primary study authors in their paper, 14 SR teams were not accessible for email contact, for similar reasons as already outlined. From the 190 emails that were successfully sent, 50 replies were received, with 2 replies deemed unclear, yielding a 25% response rate. After receiving these email responses, 25 of 48 of clear respondents (52%) confirmed they did contact study authors despite not reporting so in their papers.

The total number of SRs with confirmed initiated contact with primary RCT authors out of all included SRs was 64/329 (19.4%), with 61% (*n* = 39) reporting author contact in their paper, and 39% (*n* = 25) not reporting author contact in their paper (Figure 2).

Number of contacts initiated, number of responses received when contact was initiated and time given to primary authors to respond

Of the 64 SRs that confirmed author contact, 37 SR authors provided details on how many primary authors they contacted and the number of replies they received. The mean ± SD contacts SR authors made were 10 (10) RCT authors, and the mean ± SD replies were received 5 (6.7). The mean ± SD response rate *per* contact sent was 0.49 (0.45) or 49% (45). The waiting time for responses prior to recording a non-response was a mean ± SD of 10.1 (28.3) weeks with a median (range) of 4 (2-126) weeks (Table 1).

Type of information requested when author contact was initiated

Of the 64 SR authors who responded with details about the contacts they made, 64% (*n* = 41) reached out to clarify outcome data; 48% (*n* = 31) for inclusion/exclusion criteria; 39% (*n* = 25) for methods, design or risk of bias; and 14% (*n* = 9) for other reasons (full-text requests, data in corrections).

Factors that prevented reviewers from contacting authors

Of the 23 papers that did not mention RCT author contact in their SR and replied to confirm as such, 61% (*n* = 14) considered RCT author contact as part of their study design. When not pursuing author contact, 52% (*n* = 12) of respondents gave reasons related to resources and time; 43% (*n* = 10) poor previous experience; 26% (*n* = 6) perceived likelihood of poor response; 21% (*n* = 5) integrity or bias; and 30% (*n* = 7) other reasons (no missing data, or enough data was available, or data could be estimated).

Table 1 Summary of key results

Summary	
Total number of SRs that report author contact in their paper (n = 329), n (%)	125 (38)
Total number of SRs that confirmed author contact but didn't report it (n = 48), n (%)	25 (52)
The mean ± SD contacts made by authors per SR	10 (10) RCT authors
The mean ± SD response rate for SRs per author contact sent	0.49 (0.45) or 49% (45)
The mean ± SD and median (range) waiting time for responses prior to recording a non-response	10.1 (28.3) weeks and 4 (2-126) weeks

SR: Systematic review; RCTs: Randomized controlled trials.

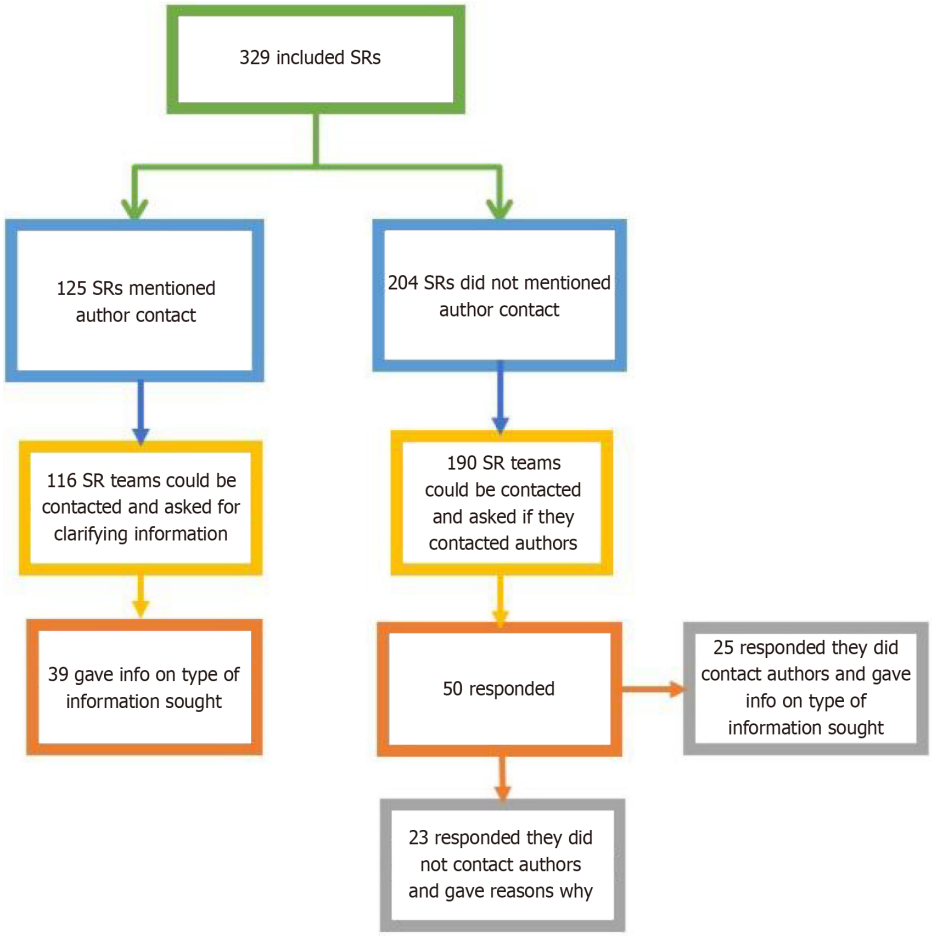


Figure 2 Graphical illustration of included systematic reviews teams' responses.

Impact factor and funding source

Neither an impact factor higher or lower than five, nor whether SR funding was from industry, public or not reported, was correlated with whether SR authors confirmed they contacted primary study authors, confirmed that they did not, or did not respond to us to confirm either (all P values > 0.05).

DISCUSSION

SRs are a core form of knowledge translation for clinicians and researchers. However, their role in guideline development and international policy is perhaps more impactful. Previous research has clearly identified that large numbers of studies are unclear when it comes to methods, such as to lead to a downgrade in judgements of quality[3], that missing data can be a risk, as it may change as studies are subsequently published in different forms[4] and this in turn can have major impacts on the certainty of subsequent outputs and guidance for the clinical community[9,10].

This is the first study to investigate whether authors of SRs are currently following guidance[7] to mitigate these risks, by routinely attempting to contact randomized controlled trial authors to clarify or supply information.

This review has found that a third of SRs explicitly planned to do this and reported it in their methods, with a further 7.5% reporting they did this but did not record it in their review. This still leaves a vast majority of reviews that did not ever attempt such contact at all, despite the risks and guidance to the contrary.

Responding review teams commented on the reasons for not planning this core element of review work, with some of the reasons being experiential and related to poor response in the past. Other reasons included perceived bias, which is not supported by organizations such as Cochrane[7], and most commonly, time. This final point is interesting. We recognize the significant investment of time and resource needed for SRs as a barrier, but for those already pursuing such works we believe maximining the value, impact and ultimate verisimilitude of findings is so central that the minimal extra effort needed would seem to justify the investment.

In what is also a novel finding, 37 review teams gave details of the effectiveness of their primary author contacts, suggesting 50% of primary authors contacted responded. This is in line with our experience as SR authors and would therefore suggest barriers of resource are perceived but not likely borne out in reality if such practice is integrated.

It is worth commenting that this practice is secondary researchers accounting for primary study issues. Therefore, a better solution would be for trial authors to include such detail, as well as editors and reviewers mandating this to be the case. But as this is a well-recognized and pervasive issue that is likely to continue given the rapid growth in academic output, such mechanisms are vital to enhance the evidence base when synthesized as a whole.

A limitation of this work is the sample size and choice. A large sample has been taken but it has been limited to recent publications and trial-focused interventional reviews. It is possible that these findings are not therefore more generalisable. However, the importance of this context is such that even if it is a limited finding, we believe its importance to still be key.

There are some limitations of this work. While a large sample was obtained, it was limited to recent publications and trial-focused interventional reviews, potentially affecting the generalisability of the findings. Additionally, the responsiveness of authors we contacted for missing information may not accurately reflect whether primary author contact was established. Furthermore, some authors who responded to our initial email did not respond to the follow-up questionnaire or provided only partial responses, possibly due to response fatigue from our two-step email approach. These factors may further impact the overall generalisability of the study. Finally, the responsiveness of authors we contacted for missing information may not accurately reflect whether primary author contact was established, with non-responses further limiting the sample size.

Future research could seek to investigate in greater detail the rate of response from authors, time taken to make such contacts and possibly methods to enhance response or enhance primary publications.

CONCLUSION

Our study provides novel insights on the current state of the field regarding author responses to requests for information on published RCTs. It does highlight how common such issues occur and the need for peer reviewers and journal editors to work to ensure that publications are transparent in such reporting. This study is limited by its focus on recent publications, which may not represent the broader spectrum of SRs. Additionally, the response rate from contacted authors may have been influenced by our specific inquiry approach, and may not reflect general responsiveness. Future research should investigate strategies to enhance primary author response rates in SRs, such as the use of standardized contact protocols or incentives for participation. Additionally, studies should assess the impact of author contact on the risk of bias assessments and the quality of evidence synthesis.

FOOTNOTES

Author contributions: Sinopoulou V co-conceived the project idea, led all stages and write-up of the paper, reviewed and approved the final version; Shah E contributed to screening, data extraction, data analysis, and drafting of the final version, reviewed and approved the final version; Gordon M co-conceived the project idea, supervised at all stages of the project, contributed to the writing, reviewed and approved the final version, and is guarantor for the data; Tony-Jimmy TE contributed to screening, data extraction and data analysis, and approved the final version.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Country of origin: United Kingdom

ORCID number: Vasiliki Sinopoulou 0000-0002-2831-9406; Eshan Shah 0009-0007-2178-6814; Morris Gordon 0000-0002-1216-5158; Tonia E Tony-Jimmy 0009-0004-8343-5209.

S-Editor: Fan M
L-Editor: A
P-Editor: Guo X

REFERENCES

1 **Moosapour H**, Saeidifard F, Aalaa M, Soltani A, Larijani B. The rationale behind systematic reviews in clinical medicine: a conceptual framework. *J Diabetes Metab Disord* 2021; **20**: 919-929 [PMID: 34178868 DOI: 10.1007/s40200-021-00773-8]

2 **Spieth PM**, Kubasch AS, Penzlin AI, Illigens BM, Barlinn K, Siepmann T. Randomized controlled trials - a matter of design. *Neuropsychiatr Dis Treat* 2016; **12**: 1341-1349 [PMID: 27354804 DOI: 10.2147/NDT.S101938]

3 **Yin Y**, Shi F, Zhang Y, Zhang X, Ye J, Zhang J. Evaluation of reporting quality of randomized controlled trials in patients with COVID-19 using the CONSORT statement. *PLoS One* 2021; **16**: e0257093 [PMID: 34555033 DOI: 10.1371/journal.pone.0257093]

4 **Sinopoulou V**, Gordon M, Moran GW, Egiz AMAM, Phlananthachai S, Rane A, Al-Tameemi AHA. Prepublication abstract-only reports compared with full-text manuscripts for randomised controlled trials in inflammatory bowel disease: a systematic review. *BMJ Open Gastroenterol* 2024; **11** [PMID: 38453251 DOI: 10.1136/bmjgast-2023-001334]

5 **Song F**, Hooper, Loke Y. Publication bias: what is it? *OAJCT* 2013 [DOI: 10.2147/oajct.s34419]

6 **Kicinski M**, Springate DA, Kontopantelis E. Publication bias in meta-analyses from the Cochrane Database of Systematic Reviews. Stat Med 2015; 34: 2781-2793 [PMID: 25988604 DOI: 10.1002/sim.6525]

7 **Li T**, Higgins JPT, Deeks JJ (editors). Chapter 5: Collecting data. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.0 (updated July 2019). Cochrane, 2019. Available from: https://www.training.cochrane.org/handbook

8 **Gordon M**, Sinopoulou V, Shah E, Ewomazino T, Tony-jimmy ET. Author contacts for systematic reviews of RCTs: A systematic review protocol. University of Central Lancashire. E-print. Available from: https://clok.uclan.ac.uk/50926/3/protocol%20author%20contact.pdf

9 **Granholm A**, Alhazzani W, Møller MH. Use of the GRADE approach in systematic reviews and guidelines. *Br J Anaesth* 2019; 123: 554-559 [PMID: 31558313 DOI: 10.1016/j.bja.2019.08.015]

10 **Papadopoulou A**, Amil-Dias J, Auth MK, Chehade M, Collins MH, Gupta SK, Gutiérrez-Junquera C, Orel R, Vieira MC, Zevit N, Atkins D, Bredenoord AJ, Carneiro F, Dellon ES, Gonsalves N, Menard-Katcher C, Koletzko S, Liacouras C, Marderfeld L, Oliva S, Ohtsuka Y, Rothenberg ME, Strauman A, Thapar N, Yang GY, Furuta GT. Joint ESPGHAN/NASPGHAN Guidelines on Childhood Eosinophilic Gastrointestinal Disorders Beyond Eosinophilic Esophagitis. *J Pediatr Gastroenterol Nutr* 2024; **78**: 122-152 [PMID: 38291684 DOI: 10.1097/MPG.0000000000003877]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 97694

DOI: 10.5662/wjm.v15.i3.97694

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Multidisciplinary management of pituitary macroadenoma

Gladness Aluyi-Osa, Ayuba Suleman, Carlo Salati, Leopoldo Spadea, Caterina Gagliano, Mutali Musa, Marco Zeppieri

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade A, Grade B

Novelty: Grade A, Grade B

Creativity or Innovation: Grade B, Grade B

Scientific Significance: Grade A, Grade C

P-Reviewer: Osman H

Received: June 5, 2024

Revised: November 18, 2024

Accepted: December 3, 2024

Published online: September 20, 2025

Processing time: 273 Days and 22.9 Hours

Gladness Aluyi-Osa, Ayuba Suleman, Mutali Musa, Department of Ophthalmology, Africa Eye Laser Center, Km 7, Benin 300105, Nigeria

Carlo Salati, Marco Zeppieri, Department of Ophthalmology, University Hospital of Udine, Udine 33100, Italy

Leopoldo Spadea, Eye Clinic, Policlinico Umberto I, "Sapienza" University of Rome, Rome 00142, Italy

Caterina Gagliano, Department of Medicine and Surgery, University of Enna "Kore", Enna 94100, Italy

Caterina Gagliano, Mediterranean Foundation "G.B. Morgagni", 95125 Catania, Italy

Mutali Musa, Department of Optometry, University of Benin, Benin 300283, Nigeria

Corresponding author: Marco Zeppieri, MD, PhD, Doctor, Department of Ophthalmology, University Hospital of Udine, p.le S. Maria della Misericordia 15, Udine 33100, Italy. mark.zeppieri@asufc.sanita.fvg.it

Abstract

BACKGROUND

Pituitary macroadenomas represent a significant challenge in clinical management due to their variable presentations and complex treatment considerations. This manuscript explores the multidisciplinary approach to understanding and managing pituitary macroadenomas, integrating neurosurgery, endocrinology, radiology, and pathology perspectives.

AIM

To summarize the literature on pituitary macroadenoma and outline the possible multidisciplinary approach in the diagnosis, management, and rehabilitation of individuals with pituitary adenomas, to add to already preexisting knowledge, in managing these cases enhancing better ocular and systemic outcomes.

METHODS

A search was conducted on an online publication database (PubMed) using the term "pituitary adenoma" including all results published over twenty years (2004-2024). Results were sorted for relevance, language, and completeness.

RESULTS

A total of 176 records were returned. The guidelines of the PRISMA 2020 sta-

tement were followed in this study. A total of 23 records were excluded due to being out of scope while a further 13 records were duplicates. Another 17 records were not available as full-length articles and were also excluded. The references of each included record was further searched for relevant publications. A total of 141 records were therefore used in this minireview.

CONCLUSION

Pituitary macroadenomas pose substantial clinical challenges due to their size and potential for significant hormonal and neurological impact, modern therapeutic strategies offer effective management options. Early detection and comprehensive treatment are essential for optimizing patient outcomes and maintaining quality of life. Continued research and advancements in medical technology are likely to further enhance the management and prognosis of this condition in the future

Key Words: Pituitary; Macroadenoma; Multidisciplinary; Hormonal dysregulation

Core Tip: The review encompasses recent advancements in diagnostic imaging techniques, surgical approaches, hormonal management, and adjuvant therapies. Furthermore, it discusses the importance of a collaborative framework involving specialists from various disciplines to optimize patient outcomes and quality of life. By synthesizing current evidence and clinical experiences, this manuscript aims to provide insights into the comprehensive management of pituitary macroadenomas and stimulate further research.

Citation: Aluyi-Osa G, Suleman A, Salati C, Spadea L, Gagliano C, Musa M, Zeppieri M. Multidisciplinary management of pituitary macroadenoma. *World J Methodol* 2025; 15(3): 97694

URL: https://www.wjgnet.com/2222-0682/full/v15/i3/97694.htm

DOI: https://dx.doi.org/10.5662/wjm.v15.i3.97694

INTRODUCTION

Pituitary adenoma, whether micro or macro depending on size, is a group of benign tumors within the skull, around the pituitary gland, which can extend to extra pituitary areas. A pituitary adenoma is referred to as micro when the size is less than 10mm, and when the size is more than 10mm it is referred to as Macroadenoma. Sizes larger than 40mm are referred to as giant tumors[1].

Pituitary adenomas effect on the visual system is dependent on several factors including, size, location, and whether it is functional or not, it is still not a fact if there is any relationship between the effect of functional and non-functional pituitary adenoma[2], with little or no effect on the motor function. Although it is a benign condition it can significantly affect vision and other systemic functions, because the pituitary gland also referred to as the master gland, helps regulate the function of other endocrine glands (glands whose secretion flows directly into the bloodstream), for the onward manifestation of its effect at effector organ or tissue or even system.

Pituitary adenoma can be said to be functional or non-functional depending on its effect on hormonal secretion; non-functional adenoma can affect vision, as seen in about 58% of cases, and the person involved may not know immediately because there is a compensation from the unaffected eye[3].

The pituitary gland is made up of two lobes, the anterior pituitary and the posterior pituitary. The anterior pituitary, constitutes a major portion of the pituitary gland, hence it is also responsible for the triggering effect on other glands within the body, including those anatomically far from it. Pituitary adenoma can cause varying levels of visual impairment, its manifestations in the eyes depending on size and location, including but not limited to optic atrophy, visual field defect, specifically bitemporal hemianopia in both eyes, due to possible compression at the chiasma level, swollen disc, reduced visual acuity, when the optic nerve (CNII) is involved, sluggish pupillary reaction and possibly raised intracranial pressure. This condition's differential includes Brain stem glioma, Arachnoid cyst, Ependymoma, Glioblastoma multiforme, *etc*.[4]. Diagnosis is strictly by matching visual or systemic findings with neuroimaging, the systemic component of it comes into play when the tumor is functional as earlier stated. Computerized tomography scan, or magnetic resonance imaging (MRI), requires a multidisciplinary approach to not only diagnosis but also care in the long run, if the affected individual must have a better quality of life[5].

As earlier said, Pituitary adenomas are one of the most common intracranial tumors, the non-functional type ordinarily will not cause or show any signs, unless there is a mass effect on structures surrounding the pituitary gland[6].

Due to the complex systemic sequelae presented in patients with pituitary adenoma, there is a need for a holistic approach to care, this includes the help of the ophthalmologist, Neurologist, Neuroopthalmologist, Radiologist, and Endocrinologist. Each of these specialties has a role they play if the management of a pituitary macroadenoma is regarded as holistic and clinical in approach. Although pituitary macroadenomas are referred to as benign tumors, they can exhibit some significant effects, both in the visual system, and the overall health of the patient, hence the importance of a

multidisciplinary approach cannot be overemphasized. This will not only potentiate the management regimen but will also hasten it, with high chances of better recovery or prognosis. In cases where pituitary macroadenomas affect the secretion of hormones, for example, the thyroid hormone secretion macroadenomas, metabolic activities like heart rate, sweating, and the like might become significantly altered[7], the ophthalmologist under this circumstance initiate the need for the patient to see the endocrinologist as soon as possible, also in individuals who have pituitary macroadenoma affecting prolactin hormone, there is bound to be sexual/reproductive changes be it male or a female[8]. Furthermore, some pituitary tumors might present like a pituitary macroadenoma, but in the real sense, it is a more metastatic condition, the help of the radiologist and neurosurgeon, comes into play here, not just for better prognosis, but sometimes to even save the life of the individual, so at the end of the day all tumors involving the pituitary gland, whether quiet or active require the action of the various above-listed professionals for proper management now and in the future. Over the years there have been various workups done in seeing to a better pituitary macroadenoma treatment from the aspect of immunity[8], to the aspect of radiotherapy[9], surgical approach[10-12], with the sole aim of better outcome, so a look at the preexisting management plan for pituitary macroadenomas and its systemic sequelae is very important. Transient visual obscuration (TVO) is rare in pituitary adenoma, there is evidence of resolved TVOs after resection of pituitary macroadenoma that was compressive[12].

MATERIALS AND METHODS

This review was done using a systematic search string on PubMed, as stated below, (("eye" [MeSH Terms] OR "eye" [All Fields]) AND ("pituitary neoplasms" [MeSH Terms] OR ("pituitary" [All Fields] AND "neoplasms" [All Fields]) OR "pituitary neoplasms" [All Fields] OR ("pituitary" [All Fields] AND "adenoma" [All Fields]) OR "pituitary adenoma" [All Fields])) AND ((ffrft [Filter]) AND (2004: 2024 [pdat])). Two authors (AG and AS) scrutinized all records for relevance. The search strategy followed the PRISMA[13] guidelines as shown in the flowchart below as Figure 1. The results were further stratified to only include relevant work between 2004 and 2024. This is shown in Figure 1 below.

RESULTS

A total of 176 records were returned. 23 records were excluded due to being out of scope while a further 13 records were duplicates. Another 17 records were not available as full-length articles and were also excluded. A further 18 articles were harvested from the references of the 123 records so stratified. A total of 141 records were therefore used in this minireview.

DISCUSSION

Overview of pituitary macroadenoma

Importance of a multidisciplinary approach: In the management of pituitary macroadenoma and tumors in general, there is a need for the utilization of a multidisciplinary approach, in the form of shared care, whether the tumor is benign or metastatic, a constant observation by various specialties involved in neoplasia, depending on the location and cause is constantly being sought for. Before now Multidisciplinary approach to care was based on consultation as requested, but recently it has very paramount for better outcomes in the long run. An important component of an effective multidisciplinary team includes better communication, standardized coordination, and improved interdisciplinary decision-making, all of this must include the patient in the care process[14,15]. Although there are barriers to effective inter-professional collaboration in the management of pituitary macroadenoma, another importance of this review is to suggest possible mechanisms of action to be taken to enhance effective shared care regimen in the management of this emerging condition, which is sometimes masqueraded in form of other conditions. Furthermore, outcomes of pituitary surgery as a result of various analyses, have been shown to demonstrate significant positive outcomes following the implementation of a multidisciplinary approach. The outcome can be measured in terms of lower complications, shorter duration of stay in health centers, and increased curative resection[16].

Post-operative management would require a multidisciplinary approach, each doctor should be brought to the status quo regarding the performed procedure to avoid complications involved in managing the patient[17].

Pathophysiology of pituitary macroadenoma: Pathophysiology of Pituitary adenoma seems not be straight forward, hence prompt recognition especially in patient presenting with cavernous sinus syndrome, particularly in an hemodialysis clinic, is important for better management outcome[18]. In patients with pituitary adenoma, early detection of a dysfunctional visual pathway, can give insight into treatment modality and reduce the risk of permanent vision loss [19]. Tumors involving the sellar and parasellar region usually present with optic nerve head neuropathy similar to those found in glaucoma patients[20].

Pituitary adenoma can occur side by side with other tumors like the clear cell meningioma, due to their close similarities in radiological assessment, these are known as collision tumors *e.g.* the Clear cell meningioma which happens to be very aggressive[21]. The phenomenon by which pituitary apoplexy occurs is connected to either a decreased blood supply or by hemorrhagic mechanism[22,23]. There have been reported cases of Pituitary adenoma being confused with

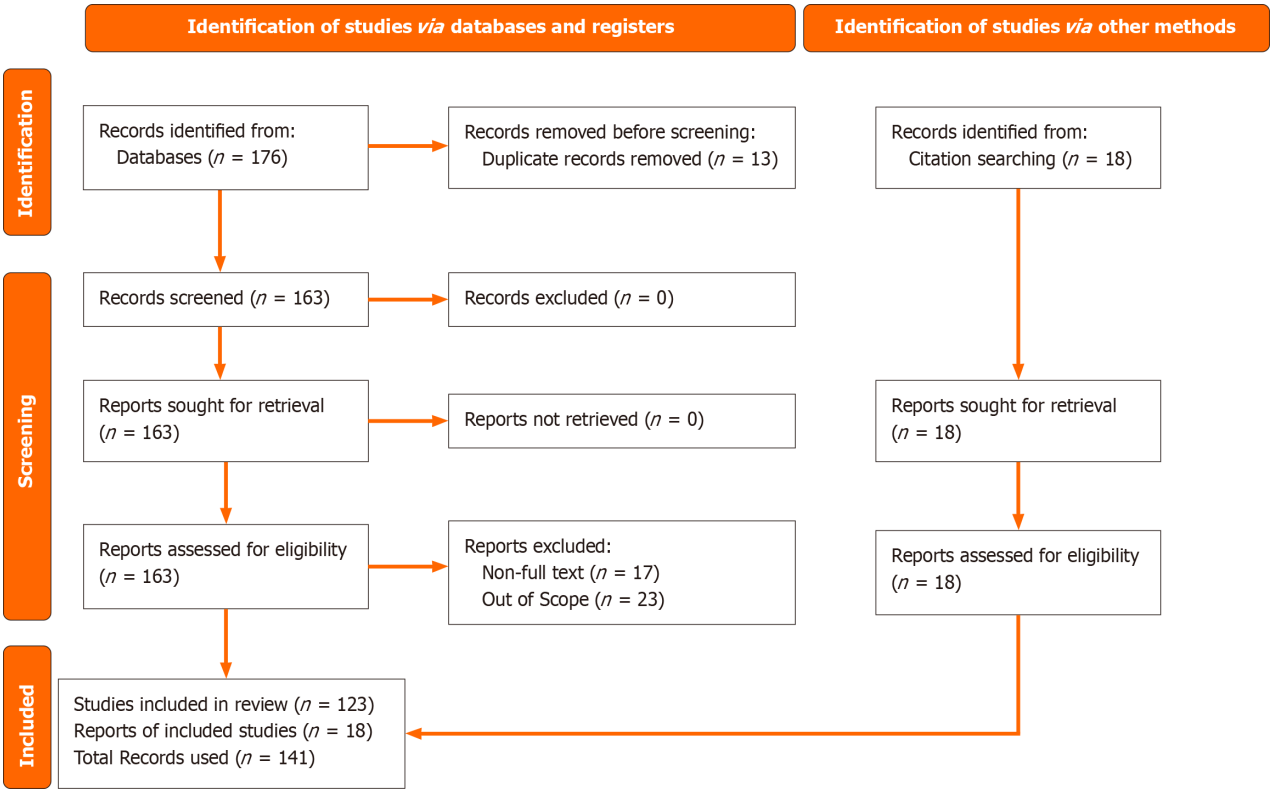


Figure 1 The search strategy followed the PRISMA.

other syndromes, like the Tolosa Hunt Syndrome[24]. Changes in vascular perfusion of the pituitary gland can be affected by uncontrolled diabetes[25]. The presence of a pituitary lesion or disease usually points to the possibility of an existing malignancy[26]. Pituitary macroadenoma should be considered in cases presenting with typical clinical signs of Foster-Kennedy syndrome[27,28].

Though rare there has been a report of metastatic gastric cancer found in the pituitary (MGCP)[29]. Acute ischemic stroke though rare has been identified as a complication of pituitary apoplexy secondary to pituitary adenoma[30]. Stable chiasma lesions have been shown to have similar visual field defects as the patient grows older hence ruling out any statistical difference in visual field defect as a result of age[31].

Tumor biology and growth patterns: The height of a pituitary adenoma from the genu of the cavernous internal carotid artery correlates better with visual outcome compared with gross tumor height[32]. Results from previous studies showed biological evidence to support, the use of specific proteostasis modulation in the management of solid tumors, with syndromic sequelae, especially tumors that involves misfolding of proteins[33].

Diagnosing cases of collision tumor is sometimes challenging, but it poses a great importance regarding post-operative care of the individual, in terms of using radiation therapy, it also helps to detect recurrence nature of tumors[34].

There is the possibility of gradual resolution of the residue of a pituitary adenoma, within the intra-cavernous sinus space, as a result of possible necrosis[35]. Involvement of orbital regions in cases involving the sinonasal anatomy usually is a sign[36].

Tumor size has been found to correlate positively with the mean deviation and correlates negatively with average sensitivity in both cases of functional and non-functional pituitary adenomas[2].

Orbital involvement in most of the sinonasal diseases indicates the extensive and aggressive nature of the pathology and many of these, even if they are not malignancies are difficult to treat[37]. This is especially true for acute fulminant and chronic invasive fungal rhinosinusitis[38].

Decisions in the early detection and management of pituitary carcinoma, it is important to establish systems that help with genomic profiling and also determination of molecular biomarkers[39]. The relationship between the existence of adrenocorticotropic hormone (ACTH) pheochromocytoma on its own or as part of the multiple endocrine Neoplasia type 1[40]. Rapidly developing headache which are accompanied by visual impairment in the female gender with sellar tumors is usually of the sellar atypical teratoid/rhabdoid tumors[41]. Tumor growth and volume have been implicated in symptoms reoccurring from time to time, In cases of highly vascularized lesions in the suprasellar space, this should give a high index of suspicion for possible Hemangioblastoma[42,43]. The E2F3 transcription factor has been fingered as one of the players in tumorgenicity, although the mechanism is still undergoing a lot of research, as seen in the work of Parisi *et al*[44], it is not a common phenomenon for pituitary infarction or hemorrhage to occur around the parasellar region[45].

Pituitary adenoma without chiasma compression but with lateral extension into the cavernous sinus can present with normal visual fields and cranial nerve three palsy with pupillary involvement[46]. Pituitary macroadenoma presenting with ophthalmoplegia without visual field defect has been shown to have a good prognosis provided it is attended to on

time[47]. Metastatic progression of pituitary macroadenoma post radiotherapy has been reported although it incidence is low[48]. The growth of adenoma during pregnancy and subsequent regression post-partum has been reported[49,50]. Pituitary apoplexy should be considered as a differential in patients suspected to have bacterial meningitis, this should be done due to the associated mortality and morbidity if missed[51].

Hormonal dysregulation and clinical manifestations: The pituitary gland secretes various hormones that help to regulate various bodily functions. Changes in the hypophyseal structure would therefore result in a lot of systemic manifestations (hormonal) such as hypothyroidism, hyperthyroidism, hypokalemia, *etc.* Persistent hypokalemia could result in ocular manifestations such as central retina vein occlusion[52]. Hyponatremia resistant to conventional therapy should point to pituitary gland macroadenoma[53]. Pituitary prolactinoma secreting growth hormone (GH) has been reported as a variant of the macroadenomas[54]. Also, suppressed immunity leading to intraocular complications has been reported in individuals with pituitary macroadenoma[55]. In cases of Hypothalamic-pituitary tumors, as seen in some genetic conditions, there is the possibility of the individual subsequently having severe visual deterioration as the person ages, which has been reported in Klinefelter's syndrome[56].

Changes seen in the trabecular meshwork in cases of excessive secretion of the adrenocorticotrophic hormone (hypercortisolism) can be reversed subsequently to its normal physiological state[57]. Pituitary macroadenoma with apoplexy has been seen in cases of coronavirus disease 2019 (COVID-19) infection, with patients presenting with sudden bilateral vision loss and fever, so this should be looked out for when attending to patients with respiratory infection and unexplained vision loss[58]. Diplopia as a result of restrictive extraocular muscle myopathy is a possible sign of acromegaly due to the presence of pituitary macroadenoma[59]. Cranial nerve III palsy is not common with pituitary macroadenoma, its presence indicates the extension of lesion to parasellar areas and cavernous regions[60]. The fact that cranial neuropathy and diabetes are occurring together, should not be a criterion for bringing forth a causal relationship, further workup is of importance in cases like this[61]. Pertinent literature regarding clinical investigations of pituitary macroadenomas is listed in Table 1.

Diagnostic modalities

Imaging techniques (MRI, computed tomography, *etc.*): Early detection of visual field pathway dysfunction, may help in treatment regimen modification for patients with pituitary macroadenoma and also help to reduce the occurrence of irreversible optic neuropathy[62]. Ganglion cell layer-internal plexiform layer assessment using optical coherence tomography (OCT) is a very important tool, with a high degree of sensitivity, in detecting lesions in the anterior portion of the visual pathway, especially in cases where chiasmatic compression exists as a result of pituitary adenoma[63]. Changes in the structure of retinal tissues have a close relationship to retinal nerve fiber thickness and the outcome of the management regimen[64,65]. It is possible for Cushing's syndrome to occur as a result of hypersecretion of ACTH, but this time it is stimulated from an orbital neuroendocrine tumor, rather than the typical Pituitary gland tumor or associated tumor[66]. The parapapillary zone of atrophy is worse and more frequent in those with large intrasellar or perisellar pituitary tumors[67].

Fast changes in the pattern electroretinogram in patients with glaucoma should spur the conduction of an MRI or computed tomography (CT) scan to rule out intra-cranial space occupying lesions[68,69]. Figure 2 and Figure 3 show examples of imaging scans. Defects in the retinal nerve fiber layer (RNFL) and macula ganglion cell complex (GCC) are seen in patients with chronic chiasma compression earlier than visual field defects. The macula GCC correlates with visual outcomes[69-72]. The assessment of contrast sensitivity can be very important in diagnosing changes at the chiasmatic level in cases of pituitary adenoma[73], Disorders of the anterior visual pathway capable of causing secondary visual disorders of the visual cortex, this can be detected through the use of MRI[74,75]. MRI is often used over CT for diagnosing pituitary adenomas because it excels in characterizing tiny pituitary sellar lesions and improving anatomical delineation prior to surgery. Additionally, MRI is recommended for post-operative monitoring[76].

Apart from the visual field changes in chiasma pathology, inattention to the temporal side on monocular testing should prompt the clinician's attention to possible chiasma pathology[77]. Cases of retinal alterations that are uncharacteristic, should be carefully evaluated for the possibility of chiasma lesions[78].

Stratus OCT has been shown useful in the diagnosis of band atrophy caused by chiasma lesions[79]. In the absence of a visual field assessment, a domain OCT can be used to differentiate lesions of the ganglion cell layer, from that typical of glaucomatous changes[80,81]. Information gathered from the OCT angiography, can be used as a prognostic factor, in determining visual outcome following surgical intervention[82,83]. Although sometimes the extent of visual field loss is related to the tumor size, but not in all cases[84]. The use of OCT in the detection of the degree of RNFL loss and associated visual field changes might have some limitations to its use[85].

The use of simple temporal depression in visual field results has been shown to aid in the diagnosis of compressive optic lesions affecting the optic chiasm which is affected by pituitary gland tumor[86,87]. Having good knowledge about identifying isolated nerve palsy can play a major role in helping to diagnose Pituitary gland-related anomalies, and it will also help direct treatment to the appropriate quarters[88].

Endocrine testing (hormonal assays): Hormonal testing can help to confirm or refute diagnoses of pituitary adenomas in a timely and minimally invasive manner.

Insulin growth factor testing is usually the first port of call. This hormone is usually secreted in times of increased GH in the bloodstream. The presence of elevated levels of both these hormones can confirm the presence of a pituitary adenoma[89]. These hormones are also useful for monitoring the progression of management for pituitary adenomas as a return to normal levels may indicate a remission[89].

Table 1 Clinical investigation of pituitary macroadenomas

Ref.	Examination	Organ	Assessment
[63-65]	OCT	Eye	Retinal layer analysis
[74-76]	MRI/CT scans	Brain	Pituitary gland imaging
[80-84]	Hormonal assays	Plasma, CSF	Fluid assays
[96-99]	Histopathological examination	Brain tissue	Hematoxylin and eosin staining, physical examination *etc.*

OCT: Optical coherence tomography; MRI: Magnetic resonance imaging; CT: Computed tomography; CSF: Cerebrospinal fluid.

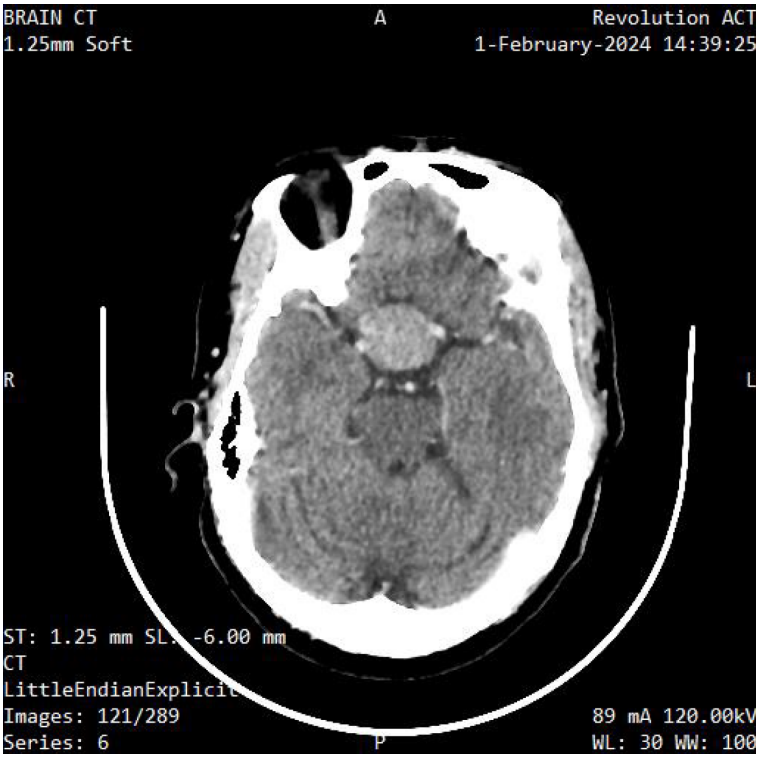


Figure 2 Sagittal imaging scans of a patient with pituitary adenoma.

Adrenocorticotropic hormone helps to regulate the adrenal steroid in the body. A raised ACTH may indicate the presence of an ACTH-secreting adenoma[90]. These types of pituitary hormones are usually more aggressive[90]. In such patients, there can be a regression into Cushing's disease[91]. Patients who present with headaches, visual changes, and reduced libido should undergo prolactin levels test, ACTH levels, and testosterone[92]. The dexamethasone/corticotrophin-releasing-hormone test is classically used to test for this. Following a 48-hour regime of 0.5 mg/kg of dexamethasone, a loading dose of 1 ug/kg body weight is given exactly 2 hours after the last dexamethasone dose followed by a 15 minutes wait before the level of plasma cortisol is measured[93]. Cortisol levels higher than 1.8 μg/dL subsequently confirm abnormally increased ACTH levels which is a sequelae of ACTH releasing pituitary adenoma[93].

Other assays that can help in the differential diagnoses of pituitary macroadenomas include gonadotroph secreting hormone assay, lactotroph secreting hormone, somatotroph secreting hormone, and thyroid-secreting hormone assays[94,95].

Histopathological evaluation: In cases of pediatric and juvenile craniopharyngioma, changes in vascular structure, are closely related to structural and functional outcomes[96,97]. Expert knowledge in the area of the anatomy of the brain and histopathologic assessment of imaging findings, are very important in differentiating various pathologies and aid in better management[98]. Proper analysis is needed to differentiate pituitary macroadenoma from sellar chondrosarcoma due to similar systemic effects[99].

Multidisciplinary management strategies

Vasospasms and fluctuation in blood pressure intraoperatively have been indicted and correlated to the existence of pituitary adenoma[100]. There is a synergistic improvement in terms of visual outcome and recovery in cases where both surgical resection and additional radiological intervention are carried out[101].

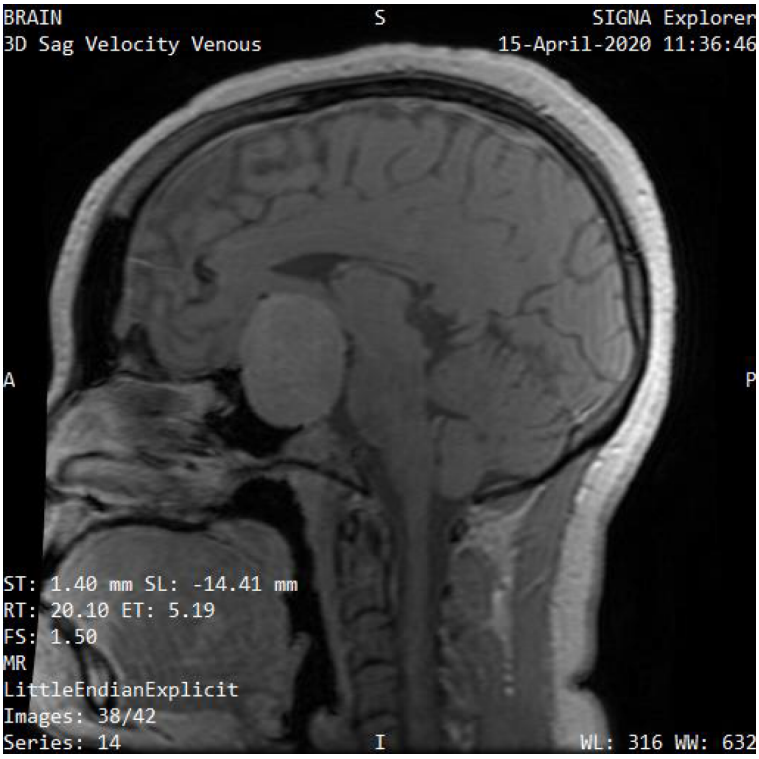


Figure 3 Radial computed tomography imaging scans of a patient with pituitary adenoma.

In cases where individuals present with similar pathognomic signs with other pathological entities, a multidisciplinary approach should be instituted[102], Autoimmune workup is necessary in cases of xanthogranulomatous hypohysitis [103]. Management can be multifaceted depending on the presenting signs and diagnostic findings[104], Been able to carry out extensive systemic workup and keeping the mind open is important in managing conditions that might be very radical, but will present initially as a calm lesion[105].

Patients presenting with prolactinoma secondary to macroadenoma could benefit from the use of pharmaceutical agents like cabergoline[106]. Different patients require different approaches, pregnant women require multidisciplinary consideration, and pregnant women taking bromocriptine for macroprolactinoma secondary to pituitary macroadenoma would require surgery due to the low data of the safety of bromocriptine to the fetus[107].

Neurosurgical considerations

Neurosurgical intervention provides a better prognosis for pituitary macroadenomas as compared to purely conservative medical management[108]. Debulking of tumor size usually leads to improvement in the patient's symptoms and signs [109]. However, a high degree of suspicion is required to screen for other coexisting disorders of the central nervous system, in cases of optic disc melanocytoma which is presenting with disproportionate signs or symptoms[110]. Hypothalomopituitary involvement in cases of neurosarcoidosis is rare, which leads to complications that seem to occur more frequently, than those seen in other neurological and or systemic syndromes[111,112]. The thickness of the RNFL has been found to not be a very important factor in determining post-surgical outcome, after a decompressive surgery [113], It is important to recognize pituitary apoplexy as a major cause of cranial nerve anomaly, as it has been fingered as a rare presenting sign of pituitary adenoma[113]. In cases of pituicytoma where there is a partial removal of the tumor, management can be completed with the help of radiotherapy[114,115]. In cases of collision tumors, a craniotomy is preferred over the transsphenoidal approach[116].

The size of the tumor plays a key role in determining the treatment regimen[117]. The minimally invasive nature of transsphenoidal and endoscopic procedures in the management of pituitary adenoma or tumors of the sellar turcica makes them the go-to procedure[118].

Timing for surgical intervention in cases of pituitary apoplexy resulting from internal carotid artery should be prompt, due to the effect of this condition on visual acuity[119]. Neuroimaging should be considered in patients with conjunctival chemosis, as Yamamuro *et al*[120], presented a case where a pituitary neuroendocrine tumor masqueraded as severe conjunctiva chemosis[120].

Transsphenoidal surgery

Transphenoidal surgeries go through the nose to reach the pituitary adenoma as against accessing *via* the skull. Visual field recovery following transsphenoidal tumor resection surgery is correlated with the absence of preoperative central visual field anomaly or even bilateral visual field defects, in patients with pituitary adenoma[121], the use of transsphenoidal technique as the surgery of choice in the management of pituitary adenoma, has been found to show improvement, in avoiding irreversible visual impairment[122-126].

Transsphenoidal surgery has been shown to cause progressive improvement in visual outcomes in patients with non-functional pituitary macroadenoma[127]. There is a report that transsphenoidal surgery for the removal of pituitary macroadenoma is associated with transient refractive error changes with the postulation that hyponatremia induced by tumor removal causes changes in the aqueous humor which in turn causes changes in the lens *via* osmosis[128]. Early neurosurgical intervention is crucial in patients with pituitary apoplexy[129]. Pituitary apoplexy is a complication of pituitary adenoma in most cases[130]. Decreased perfusion to the optic disc and retina caused by compressive lesions has been shown to possibly further progress after decompression procedures[131].

Endoscopic techniques

Extended intracavernous surgery using endoscopy and a measured, selective resection of the medial wall of the cavernous sinus was reported to be viable for the management of invasive secreting pituitary adenomas[132]. In another case, no tumor recurrence and the total removal of the aneurysm was completed at about 6 months of follow-up, using the endoscopic endonasal surgery technique[133]. Following Endoscopic surgery assessment of OCT angiography showed improvement in the density of retinal fibers[134-136]. Endoscopic endonasal approaches to the removal of pituitary macroadenoma or tuberculum sellae tumors are safe and less invasive than craniotomy[137,138].

Radiation therapy

Radiological therapy involves the use of ionizing radiation to manage diseases. These are commonly used against cancer cells to kill or restrict their multiplication. There seems to be no significant relationship between the amount of radiation delivered and its effect on some ocular parameters like the RNFL and the Endothelial cell density, otherwise, changes are usually related to the tumor suppression mechanism[139]. The use of Gamma knife Radiosurgery has been shown to come out with lesser side effects and the patient can return to their normal daily activities soon afterward[140].

Stereotactic radiosurgery

Outcomes of proton therapy have been linked to the possible technique in unlocking the effectiveness or benefits of proton stereotactic radiosurgery[141]. Aneurysm can be a complication following stereotactic surgery, and it can happen many years post-surgery[142]. Fractionated stereotactic therapy has been shown to reduce tumor size without toxicity [143] There have been incidences of the development of central nervous system lymphoma post-irradiation[144]. Stereotactic radiotherapy has been proven to give better doses to the surrounding extra cranium than conventional radiotherapy[145].

Medical suppression of hormonal secretions

Physiologic pituitary gland enlargement following pregnancy has been treated using bromocriptine[146]. Fan *et al*[147] reported on a case of pituitary adenoma which regressed and completely shrunk after management with bromocriptine [147]. Bromocriptine is a dopamine receptor agonist that helps to reduce the amount of prolactin in the body, thereby mitigating the progression of prolactin-linked-adenomas[148].

Hormonal replacement therapy

One of the major life-threatening complications of using the gonadotropin-releasing hormone agonist, is Pituitary apoplexy[149,150], Pasireotide which is a somatostatin analogue has been shown to help in the reduction of pituitary mass as seen in a case of Cushing syndrome[151], where a 14-year-old girl presented with headache, unilateral right eye ptosis and secondary amenorrhea. Baagar *et al*[151] also successfully managed a case of pituitary adenoma using Pasireotide[151].

Excluding thyroiditis, the majority of the endocrine dysfunction or dysregulation usually becomes permanent regardless of the use of immune checkpoint inhibitors[152,153].

The effect of high prolactin levels on the tear film is duration-dependent[154]. Studies have shown that the eye might become very sensitive, due to prolonged exposure to GH and the insulin-like growth factor-1[155], Studies have shown that the effect of retinoic acid anabolic androgenic steroids and their relationship with the prolactin-secreting pituitary adenoma needs to be studied more in other to get an idea of what links these parameters together[156]. Carbagoline use can be very effective in terms of visual improvement and reduction in tumor size in cases of some pituitary adenomas, that are functional[157]. Prompt use of Carbagoline resulted in swift recovery of visual acuity[158].

Challenges faced and lessons learned

A drop in visual acuity may be the first sign or symptom noticed in cases of pituitary adenomas. Visual acuity usually improves spontaneously post-operatively, although rates may differ[159,160]. Basic tests such as frequency doubling technique have been shown effective in pituitary macroadenoma diagnosis[161].

Contrast sensitivity has been found to vary with various factors, including luminance level, neural mechanism, grating motion, and shape. It is worthy of note that contrast sensitivity can be influenced by factors such as refractive status, surgeries *e.g.* cataract surgery or even refractive surgery, diabetic eye changes, and Pituitary adenomas[162], RNFL thickness and optic disc can be very important indicators in terms of prognosis, Severe scotoma is found more in the upper quadrant of the temporal field, and also in the lower nasal hemifield[163]. Patients with tumors affecting the extraocular muscle may present with complaints at the first visit to an eye hospital, quickly identifying these conditions is necessary for their management, both in the short and long run[164] for visual outcome evaluation, following pituitary adenoma surgical procedure[165]. Eyecare practitioners should have a very high level of suspicion for internal carotid artery aneurism, as a differential for any form of sellar mass[166]. Evidence support the fact that mutation in specific

genes can cause early onset Cushing disease, with invariably increased size of adrenocorticotrophic pituitary adenoma [166]. Radial peripapillary capillary density is a stronger indicator for determining outcome in terms of visual field recovery[167]. Visual field constriction that appears to be rapid even in the presence of other ocular morbidity, should prompt immediate radiological assessment. A high index of suspicion must be correlated with appropriate and prompt radiological assessment[168], The GCC and ganglion cell layer thickness parameters can help predict the integrity of the central visual field of patients with pituitary tumors[169,170]. Care and due diligence must be ensured to avoid misdiagnosis, in their case series Choudhari *et al*[171], showed the misdiagnosing of pituitary adenoma as NTG[171].

Future directions and innovations

More work on the aspect of the effect of the COVID-19 vaccine on the effect of pituitary adenoma needs to be done, The COVID-19 vaccine has been implicated in the possible cause for the increase in the size of the pituitary gland, although more work needs to be done[172]. The use of BRAF and MEK inhibitors represent or stand a chance in the future, in the treatment of capillary craniopharyngioma[173]. Differentiating some forms of sellar region tumor possesses some challenges, hence newer methods such as the use of randomics and deep learning(machine learning are the newer methods to give better differentiating outcomes, with subsequent effects on the management plan and expected outcome [174]. The use of intraoperative MRI together with a transsphenoidal approach is an emerging technique found to be associated with significantly reduced complications[175]. Some records could not be retrieved as they did not meet the selection criteria

CONCLUSION

In conclusion, the management of pituitary macroadenomas exemplifies the necessity of a multidisciplinary approach in modern medicine. These tumors, due to their potential to cause significant endocrine dysfunction and compress adjacent neural structures, demand comprehensive and coordinated care. Collaboration among endocrinologists, neurosurgeons, ophthalmologists, radiologists, and pathologists is pivotal for accurate diagnosis, effective treatment planning, and monitoring of patient outcomes. Advanced imaging techniques and tailored surgical strategies, combined with targeted pharmacotherapy, have significantly enhanced the prognosis for patients with pituitary macroadenomas. However, ongoing research is essential to refine these interventions, reduce recurrence rates, and better understand the molecular and genetic underpinnings of these tumors. By embracing a multidisciplinary framework, healthcare professionals can ensure holistic and patient-centered care, ultimately improving the quality of life for individuals affected by this condition.

ACKNOWLEDGMENT

We want to thank Dr. G. O. Okoye (MD, PhD), Founder of Africa Eye Laser Centre, Benin City, Edo State, Nigeria, who provided us with the figures used in this paper.

FOOTNOTES

Author contributions: Aluyi-Osa G, Saleman A, Musa M and Zeppieri M wrote the outline; Aluyi-Osa G, Suleman A, and Musa M did the research and writing of the manuscript; Salati C, Spadea L, Gagliano C, Musa M and Zeppieri M assisted in the writing of the draft and final paper; Zeppieri M was responsible for the conception and design of the study and completed the English and scientific editing; Aluyi-Osa G, Suleman A, Salati C, Spadea L, Gagliano C, Musa M and Zeppieri M assisted in the editing, making critical revisions of the manuscript and viewing all versions of the manuscript. All authors provided the final approval of the article.

Conflict-of-interest statement: The authors declare that there are no conflicts of interest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Country of origin: Italy

ORCID number: Carlo Salati 0000-0003-4736-5296; Leopoldo Spadea 0000-0002-1190-3956; Mutali Musa 0000-0001-7486-8361; Marco Zeppieri 0000-0003-0999-5545.

S-Editor: Liu H
L-Editor: A

P-Editor: Guo X

REFERENCES

1 **Russ S**, Anastasopoulou C, Shafiq I. Pituitary Adenoma. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2024 [PMID: 32119338]

2 **Qin J**, Li K, Wang X, Bao Y. A comparative study of functioning and non-functioning pituitary adenomas. *Medicine (Baltimore)* 2021; **100**: e25306 [PMID: 33832102 DOI: 10.1097/MD.0000000000025306]

3 **Abouaf L**, Vighetto A, Lebas M. Neuro-ophthalmologic exploration in non-functioning pituitary adenoma. *Ann Endocrinol (Paris)* 2015; **76**: 210-219 [PMID: 26070465 DOI: 10.1016/j.ando.2015.04.006]

4 **Hlaváč M**, Sommer F, Karpel-Massler G, Wirtz R, Hoffmann T, Paľa A. [Differential diagnosis and treatment of pituitary adenomas]. *HNO* 2019; **67**: 307-318 [PMID: 30790007 DOI: 10.1007/s00106-019-0629-3]

5 **Chapman PR**, Singhal A, Gaddamanugu S, Prattipati V. Neuroimaging of the Pituitary Gland: Practical Anatomy and Pathology. *Radiol Clin North Am* 2020; **58**: 1115-1133 [PMID: 33040852 DOI: 10.1016/j.rcl.2020.07.009]

6 **Waqar F**, Arif A, Muazzam A, Khan A. Pituitary Adenoma With Apoplexy Presenting As Unilateral Third Nerve Palsy. *Cureus* 2023; **15**: e40555 [PMID: 37465780 DOI: 10.7759/cureus.40555]

7 **Trukhina DA**, Przhiyalkovskaya EG, Belaya ZE, Grigoriev AY, Azizyan VN, Mamedova EO, Rozhinskaya LY, Lapshina AM, Pigarova EA, Dzeranova LK, Platonova NM, Troshina EA, Melnichenko GA. [Thyrotropin-secreting pituitary adenomas: clinical features and results of treatment in 45 patients]. *Probl Endokrinol (Mosk)* 2023; **70**: 23-36 [PMID: 38796758 DOI: 10.14341/probl13325]

8 **Husebye ES**, Castinetti F, Criseno S, Curigliano G, Decallonne B, Fleseriu M, Higham CE, Lupi I, Paschou SA, Toth M, van der Kooij M, Dekkers OM. Endocrine-related adverse conditions in patients receiving immune checkpoint inhibition: an ESE clinical practice guideline. *Eur J Endocrinol* 2022; **187**: G1-G21 [PMID: 36149449 DOI: 10.1530/EJE-22-0689]

9 **Xia Y**, Ma X, Griffiths BB, Luo Y. Neurosurgical anesthesia for a pregnant woman with macroprolactinoma: A case report. *Medicine (Baltimore)* 2018; **97**: e12360 [PMID: 30212994 DOI: 10.1097/MD.0000000000012360]

10 **Wilk A**, Zielinski G, Witek P, Koziarski A. Outcome Assessment After Surgical Treatment of Tuberculum Sellae Meningiomas- A Preliminary Report. *Turk Neurosurg* 2016; **26**: 824-832 [PMID: 27560535 DOI: 10.5137/1019-5149.JTN.14160-15.1]

11 **Tanriverdi F**, Karaca Z, Oner A, Durak AC, Selcuklu A, Unluhizarci K, Kelestimur F. Complete surgical resolution of bilateral total opthalmoplegia without visual field defect in an acromegalic patient presented with pituitary apoplexy. *Endocr J* 2007; **54**: 681-684 [PMID: 17785921 DOI: 10.1507/endocrj.k07-008]

12 **Ryden NA**, Lam H, Judge C, Venteicher AS, Lee MS. Transient Visual Obscurations Without Papilloedema as the Heralding Symptom of Chiasmal Compression. *Neuroophthalmology* 2023; **47**: 106-109 [PMID: 36891405 DOI: 10.1080/01658107.2022.2127790]

13 **Hansford HJ**, Richards GC, Page MJ, Sharp MK, Lee H, Cashin AG. Reporting health and medical research. *BMJ Evid Based Med* 2024; **29**: 358-362 [PMID: 38453420 DOI: 10.1136/bmjebm-2023-112563]

14 **White A**, Junior de Andrade E, Kshettry VR, Sindwani R, Recinos PF. Preoperative Workup for Patients with Pituitary Lesions. *Otolaryngol Clin North Am* 2022; **55**: 233-246 [PMID: 35256177 DOI: 10.1016/j.otc.2021.12.001]

15 **Halilioglu O**, Kuruoglu E, Ozkaya HM, Keskin FE, Gunaldi O, Oz B, Gazioglu N, Kadioglu P, Tanriover N. Multidisciplinary Approach for Acromegaly: A Single Tertiary Center's Experience. *World Neurosurg* 2016; **88**: 270-276 [PMID: 26806060 DOI: 10.1016/j.wneu.2015.12.092]

16 **Colliander R**, Sharma S, Shlobin NA, Fernandez LG, LoPresti MA, Lam S, DeCuypere M. Visual outcomes after treatment of craniopharyngioma in children: A systematic review. *Childs Nerv Syst* 2024; **40**: 1641-1659 [PMID: 38416204 DOI: 10.1007/s00381-024-06328-5]

17 **Nioi M**, Napoli PE, Ferreli F. Fatal Iatrogenic Pituitary Apoplexy after Surgery for Neuroophthalmological Disorder. *Anesthesiology* 2019; **130**: 822 [PMID: 30664061 DOI: 10.1097/ALN.0000000000002584]

18 **Jamal Y**, Camacho Y, Hanft S, Chiarolanzio P, Goldberg MD, Mullally JA. A Case of Pituitary Apoplexy and Cavernous Sinus Syndrome during Hemodialysis. *Case Rep Endocrinol* 2023; **2023**: 3183088 [PMID: 37152694 DOI: 10.1155/2023/3183088]

19 **Lachowicz E**, Lubiński W. The clinical value of the multi-channel PVEP and PERG in the diagnosis and management of the patient with pituitary adenoma: a case report. *Doc Ophthalmol* 2018; **137**: 37-45 [PMID: 29968203 DOI: 10.1007/s10633-018-9647-9]

20 **Qu Y**, Wang YX, Xu L, Zhang L, Zhang J, Zhang J, Wang L, Yang L, Yang A, Wang J, Jonas JB. Glaucoma-like optic neuropathy in patients with intracranial tumours. *Acta Ophthalmol* 2011; **89**: e428-e433 [PMID: 21332674 DOI: 10.1111/j.1755-3768.2011.02118.x]

21 **Chatain GP**, Chee K, Driscoll M, Kleinschmidt-DeMasters BK, Lillehei KO. Pituitary Adenoma Coexistent with Sellar Clear Cell Meningioma Unattached to the Dura: Case Report and Treatment Considerations. *J Neurol Surg Rep* 2024; **85**: e1-e10 [PMID: 38213880 DOI: 10.1055/s-0043-1777792]

22 **Tanios G**, Mungo NA, Kapila A, Bajaj K. Pituitary apoplexy: a rare complication of leuprolide therapy in prostate cancer treatment. *BMJ Case Rep* 2017; **2017** [PMID: 28710301 DOI: 10.1136/bcr-2016-218514]

23 **Özçetin M**, Karacı M, Toroslu E, Edebali N. A pediatric case of pituitary macroadenoma presenting with pituitary apoplexy and cranial nerve involvement: case report. *Turk Pediatri Ars* 2016; **51**: 162-165 [PMID: 27738402 DOI: 10.5152/TurkPediatriArs.2016.1945]

24 **Świątkowska-Stodulska R**, Stodulski D, Babińska A, Piskunowicz M, Sworczak K. Bilateral Tolosa-Hunt syndrome mimicking pituitary adenoma. *Endocrine* 2017; **58**: 582-586 [PMID: 29032531 DOI: 10.1007/s12020-017-1422-2]

25 **Mittal A**, Mishra S, Yadav K, Rajput R. Uncontrolled diabetes as a rare presenting cause of pituitary apoplexy. *BMJ Case Rep* 2019; **12** [PMID: 30824466 DOI: 10.1136/bcr-2018-228161]

26 **Mansoor Q**, Carey PE, Adams W. A rare ophthalmic presentation of pituitary metastases. *BMJ Case Rep* 2012; **2012** [PMID: 22665555 DOI: 10.1136/bcr.11.2011.5145]

27 **Ayele B**, Mengesha A, Wotiye A, Alemayehu Y. Giant Pituitary Adenoma Presenting with Foster-Kennedy Syndrome in a 21-Year Old Ethiopian Patient: A Rarely Reported Phenomenon: A Case Report. *Ethiop J Health Sci* 2020; **30**: 311-314 [PMID: 32165821 DOI: 10.4314/ejhs.v30i2.19]

28 **Musa M**, Aluyi-Osa G, Zeppieri M. Foster Kennedy Syndrome (FKS): A Case Report. *Clin Pract* 2022; **12**: 527-532 [PMID: 35892442 DOI:

10.3390/clinpract12040056]
29 **Yang C**, Zhang H, Zhang S, Liu L, Ma B, Lou J, Sun X, Zhang B. Oculomotor Paralysis, Postorbital Pain, and Hypopituitarism as First Presentations of Metastatic Gastric Cancer in the Pituitary Flourished by Internal Carotid Aneurysm: A Case Report. *Medicine (Baltimore)* 2015; **94**: e2317 [PMID: 26683972 DOI: 10.1097/MD.0000000000002317]
30 **Pasha SA**, Ranganthan LN, Setty VK, Reddy R, Ponnuru DA. Acute Ischaemic Stroke as a Manifestation of Pituitary Apoplexy in a Young Lady. *J Clin Diagn Res* 2017; **11**: OD03-OD05 [PMID: 28658829 DOI: 10.7860/JCDR/2017/25046.9782]
31 **Rudolph T**, Frisén L. Influence of ageing on visual field defects due to stable lesions. *Br J Ophthalmol* 2007; **91**: 1276-1278 [PMID: 17389744 DOI: 10.1136/bjo.2006.112508]
32 **Ng BCF**, Mak CH, Steffi CSY, Wing SK, Shing TT, Ching CF. A Factorial Analysis on Visual Outcomes of Transsphenoidal Surgery for Pituitary Macroadenoma. *Asian J Neurosurg* 2022; **17**: 280-285 [PMID: 36120608 DOI: 10.1055/s-0042-1751011]
33 **Chittiboina P**, Mandal D, Bugarini A, Asuzu DT, Mullaney D, Mastorakos P, Stoica S, Alvarez R, Scott G, Maric D, Elkahloun A, Zhuang Z, Chew EY, Yang C, Linehan M, Lonser RR. Proteostasis Modulation in Germline Missense von Hippel Lindau Disease. *Clin Cancer Res* 2023; **29**: 2199-2209 [PMID: 37018064 DOI: 10.1158/1078-0432.CCR-22-3651]
34 **Aydin MV**, Yangi K, Toptas E, Aydin S. Skull Base Collision Tumors: Giant Non-functioning Pituitary Adenoma and Olfactory Groove Meningioma. *Cureus* 2023; **15**: e44710 [PMID: 37809125 DOI: 10.7759/cureus.44710]
35 **Nadkarni T**, Desai K, Goel A. Spontaneous resolution of residual pituitary adenoma. Case report. *Neurol Med Chir (Tokyo)* 2005; **45**: 315-317 [PMID: 15973066 DOI: 10.2176/nmc.45.315]
36 **Bulte CA**, Hoegler KM, Khachemoune A. Collision tumors: A review of their types, pathogenesis, and diagnostic challenges. *Dermatol Ther* 2020; **33**: e14236 [PMID: 32852089 DOI: 10.1111/dth.14236]
37 **Fan X**, Liu T, Zhang Z, Sun J, Niu N, Mao C, Wang F, Li J, Zhou D, Cao X, Jin Z, Feng F. Comparison of neuroimaging features of histiocytic neoplasms with central nervous system involvement: a retrospective study of 121 adult patients. *Eur Radiol* 2023; **33**: 8031-8042 [PMID: 37191919 DOI: 10.1007/s00330-023-09724-8]
38 **Zheng XQ**, Zhou X, Yao Y, Deng K, You H, Duan L, Zhu HJ. Acromegaly complicated with fulminant pituitary apoplexy: clinical characteristic analysis and review of literature. *Endocrine* 2023; **81**: 160-167 [PMID: 37195580 DOI: 10.1007/s12020-023-03379-7]
39 **Xu L**, Khaddour K, Chen J, Rich KM, Perrin RJ, Campian JL. Pituitary carcinoma: Two case reports and review of literature. *World J Clin Oncol* 2020; **11**: 91-102 [PMID: 32133278 DOI: 10.5306/wjco.v11.i2.91]
40 **Rebrova DV**, Grigorova SI, Vorokhobina NV, Zgoda EA, Novokshonov KY, Feofanova SG, Rusakov VF, Krasnov LM, Fedorov EA, Chinchuk IK, Shikhmagomedov SS, Pushkaruk AA, Sleptsov IV. [Corticotropin-producing pheochromocytoma in multiple endocrine neoplasia type 1]. *Probl Endokrinol (Mosk)* 2023; **69**: 55-64 [PMID: 37968952 DOI: 10.14341/probl13260]
41 **Yu R**. Sellar Mass in 2 Patients With Acute-Onset Headache and Visual Symptoms: Not Your Usual Pituitary Adenoma. *AACE Clin Case Rep* 2023; **9**: 197-200 [PMID: 38045795 DOI: 10.1016/j.aace.2023.09.004]
42 **Alvarez R**, Mastorakos P, Hogan E, Scott G, Lonser RR, Wiley HE, Chew EY, Chittiboina P. Retrobulbar Hemangioblastomas in von Hippel-Lindau Disease: Clinical Course and Management. *Neurosurgery* 2021; **88**: 1012-1020 [PMID: 33442737 DOI: 10.1093/neuros/nyaa565]
43 **Li Z**, Feng T, Teng H, Hu Y, Yao Y, Liu Y. Suprasellar hemangioblastoma without von Hippel-Lindau disease: a case report and literature review. *Int J Clin Exp Pathol* 2015; **8**: 7553-7558 [PMID: 26261668]
44 **Parisi T**, Yuan TL, Faust AM, Caron AM, Bronson R, Lees JA. Selective requirements for E2f3 in the development and tumorigenicity of Rb-deficient chimeric tissues. *Mol Cell Biol* 2007; **27**: 2283-2293 [PMID: 17210634 DOI: 10.1128/MCB.01854-06]
45 **Sivaraju L**, Hegde VS, Kiran NA, Ghosal N, Hegde AS. Pituitary apoplexy presenting as a peripheral rim enhancing parasellar mass lesion with dural enhancement along the tentorium. *Neuroradiol J* 2017; **30**: 561-567 [PMID: 28581357 DOI: 10.1177/1971400917690765]
46 **Natarajan D**, Tatineni S, Ponnapalli SP, Sachdeva V. Pituitary adenoma presenting as acute onset isolated complete third cranial nerve palsy without vision changes. *BMJ Case Rep* 2020; **13** [PMID: 32587114 DOI: 10.1136/bcr-2019-232490]
47 **Zoli M**, Milanese L, Faustini-Fustini M, Guaraldi F, Asioli S, Zenesini C, Righi A, Frank G, Foschini MP, Sturiale C, Pasquini E, Mazzatenta D. Endoscopic Endonasal Surgery for Pituitary Apoplexy: Evidence On a 75-Case Series From a Tertiary Care Center. *World Neurosurg* 2017; **106**: 331-338 [PMID: 28669873 DOI: 10.1016/j.wneu.2017.06.117]
48 **Lall RR**, Shafizadeh SF, Lee KH, Mao Q, Mehta M, Raizer J, Bendok BR, Chandler JP. Orbital metastasis of pituitary growth hormone secreting carcinoma causing lateral gaze palsy. *Surg Neurol Int* 2013; **4**: 59 [PMID: 23646269 DOI: 10.4103/2152-7806.110658]
49 **Lee HR**, Song JE, Lee KY. Developed diplopia and ptosis due to a nonfunctioning pituitary macroadenoma during pregnancy. *Obstet Gynecol Sci* 2014; **57**: 66-69 [PMID: 24596820 DOI: 10.5468/ogs.2014.57.1.66]
50 **Vosoughi AR**, Tyndel F, Suthiphosuwan S, Micieli JA. Post-partum Resolution of Bitemporal Hemianopia with Persisting Pituitary Adenoma. *Can J Neurol Sci* 2024; **51**: 314-316 [PMID: 36329658 DOI: 10.1017/cjn.2022.314]
51 **Wong SH**, Das K, Javadpour M. Pituitary apoplexy initially mistaken for bacterial meningitis. *BMJ Case Rep* 2013; **2013** [PMID: 24014324 DOI: 10.1136/bcr-2013-009223]
52 **Kalaria TR**, Chopra R, Ayuk J, Buch H. Retinal vein occlusion as the presenting feature of Cushing's syndrome. *BMJ Case Rep* 2021; **14** [PMID: 33495181 DOI: 10.1136/bcr-2020-238204]
53 **Madhusudhan S**, Madhusudhan TR, Haslett RS, Sinha A. Pituitary apoplexy following shoulder arthroplasty: a case report. *J Med Case Rep* 2011; **5**: 284 [PMID: 21729259 DOI: 10.1186/1752-1947-5-284]
54 **Besouw MT**, Levtchenko EN, Willemsen MA, Noordam K. Growth hormone producing prolactinoma in juvenile cystinosis: a simple coincidence? *Pediatr Nephrol* 2008; **23**: 307-310 [PMID: 17638022 DOI: 10.1007/s00467-007-0543-x]
55 **Lee EK**, Kim JH, Yu HG. Candida albicans endophthalmitis in a patient with a non-functioning pituitary adenoma evolving into Cushing's disease: A case report. *Med Mycol Case Rep* 2014; **6**: 37-41 [PMID: 25379398 DOI: 10.1016/j.mmcr.2014.09.001]
56 **Beisti Ortego A**, De Arriba Muñoz A, Ferrer Lozano M, Martínez de Zabarte Fernández JM, Calvo Escribano C, Labarta Aizpún JI. [Hypogonadotropic hypogonadism in Klinefelter syndrome and hypothalamic-pituitary tumor]. *Arch Argent Pediatr* 2015; **113**: e6-e9 [PMID: 25622177 DOI: 10.5546/aap.2015.e6]
57 **Griffin S**, Boyce T, Edmunds B, Hills W, Grafe M, Tehrani S. Endogenous hypercortisolism inducing reversible ocular hypertension. *Am J Ophthalmol Case Rep* 2019; **16**: 100573 [PMID: 31768472 DOI: 10.1016/j.ajoc.2019.100573]
58 **Katti V**, Ramamurthy LB, Kanakpur S, Shet SD, Dhoot M. Neuro-ophthalmic presentation of COVID-19 disease: A case report. *Indian J Ophthalmol* 2021; **69**: 992-994 [PMID: 33727476 DOI: 10.4103/ijo.IJO_3321_20]
59 **Heireman S**, Delaey C, Claerhout I, Decock CE. Restrictive extraocular myopathy: a presenting feature of acromegaly. *Indian J Ophthalmol*

2011; **59**: 517-519 [PMID: 22011505 DOI: 10.4103/0301-4738.86330]

60 **Gaballa S**, Lindsay J, AlJaf A, Hlaing KM, Patel K. Acute Unilateral Oculomotor Nerve Palsy as the Initial Presenting Sign of Nonfunctioning Apoplectic Gonadotroph Adenoma. *Cureus* 2020; **12**: e8819 [PMID: 32742834 DOI: 10.7759/cureus.8819]

61 **Zahedi M**, Hizomi Arani R, Tohidi M, Haghighi S, Mehrpour M, Hadaegh F. Nasopharyngeal B-cell lymphoma with pan-hypopituitarism and oculomotor nerve palsy: a case report and review of the literature. *BMC Endocr Disord* 2020; **20**: 163 [PMID: 33143716 DOI: 10.1186/s12902-020-00644-y]

62 **Lachowicz E**, Lubiński W. The importance of the electrophysiological tests in the early diagnosis of ganglion cells and/or optic nerve dysfunction coexisting with pituitary adenoma: an overview. *Doc Ophthalmol* 2018; **137**: 193-202 [PMID: 30374652 DOI: 10.1007/s10633-018-9659-5]

63 **Agarwal R**, Jain VK, Singh S, Charlotte A, Kanaujia V, Mishra P, Sharma K. Segmented retinal analysis in pituitary adenoma with chiasmal compression: A prospective comparative study. *Indian J Ophthalmol* 2021; **69**: 2378-2384 [PMID: 34427226 DOI: 10.4103/ijo.IJO_2086_20]

64 **Wang X**, Chou Y, Zhu H, Xing B, Yao Y, Lu L, You H, Gan L, Wang M, Ma J, Zhong Y. Retinal Microvascular Alterations Detected by Optical Coherence Tomography Angiography in Nonfunctioning Pituitary Adenomas. *Transl Vis Sci Technol* 2022; **11**: 5 [PMID: 34985507 DOI: 10.1167/tvst.11.1.5]

65 **Lee GI**, Park KA, Oh SY, Kong DS, Hong SD. Inner and outer retinal layer thickness alterations in pediatric and juvenile craniopharyngioma. *Sci Rep* 2021; **11**: 2840 [PMID: 33531536 DOI: 10.1038/s41598-021-82107-5]

66 **Tan H**, Chen D, Yu Y, Yu K, He W, Cai B, Jiang S, Tang Y, Tong N, An Z. Unusual ectopic ACTH syndrome in a patient with orbital neuroendocrine tumor, resulted false-positive outcome of BIPSS:a case report. *BMC Endocr Disord* 2020; **20**: 116 [PMID: 32736557 DOI: 10.1186/s12902-020-00590-9]

67 **Wang YX**, Xu L, Lu W, Liu FJ, Qu YZ, Wang J, Jonas JB. Parapapillary atrophy in patients with intracranial tumours. *Acta Ophthalmol* 2013; **91**: 521-525 [PMID: 22632415 DOI: 10.1111/j.1755-3768.2012.02454.x]

68 **Ventura LM**, Venzara FX 3rd, Porciatti V. Reversible dysfunction of retinal ganglion cells in non-secreting pituitary tumors. *Doc Ophthalmol* 2009; **118**: 155-162 [PMID: 18670795 DOI: 10.1007/s10633-008-9143-8]

69 **Molitch ME**. Nonfunctioning pituitary tumors. *Handb Clin Neurol* 2014; **124**: 167-184 [PMID: 25248587 DOI: 10.1016/B978-0-444-59602-4.00012-5]

70 **Lukewich MK**, Micieli JA. Chronic chiasmal compression and persistent visual field defect without detectable changes in optical coherence tomography of the macular ganglion cell complex. *Am J Ophthalmol Case Rep* 2019; **16**: 100533 [PMID: 31467999 DOI: 10.1016/j.ajoc.2019.100533]

71 **Yum HR**, Park SH, Park HY, Shin SY. Macular Ganglion Cell Analysis Determined by Cirrus HD Optical Coherence Tomography for Early Detecting Chiasmal Compression. *PLoS One* 2016; **11**: e0153064 [PMID: 27049647 DOI: 10.1371/journal.pone.0153064]

72 **Cennamo G**, Auriemma RS, Cardone D, Grasso LF, Velotti N, Simeoli C, Di Somma C, Pivonello R, Colao A, de Crecchio G. Evaluation of the retinal nerve fibre layer and ganglion cell complex thickness in pituitary macroadenomas without optic chiasmal compression. *Eye (Lond)* 2015; **29**: 797-802 [PMID: 25853400 DOI: 10.1038/eye.2015.35]

73 **Kasputytė R**, Slatkevičienė G, Liutkevičienė R, Glebauskienė B, Bernotas G, Tamašauskas A. Changes of visual functions in patients with pituitary adenoma. *Medicina (Kaunas)* 2013; **49**: 132-137 [PMID: 23893057]

74 **Sun M**, Zhang Z, Ma C, Chen S, Chen X. Quantitative analysis of retinal layers on three-dimensional spectral-domain optical coherence tomography for pituitary adenoma. *PLoS One* 2017; **12**: e0179532 [PMID: 28628662 DOI: 10.1371/journal.pone.0179532]

75 **Song X**, Wang G, Zhang T, Feng L, An P, Zhu Y. Functional magnetic resonance imaging evaluation of visual cortex activation in patients with anterior visual pathway lesions. *Neural Regen Res* 2012; **7**: 692-696 [PMID: 25745465 DOI: 10.3969/j.issn.1673-5374.2012.09.009]

76 **Guy RL**, Benn JJ, Ayers AB, Bingham JB, Lowy C, Cox TC, Sonksen PH. A comparison of CT and MRI in the assessment of the pituitary and parasellar region. *Clin Radiol* 1991; **43**: 156-161 [PMID: 2013189 DOI: 10.1016/s0009-9260(05)80470-2]

77 **Fledelius HC**. Temporal visual field defects are associated with monocular inattention in chiasmal pathology. *Acta Ophthalmol* 2009; **87**: 769-775 [PMID: 18976316 DOI: 10.1111/j.1755-3768.2008.01328.x]

78 **Lavaque AJ**, Yilmaz T, Cordero-Coma M. Localized bi-nasal macular edema in optic chiasmal syndrome. *Indian J Ophthalmol* 2013; **61**: 351-353 [PMID: 23548317 DOI: 10.4103/0301-4738.97079]

79 **Leal BC**, Moura FC, Monteiro ML. Retinal nerve fiber layer loss documented by Stratus OCT in patients with pituitary adenoma: case report. *Arq Bras Oftalmol* 2006; **69**: 251-254 [PMID: 16699679 DOI: 10.1590/s0004-27492006000200021]

80 **Yang L**, Qu Y, Lu W, Liu F. Evaluation of Macular Ganglion Cell Complex and Peripapillary Retinal Nerve Fiber Layer in Primary Craniopharyngioma by Fourier-Domain Optical Coherence Tomography. *Med Sci Monit* 2016; **22**: 2309-2314 [PMID: 27372909 DOI: 10.12659/msm.896221]

81 **Shon K**, Sung KR. Assessment of macular ganglion cell loss patterns in neurologic lesions that mimic glaucoma. *Korean J Ophthalmol* 2014; **28**: 314-322 [PMID: 25120340 DOI: 10.3341/kjo.2014.28.4.314]

82 **Ergen A**, Kaya Ergen S, Gunduz B, Subasi S, Caklili M, Cabuk B, Anik I, Ceylan S. Retinal vascular and structural recovery analysis by optical coherence tomography angiography after endoscopic decompression in sellar/parasellar tumors. *Sci Rep* 2023; **13**: 14371 [PMID: 37658097 DOI: 10.1038/s41598-023-40956-2]

83 **Cennamo G**, Solari D, Montorio D, Scala MR, D'Andrea L, Tranfa F, Cavallo LM. The role of OCT- angiography in predicting anatomical and functional recovery after endoscopic endonasal pituitary surgery: A 1-year longitudinal study. *PLoS One* 2021; **16**: e0260029 [PMID: 34855775 DOI: 10.1371/journal.pone.0260029]

84 **Boland MV**, Lee IH, Zan E, Yousem DM, Miller NR. Quantitative Analysis of the Displacement of the Anterior Visual Pathway by Pituitary Lesions and the Associated Visual Field Loss. *Invest Ophthalmol Vis Sci* 2016; **57**: 3576-3580 [PMID: 27388050 DOI: 10.1167/iovs.16-19410]

85 **Johansson C**, Lindblom B. The role of optical coherence tomography in the detection of pituitary adenoma. *Acta Ophthalmol* 2009; **87**: 776-779 [PMID: 18771481 DOI: 10.1111/j.1755-3768.2008.01344.x]

86 **Takahashi M**, Goseki T, Ishikawa H, Hiroyasu G, Hirasawa K, Shoji N. Compressive Lesions of the Optic Chiasm: Subjective Symptoms and Visual Field Diagnostic Criteria. *Neuroophthalmology* 2018; **42**: 343-348 [PMID: 30524487 DOI: 10.1080/01658107.2018.1438477]

87 **Kummararaj G**, Balaji V, Kummararaj S, Venugopal NP. Full-field perimetry for evaluation of glaucomatous (presumed) cup. *Indian J Ophthalmol* 2012; **60**: 581-582 [PMID: 23202411 DOI: 10.4103/0301-4738.99858]

88 **Koylu B**, Firlatan B, Sendur SN, Oguz SH, Dagdelen S, Erbas T. Giant growth hormone-secreting pituitary adenomas from the endocrinologist's perspective. *Endocrine* 2023; **79**: 545-553 [PMID: 36318446 DOI: 10.1007/s12020-022-03241-2]

89 **Graffeo CS**, Donegan D, Erickson D, Brown PD, Perry A, Link MJ, Young WF, Pollock BE. The Impact of Insulin-Like Growth Factor Index

and Biologically Effective Dose on Outcomes After Stereotactic Radiosurgery for Acromegaly: Cohort Study. *Neurosurgery* 2020; **87**: 538-546 [PMID: 32267504 DOI: 10.1093/neuros/nyaa054]

90 **Zoli M**, Faustini-Fustini M, Mazzatenta D, Marucci G, De Carlo E, Bacci A, Pasquini E, Lanzino G, Frank G. ACTH adenomas transforming their clinical expression: report of 5 cases. *Neurosurg Focus* 2015; **38**: E15 [PMID: 25639317 DOI: 10.3171/2014.11.FOCUS14679]

91 **Sun X**, Lu L, Feng M, Fan Y, Bao X, Dai C, Deng K, Guo D, Yao Y, Zhu H, Wang R. Cushing Syndrome Caused by Ectopic Adrenocorticotropic Hormone-Secreting Pituitary Adenomas: Case Report and Literature Review. *World Neurosurg* 2020; **142**: 75-86 [PMID: 32592962 DOI: 10.1016/j.wneu.2020.06.138]

92 **Phillips J**, East HE, French SE, Melcescu E, Hamilton RD, Nicholas WC, Fratkin JF, Parent AD, Luzardo G, Koch CA. What causes a prolactinoma to be aggressive or to become a pituitary carcinoma? *Hormones (Athens)* 2012; **11**: 477-482 [PMID: 23422771 DOI: 10.14310/horm.2002.1380]

93 **Yanovski JA**, Cutler GB Jr, Chrousos GP, Nieman LK. The dexamethasone-suppressed corticotropin-releasing hormone stimulation test differentiates mild Cushing's disease from normal physiology. *J Clin Endocrinol Metab* 1998; **83**: 348-352 [PMID: 9467539 DOI: 10.1210/jcem.83.2.4568]

94 **Tsukaguchi R**, Hasebe M, Honjo S, Hamasaki A. Ovarian Hyperstimulation Syndrome Caused by Functional Gonadotroph Pituitary Adenoma. *JCEM Case Rep* 2023; **1**: luad087 [PMID: 37908987 DOI: 10.1210/jcemcr/luad087]

95 **Horiguchi K**. The molecular biology of thyrotroph pituitary neuroendocrine tumors. *Endocr J* 2023; **70**: 135-139 [PMID: 36653153 DOI: 10.1507/endocrj.EJ22-0514]

96 **Lee GI**, Kim Y, Park KA, Oh SY, Kong DS, Hong SD. Parafoveal and peripapillary vessel density in pediatric and juvenile craniopharyngioma patients. *Sci Rep* 2022; **12**: 5355 [PMID: 35354881 DOI: 10.1038/s41598-022-09391-7]

97 **Liu G**, Su L, Xiang Y, Liu Y, Zhang S. Coexistence of craniopharyngioma and meningioma: Two rare cases and literature review. *Medicine (Baltimore)* 2020; **99**: e23183 [PMID: 33327235 DOI: 10.1097/MD.0000000000023183]

98 **Larkin S**, Ansorge O. Pathology And Pathogenesis Of Pituitary Adenomas And Other Sellar Lesions. 2017 Feb 15. In: Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000– [PMID: 28402620]

99 **Cao J**, Li G, Sun Y, Hong X, Huang H. Sellar chondrosarcoma presenting with amenorrhea: A case report. *Medicine (Baltimore)* 2018; **97**: e11274 [PMID: 29979394 DOI: 10.1097/MD.0000000000011274]

100 **Joo C**, Ha G, Jang Y. Pituitary apoplexy following lumbar fusion surgery in prone position: A case report. *Medicine (Baltimore)* 2018; **97**: e0676 [PMID: 29742711 DOI: 10.1097/MD.0000000000010676]

101 **Patel KR**, Zheng J, Tabar V, Cohen MA, Girotra M. Extended Survival After Surgical Resection for Pituitary Metastases: Clinical Features, Management, and Outcomes of Metastatic Disease to the Sella. *Oncologist* 2020; **25**: e789-e797 [PMID: 31784491 DOI: 10.1634/theoncologist.2019-0520]

102 **Paschou SA**, Tzioras K, Trianti V, Lyra S, Lioutas VA, Seretis A, Vryonidou A. Young adult patient with headache, fever and blurred vision. *Hormones (Athens)* 2016; **15**: 548-550 [PMID: 28222415 DOI: 10.14310/horm.2002.1701]

103 **Salhi S**, Oueslati I, Mouelhi Y, Zehani A, Kchir N, Kamoun E, Yazidi M, Chihaoui M. Secondary xanthogranulomatous hypophysitis mimicking a pituitary macroadenoma: a case report. *J Int Med Res* 2024; **52**: 3000605231223033 [PMID: 38190975 DOI: 10.1177/03000605231223033]

104 **Clapp AN**, DePold Hohler A. A case of parasympathetic hyperactivity and associated Parry-Romberg syndrome. *SAGE Open Med Case Rep* 2021; **9**: 2050313X211034351 [PMID: 34367643 DOI: 10.1177/2050313X211034351]

105 **Gao H**, Wu S, Zhang X, Xie T. Minimally invasive follicular thyroid carcinoma mimicking pituitary adenoma: a case report. *Int J Clin Exp Pathol* 2019; **12**: 3949-3952 [PMID: 31933788]

106 **Dutta D**, Ahuja A, Sharma L, Bhardwaj M, Kulshreshtha B. Macular amyloidosis complicating macroprolactinoma--a novel clinical association. *Endokrynol Pol* 2015; **66**: 555-558 [PMID: 26662655 DOI: 10.5603/EP.2015.0068]

107 **Gondim J**, Ramos Júnior F, Pinheiro I, Schops M, Tella Júnior OI. Minimally invasive pituitary surgery in a hemorrhagic necrosis of adenoma during pregnancy. *Minim Invasive Neurosurg* 2003; **46**: 173-176 [PMID: 12872196 DOI: 10.1055/s-2003-40734]

108 **Saktiwarawat K**, Tunthanathip T, Oearsakul T, Taweesomboonyat C. Comparing neuroendocrine recovery between surgical and conservative management in pituitary apoplexy patients: a propensity score-matched analysis. *Neurosurg Rev* 2024; **47**: 236 [PMID: 38802695 DOI: 10.1007/s10143-024-02461-6]

109 **Huynh N**, Stemmer-Rachamimov AO, Swearingen B, Cestari DM. Decreased vision and junctional scotoma from pituicytoma. *Case Rep Ophthalmol* 2012; **3**: 190-196 [PMID: 22740829 DOI: 10.1159/000339242]

110 **Attiku Y**, Rishi P, Bassi S. Coexisting Optic Disc Melanocytoma and Pituitary Adenoma. *Ocul Oncol Pathol* 2019; **5**: 319-322 [PMID: 31559242 DOI: 10.1159/000496149]

111 **Hassani FD**, Fadli M, El Abbadi N. [Pituitary sarcoidosis mimicking pituitary adenoma: case report and literature review]. *Pan Afr Med J* 2019; **33**: 92 [PMID: 31489070 DOI: 10.11604/pamj.2019.33.92.17881]

112 **Póczoš P**, Kremláček J, Česák T, Macháčková M, Jirásková N. The use of optical coherence tomography in chiasmal compression. *Cesk Slov Oftalmol* 2019; **75**: 120-127 [PMID: 31779460 DOI: 10.31348/2019/3/2]

113 **Cho WJ**, Joo SP, Kim TS, Seo BR. Pituitary apoplexy presenting as isolated third cranial nerve palsy with ptosis : two case reports. *J Korean Neurosurg Soc* 2009; **45**: 118-121 [PMID: 19274125 DOI: 10.3340/jkns.2009.45.2.118]

114 **Zaki U**, Shakeel AS, Rauf Y, Raza M. Pituicytoma: A rare tumor of the sella. A case report and review of literature for diagnosis and management. *Surg Neurol Int* 2023; **14**: 220 [PMID: 37404513 DOI: 10.25259/SNI_248_2023]

115 **Yin S**, Zhou P, Li Q, Jiang S. Intrasellar Clear Cell Meningioma Mimicking Invasive Pituitary Adenoma: A Case Report and Review of the Literature. *Turk Neurosurg* 2015; **25**: 976-979 [PMID: 26617154 DOI: 10.5137/1019-5149.JTN.11847-14.1]

116 **Jin G**, Hao S, Xie J, Mi R, Liu F. Collision tumors of the sella: coexistence of pituitary adenoma and craniopharyngioma in the sellar region. *World J Surg Oncol* 2013; **11**: 178 [PMID: 23919255 DOI: 10.1186/1477-7819-11-178]

117 **Lynch GL**, Broome MR, Scagliotti RH. What is your diagnosis? Mass originating from the pituitary fossa. *J Am Vet Med Assoc* 2006; **228**: 1681-1682 [PMID: 16740067 DOI: 10.2460/javma.228.11.1681]

118 **Jugović D**, Spazzapan P, Porčnik A, Prestor B. TRANS-ENDOSCOPIC TREATMENT OF CRANIOPHARYNGIOMA AND RECOVERY FROM BLINDNESS IN ADULT PATIENT - A CASE REPORT. *Acta Clin Croat* 2020; **59**: 549-554 [PMID: 34177068 DOI: 10.20471/acc.2020.59.03.22]

119 **Chokyu I**, Tsuyuguchi N, Goto T, Chokyu K, Chokyu M, Ohata K. Pituitary apoplexy causing internal carotid artery occlusion--case report. *Neurol Med Chir (Tokyo)* 2011; **51**: 48-51 [PMID: 21273745 DOI: 10.2176/nmc.51.48]

120 **Yamamuro S**, Yoshino A, Nishide T, Negishi H, Kumagawa T. A case report of pituitary neuroendocrine tumor manifesting as severe conjunctival chemosis. *BMC Ophthalmol* 2023; **23**: 479 [PMID: 37993825 DOI: 10.1186/s12886-023-03224-5]

121 **Lee DK**, Sung MS, Park SW. Factors Influencing Visual Field Recovery after Transsphenoidal Resection of a Pituitary Adenoma. *Korean J Ophthalmol* 2018; **32**: 488-496 [PMID: 30549473 DOI: 10.3341/kjo.2017.0094]

122 **Hug NF**, Purger DA, Moss HE, Dodd RL. Pituitary macroadenoma causing vision loss in Wyburn-Mason syndrome: illustrative case. *J Neurosurg Case Lessons* 2022; **4** [PMID: 36572974 DOI: 10.3171/CASE22236]

123 **Santos CDSE**, Filho LMDCL, Santos CAT, Neill JS, Vale HF, Kurnutala LN. Pituitary tumor resection in a patient with SARS-CoV-2 (COVID-19) infection. A case report and suggested airway management guidelines. *Braz J Anesthesiol* 2020; **70**: 165-170 [PMID: 32834194 DOI: 10.1016/j.bjane.2020.05.003]

124 **Tagoe NN**, Essuman VA, Bankah P, Dakurah T, Hewlett VK, Akpalu J, Ndanu TA. Visual Outcome of Patients with Pituitary Adenomas Following Surgery and Its Contributory Factors at a Tertiary Hospital in Ghana. *Ethiop J Health Sci* 2019; **29**: 895-902 [PMID: 30700957 DOI: 10.4314/ejhs.v29i1.11]

125 **Wang J**, Song DL, Deng L, Sun SY, Liu C, Gong DS, Wang Y, Xu QW. Extraventricular neurocytoma of the sellar region: case report and literature review. *Springerplus* 2016; **5**: 987 [PMID: 27398267 DOI: 10.1186/s40064-016-2650-2]

126 **Choi KY**, Choi S, Jeong S, Won TB. Successful Endoscopic Transsphenoidal Approach Treatment of Sphenoid Sinus Organized Hematoma Causing Visual Deficit: A Case Report. *Medicina (Kaunas)* 2023; **59** [PMID: 37893520 DOI: 10.3390/medicina59101802]

127 **Dekkers OM**, de Keizer RJ, Roelfsema F, Vd Klaauw AA, Honkoop PJ, van Dulken H, Smit JW, Romijn JA, Pereira AM. Progressive improvement of impaired visual acuity during the first year after transsphenoidal surgery for non-functioning pituitary macroadenoma. *Pituitary* 2007; **10**: 61-65 [PMID: 17318437 DOI: 10.1007/s11102-007-0007-0]

128 **Ishikawa H**, Akura J, Uchida K, Ikeda N, Ikeda T, Borlongan CV, Mimura O. A case with transient refractive change after removal of pituitary tumor. *BMC Ophthalmol* 2013; **13**: 65 [PMID: 24180221 DOI: 10.1186/1471-2415-13-65]

129 **Seuk JW**, Kim CH, Yang MS, Cheong JH, Kim JM. Visual outcome after transsphenoidal surgery in patients with pituitary apoplexy. *J Korean Neurosurg Soc* 2011; **49**: 339-344 [PMID: 21887391 DOI: 10.3340/jkns.2011.49.6.339]

130 **Pokhrel B**, Khanal S, Chapagain P, Sedain G. Pituitary Apoplexy Complicated by Cerebral Infarction: A Case Report. *JNMA J Nepal Med Assoc* 2021; **59**: 723-726 [PMID: 34508512 DOI: 10.31729/jnma.6120]

131 **Lee GI**, Park KA, Oh SY, Kong DS. Changes in parafoveal and peripapillary perfusion after decompression surgery in chiasmal compression due to pituitary tumors. *Sci Rep* 2021; **11**: 3464 [PMID: 33568736 DOI: 10.1038/s41598-021-82151-1]

132 **Lefevre E**, Chasseloup F, Hage M, Chanson P, Buchfelder M, Kamenický P. Clinical and therapeutic implications of cavernous sinus invasion in pituitary adenomas. *Endocrine* 2024; **85**: 1058-1065 [PMID: 38761347 DOI: 10.1007/s12020-024-03877-2]

133 **Gu Y**, Zhong X, Gao Y, He L. Endoscopic endonasal approach for simultaneously treating a pituitary adenoma coexisting with a paraclinoid aneurysm: illustrative case. *J Neurosurg Case Lessons* 2022; **3**: CASE22130 [PMID: 35733842 DOI: 10.3171/CASE22130]

134 **Wei P**, Falardeau J, Chen A, Wang J, Liu L, Jia Y, Huang D. Optical coherence tomographic angiography detects retinal vascular changes associated with pituitary adenoma. *Am J Ophthalmol Case Rep* 2022; **28**: 101711 [PMID: 36164560 DOI: 10.1016/j.ajoc.2022.101711]

135 **Kurian DE**, V R, Horo S, Chacko AG, Prabhu K, Mahasampath G, Korah S. Predictive value of retinal nerve fibre layer thickness for postoperative visual improvement in patients with pituitary macroadenoma. *BMJ Open Ophthalmol* 2022; **7** [PMID: 36161840 DOI: 10.1136/bmjophth-2021-000964]

136 **Cennamo G**, Solari D, Montorio D, Scala MR, Melenzane A, Fossataro F, Somma T, Tranfa F, Cavallo LM. Early vascular modifications after endoscopic endonasal pituitary surgery: The role of OCT-angiography. *PLoS One* 2020; **15**: e0241295 [PMID: 33119707 DOI: 10.1371/journal.pone.0241295]

137 **Thirumala PD**. Visual evoked potentials for visual function monitoring during endoscopic sphenoidal surgery: Advancement and challenges. *Neurol India* 2018; **66**: 958-959 [PMID: 30038078 DOI: 10.4103/0028-3886.237010]

138 **Mahvash M**, Igressa A, Pechlivanis I, Weber F, Charalampaki P. Endoscopic endonasal transsphenoidal approach for resection of a coexistent pituitary macroadenoma and a tuberculum sellae meningioma. *Asian J Neurosurg* 2014; **9**: 236 [PMID: 25685225 DOI: 10.4103/1793-5482.146629]

139 **La Rosa A**, Wroe A, Fellows Z, Kotecha R. Proton Radiosurgery: Current Concepts and Limitations for CNS Radiosurgery. *Neurol India* 2023; **71**: S174-S182 [PMID: 37026350 DOI: 10.4103/0028-3886.373636]

140 **Orski M**, Tarnawski R, Wylęgała E, Tarnawska D. The Impact of Robotic Fractionated Radiotherapy for Benign Tumors of Parasellar Region on the Eye Structure and Function. *J Clin Med* 2023; **12** [PMID: 36675334 DOI: 10.3390/jcm12020404]

141 **Chao ST**, Thakkar VV, Barnett GH, Vogelbaum MA, Angelov L, Weil RJ, Rasmussen P, Reuther AM, Jamison B, Neyman G, Suh JH. Prospective study of the short-term adverse effects of gamma knife radiosurgery. *Technol Cancer Res Treat* 2012; **11**: 117-122 [PMID: 22335405 DOI: 10.7785/tcrt.2012.500240]

142 **Wang PW**, Chung MH, Feng SW, Liao HC, Wu YC, Hueng DY, Yang YJ, Ju DT. Case report: Ruptured internal carotid artery fusiform aneurysm mimicking pituitary apoplexy after stereotactic radiosurgery. *Front Neurol* 2023; **14**: 1219372 [PMID: 37602248 DOI: 10.3389/fneur.2023.1219372]

143 **Puataweepong P**, Dhanachai M. Rapid and significant reduction in size of pituitary adenoma in children treated with fractionated stereotactic radiation therapy: a case report. *Case Rep Endocrinol* 2011; **2011**: 187839 [PMID: 22937278 DOI: 10.1155/2011/187839]

144 **Papanastasiou L**, Pappa T, Dasou A, Kyrodimou E, Kontogeorgos G, Samara C, Bacaracos P, Galanopoulos A, Piaditis G. Case report: Primary pituitary non-Hodgkin's lymphoma developed following surgery and radiation of a pituitary macroadenoma. *Hormones (Athens)* 2012; **11**: 488-494 [PMID: 23422773 DOI: 10.14310/horm.2002.1382]

145 **Ram TS**, Ravindran PB, Viswanathan FR, Viswanathan PN, Pavamani SP. Extracranial doses in stereotactic and conventional radiotherapy for pituitary adenomas. *J Appl Clin Med Phys* 2006; **7**: 96-100 [PMID: 17533326 DOI: 10.1120/jacmp.v7i2.2203]

146 **Ennaifer H**, Jemel M, Kandar H, Grira W, Kammoun I, Salem LB. Developed diplopia due to a pituitary macroadenoma during pregnancy. *Pan Afr Med J* 2018; **29**: 39 [PMID: 29875921 DOI: 10.11604/pamj.2018.29.39.12706]

147 **Fan J**, Shen H, Mo J, Zhang J. Complete Shrinking of Mixed Growth Hormone and Prolactin-Secreting Pituitary Adenoma With Bromocriptine Therapy Alone. *J Craniofac Surg* 2024; **35**: e620-e622 [PMID: 38710062 DOI: 10.1097/SCS.0000000000010227]

148 **Chen Z**, Shou X, Ji L, Cheng H, Shen M, Ma Z, He W, Ye Z, Zhang Y, Qiao N, Zhang Q, Wang Y. Presurgical Medical Treatment in Prolactinomas: Surgical Implications and Pathological Characteristics From 290 Cases. *J Clin Endocrinol Metab* 2024; **109**: 1433-1442 [PMID: 38163969 DOI: 10.1210/clinem/dgad758]

149 **Triviño V**, Fidalgo O, Juane A, Pombo J, Cordido F. Gonadotrophin-releasing hormone agonist-induced pituitary adenoma apoplexy and casual finding of a parathyroid carcinoma: A case report and review of literature. *World J Clin Cases* 2019; **7**: 3259-3265 [PMID: 31667176 DOI: 10.12998/wjcc.v7.i20.3259]

150 **Kim H**, Lee JH, Choi SJ, Kim WK, Lee JS, Lee KH. Analysis of fatal intracranial hemorrhage in 792 acute leukemia patients. *Haematologica* 2004; **89**: 622-624 [PMID: 15136234]

151 **Baagar KA**, Sadiq A, Khan AA, Dabbous Z, Rohani Z. Successful medical management of a pituitary macroadenoma with features of resistant acromegaly and hyperprolactinemia using pasireotide. *Qatar Med J* 2024; **2024**: 17 [PMID: 38654814 DOI: 10.5339/qmj.2024.17]

152 **Regazzo D**, Avallone S, MacSweeney CP, Sergeev E, Howe D, Godwood A, Bennett KA, Brown AJH, Barnes M, Occhi G, Barbot M, Faggian D, Tropeano MP, Losa M, Lasio G, Scaroni C, Pecori Giraldi F. A novel somatostatin receptor ligand for human ACTH - and GH - secreting pituitary adenomas. *Eur J Endocrinol* 2024; **190**: K8-K16 [PMID: 38123488 DOI: 10.1093/ejendo/lvad171]

153 **Kotwal A**, Kennedy R, Kikani N, Thosani S, Goldner W, Shariff A. Endocrinopathies Associated With Immune Checkpoint Inhibitor Use. *Endocr Pract* 2024; **30**: 584-591 [PMID: 38554775 DOI: 10.1016/j.eprac.2024.03.023]

154 **Doğan C**, Güleser ÜY, Kılıçarslan O, Mergen B, Açbay Ö, İskeleli G. The Effect of Prolactinoma on Tear Film Function. *Turk J Ophthalmol* 2022; **52**: 374-378 [PMID: 36578179 DOI: 10.4274/tjo.galenos.2022.98752]

155 **Skrzypiec I**, Wierzbowska J, Sobol M, Zieliński G. Corneal Tonometric and Morphological Changes in Patients with Acromegaly. *J Clin Med* 2022; **11** [PMID: 36431227 DOI: 10.3390/jcm11226750]

156 **Barbosa AP**, Oliveira FR, Rocha FJD, Muglia VF, Rocha EM. Lacrimal gland atrophy and dry eye related to isotretinoin, androgen, and prolactin: differential diagnosis for Sjögren's syndrome. *Arq Bras Oftalmol* 2021; **84**: 78-82 [PMID: 33470346 DOI: 10.5935/0004-2749.20210012]

157 **Kanj U**, Lee SS, Wattegama M, Chavda S, Karavitaki N, Batra R. Foster Kennedy syndrome secondary to a giant prolactinoma with a remarkable response to cabergoline. *Endocrinol Diabetes Metab Case Rep* 2022; **2022** [PMID: 36017805 DOI: 10.1530/EDM-22-0261]

158 **Shibue K**, Yamakawa M, Nishida N, Hamasaki A. Resolution of Visual Field Defect in Macroprolactinoma After Treatment With Cabergoline. *Cureus* 2022; **14**: e25548 [PMID: 35783884 DOI: 10.7759/cureus.25548]

159 **Uvelius E**, Valdemarsson S, Bengzon J, Hammar B, Siesjö P. Visual acuity in patients with non-functioning pituitary adenoma: Prognostic factors and long-term outcome after surgery. *Brain Spine* 2023; **3**: 102667 [PMID: 38020979 DOI: 10.1016/j.bas.2023.102667]

160 **Butenschoen VM**, Schwendinger N, von Werder A, Bette S, Wienke M, Meyer B, Gempt J. Visual acuity and its postoperative outcome after transsphenoidal adenoma resection. *Neurosurg Rev* 2021; **44**: 2245-2251 [PMID: 33040306 DOI: 10.1007/s10143-020-01408-x]

161 **Monteiro ML**, Moura FC, Cunha LP. Frequency doubling perimetry in patients with mild and moderate pituitary tumor-associated visual field defects detected by conventional perimetry. *Arq Bras Oftalmol* 2007; **70**: 323-329 [PMID: 17589707 DOI: 10.1590/s0004-27492007000200024]

162 **Kaur K**, Gurnani B. Contrast Sensitivity. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2024 [PMID: 35593849]

163 **Kotoda Y**, Kotoda M, Ogiwara M, Kinouchi H, Iijima H. Left-Right and Upper-Lower Light Sensitivity Asymmetry in Visual Field Defects Caused by Pituitary Adenoma: A Retrospective Observational Study. *Clin Ophthalmol* 2020; **14**: 317-324 [PMID: 32099316 DOI: 10.2147/OPTH.S234422]

164 **Zheng J**, Chen W, Huang D, Wang Y, Zheng D, Zhou L, Brelén ME, Huang Z. Ocular symptoms as the initial clinical manifestations in patients with extraocular tumors. *Ann Transl Med* 2021; **9**: 497 [PMID: 33850894 DOI: 10.21037/atm-21-830]

165 **Iqbal M**, Irfan S, Goyal JL, Singh D, Singh H, Dutta G. An Analysis of Retinal Nerve Fiber Layer Thickness before and after Pituitary Adenoma Surgery and its Correlation with Visual Acuity. *Neurol India* 2020; **68**: 346-351 [PMID: 32189695 DOI: 10.4103/0028-3886.280634]

166 **Bao XD**, Lu L, Zhu HJ, Yao Y, Feng M, Wang RZ, Zhai X, Fu Y, Gong FY, Lu ZL. Concurrent mutations of germline GPR101 and somatic USP8 in a pediatric giant pituitary ACTH adenoma: a case report. *BMC Endocr Disord* 2022; **22**: 152 [PMID: 35668434 DOI: 10.1186/s12902-022-01058-8]

167 **Yoneoka Y**, Hatase T, Watanabe N, Jinguji S, Okada M, Takagi M, Fujii Y. Early morphological recovery of the optic chiasm is associated with excellent visual outcome in patients with compressive chiasmal syndrome caused by pituitary tumors. *Neurol Res* 2015; **37**: 1-8 [PMID: 24938320 DOI: 10.1179/1743132814Y.0000000407]

168 **Nganga HK**, Lubanga RP. Pituitary macroadenoma presenting with pituitary apoplexy, acromegaly and secondary diabetes mellitus - a case report. *Pan Afr Med J* 2013; **15**: 39 [PMID: 24062868 DOI: 10.11604/pamj.2013.15.39.2054]

169 **Santorini M**, Ferreira De Moura T, Barraud S, Litré CF, Brugniart C, Denoyer A, Djerada Z, Arndt C. Comparative Evaluation of Two SD-OCT Macular Parameters (GCC, GCL) and RNFL in Chiasmal Compression. *Eye Brain* 2022; **14**: 35-48 [PMID: 35282333 DOI: 10.2147/EB.S337333]

170 **Koçer AM**, İlhan B, Güngör A. Intracranial Mass Lesion in a Patient Being Followed up for Amblyopia. *Turk J Ophthalmol* 2020; **50**: 317-320 [PMID: 33342203 DOI: 10.4274/tjo.galenos.2020.36360]

171 **Choudhari NS**, Neog A, Fudnawala V, George R. Cupped disc with normal intraocular pressure: the long road to avoid misdiagnosis. *Indian J Ophthalmol* 2011; **59**: 491-497 [PMID: 22011496 DOI: 10.4103/0301-4738.86320]

172 **Srimanan W**, Panyakorn S. COVID-19 Vaccine-Induced Expansion of Pituitary Adenoma: A Case Report. *Cureus* 2023; **15**: e50685 [PMID: 38229808 DOI: 10.7759/cureus.50685]

173 **Shah SN**, Kaki PC, Shah SS, Shah SA. Concurrent Radiation and Targeted Therapy for Papillary Craniopharyngioma: A Case Report. *Cureus* 2023; **15**: e40190 [PMID: 37431357 DOI: 10.7759/cureus.40190]

174 **Jiang C**, Zhang W, Wang H, Jiao Y, Fang Y, Feng F, Feng M, Wang R. Machine Learning Approaches to Differentiate Sellar-Suprasellar Cystic Lesions on Magnetic Resonance Imaging. *Bioengineering (Basel)* 2023; **10** [PMID: 38002419 DOI: 10.3390/bioengineering10111295]

175 **Baumann F**, Schmid C, Bernays RL. Intraoperative magnetic resonance imaging-guided transsphenoidal surgery for giant pituitary adenomas. *Neurosurg Rev* 2010; **33**: 83-90 [PMID: 19823884 DOI: 10.1007/s10143-009-0230-4]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 98132

DOI: 10.5662/wjm.v15.i3.98132

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Impact of cognitive rehabilitation interventions on memory improvement in patients after stroke: A systematic review

Rebecca Mathew, Komudi Sapru, Dhruv Nihal Gandhi, Tahoora Abdul Nasir Surve, Devina Pande, Anushri Parikh, Rebecca Bhairavdutt Sharma, Ravneet Kaur, Md Al Hasibuzzaman

Specialty type: Medicine, research and experimental

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade A, Grade B, Grade D

Novelty: Grade A, Grade B, Grade C

Creativity or Innovation: Grade A, Grade B, Grade C

Scientific Significance: Grade A, Grade B, Grade C

P-Reviewer: Chakraborty R; Lu JY; Mohamed Mahmoud MI

Received: June 18, 2024

Revised: October 7, 2024

Accepted: December 18, 2024

Published online: September 20, 2025

Processing time: 260 Days and 18.8 Hours

Rebecca Mathew, Instructor Nursing, Fatima College of Health Sciences, Ajman 3798, United Arab Emirates

Komudi Sapru, Department of Medicine, Saraswati Medical College, Unnao 55905, Uttar Pradesh, India

Dhruv Nihal Gandhi, Department of Internal Medicine, KJ Somaiya Medical College and Research Center, Mumbai 400022, India

Tahoora Abdul Nasir Surve, Devina Pande, Rebecca Bhairavdutt Sharma, Department of Medicine, KJ Somaiya Medical College, Mumbai 400022, India

Anushri Parikh, Department of Medicine, Medical College Baroda, Vadodara 33872, India

Ravneet Kaur, Department of Medicine, Lady Hardinge Medical College, Delhi 33872, India

Md Al Hasibuzzaman, Department of Medicine, Niramoy Hospital, Panchagarh 5010, Bangladesh

Corresponding author: Md Al Hasibuzzaman, MBBS, Department of Medicine, Niramoy Hospital, Panchagarh 5010, Bangladesh. al.hasibuzzaman.hasib@gmail.com

Abstract

BACKGROUND

Cognitive impairment is a major cause of disability in patients who have suffered from a stroke, and cognitive rehabilitation interventions show promise for improving memory.

AIM

To examine the effectiveness of virtual reality (VR) and non-VR (NVR) cognitive rehabilitation techniques for improving memory in patients after stroke.

METHODS

An extensive and thorough search was executed across five pertinent electronic databases: Cumulative Index to Nursing and Allied Health Literature; MEDLINE (PubMed); Scopus; ProQuest Central; and Google Scholar. This systematic review was conducted following the preferred reporting items for systematic reviews and meta-analyses guideline. Studies that recruited participants who experienced a stroke, utilized cognitive rehabilitation interventions, and published in the last 10

years were included in the review.

RESULTS

Thirty studies met the inclusion criteria. VR interventions significantly improved memory and cognitive function (mean difference: 4.2 ± 1.3, $P < 0.05$), whereas NVR (including cognitive training, music, and exercise) moderately improved memory. Compared with traditional methods, technology-driven VR approaches were particularly beneficial for enhancing daily cognitive tasks.

CONCLUSION

VR and NVR reality interventions are beneficial for post-stroke cognitive recovery, with VR providing enhanced immersive experiences. Both approaches hold transformative potential for post-stroke rehabilitation.

Key Words: Cognitive rehabilitation; Memory improvement; Stroke; Technology; Virtual reality

Core Tip: Virtual reality (VR) and non-VR cognitive rehabilitation interventions show promise in enhancing memory and overall cognitive function in patients who experienced a stroke. In this systematic review of 30 studies, VR-based approaches demonstrated significant cognitive improvements, leveraging immersive experiences that simulate real-world tasks to aid recovery. Non-VR interventions, including brain stimulation, and cognitive training have also been shown to be effective in improving memory and quality of life. By providing insights into these diverse strategies, this review underscored the transformative potential of tailored cognitive rehabilitation techniques in post-stroke recovery.

Citation: Mathew R, Sapru K, Gandhi DN, Surve TAN, Pande D, Parikh A, Sharma RB, Kaur R, Hasibuzzaman MA. Impact of cognitive rehabilitation interventions on memory improvement in patients after stroke: A systematic review. *World J Methodol* 2025; 15(3): 98132

URL: https://www.wjgnet.com/2222-0682/full/v15/i3/98132.htm

DOI: https://dx.doi.org/10.5662/wjm.v15.i3.98132

INTRODUCTION

Cerebrovascular accidents or strokes are major causes of morbidity worldwide and impose a substantial burden on individuals, families, and healthcare systems[1]. Stroke is among the primary contributors to mortality and disability worldwide[2,3]. Cognitive impairment following a stroke is the most prevalent type of disability[4]. In the realm of neurological rehabilitation, the aftermath of a stroke presents a formidable challenge, necessitating comprehensive strategies to mitigate the multifaceted impact on an individual's physical, psychological, and cognitive well-being[5]. While considerable progress has been made in the acute management of stroke, the consequences of stroke-related cognitive impairments continue to delude both clinicians and researchers[6]. Cognitive deficits, including memory loss, attention difficulties, and executive dysfunction, often persist in patients who experienced a stroke, with some studies suggesting that the rate of dementia is approximately 30% at 1-year post-stroke. As the population continues to age and stroke incidence remains a significant global health issue, the imperative to optimize recovery strategies has never been more pronounced[1,7].

Cognitive rehabilitation, a therapeutic approach aimed at improving cognitive functioning and daily living skills through structured interventions, has emerged as a promising avenue to address these enduring challenges[8,9]. This is a dynamic and evolving field and has emerged as a beacon of hope for individuals who experienced a stroke and are grappling with memory impairments[10]. The principal goals of post-stroke cognitive rehabilitation are to improve cognitive functioning to allow individuals to participate in everyday activities on their own, reduce the severity and impact of impairment, and improve overall quality of life. These requirements can change as patients progress, receive support from caregivers, or move through various stages of recovery following a stroke[11].

The efficacy of various cognitive rehabilitation interventions in post-stroke recovery has garnered increasing attention in the last few decades. This systematic review aimed to comprehensively evaluate the existing body of evidence to shed light on the benefits and limitations of cognitive rehabilitation interventions for patients who experienced a stroke in terms of memory improvement. Several factors explain the rationale for undertaking this review. First, stroke survivors frequently experience cognitive deficits. Personalized rehabilitation strategies are needed since these impairments can impact a variety of cognitive areas, including memory, attention, language, executive function, and visuospatial abilities [12]. Second, the diversity of available cognitive rehabilitation techniques and interventions complicates the decision-making process for clinicians and rehabilitation specialists. A rigorous evaluation of the literature is needed to guide evidence-based clinical practice.

Stroke-induced memory impairment can range from mild forgetfulness to severe amnesia, impacting not only individuals but also their caregivers and the broader healthcare system[13]. Understanding the potential benefits of cognitive rehabilitation interventions for memory improvement is pivotal, as it may pave the way for more tailored and effective post-stroke rehabilitation strategies[11]. Recent technological advancements have led to the development of innovative rehabilitation programs, such as computer-based cognitive training and virtual reality (VR) simulations, which offer interactive and engaging environments for patients[14]. These modern approaches stand in contrast to traditional methods that often involve paper-and-pencil exercises or therapist-guided recall and memory drills. While traditional methods are generally more accessible and cost-effective, they may lack the adaptability and immersive engagement provided by computer-based and VR programs[15]. Conversely, modern approaches can offer more personalized and data-driven experiences, potentially enhancing patient motivation and participation. However, they may be limited by accessibility issues and higher costs, particularly in resource-limited settings[16]. Evaluating the effectiveness of these modern approaches compared with traditional methods was critical to this review.

In this comprehensive analysis, we scrutinized the existing body of research to explore the outcomes of cognitive rehabilitation programs specifically designed to target memory deficits in patients who experienced stroke. By critically assessing the available evidence, we provided clinicians, researchers, and caregivers with valuable insights into the effectiveness of these interventions. Ultimately, this systematic review aimed to provide valuable insights into optimizing post-stroke cognitive rehabilitation strategies, contributing to improved patient outcomes and better quality of life for individuals affected by stroke-related cognitive dysfunction.

Review question

What is the impact of cognitive rehabilitation interventions, including memory training, neurorehabilitation, mindfulness, and VR, on memory improvement in patients who experienced a stroke?

MATERIALS AND METHODS

The preferred reporting items for systematic reviews and meta-analyses statement was followed in the conduct and reporting of this systematic review[17]. This protocol was registered in the PROSPERO database (CRD42023454330) in August 2023.

Databases and search strategy

An extensive and thorough search was executed across pertinent electronic databases, such as the Cumulative Index to Nursing and Allied Health Literature, MEDLINE (PubMed), Scopus, ProQuest Central, and Google Scholar, to identify additional relevant studies. The gray literature and references of the included studies were also searched. The search method combined keywords and medical subject headings (MeSH) terms relevant to the "population, intervention, control, and outcome" components of patients who experienced stroke, cognitive interventions, and memory improvement.

The search strategy was based on subject-specific header indices within each database, incorporating MeSH terms as well as their corresponding synonyms (keywords). Truncations, wildcards, and additional terms were selected to accurately represent the focus of the review. The Boolean operators 'AND' and 'OR' were used to combine these search terms. The search strategy, which combined MeSH terms and keywords for MEDLINE (PubMed), is outlined in Table 1 and was adapted to adhere to the specific syntax requirements of each database.

Two reviewers individually searched all titles and abstracts for studies that were pertinent to the review. The full texts of all relevant papers were retrieved and examined by the same reviewers, with conflicts resolved by consensus. Another author was assigned as the third reviewer to resolve any unresolved differences. The entire list of articles retrieved was imported into Rayyan software to remove duplicates and scrutinize the title and abstract.

Selection criteria

The specific inclusion criteria were as follows: Studies involving patients who experienced a stroke as the target population; studies focusing on cognitive rehabilitation interventions, including memory training, neurorehabilitation, mindfulness, and VR; studies assessing memory improvement as an outcome measure; randomized controlled trials, quasi-experimental, and observational studies; and studies published in English and those published within the last 10 years.

The exclusion criteria were studies not involving patients who experienced a stroke; studies focusing solely on non-cognitive interventions (*e.g.*, physical rehabilitation); studies without a clear focus on memory improvement; studies without relevant outcome measures or lacking proper methodology; studies not available in the English language; and studies published before 2014.

Data extraction

The reviewers independently retrieved the following data and compiled it into a standard data extraction form: Study author; year; country; study purpose; setting; study design; participant characteristics; sample size; data collection method; type of cognitive rehabilitation; interventions; and significant results reported.

Table 1 Search strategy for the MEDLINE database

Search terms for MEDLINE database		
P (population)	Patients who experienced stroke	"Post-stroke patients" OR "Stroke survivors" OR "Cerebrovascular accident patients" OR "Post-cerebrovascular accident individuals" OR "Patients with post-stroke sequelae" OR "Rehabilitated stroke patients" OR "Stroke recovery" OR "Post-stroke rehabilitation" OR "CVA survivors" OR "Hemiplegic patients post-stroke"
I (intervention)	Cognitive rehabilitation	"Cognitive rehabilitation" OR "Cognitive training" OR "Neurorehabilitation" OR "Cognitive therapy" OR "Cognitive retraining" OR "Cognitive intervention" OR "Brain injury rehabilitation" OR "Stroke rehabilitation" OR "Neuropsychological rehabilitation" OR "Memory rehabilitation" OR "Cognitive skill training"
O (outcome)	Memory improvement	"Memory enhancement" OR "Improving memory" OR "Memory boost" OR "Enhanced cognitive function" OR "Cognitive improvement" OR "Memory recovery" OR "Memory rehabilitation" OR "Memory training" OR "Cognitive enhancement" OR "Cognitive training for memory"
		Sets "1-3" will be combined with 'AND'

Risk of bias assessment

The risk of bias in the included studies was assessed using the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions. The randomized trials were specifically evaluated for bias *via* version 2 of the Cochrane risk of bias assessment[18]. The risk of bias tool for nonrandomized studies of interventions was used to evaluate quasi-experimental and nonrandomized trials[19]. The methodological quality of this systematic review was appraised using the AMSTAR 2 tool, ensuring adherence to established guidelines for rigor and transparency in systematic review reporting (Supplementary material).

Data synthesis

The data were systematically extracted from each included study and collated in tabular form (Table 2). This included details on study characteristics (*e.g.*, author, publication year, study design), participant demographics (inclusion criteria), intervention specifics (*e.g.*, type of cognitive rehabilitation, duration), and outcome measures related to memory improvement in patients who experienced a stroke. The review provided a narrative synthesis of the data extracted from the included studies, organized under several key headings. This offered a comprehensive understanding of the patterns, trends, and variations in the research findings, shedding light on the impact of cognitive rehabilitation interventions on memory improvement in patients who experienced a stroke.

RESULTS

The search extracted a total of 165 studies, with 72 duplicates eliminated, yielding 93 titles and abstracts that were assessed for eligibility. Following the screening of titles and abstracts, 51 full-text studies were reviewed for eligibility. Thirty of the full texts matched the inclusion criteria for the systematic review. Figure 1 depicts the flow diagram of the study selection process.

Study characteristics

The characteristics of the included studies considered in this review are summarized in Table 2.

Quality assessment of the included studies

Only one study exhibited a high risk of bias in terms of methodological assessment[20]. All studies demonstrated a low risk of bias in the dimensions of bias due to deviations from intended interventions (D2) and bias due to a lack of data in the results (D3). However, issues with the randomization procedure were reported in seven studies[21-27], potentially impacting the internal validity of these studies by increasing the risk of selection bias. Proper randomization is crucial for ensuring that any observed effects can be confidently attributed to the intervention rather than underlying differences between study groups. Additionally, two studies raised concerns about measurement bias in the assessment of outcome bias (D4), which could affect the reliability of outcome measurements and lead to inaccurate conclusions about the effectiveness of the intervention[21,28].

Three studies were reviewed with noted issues in the quality assessment of the stated outcome (D5), indicating potential variability in outcome reporting or inconsistency in measurement tools, which may influence the comparability of results across studies[29-31]. One study revealed a significant risk of outcome bias, suggesting that its findings should be interpreted cautiously, as they may reflect measurement inaccuracies or selective outcome reporting[28]. Figures 2 and 3 provide a visual summary of the risk of bias assessment across all included studies, highlighting the areas where methodological weaknesses were most prevalent.

Efficacy of VR-based cognitive rehabilitation intervention

In the field of cognitive rehabilitation for patients who experienced a stroke, VR technology has offered innovative solutions. Wilson *et al*[32] used interactive VR sessions where patients engaged in goal-oriented and exploratory tasks, targeting both unimanual and bimanual movements. It consisted of four goal-based and three exploratory movement

Table 2 Summary of characteristics of included studies

Ref.	Country	Aim of the study	Study design	Participant characteristics, sample size	Data collection method	Interventions characteristics	Key finding reported by author(s)
Wilson *et al* [32], 2021	Australia	To test whether the intensive use of a home-based virtual rehabilitation system can improve cognitive and functional outcomes in patients recovering from stroke	Parallel randomized control trial	The patients who met the following inclusion criteria were included in the study: Those who had upper-limb weakness due to a confirmed unilateral stroke; expressed the intention to undergo rehabilitation; English speakers who could sit and maintain posture unassisted and possessed at least a minimal upper limb movement range as assessed by an occupational therapist. Excluded were patients with prior neurological disorders (except stroke); psychiatric or developmental disorders; visual impairments preventing task completion; and those under 18 years of age. n = 19	Box and Block test. 9-Hole Peg Test. MoCA. SIS. Neurobehavioral function inventory	Patients who experienced a stroke received EDNA training at home for 30-min sessions, with a minimum of three and a maximum of four sessions each week over 8 weeks. The training included four goal-based and three exploratory movement tasks involving handheld objects or tangible user interfaces on a tablet. The control group engaged in 30-min sessions of a GRASP program for 8 weeks. In addition to conventional rehabilitation therapy, they participated in arm and hand exercises	Cognitive outcomes showed significant differences pretest and post-test for the EDNA group. The magnitude in MoCA improvement was moderate to large with the effect size g = 0.70 (t = 2.31, P = 0.036)
Jaywant *et al* [35], 2023	New York	To elucidate the formulation of a combined executive function intervention in patients experiencing chronic stroke that integrated CCT with MST	Non-randomized pilot study	Inclusion criteria were first-time stroke more than 6 months before enrollment, English speakers, having an evidence of cognitive difficulties, willingness to participate for the full study duration, proficiency with a computer keyboard and mouse, not concurrently receiving other cognitive rehabilitation services and able to perform basic self-care functions. Patients with other neurological disorders, severe mental illness, alcohol/substance use disorder, severe depression requiring psychiatric care, dementia, or dependence in self-care activities due to cognitive deficits were not included. n = 3	Client Satisfaction Questionnaire-8. Credibility and Expectancy Questionnaire. WAIS. WMS. Symbol-Digit Modalities Test. TMT A and B. Paced Auditory Serial Addition Test. Behavior Rating Inventory of Executive Function. WCPA	An intervention was developed that integrated CCT and MST to target executive functions and train for the transfer to daily C-IADLs. Rehacom was chosen as the software for CCT. The multicontext approach was used for the MST component	Participant P1 self-reported a slight improvement in everyday executive functioning on the BRIEF but demonstrated a slight decline in performance on neuropsychological measures and slightly worse performance on the WCPA. Participants P2 and P3 both demonstrated an improvement in select neuropsychological tests and the WCPA
Jung *et al*[36], 2021	South Korea	To determine the efficacy of computer-assisted rehabilitation techniques in patients of stroke and TBI and to compare the patterns of cognitive function recovery in both these groups	Retrospective cohort study	32 patients who were diagnosed with stroke or TBI using CT and magnetic resonance imaging and those patients with impaired cognition (MMSE score of ≤ 27) were enrolled. Patients with the presence of a previous central nervous system lesion, such as TBI, stroke, brain tumor, and epilepsy, an impossible one-step	Computerized neuropsychological test. MMSE. MBI	Participants underwent 30 sessions of computer-assisted cognitive rehabilitation (Comcog) five times a week. Comcog system uses 10 training activities: 2 auditory processing tasks that assess response time during auditory stimulation; 2 visual processing tasks that assess response time during visual	A significant improvement was observed in MMSE (P = 0.000), MBI (P = 0.000), intelligence quotient (P = 0.002), and all computerized neuropsychological test components except for the word color test in the stroke group. When comparing

				obey command due to higher brain dysfunction (aphasia or hemispatial neglect), poor cooperation, the presence of a visual or hearing impairment that interfered with cognitive rehabilitation, and unstable vital signs were excluded from the study		stimulation; 2 selective attention tasks that track attention in distraction; 3 working memory tasks that assess recognition and recall memory using visual, auditory, and multisensory stimulation; and 1 emotional attention task that assesses responses to pleasant or unpleasant stimulation	the TBI and stroke groups, it was noted that all parameters, except for digit span forward, visual learning, word color test, and MMSE, had higher mean values in the stroke group. Significantly higher values were seen in the stroke group for visual span forward and card sorting test
Kober *et al* [37], 2015	Austria	To evaluate an adaptive human-computer interface in improving cognitive function in patients who experienced a stroke	Non-randomized prospective study	24 patients who experienced first-time stroke with any site of brain lesion and motor deficit were included in the study. Patients with visual hemi-neglect, dementia, psychiatric disorders such as depression or anxiety, other concomitant neurological disorders, aphasia, or insufficient motivation and cooperation were excluded from the study	Long-term memory was tested using CVLT and Visual and Verbal Memory Test 2. Short-term memory was tested using Corsi Block Tapping Test, Digit span test, CVLT, VVM2. Working memory was tested using Corsi Block Tapping Test backwards task and digit span test	For both NF training protocols, electroencephalography signal was recorded using a 10-channel amplifier with a sampling frequency of 256 Hz. Up to ten NF training sessions were carried out on different days three to five times per week. Each session lasted approximately 45 min and consisted of seven runs of 3 min each	After NF training, the sensorimotor rhythm patient group showed significant performance improvements in parameters of the CVLT assessing verbal short-term and long-term memory compared to the pre-assessment. Sensorimotor rhythm patients showed a numerical performance improvement in visual-spatial short-term memory
Li *et al*[38], 2022	China	To determine if left dorsolateral prefrontal cortex iTBS can improve cognitive function in patients who experienced a stroke	Prospective single center randomized pseudocontrolled trial with double blinding	58 patients who met the inclusion criteria in which stroke was confirmed by CT or magnetic resonance imaging, 18 to 65-year-old patients, post stroke cognitive impairment diagnosis, absence of visual or hearing impairment, ability to complete the assessment and training, vitally stable and have signed informed consent for iTBS treatment were included. Patients with cognitive dysfunction caused by cranio-cerebral trauma or neurological diseases, aphasia, unstable arrhythmias, or other serious physical conditions, contrain-dications of magnetic stimulation, history of seizures, patients in critical condition were excluded from the study	MMSE. Oxford cognitive screen. Event-related potential P300 pre and post intervention	Stimulation was done by using a transcranial magnetic stimulator (nagneuro 60 type stimulator) and a figure-of-eight coil. In the iTBS group, three continuous pulses at 50 Hz were repeated at 5 Hz (2 s on, 8 s off) for a total of 192 s and 600 pulses. In the sham stimulation group, coil was rotated by 90° so it sat perpendicular to the target area, and the minimum stimulation was generated. Stimulation parameters and site matched those of the iTBS group	Post-intervention MMSE scores showed a statistically significant increase from the baseline. After iTBS intervention, there was significant improvement in the overall cognitive function, executive function, and memory function
Haire *et al* [42], 2021	Canada	To assess the outcome of TIMP interventions on improvement of cognitive and affective outcomes relative to baseline	Randomized controlled trial	30 participants who were chronic post-stroke and community-dwelling were randomized to one of three experimental groups who met the	TMT-part B. The forward DST. The General Self-Efficacy Scale. The Multiple Affect Adjective Check List-Revised. The	The interventions used differed among the groups of participants: Group 1: 45 min of active TIMP training. Group 2: 30 min of TIMP followed by 15	TIMP + motor imagery seemed to improve cognitive adaptability in individuals with chronic post-stroke conditions,

		in patients who experienced a stroke		following inclusion criteria: (1) Hemiparesis/unilateral stroke sustained more than 6 months prior to enrollment in the study; (2) Presence of at least minimal movement of the affected limb; (3) Age: 30-79 years; and (4) Ability to understand and follow simple instructions. Those patients who had a presence of comorbid neurological disorder and were actively participating in an upper extremity rehabilitation program at the same time as the study period were excluded from the study	Self-Assessment Manikin	min of cued motor imagery, involving listening to a metronome beat set for each exercise while engaging in motor imagery. Group 3: 30 min of TIMP followed by 15 min of motor imagery without external cues. Exercises focused on training of gross and fine motor control using acoustic and electronic instruments, which were selected and positioned to meet individual needs	potentially attributable to the reinforcement of mental constructs *via* motor imagery after engaged training
Abd-Elaziz *et al*[47], 2015	Egypt	To measure the effect of cognitive rehabilitation of elderly patients with a history of stroke on their cognitive function and ADL	Quasi experimental research design	70 elderly patients who were aged 60 years and above with post stroke dementia with a history of stroke at least three months prior to study documented by CT or magnetic resonance imaging brain and with a stable medical status were recruited for the study. Patients with the presence of additional severe medical conditions preventing active rehabilitation, aphasia, agnosia, disturbed conscious level and those on antipsychotic drugs, antiepileptic, and anticoagulant drugs were excluded from the study	MMSE: Including five items (orientation, registration, attention and calculation, recall, and language. Digit span (forward and backward). Logical memory. Geriatric Depression Scale. Barthel index	The program consisted of three theoretical session about health education for diabetes mellitus, hypertension, and prevention of recurrent stroke and five practical sessions about spatial memory, attention and concentration, visual attention, fish face task, N400 task	Training programs significantly benefited cognitive function in elderly patients who experienced a stroke. Significant differences were observed in pretest, post-test, and follow-up assessments in the studied group, including MMSE, logical memory, digit span forward, and digit span backward (P value = 0.000). At baseline, group I had a mean logical memory of 4.65 ± 2.37, and group II had 4.37 ± 1.92. After the program, group I showed slight improvement, with a mean of 6.62 ± 2.12
Hellgren *et al* [28], 2015	Sweden	Aimed at investigating the effects of computerized working motor training on working motor skills, cognitive tests, activity performance, and estimated health and whether the effects of computerized working motor training can be attributed to gender or time since injury	Randomized controlled trial-crossover design	Inclusion criteria: (1) Age between 20-65 years; (2) With subjective working motor impairment; (3) Significantly impaired WAIS WM index compared with index of verbal comprehension; and (4) Presence of motivation for training. Exclusion criteria: (1) Intelligence quotient ≤ 70 as measured with WAIS-III/WAIS-IV; (2) Depression; and (3) Perceptual or motor difficulties that make the computerized WM training impossible. n = 48	Neuropsychological tests focused on verbal and visual working memory: Paced Auditory Serial Attention Test; forward and backward block repetition; and Listening Span Task. EQ-5D questionnaire and the interviews based on the Canadian occupational performance measure	The computerized WM training program Cogmed was used. It consisted of various visuospatial and verbal working memory tasks. The difficulty of each task was adapted to each patient's WM capacity. After completing the 25 training sessions, each individual was assigned a WM index. Each session was 45-60 min of intense exercise including one break with the exercise intensity varying between 4-5 days/week for 5-7 weeks. All participants were trained in pairs or in groups of three, and both individual	After the program ended 20 weeks later, the group showed significant improvements on all neuropsychological tests (P < 0.001). There was a marked positive change in the working memory index (P < 0.001), with each patient showing improvement during the training. Computerized working memory training can enhance cognitive abilities and daily life performance for those with acquired brain

						performances and group performances were analyzed and presented at a 20-week follow-up session	injuries, particularly when started early in rehabilitation
Fishman *et al* [48], 2021	Canada	To determine whether a simple intervention targeting goal setting could improve cognitive performance across commonly affected domains (attention/working memory, and verbal memory) in the chronic phase of stroke	Randomized controlled trial-single-blind, parallel-design	72 stroke survivors were randomly assigned to experimental and control group who met the inclusion criteria such as English speaking, nonaphasic individuals between 35 and 90 years of age with definite ischemic stroke diagnoses based on CT and magnetic resonance imaging scans at least 3 months post stroke. Participants with severe aphasia or dementia were excluded from the study	Controlled Oral Word Association Test. CVLT-II. Semantic fluency test. DST. TMT. Verbal fluency tasks	For each task, half of the participants were asked to establish a goal to increase their performance by 20%; the researcher gave them a specific number to aim for. CVLT encouraged participants to set goals for their word learning task. If they successfully recalled at least 10 words, they were to recall 1 extra word; if they correctly recalled fewer than 10 words, they were to recollect 2 additional words	Goal setting improved cognitive performance in stroke survivors. Participants with goal-setting instructions showed better executive function, attention/working memory, and learning compared to those with standard instructions. They exceled in tasks involving verbal executive function, attention/working memory, and verbal learning
Fernandez-Gonzalo *et al* [21], 2016	Sweden	To validate the effectiveness of resistance exercise in individuals with greater physical capabilities, focusing on various cognitive functions such as working memory, verbal fluency tasks and attention	Pilot randomized controlled trial	Participants who were included in the study were individuals who had experienced a stroke, over 40 years of age, at least 6 months post-stroke, had mild to moderate hemiparetic gait, and could perform closed-chain exercises with the prescribed device. Exclusion criteria included unstable angina, congestive heart failure, severe arterial disease, major depression, dementia (scoring < 24 on the MMSE), difficulty understanding instructions, or chronic pain. n = 32	Digit span forward. Spatial span forward - WMS-III. Conners Continuous Performance Test-II. Stroop Color and Word Test. Rey Auditory Verbal Learning Test. Verbal fluency test. Semantic fluency	Participants underwent unilateral resistance exercise training using the more-affected leg on a flywheel leg press. This training occurred 2 days per week, with over 48 h of rest between sessions, for a period of 12 weeks. The training sessions consisted of a standardized warm-up, followed by 4 sets of 7 maximal repetitions designed to induce ECC overload as validated in previous stroke studies. The training involved pushing with maximal effort during the entire range of motion in the concentric phase, resisting inertial force during the first part of the eccentric phase, and applying maximal effort to stop movement at about 70° knee flexion	The training group showed enhancements in cognitive function. This included improved verbal fluency and executive functions, as measured by the verbal fluency test and TMT
Faria *et al*[22], 2016	Portugal	To check the effectiveness and benefits of virtual reality-based cognitive rehabilitation through simulated ADL *via* Reh@City	Randomized controlled trial	Patients who experienced a stroke were randomly assigned to intervention and control group. Inclusion criteria: MMSE > 15; ability to read and write; capacity to sit; no hemi-spatial neglect; and motivation to engage in the study. Patients with moderate or severe language comprehension deficits were excluded	Addenbrooke cognitive examination, attention, memory, verbal fluency, language, and visuo-spatial abilities. TMT A and B. Picture arrangement test. SIS 30. System Usability Scale	Virtual reality-based cognitive intervention used a city simulation called Reh@City, which featured a three-dimensional environment with streets, sidewalks, buildings, parks, and moving cars. Reh@City required patients to complete common daily tasks at four frequently visited locations: A supermarket; a post office; a bank; and a pharmacy. The system offered visual feedback with time and point counters, rewarding	The study revealed that the virtual reality-based cognitive rehabilitation had a significantly positive impact on cognitive functions like global cognitive performance, attention, memory, visuospatial skills, and executive functions. It also led to enhancements in subjective health status, physical functioning, and overall recovery.

						patients for completing objectives and intermediate tasks while deducting points for mistakes or using the help button. This mainly targeted executive functions, encouraging problem-solving, planning, and reasoning skills	These results indicated that the virtual reality-based cognitive rehabilitation intervention had a positive effect on cognitive and functional outcomes for patients who experienced a stroke. Notably, memory within the experimental group saw significant improvement (Z = -2.081, P = 0.037, r = 0.69)
Faria *et al*[23], 2020	Portugal	To compare the effectiveness of two cognitive rehabilitation interventions in improving cognitive function and self-perceived cognitive deficits in patients experiencing chronic stroke	Randomized controlled trial	Participants who were included in the study were those under 75 years, in 6 months post-stroke chronic phase, with no hemi-spatial neglect, ability to sit, and motivated to take part in the study. Patients whose MoCA scores fell more than two standard deviations below the average score, with severe depressive symptoms, and those who had received occupational therapy within the 2 months leading up to the study were not included	Neuropsychological assessment. MoCA. TMT A and B. Verbal paired associates from the WMS-III-memory assessment. Digit span (forward and backward recall conditions). Symbol search. Digit symbol coding. Patient-Reported Evaluation of Cognitive State	The task generator is a tool for creating personalized cognitive rehabilitation programs consisting of 11 tasks. After assessing each participant's cognitive abilities using the MoCA, a training program was generated. The virtual reality-based Reh@City v2.0 intervention took the same task generator tasks and placed them in a virtual city. Patients had to complete cognitive tasks related to everyday activities like shopping or reading the newspaper. This virtual city included billboards and products from real places in Portugal to make the tasks relatable to the real world	The task generator intervention enhanced orientation on the MoCA, along with specific processing speed and verbal memory. The task generator group only showed significant improvements in retention scores, both immediately after the intervention and at follow-up. In the learning and memory test, the Reh@City v2.0 group significantly improved retention and recognition scores after the intervention. Reh@City v2.0 performed better in general cognitive function, visuospatial ability, and executive functions and showed significant and substantial improvements in verbal memory and processing speed
Liu *et al*[24], 2022	China	To evaluate the effectiveness of an immersive virtual reality puzzle game as a rehabilitation therapy for elderly patients who experienced a stroke with cognitive issues with primary focus is on enhancing executive function and visual-spatial attention	Pilot randomized controlled trial	30 elderly patients with post-stroke cognitive impairment in the age group between 60 and 90 years old, having MoCA score between 18 and 26, Fugl-Meyer motor scale exceeding 85 for at least one upper and lower limb. Patients who were challenging to evaluate, examine, or could not follow instructions and with severe hearing or visual impairment, mental disorders, or a history of epilepsy or vertigo were excluded from the study	MoCA scale. TMT-A. Digit symbol substitution test. DST-forward and DST-backward. Verbal fluency test. MBI	The control group underwent traditional cognitive training, which included activities such as processing speed and attention training, memory training, computational ability training, and problem-solving ability training. The intervention group used an immersive virtual reality system for training, which included life skills training, exergames, and entertaining games, totaling 16 different games. Both groups received an extra 15 min of	Virtual reality-based puzzle games can enhance cognitive function in elderly patients who experienced a stroke, including overall cognition, memory, attention, and daily living skills. Significant improvements were observed memory improvement in patients who experienced a stroke and underwent immersive virtual reality-based training forward

						intervention each day, 6 days a week, for 6 weeks. Cognitive function was evaluated before and after the 6-week treatment for all participants. Self-report questionnaires were given only to the IVRG group after 6 weeks of training	DST (Z = 0.78, P = 0.435 > 0.05) backward DST (Z = 0.347, P = 0.728 > 0.05)
Maier *et al* [20], 2020	Spain	To assess if adaptive conjunctive cognitive training in patients experiencing chronic stroke improved attention, memory, spatial awareness, and depressive mood compared to standard cognitive tasks, while considering comorbidities	Randomized controlled pilot trial	30 patients experiencing chronic stroke were randomized into control and intervention group who were in the age group of 45 to 75 years, had a cognitive impairment, and absence of severe upper limb motor disability. Patients with severe cognitive impairment and impairments like spasticity, communication disabilities, hemianopia, physical impairments, or severe mental health problems were excluded from the study	Averaged standardized composite scores. Neuropsychological test battery. Corsi Block Tapping Test Backward (Corsi B). RAVLT immediate. Delayed recall (RAVLT D). WAIS digit span backward (WAIS B)	The rehabilitation gaming system was used for daily cognitive training in a study where participants were split into an experimental group and a control group. The experimental group underwent a 6-week adaptive conjunctive cognitive training using the rehabilitation gaming system. The control group worked on standard cognitive tasks at home over the same 6-week period. Cognitive assessments, including executive function, spatial awareness, attention, and memory, were conducted at three points: Baseline, after 6 weeks, and during an 18-week follow-up	The experimental group demonstrated noteworthy enhancements in attention and spatial awareness, whereas the control group displayed memory improvement but not in other areas. Virtual reality-based cognitive training shows potential for patients who experienced a stroke, particularly those dealing with depression
Marangolo *et al*[41], 2018	Italy	To investigate the effects of tDCS on language recovery in aphasic individuals	Randomized controlled trial-crossover, double-blind design	The study involved 12 participants (6 males and 6 females) with left-brain damage and chronic aphasia. Inclusion criteria were patients who were native Italian speakers, right-handed before their brain injury, had experienced a single left-hemispheric stroke at least 6 months prior, possessed mild affluent aphasia without articulatory difficulties, possessed basic comprehension skills, and had no attention or memory deficits that could affect their performance	Standardized language tests (the Battery for the Analysis of Aphasic Disorders test). Neuropsychological battery of tests-working memory (*i.e.* digit span)	tDCS was applied over the right cerebellar cortex for 20 min. Each stimulation condition consisted of five consecutive daily sessions over 1 week with a 6-day gap between sessions. During the tDCS sessions, participants completed specific language tasks, such as naming pictures and generating verbs in response to presented nouns. In both tasks, the examiner manually documented the participant's responses on a separate sheet. If the participant did not provide a response within the 20-s timeframe, the program automatically displayed the next picture or noun	The study suggested that cathodal cerebellar tDCS coupled with language training could improve verb retrieval in individuals with aphasia. Notably, the improvement was more pronounced in the cognitively demanding verb generation task. These findings indicated the potential therapeutic benefits of cerebellar stimulation for aphasia treatment, particularly in tasks involving executive and memory components
Oliveira *et al* [33], 2022	Portugal	To evaluate a virtual reality-based approach for aiding cognitive recovery in patients who experienced a stroke	Single-arm pre-post design	30 sub-acute patients who experienced a stroke over the age 18 with no impairments and history of psychiatric, neurological disorders or substance abuse, and having sufficient cognitive and language abilities and willing to participate. Those	MoCA. Frontal assessment battery. WMS-I. Color Trails Test	The Systemic Lisbon Battery is a virtual reality program set in a city where patients engage in various activities such as brushing teeth, showering, selecting clothes, arranging shoes, following recipes, recalling news or shopping in a virtual	The results indicated superior performance in assessments of overall cognitive function, executive abilities, attention, and memory. Memory [WMS memory quotient: t(25) = -3.297; P < 0.01]. Modified

				patients who could not complete at least 6 training sessions (*i.e.* 180 min of intervention) were excluded from the study		shop. Each session incorporates spatial orientation and memory by recalling door numbers and street details. The intervention plan was structured by difficulty and targeted cognitive domains, with interactions using a computer mouse. For the completely dependent patients, the psychologist controlled the mouse based on patient instructions. Each patient completed 7 sessions, each lasting approximately 30 min	reliable change index analysis indicated that 15% improved in memory. Virtual reality exercises focusing on everyday activities can offer short-term cognitive rehabilitation benefits for patients who experienced a stroke
Withiel *et al* [45], 2019	Australia	To assess the effectiveness of group compensatory memory skills training and CCT in rehabilitating memory after stroke	Randomized controlled trial	65 participants were randomized into two interventional and one waitlist control group. Those with a history of stroke confirmed by neurological examination and brain imaging at least 3 months previously were included in the study. Patients with physical impairment and with severe cognitive or communication deficits were excluded	The RAVLT -verbal and visual learning. Brief visuospatial memory test-revised–memory. Royal Prince Alfred Prospective Memory Test-prospective memory. Symbol Span Test-spatial memory. Digit span backward-verbal working memory. Everyday Memory Questionnaire-Revised. Part A of the Comprehensive Assessment of Prospective Memory	An adapted version of the manualized memory group program, "Making the Most of your Memory: An Everyday Memory Skills Program," was used. It consisted of six 2-h sessions, conducted weekly at a university psychology training clinic. An experienced neuropsychologist led the sessions with two provisional psychologists' assistance. Lumosity™, is an adaptable CCT program. The training regimen consisted of 30 min per day, 5 days a week, for 6 weeks. After the project concluded, participants on the waitlist were given the option to select a memory intervention	65 community-dwelling stroke survivors took part (24 in the memory group, 22 in CCT, and 19 in the wait-list control). The memory group showed more significant progress in memory-related goals and internal strategy use at the 6-week follow-up compared to computerized training and wait-list control. Memory skills groups, rather than computerized training, may assist community-dwelling stroke survivors in reaching their functional memory goals
Park and Lee [29], 2018	South Korea	To compare CMDT with AMST based on its effects on increasing attention, memory, and cognitive functioning when used in the rehabilitation of individuals with chronic stroke	Pilot randomized controlled trial	30 participants were included in the study and randomly assigned to the experimental and control group who were diagnosed stroke with cerebral hemorrhage or cerebral infarction, MMSE-K score ≥ 21, able to follow verbal instructions and having dual task capability. Patients with dementia, history of seizure, high blood pressure or angina, and visual or auditory impairments that would interfere with task performance were excluded	TMT-A. TMT-B. ST. DST. CMDT and AMST of the experimental group using a metronome	The control group received three sessions of CMDT every week for 6 weeks and included motor tasks associated with balance and posture while sitting and standing, which were performed simultaneously with cognitive tasks related to attention, memory, and cognitive function. Tasks included counting backward from a number while sitting up and naming the days of the week in reverse order during trunk rotation. The interventional group received CMDT + AMST in a different room than the control group but in the same manner as the control group. AMST used the interactive metronome (IM pro 9.0) and involved various motor tasks. These tasks	In the interventional group, significant changes occurred in multiple test scores: TMT-A and TMT-B (P = 0.001), DST-forward and DST-backward (P = 0.001), ST-word, and ST-color (P = 0.001). Combined (CMDT + AMST) intervention was more effective in improving cognitive function, attention, and memory in patients with stroke than CMDT alone

						included tapping both hands while making a semi-circular movement and pressing the right or left trigger in response to the reference sound	
Park and Park [30], 2015	South Korea	To study the effects of CoTrans (computer-based cognitive rehabilitation program) on the cognitive function and visual perception of patients with acute stroke	Randomized controlled trial	30 participants were included in the study and randomly assigned to the experimental or control group who had history of no more than one stroke with an onset duration of < 3 months; MMSE score of ≤ 23; has the ability to understand instructions and use the controller with the unaffected upper limb and not having unilateral hemispatial neglect and hemianopsia	Lowenstein Occupational Therapy Cognitive Assessment. Motor-free Visual Perception Test-3	The control group received conventional cognitive rehabilitation with emphasis on visual perception ability using pencil and paper. The experimental group received a Korean Computer-based cognitive rehabilitation program with CoTrans program using a joystick and a large button focusing on visual perception, attention, memory, orientation, sequencing, and categorization	Computer based cognitive rehabilitation with CoTrans may contribute toward the recovery of cognitive function and visual perception in patients with acute stroke. The improvement in Lowenstein Occupational Therapy Cognitive Assessment and Motor-free Visual Perception Test was higher in the experimental group than in the control group subjects after 20 sessions. A statistically significant difference was observed between the two groups at the end of treatment
Patani[44], 2020	India	To determine how neurobic exercises affect the memory of patient who experienced a stroke	Randomized controlled trial	The study included 40 participants aged 50 to 80, of both genders, diagnosed with stroke, MMSE score > 22, higher Brunnstrom's recovery stage and Barthel index score > 12. Patients with neuromusculoskeletal condition, other psychiatric illness, hearing and visual deficit, hemodynamic instability with uncontrolled hypertension and other progressive metabolic diseases were excluded	MoCA scale. SIS	Neurobic exercises are a distinctive brain workout program that combines physical senses such as vision, hearing, taste, smell, and touch, along with emotional senses in a regularly changing routine	The post-treatment mean MoCA score in the experimental group was 18.35 ± 4.36, in comparison, the conventional group had a mean MOCA score of 11.70 ± 3.31. With the use of SIS and MoCA, neurobic exercises significantly improved memory
Prokopenko *et al*[31], 2013	Russia	To evaluate the effectiveness of novel computerized correction programs for cognitive neurorehabilitation	Randomized controlled trial	Inclusion criteria: Patients with cognitive problems following stroke; having mild dementia, without significant speech problems or epilepsy; and in the acute and early restorative period of stroke. Exclusion criteria: Patients with MMSE < 20; medically unstable; were not fluent in Russian or had speech problems. *n* = 43 participants (experimental group: 24 participants; Control group: 19 participants)	MoCA. Schulte's tables (for attention deficit estimation)	The experimental group received training with computer programs 30 min/day for 2 weeks in addition to standard treatment. Visual and spatial memory training involves remembering the positions of images in a five-by-five square with an increasing number of objects (images of books). The patient clicks on the cells to recall the image positions. The number of objects to remember increases with correct performance until two mistakes are made. Information about speed and correctness	The intervention group displayed a noteworthy enhancement in cognitive function, as indicated by the MMSE, frontal assessment battery, clock drawing test, Schulte's test, and MoCA (with a significance level of $P < 0.01$), following the treatment course

						of answers and the amount of information memorized is displayed on the screen. Other computerized tasks in the cognitive correction program include remembering symbol sequences, arranging clock hands, and serial counting	
Song and Fu [59], 2022	China	To investigate the impact of cognitive impairment rehabilitation on the cognitive performance of older patients who experienced a stroke	Retrospective study	120 patients with the first onset of stroke, having stable vital signs were randomly assigned to the study and control group. Patients with complicated cerebrovascular conditions, cognitive impairment, physical limitations, intellectual abnormalities, malignant tumors were excluded	MoCA	The study group received additional rehabilitation for cognitive impairment. Patients with MoCA scores below 26 had enhanced cognitive training. This training included exercises for time, space, and character discrimination, where patients were shown familiar individuals, places, and the current time and asked to distinguish them independently. Number practice aimed to help patients understand basic numbers, sort and calculate them, enhancing logical thinking. Training for language and memory abilities involved increased communication with family members, memory stimulation, daily reading sessions, and exercises for reasoning abilities like describing and categorizing various daily items in the ward	The scores for the study group cognitive function, simple intelligence state, and neurological deficiency were higher than the scores of the control group following the rehabilitation treatment (P < 0.05)
Studer *et al* [34], 2021	Germany	To examine if patients' commitment to daily self-directed training could be improved through precommitment	Randomized controlled trial	95 adult patients who experienced a stroke with visuospatial memory impairments were recruited and randomly allocated into three groups: A precommitment, control and standard therapy only group. Patients with moderate or severe aphasia, dementia, severe deficits in multiple cognitive domains, inability to provide consent and multi-resistant bacteria were excluded	Wechsler spatial span test. Verbal learning and memory test	Wizard (Peak) offers tablet-based training for visuospatial working memory. In the game, geometrical figures are hidden under cards. During each round, the cards are revealed one by one in a random order, and the player must select the correct hiding place using the touchscreen. The game has a narrative involving a Wizard who needs items like strength, weapons, and trophies to battle monsters. Tokens are earned through successful trials and lost when mistakes are made. The game adjusts its difficulty based on the player's performance and is played on tablet computers in a designated room with technical support. After each training session, patients rated their enjoyment of the	Patients who conducted Wizard training showed a significantly larger pre-post change in the Wechsler spatial span test backward scores than those who were not offered [$F(1,80) = 12.947$, $P_{corr} = 0.002$, $d = 0.72$]. Wizard training was associated with a larger improvement in verbal learning capacity on the verbal learning and memory test, and the degree of improvement correlated positively with the training dose. Self-directed training with the Wizard game improved working memory functions of the impaired patients who experienced a

						Wizard game on a Likert scale from 1 to 7	stroke
Tsai *et al*[39], 2020	Taiwan	To investigate the comparative effects of repetitive transcranial magnetic stimulation and iTBS in patients with left hemispheric stroke on patients' global, memory, attention, language and visuospatial cognitive function	Randomized, controlled, double-blind study	44 patients were randomly assigned to rTMS, iTBS and sham groups diagnosed with ischemic or hemorrhagic stroke with cognitive impairment, no history of seizure, intracranial occupying lesion, use of antidepressants or neurostimulators. Patients with unstable cardiac dysrhythmia, fever, infection, hyperglycemia, epilepsy or previous administration of tranquilizers, neurostimulators or other medication that significantly affected the cortical motor threshold and those with metallic intracranial devices, pacemakers or other electronic devices in their bodies were excluded	RBANS. Beck Depression Inventory	The iTBS treatment consisted of 3 pulses of 50 Hz bursts repeated at 5 Hz for a total of 190 seconds (600 pulses). The 5 Hz rTMS protocol was applied at an intensity of 80% of the resting motor threshold, with 2 strains at an interval of 8 seconds, repeated every 10 seconds for a total of 10 minutes (600 pulses). Each patient received 10 days of rTMS treatment, administered in the morning from Monday to Friday for 2 consecutive weeks. For the control group, a placebo coil (Magstim) for the sham stimulation was used, which delivered less than 5% of the magnetic output with an audible click on discharge	After 10 rTMS sessions, the 5 Hz rTMS group showed significant increases in total RBANS score (P = 0.003) and improved delayed memory (P = 0.007). The iTBS group exhibited significant increases in total RBANS score (P = 0.001) and enhancements in immediate memory (P = 0.006), language (P = 0.005), and delayed memory (P = 0.008). Both iTBS and 5 Hz rTMS improved global cognition and memory without affecting mood and were effective and safe for treating patients who experienced left-brain stroke and enhanced memory function
Aben *et al* [25], 2014	Netherlands	To ascertain how a novel MSE training program affects the MSE of patients who experienced a stroke, depression, and quality of life over the long run	Multicenter randomized controlled trial	153 patients between the age group of 18 and 80 years, living independently, 18 months or more post onset after stroke and reported subjective memory complaints. Patients with progressive neurological disorders such as dementia or multiple sclerosis, alcohol or drug abuse, subdural hematomas, or subarachnoid hemorrhages	Metamemory in adult questionnaire	The MSE training, adapted for patients who experienced a stroke from a program by Verhey and Ponds, consisted of three main parts: An introduction about memory and stroke, training on internal and external memory strategies, and psychoeducation on how mood, anxiety, and memory-related worries affect memory complaints. It involved nine 1-h sessions conducted twice a week, including training booklets and homework assignments. The control group did not receive therapeutic interventions but was educated about stroke causes and consequences. They had nine 1-h sessions, similar to the MSE group, but did not receive homework assignments. A trained psychologist led both groups	MSE improved significantly over the intervention period in the experimental group compared with the control group (P = 0.010; Cohen's d = 0.48). In younger patients in the experimental group, MSE improved significantly more than the MSE score in the control group (B = 0.56; P < 0.003)
Chiu *et al*[46], 2021	Taiwan	To assess how a home-based reablement program impacts various rehabil-itation outcomes in patients who	Single-blind randomized clinical trial	24 participants were randomly assigned to interventional and control group who were above the age of 20, having modified Rankin scale score of 2-	Fugl-Meyer Assessment for the upper extremity. SIS	The home-reablement group underwent goal-oriented training for ADLs for 50 min a day, once a week for 6 weeks. During the initial week, the	Individuals who had experienced a stroke showed the possibility of improving their motor function, ADL and instru-

		experienced a stroke		4 points, and could maintain a sitting position for at least 30 min in a wheelchair or bed without any assistance, and have the ability to follow instructions and cooperate with the procedures. Patients with orthopedic disorder, progressive disease, and peripheral nerve injury were excluded		occupational therapist leading the program focused on 2 to 3 ADLs that the participants considered important but challenging to perform. The therapist not responsible for the assessments gauged the participants' desire for improvement and assessed their current abilities in carrying out these ADLs. From the second to the sixth week, the occupational therapist taught the participants how to perform these ADLs effectively and provided them with strategies like task analysis, task modification, and simplifying the work process	mental ADL, emotional well-being, memory, and participation in various activities through their participation in a home-reablement program
Baylan *et al* [43], 2020	United Kingdom	To evaluate the viability and approval of integrating short mindfulness training into a music listening program for individuals recovering from a stroke	Randomized clinical trial	English-speaking adults who are native speakers, aged 18 to 80 years during the first 11 months of recruitment, and in the acute stage after being clinically and/or radiologically diagnosed with an ischemic stroke are included in this study. Patients with comorbid progressive neurological or neurodegenerative condition, major psychiatric disorder, history of major substance abuse problems, clinically unstable, unable to give informed consent or unable to cooperate are excluded. $N = 72$ were recruited and randomly allocated into mindful music listening ($n = 23$), music listening alone ($n = 24$) and audiobook listening ($n = 25$)	MoCA. Test of Everyday Attention. BIRT Memory and Information Processing Battery. WAIS. WMS. Controlled Oral Word Association Test	Participants got an iPod Nano and were told to listen to their selected material daily for at least an hour, aiming for a total of 56 h over the 8 weeks and were instructed to keep a daily written record of their listening to measure adherence. In the mindful-music group, they received a recording with a brief mindfulness exercise to complete daily before listening to music for the first three weeks. These were short exercises focused on mindfulness elements. Participants were guided to let thoughts pass and refocus if distracted. At the fourth visit, another brief exercise (following the breath) was introduced for the next three weeks. For the final two weeks, participants could choose which exercise to do. During the last visit, post-intervention listening plans were discussed, and the mindful-music group received a CD or recording of the mindfulness exercises	Mindful music listening is feasible and acceptable post-stroke. The participants' self-reported positive cognitive effects were primarily related to memory and attention. The music groups, not the audiobook group, reported experiencing memory reminiscence. The mindful music group specifically mentioned an enhanced ability to refocus their mind after it wandered. This indicates improved attentional control or attentional switching
Adomavičienė *et al*[26], 2019	Lithuania	To assess how new technology influences functional status, cognitive abilities, and upper limb motor outcomes in stroke rehabilitation	Randomized prospective clinical trial	60 patients who experienced a stroke aged between 60-74 years old, having stroke-affected arm paresis, disturbed deep and superficial sensations, and MMSE score > 21 points were included in the study. Participants with stroke-affected arm paralysis, aphasia,	Fugl-Meyer Assessment upper extremity. Modified Ashworth scale. Box and Block Test. Hand Tapping Score Test. Modified Functional Independence Measure. Addenbrooke's cognitive	The conventional post-stroke rehabilitation program lasted for 3-4 h daily, 5 days a week, including various therapies. Training with the new technological devices (Kinect or Armeo robot) took place for 45 min a day, totaling ten sessions. Training sessions involved motor tasks	The Armeo group showed greater overall cognitive changes, particularly in attention and executing complex commands like drawing two pentagons. These differences were statistically significant ($P <$

				painful shoulder syndrome and hypertonic stroke affected arm were excluded from the study	examination-revised	and short rest periods. The exercise program was tailored to each patient, and they received individual supervision from an occupational therapist. Patients sat in a chair or wheelchair with seat belts for safety and were actively engaged in the exercises. The clinician assessed arm impairment, motor function recovery, and any complications at the beginning of each training session	0.05). The Addenbrooke's cognitive examination-revised testing results indicated more substantial enhancements in memory, fluency, and visuospatial abilities within the Armeo group ($P <$ 0.05)
Yin *et al*[40], 2020	China	To determine the impact of rTMS intervention on patients with post-stroke cognitive impairment to behavioral improvements, including ADL and executive and memory function	Randomized controlled trial	34 patients with post-stroke cognitive impairment, aged between 30-75 years; MoCA < 26, with stable vital signs, normal cognitive function before stroke, and no severe aphasia were included for the review. Patients with complete left prefrontal cortex injury, transcranial surgery or skull defect, metal or cardiac pacemaker implants, history of brain tumor, brain trauma, seizures, cognitive function recession and affective disorder were excluded from the trial	MoCA. Victoria Stroop test. Rivermead Behavior Memory Test	rTMS treatment was conducted using a MagPro X100 magnetic stimulator and a standard figure-of-eight air-cooled coil. A 10-Hz rTMS was applied at 80% of the resting motor threshold. Patients received treatments once a day, 5 days per week for 4 weeks. After rTMS treatments, they underwent a 30-min computer-assisted cognitive rehabilitation program covering various cognitive skills like attention, executive function, memory, calculation, language, and visuospatial skills	A two-way repeated measures ANOVA of the RBMT indicated a significant interaction effect between time and group in terms of memory ability ($F = 5.2$, df = 2, $P =$ 0.008). After two and four weeks of therapy, pairwise comparisons revealed a substantial rise in the RBMT score for the rTMS group ($P <$ 0.001)
Yun *et al*[27], 2015	South Korea	To determine if cognitive function of patients who experienced a stroke can be enhanced by tDCS	Prospective, double-blinded, randomized case-control study	45 patients who experienced a stroke were randomized into two interventional (left-FTAS and right-FTAS) and one control group who had no temporal lobe damage on magnetic resonance imaging and had been diagnosed as acute or sub-acute within six months of their stroke. Patients with apraxia, aphasia, seizure and history of craniotomy were excluded	Korean-MMSE. Computerized neurocognitive function tests. Visual and auditory CPT. DSTs-Forward and backward. Verbal learning tests. Korean version of the MBI	In the tDCS groups, anodal electrodes were placed in alignment with the 10-20 international electroencephalography system. The left-FTAS group had the electrode at T3, while the right-FTAS group had it at T4. Patients in both groups underwent tDCS treatment lasting 30 min, administered five times weekly over a period of 3 weeks. In the sham group, the same method of affixing sponge electrodes was used as in the left-FTAS group, but no electric current was applied. The cognitive rehabilitation program implemented in the study was ComCog, focusing on enhancing attention and memory in patients with cognitive disorders	Left-FTAS group performed significantly better on the Korean-MMSE, the verbal learning test-delayed recall, the visual span test, and the backward DST. In the verbal learning test, the right-FTAS group demonstrated improvement in delayed recall. In the backward visual span test, the sham group performed better. The left-FTAS group had a significant improvement in auditory memory, according to a comparison of pre- and post-treatment data for each group

ADLs: Activities of daily living; AMST: Auditory motor synchronization training; BIRT: Brain Injury Rehabilitation Trust; BRIEF: Behavior Rating Inventory of Executive Function; CCT: Computerized cognitive training; C-IADL: Compensation-instrumental activities of daily living; CMDT: Cognitive-motor dual-task training; CVLT: California Verbal Learning Test; DST: Digit span test; ECC: Extraction-contraction coupling; FTAS: Fronto-temporal Anode Stimulation; GRASP: Graded Repetitive Arm Supplementary Program; iTBS: Intermittent theta burst stimulation; IVRG: Immersive virtual reality group; MBI: Modified Barthel index; MMSE: Mini-Mental State Examination; MMSE-K: Mini Mental Status Examination-Korean; MoCA: Montreal Cognitive Assessment; MSE: Memory self-efficacy;

MST: Metacognitive strategy training; NF: Neurofeedback; RAVLT: Rey Auditory Verbal Learning test; RBANS: Repeatable Battery for the Assessment of Neuropsychological Status; rTMS: Repetitive transcranial magnetic stimulation; SIS: Stroke impact scale; ST: Stroop test; TBI: Traumatic brain injury; tDCS: Transcranial direct current stimulation; TIMP: Therapeutic instrumental music performance; TMT: Trail Making Test; VVM: Visual and Verbal Memory Test; WAIS: Wechsler Adult Intelligence Scale; WCPA: Weekly Calendar Planning Activity; WM: Working Memory; WMS: Wechsler Memory Scale.

tasks involving the manipulation of handheld objects or tangible user interfaces over the surface of a tablet. Unimanual movements involving the weaker and stronger hand were first tested, followed by bimanual movements for the exploratory tasks. These tasks challenged participants with activities ranging from reaching fixed targets to creatively engaging with virtual objects. The cognitive outcomes of the experimental group differed significantly between the pretest and post-test. The magnitude of improvement in the Montreal Cognitive Assessment score was moderate to large, with an effect size of $g = 0.70$ ($t = 2.31$, $P = 0.036$).

In a controlled trial, Faria *et al*[22] used a virtual city simulation to train patients in daily living tasks, establishing a bridge between the virtual and real worlds and increasing independence in daily life activities. The environmental setting was three-dimensional, featuring streets, sidewalks, buildings, parks, and moving cars. Patients were expected to perform routine everyday tasks at four frequently visited locations: A supermarket; a post office; a bank; and a pharmacy. On the basis of the outcomes of this study, VR-based cognitive rehabilitation considerably improved cognitive functions such as global cognitive performance, attention, memory, visuospatial skills, and executive functions. It also improved subjective health status, physical function, and total recovery. Remarkably, memory in the experimental group improved significantly ($Z = 2.081$, $P = 0.037$, $r = 0.69$).

In a randomized trial, Faria *et al*[23] evaluated the efficacy of two cognitive rehabilitation interventions for improving memory in patients who experienced a stroke: Personalized and adapted paper-and-pencil training (task generator) and a content-equivalent, ecologically valid VR-based simulation of activities of daily living. Patients were required to perform cognitive tasks associated with daily duties such as reading the newspaper or going shopping. Notably, in the learning and memory test, this intervention outperformed the other interventions in terms of improving general cognitive functioning, visuospatial ability, and executive skills.

Another novel finding was observed in a pre-post design study by Oliveira *et al*[33], where they evaluated a VR-based approach that mimicked the challenges of daily life activities, with a focus on ecological principles, for assisting in cognitive recovery in patients who experienced a stroke. The primary intervention tool was the Systemic Lisbon Battery, a VR program that simulates daily cognitive tasks in a virtual city. Patients interacted with it *via* a computer mouse, and patient interactions with the virtual environment were rated by clinicians. The *t*-tests revealed statistically significant changes, indicating that these scores improved in memory at the post-treatment assessment [WMS memory quotient: $t(25) = -3.297$; $d = 0.39$; *P* value = 0.01], and the modified reliable change index demonstrated significant improvements in memory and attention abilities. This VR cognitive rehabilitation training, which focused on ordinary tasks, may provide patients who experienced a stroke with short-term cognitive rehabilitation benefits[33].

A similar conclusion was reached by Adomavičienė *et al*[26] who conducted a randomized prospective clinical trial focused on how new technology (Kinect or Armeo robots) influenced functional status, cognitive ability, and upper limb motor outcomes in stroke rehabilitation. Memory improvements were demonstrated when a VR-based intervention was compared with conventional treatment, indicating the benefit of VR serious games for boosting attention and memory tasks in daily activities of patients who experienced a stroke.

Liu *et al*[24] used an immersive VR (IVR)-based puzzle game as rehabilitation therapy with a focus on improving executive function and visual-spatial attention. The training content of the IVR group was divided into three categories: Life skills training; exergames; and enjoyable games. The difficulty level of each game was graded out of five stars, with one being the easiest and five being the most challenging. Individuals wore head-mounted screens for training and began with a one-star difficulty level that subsequently increased. IVR technology substantially improved executive performance and spatial orientation. Patients who experienced a stroke and received IVR-based training exhibited significant improvements in memory on both the forward digit span test ($Z = 0.78$, $P = 0.435 > 0.05$) and the backward digit span test ($Z = 0.347$, $P = 0.728 > 0.05$).

Additionally, VR-based interventions also exhibited therapeutic benefits for elderly patients who experienced a stroke, significantly impacting cognitive function and daily living activities. These improvements were more significant when working memory training was initiated early in the rehabilitation process[28]. VR-based cognitive training also showed promise for patients who experienced a stroke and depression, as subgroup analysis revealed lower depression levels and better cognitive improvements with a focus on attention and memory[20]. The cumulative findings highlighted the promising potential of VR-based cognitive rehabilitation interventions in enhancing cognitive function, memory, attention, and overall quality of life among patients who experienced a stroke, offering an innovative and engaging approach to stroke rehabilitation.

Efficacy of computer-based cognitive rehabilitation interventions

Computer-based approaches have also made significant advances in cognitive rehabilitation for patients who experienced a stroke. A randomized controlled trial by Hellgren *et al*[28] employed the Cogmed program, a computerized working memory training tool that consists of various visuospatial and verbal working memory tasks. Patients engaged in various working motor tasks adapted to their capacities. The study demonstrated that computer-based training could significantly improve motor skills, cognitive performance, and overall activity execution in patients who experienced a stroke.

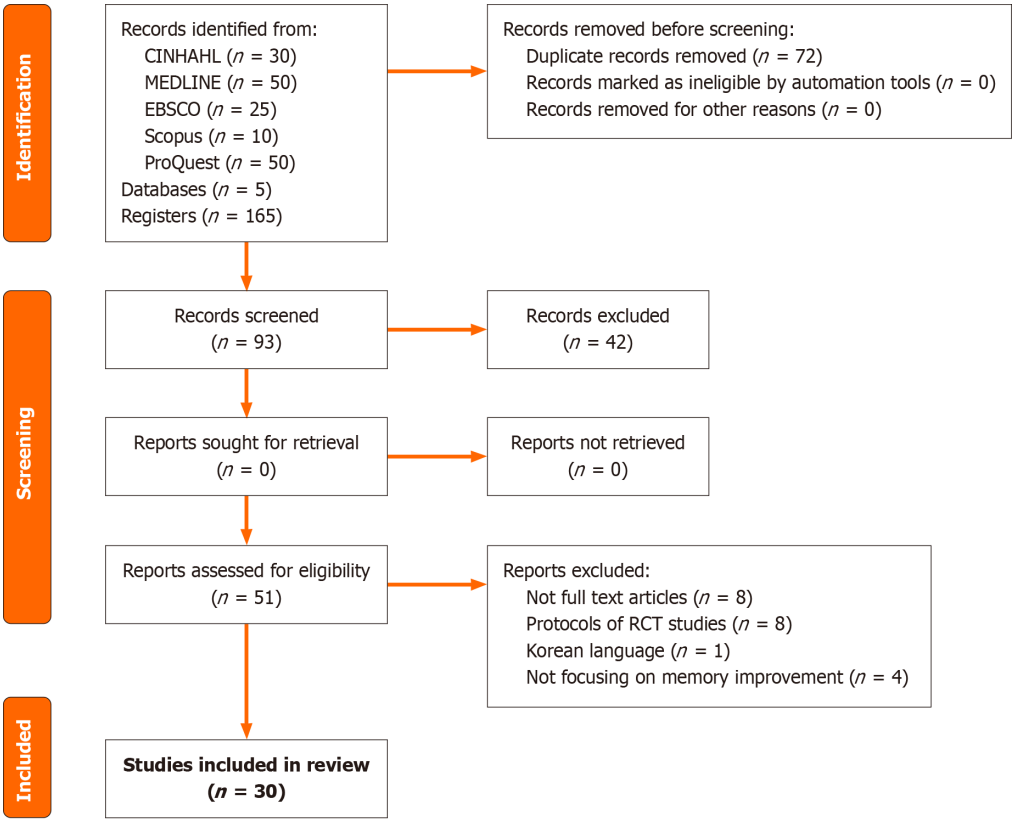


Figure 1 Preferred reporting items for systematic reviews and meta-analyses flow chart of study selection. RCT: Randomized clinical trial.

Similarly, Park and Park[30] conducted a controlled trial to assess the efficacy of the Korean computer-based application CoTrans for cognitive rehabilitation. Visual perception, attention, memory, orientation, sequencing, and categorization were all addressed in this program. The Lowenstein Occupational Therapy Cognitive Assessment and Motor-free Visual Perception Test-3 improved significantly in this study.

In another study conducted by Maier *et al*[20], a rehabilitation gaming system (RGS) for daily cognitive training of patients experiencing chronic stroke was used. The goal of this study was to assess whether adaptive conjunctive cognitive training improved attention, spatial awareness, and depressive mood compared with standard cognitive tasks while considering comorbidities.

The RGS framework entailed patients assuming the form of a virtual avatar on a computer screen and participating in conjunctive cognitive training scenarios such as the complex spheroids scenario, which trained basic attention and memory capacities. In the star constellations scenario, patients had to memorize a particular subset of stars in a constellation and then recreate them after a delay period in this visuospatial short-term memory test. The difficulty level of the four task parameters was adjusted to teach spatial attention, spatial memory, working memory, and memory-delayed recall.

The delay time countdown was used as a non-spatial alerting method to train sustained attention. In the quality controller scenario, the patients had to perform two tasks at the same time. They removed doughnuts from a fryer after their cooking time reached the right workspace, and they discovered defective candies on a conveyor belt in the left workspace. The difficulty level of the five task parameters was adjusted to train alertness, visual search ability, selective and sustained attention, inhibition of prepotent reactions, behavior initiation, and response preparation. This scenario also highlighted skills such as divided attention, multitasking, and problem-solving. The experimental group had significant improvements in attention and spatial awareness, whereas the control group simply demonstrated memory improvement.

In a clinical trial conducted by Studer *et al*[34], patients with visuospatial working memory problems were told to perform 30 min of independently driven repetitive cognitive training every day, utilizing the cognitive training game 'Wizard,' in addition to their standard therapy, for a 2-week intervention period. Wizard (Peak) provided tablet-based gamified training for visuospatial memory. Geometrical figures were concealed behind cards in this game. In each round, the cards were turned over one at a time in a randomized order to reveal the hidden figure. One figure was displayed at a time, and the player designated the hiding location by touch screen selection. The Wizard game, as an example of gamified cognitive training, significantly improved working memory abilities in patients who experienced a stroke. Patients who received Wizard instruction improved significantly more than those who did not [$F(1,80) = 12.947$, $P_{corr} = 0.002$, $d = 0.72$]. Wizard training was associated with increased improvement in verbal learning capacity on the verbal learning and memory test and in the level of progress. Self-directed training *via* the Wizard game improved the working memory functions of patients who experienced a stroke.

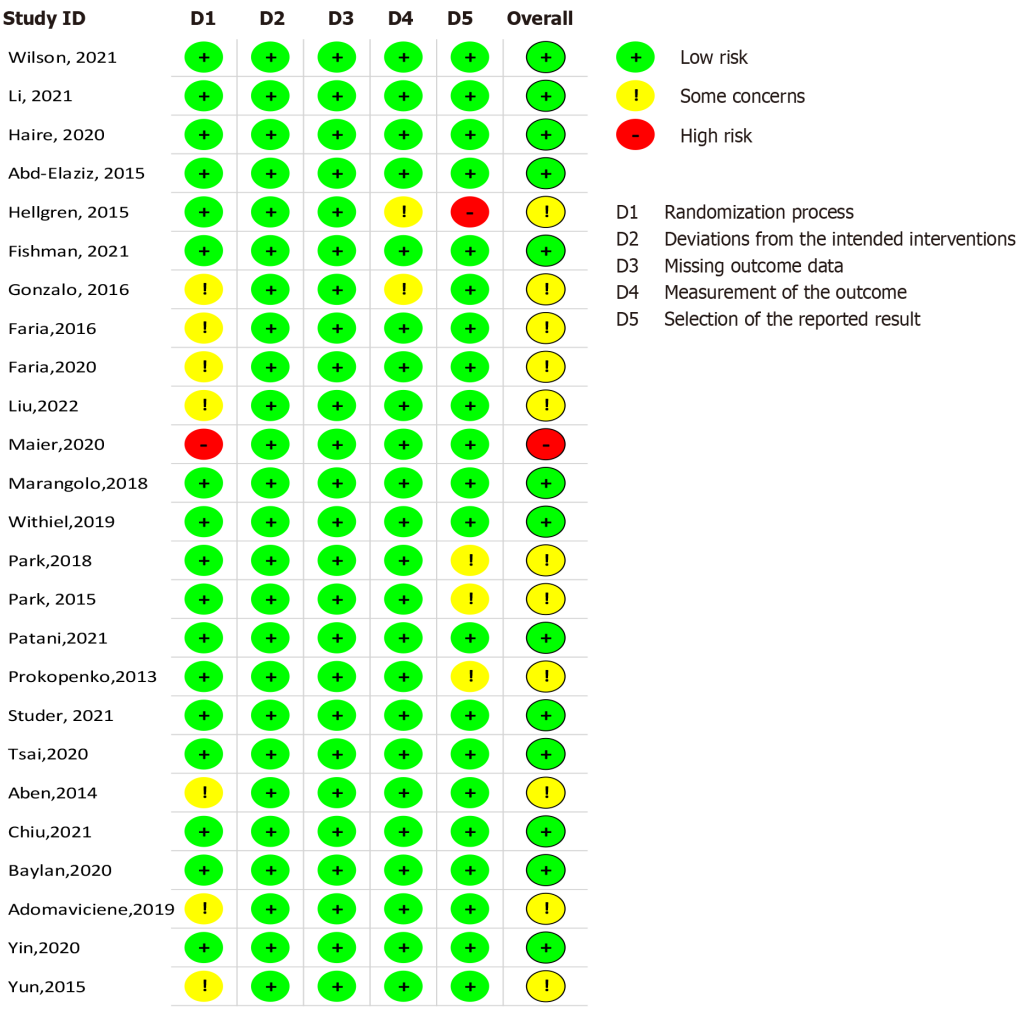


Figure 2 Risk of bias assessment for individual studies in post-stroke cognitive rehabilitation interventions.

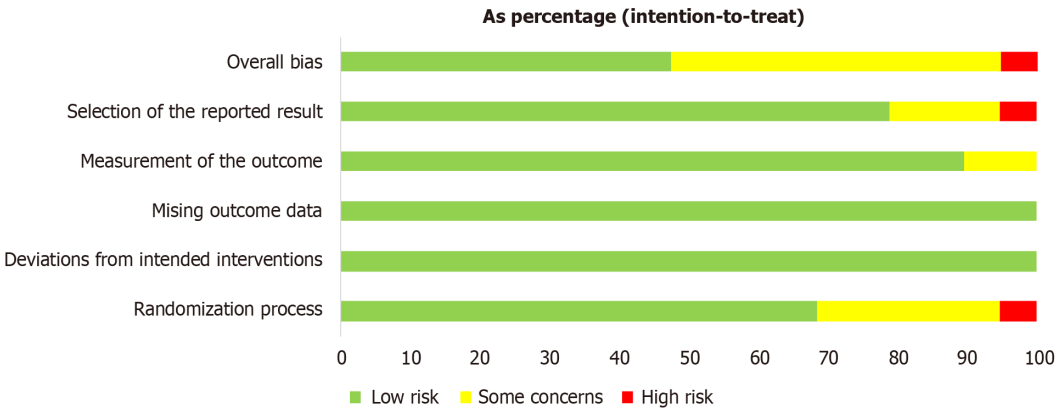


Figure 3 Risk of bias summary.

Jaywant *et al*[35] proposed a new cognitive rehabilitation technology in their study. The development of a combined executive function intervention in patients experiencing chronic stroke combined computerized cognitive training (CCT) with metacognitive strategy training (MST) to target executive functions and training for transfer to daily compensation-instrumental activities of daily living. Rehacom was selected as the software for the CCT, and the MST component was created *via* the multicontext method. It included activities to improve attention, working memory, and executive function. These exercises were designed to train low-level executive functions such as attention to detail and processing speed before progressing to higher-level executive functions such as attention, planning, organizing, and problem-solving. The exercises were modified in response to the patient's performance. The CCT sessions were preceded and followed by guided questioning. These questions were aimed to help participants anticipate obstacles, identify strategies, and make connections between Rehacom exercises and everyday compensation-instrumental activities of daily living. The

workbook contained answers to these questions. Workbook tasks were required for both the CCT and MST. Combining the CCT and MST resulted in improved cognitive performance and high participant satisfaction.

Jung *et al*[36] also used computer-assisted cognitive rehabilitation. Ten training activities were used: Two tasks for auditory processing that measured reaction times to auditory stimulation; two tasks for visual processing that measured reaction times to visual stimulation; two tasks for focused attention that evaluated close attention in distractions; three tasks for working memory that measured memory recognition and recall *via* auditory, visual, and multisensory stimulation; and one task for emotional attention that measured reactions to pleasurable or unpleasant stimulation. According to comparative studies including patients with traumatic brain injury and stroke, improved cognitive function after computer-assisted cognitive rehabilitation was observed in patients who experienced a stroke, with focal involvement benefiting the most. Except for the word color test in the stroke group, the Mini-Mental State Examination (MMSE) ($P = 0.000$), modified Barthel index ($P = 0.000$), intelligence quotient ($P = 0.002$), and all other components of the computerized neuropsychological exam improved significantly. With the exception of the digit span forward test, visual learning test, word color test, and MMSE, all the measures had higher mean values in the stroke group than in the traumatic brain injury group. The stroke group presented significantly higher scores on the visual span forward and card sorting tests.

Efficacy of non-VR-based cognitive rehabilitation intervention

Non-VR (NVR) cognitive rehabilitation interventions play a crucial role in assisting patients who experienced a stroke in their path to cognitive recovery. Among these approaches are electroencephalography (EEG)-based neurofeedback (NF), where EEG signals are recorded to help patients self-regulate specific brain frequencies through audio-visual feedback and rewards. The results of NF training varied, with the sensorimotor rhythm patient group experiencing memory improvement in California Verbal Learning Test parameters for evaluating linguistic short- and long-term memory. These patients demonstrated a numerical improvement in visual-spatial short-term memory[37].

Furthermore, several studies from various countries have investigated the impact of transcranial magnetic stimulation (TMS) techniques, such as intermittent theta burst stimulation (iTBS) and repetitive TMS, on specific brain areas, such as the prefrontal cortex on the dorsolateral side, to improve cognitive skills[38-40]. iTBS targeting the left dorsolateral prefrontal cortex effectively enhanced cognitive, memory, and executive function. Therefore, this intervention had a positive effect on post-stroke cognitive impairment[38].

Transcranial direct current stimulation (tDCS), which involves the placement of anodal electrodes at specific scalp locations combined with a cognitive rehabilitation program, has also been examined. The participants in the tDCS sessions conducted language tasks such as naming pictures and developing verbs to describe nouns that were given. The examiner manually recorded each participant's response on a separate page for both tasks. After 20 s, if the participant did not respond, the program automatically moved on to the next image or term[27].

Combining cathodal cerebellar tDCS with language training improved cognitive performance, particularly in cognitively demanding tasks. Notably, the verb generation task, which requires a high level of cognitive function, showed greater improvement. These results point to the potential therapeutic advantages of cerebellar stimulation for the treatment of aphasia, especially in regard to activities requiring executive and memory functions[41].

Musical interventions (MIs) have shown promise in enhancing cognitive and motor functions, whether through playing musical instruments, cued motor imagery, or therapeutic instrumental music performance. The Therapeutic Instrument Music Performance and MI intervention improved cognitive adaptability, potentially through MI reinforcement, albeit without direct correlation with improvements in cognitive function. The utilization of musical instruments in active training seems to elicit favorable emotional responses, although these alterations in emotional reactions are not correlated with changes in cognitive function. Mindful music listening, a unique approach, was introduced, with patients listening to music daily to assess its impact on cognitive function and quality of life. The participants self-reported an improvement in the memory and attention domains of cognition with mindful music listening over audiobook listening[42,43].

Exercise-based cognitive rehabilitation integrates physical exercise with cognitive tasks to promote attention, memory, and cognitive functioning in patients who experienced a stroke[25,44]. Neurobics exercises, which combine physical senses and brain exercises, have been examined for their potential effects on post-stroke cognitive recovery[44]. Eccentric-overload flywheel resistance training resulted in significant improvements in cognitive function, muscle adaptations, balance, gait performance, and quality of life. The results of this study may be explained by extraction-contraction coupling muscle actions that stimulate certain brain areas, such as the cingulate cortex, which is linked to brain circuits for tasks such as working memory, verbal fluency, and pain perception. This included improved verbal fluency and executive functions, as measured by the verbal fluency test and Trail-Making Test[21].

Memory skills training and cognitive training have been at the forefront, with various interventions such as group compensatory memory skills training, CCT, memory self-efficacy training, and home-based rehabilitation programs, all aimed at enhancing cognitive ability and improving overall quality of life[25,45,46]. "Making the Most of your Memory: An Everyday Memory Skills Program," a manualized memory group program, was utilized in a randomized controlled trial. Weekly sessions lasting 2 h each were held in a university psychology training clinic. The sessions were facilitated by an experienced neuropsychologist assisted by two temporary psychologists. Memory skill groups surpassed CCT in achieving memory-related goals and internal memory strategy utilization. Compared with the computerized training and wait-list control groups, the memory group demonstrated more substantial progress in memory-related goals and internal strategy use at the 6-week follow-up. Rather than computerized training, memory skill groups may help community-dwelling stroke survivors achieve their functional memory objectives[45].

Compared with cognitive-motor dual-task training (CMDT) alone, the combined intervention involving CMDT and action observation-based motor simulation training was more effective at enhancing cognitive function, attention, and memory in patients who experienced a stroke. The multiple test scores of the Trail-Making Tests A and B (P = 0.001), the digit span test-forward and backward (P = 0.001), and the Stroop test-word and color (P = 0.001) showed substantial changes in the intervention group[29].

Training programs for elderly patients who experienced a stroke revealed therapeutic effects on cognitive function and daily living activities. Abd-Elaziz *et al*[47] developed a cognitive rehabilitation program that consisted of theoretical sessions about health education for diabetes mellitus, hypertension, and prevention of recurrent stroke and practical sessions about spatial memory, attention and concentration, and visual concentration. The pre, post, and follow-up test results (MMSE, logical memory, Digit Spill Backward, and Digit Spill Forward) were significantly different across the study groups (P value = 0.000). The mean logical memory score of group I was 4.65 ± 2.37 at baseline, whereas that of group II was 4.37 ± 1.92. Following the training, group I had a mean score of 6.62 ± 2.12, indicating a slight improvement.

In stroke survivors, goal setting enhances cognitive function. Fishman *et al*[48] tested whether a simple intervention targeting goal setting could improve cognitive performance across commonly affected domains in the chronic phase of stroke. A broader range of cognitive domains, including executive function (primary outcome), attention/working memory, verbal memory (secondary outcomes), and validated cognitive measures are included in the assessment. Half of the participants were asked to set a target to improve their performance by 20% for each activity; the researcher provided them with a precise amount to aim for. The California Verbal Learning Test encouraged participants to make objectives for their word-learning assignment. They were to remember one more word if they could correctly recall ten or more words and two more words if they could correctly recall fewer than ten words. When participants with goal-setting instructions were compared with those with normal instructions, the former demonstrated superior executive function, attention/working memory, and learning. In particular, verbal learning, attention/working memory, and verbal executive function tests were all areas in which the goal-setting group performed exceptionally well.

Post-treatment cognitive neurorehabilitation has varying effects on different patient groups, with anodal stimulation to the left frontotemporal lobe improving auditory memory, highlighting the specificity of interventions for cognitive domains[27]. These NVR-based interventions encompass a rich variety of techniques and methodologies to support patients who experienced a stroke in their cognitive rehabilitation journey. They emphasized the importance of personalized and multimodal approaches, which target specific cognitive domains and foster overall cognitive enhancement without relying on VR technology.

Comparative analysis of interventions

The included studies offered comparative insight into the relative effectiveness of different cognitive rehabilitation strategies. Compared with NVR approaches, VR and computer-based interventions generally report greater efficacy in terms of effect sizes and statistical significance. For example, Studer *et al*[34] reported significant working memory improvements with a gamified training intervention (Wizard game), with a large effect size [$F(1,80) = 12.947$, $P_{corr} = 0.002$, $d = 0.72$]. While direct comparisons across intervention types were not always feasible owing to variability in study designs and outcome measures, the majority of interventions yielded statistically significant improvements in cognitive domains critical to post-stroke recovery.

DISCUSSION

In patients who experienced stroke and underwent cognitive rehabilitation, both VR and NVR interventions have made significant strides in enhancing cognitive function, memory, and overall quality of life. VR-based interventions offer immersive and interactive experiences that bridge the gap between the virtual and real worlds, resulting in significant improvements in global cognitive functioning, attention, memory, visuospatial abilities, and executive functions. They have also shown positive effects on daily living activities, especially when they are initiated early in the rehabilitation process. NVR-based approaches also provide a diverse range of techniques, including cognitive training, brain stimulation, MIs, exercise-based rehabilitation, and goal setting, all of which have been demonstrated to be effective in improving cognitive performance. The choice between VR-based and NVR-based interventions depends on individual patient needs and preferences, and a multifaceted, personalized approach holds great promise in enhancing post-stroke cognitive recovery.

Efficacy of VR-based cognitive rehabilitation intervention

Our review of the literature revealed that VR holds tremendous promise for cognitive rehabilitation in patients who experienced a stroke. Through a comprehensive analysis of various studies, we found that VR-based interventions offer innovative and engaging approaches to improve cognitive and motor functions in patients who experienced a stroke. Our examination of the home-based VR rehabilitation system EDNA-22 revealed that interactive VR sessions can effectively target both unimanual and bimanual movements, which are vital for patients aiming to regain their independence in daily life. Research, including studies by the EDNA group, has shown that VR interventions lead to substantial cognitive improvements, with a particular focus on memory, attention, and executive functions[32].

VR-based interventions are especially beneficial for elderly patients who experienced a stroke, and early initiation of working memory training has positive impacts. The introduction of the virtual city simulation (Reh@City) also provided a bridge between the virtual world and the real world and empowered patients to perform daily tasks in a more engaging and ecologically valid context. These interventions have improved global cognitive function, attention, memory,

visuospatial skills, and executive functions, suggesting a promising approach for stroke rehabilitation[22].

When comparing VR-based interventions with traditional paper-and-pencil training, our review indicated that VR was significantly more effective in enhancing cognitive function and self-perceived cognitive deficits among patients who experienced a stroke. The incorporation of real-world elements into VR simulations enhances the realism of the exercises, making them more engaging and meaningful for patients[23]. We also noted the adaptability of VR-based rehabilitation, especially through immersive puzzle games with adjustable difficulty levels. This adaptability allows for tailoring rehabilitation programs to individual patient needs, a critical advantage for achieving positive outcomes[24].

Our review highlighted the benefits of integrating new VR technology tools such as Kinect and the Armeo robot into post-stroke rehabilitation. These tools positively influence functional status, cognitive ability, and upper limb motor outcomes, highlighting the diverse potential of VR-based interventions in enhancing rehabilitation efforts[26]. Another systematic review and meta-analysis investigated the use of VR in support of exercise therapy. Notably, however, the benefits of VR-supported exercise therapy were diminished once the intervention concluded[49].

Comparative research indicated that both patients who experienced a stroke and those with traumatic brain injuries benefited from computer-assisted cognitive rehabilitation, with patients who experienced a stroke showing improvements in various cognitive metrics, such as MMSE, modified Barthel index, intelligence quotient, and components of neuropsychological exams[36]. Interactive cognitive training, as exemplified by the Wizard game, enhances working memory functions while making the rehabilitation process enjoyable and motivating[34]. Furthermore, VR-based cognitive rehabilitation is particularly beneficial for patients who experienced a stroke and depression[20].

Our review suggested that VR-based cognitive rehabilitation can provide short-term cognitive benefits to patients who experienced a stroke, including improvements in global cognition, memory, attention, and executive functions. These interventions have the potential to enhance cognitive function and overall quality of life among stroke survivors, offering an innovative and engaging approach to stroke rehabilitation. These findings are consistent with a review conducted by Parisi *et al*[50], in which the effectiveness of multisensory technologies for post-stroke cognitive rehabilitation was explored. The study revealed that these technologies, including VR with or without motion tracking, exhibited notable potential for enhancing cognitive rehabilitation. They were found to be particularly effective in improving attention, spatial cognition, global cognition, and memory compared with conventional treatments. However, a systematic review and meta-analysis conducted by Zhang *et al*[51] did not yield significant evidence supporting the advantages of VR interventions over traditional rehabilitation approaches in improving cognition among patients who experienced a stroke.

A bibliometric analysis conducted by He *et al*[52] confirmed that the use of VR tasks for the assessment and instruction of episodic memory in elderly patients who experienced a stroke is an emerging field in geriatric research. VR-based cognitive rehabilitation offers an immersive experience along with more natural interactions with the environment as a whole. Studies have demonstrated that more IVR systems improve episodic memory performance[52]. The present results are consistent with those of another systematic review conducted by Despoti *et al*[53], confirming that there was a significant increase in the working memory of patients who experienced a stroke as well as in verbal and visual memory when cognitive training was carried out in a fully immersive environment.

Efficacy of computer-based cognitive rehabilitation interventions

The field of cognitive rehabilitation for patients who experienced a stroke has undergone remarkable advancements through computer-based interventions, complementing the innovative solutions provided by VR technology. Computer-based cognitive rehabilitation (CBCR) interventions have shown significant promise in enhancing cognitive and motor functions in patients who experienced a stroke. Findings from various studies highlighted the effectiveness of these interventions. The Cogmed program demonstrated improvements in motor skills and cognitive performance through tailored working memory tasks[28]. The CoTrans program offered a comprehensive approach, targeting multiple cognitive aspects, such as visual perception, attention, and memory. Cognitive rehabilitation through CBCR with CoTrans has improved cognitive function and visual perception, particularly within the CBCR group[30].

The RGS provides multifaceted cognitive training scenarios that improve attention, spatial awareness, and mood[20]. The Wizard cognitive training game enhanced visuospatial working memory[34]. A combined approach that integrates CCT with MST is aimed at improving executive function and its transfer to daily activities[27,35]. Computer-assisted cognitive rehabilitation (Comcog) improved cognitive function and visual perception[43].

The results of this review are consistent with those of two other scoping reviews, highlighting that computerized cognitive rehabilitation has demonstrated efficacy when used in conjunction with other techniques[54,55]. The findings of a study by Nie *et al*[56] also revealed that computer-assisted cognitive rehabilitation significantly enhanced the global cognition of patients with post-stroke cognitive impairment. This approach has proven effective in enhancing both cognitive function and the ability to perform daily activities among patients with post-stroke cognitive impairment.

Computer-based interventions offer tailored, multifaceted approaches to cognitive recovery, further highlighting the transformative impact of technology in the field of post-stroke rehabilitation. While VR solutions provide immersive and interactive experiences that bridge the virtual and real worlds, computer-based training programs provide versatile tools for cognitive improvement and functional enhancement.

Efficacy of NVR-based cognitive rehabilitation intervention

NVR cognitive rehabilitation interventions offer diverse and innovative techniques to facilitate the cognitive recovery of patients who experienced a stroke. These approaches encompass a wide array of strategies that do not rely on VR technology, emphasizing personalized and multimodal solutions. Notable findings include the integration of CCT with MST, resulting in improved cognitive performance and high participant satisfaction, highlighting the potential of this combined approach[35]. EEG-based NF has emerged as a promising avenue, utilizing EEG signals to enable patients to

self-regulate specific brain frequencies through audio-visual feedback and rewards. NF training, despite varying outcomes, results in memory enhancements, particularly in verbal short-term and long-term memory, within the sensorimotor rhythm patient group, indicating domain-specific cognitive benefits[37].

Furthermore, studies from various countries have explored the potential of TMS techniques, such as iTBS and repetitive TMS, to target specific brain areas, such as the dorsolateral prefrontal cortex, and enhance cognitive ability[38-40]. Interventions such as iTBS targeting the left dorsolateral prefrontal cortex have proven effective in enhancing cognitive, memory, and executive function in patients who experienced a stroke, emphasizing the potential for brain-targeted interventions[38]. tDCS has also been integrated, involving the placement of anodal electrodes at specific scalp locations and combining them with a cognitive rehabilitation program. The cognitive tasks undertaken in tDCS sessions, such as naming pictures and describing nouns with verbs, highlight the versatility of these interventions[27]. Combining cathodal cerebellar tDCS with language training has demonstrated promise in enhancing cognitive performance, especially in cognitively demanding tasks such as verb generation, highlighting the therapeutic potential of cerebellar stimulation for activities requiring executive and memory functions[41]. Post-treatment cognitive neurorehabilitation using anodal stimulation to the left frontotemporal lobe has improved auditory memory, indicating the specificity of interventions for cognitive domains[27]. The findings of a systematic review conducted by Gong *et al*[57] are consistent with our findings. They reported that TMS is safe and efficiently aids in the cognitive recovery of patients who have suffered from post-stroke cognitive impairment. A few brief side effects may occur during therapy, but these are all expected to be below the patient's tolerance range and not have a major detrimental impact on the patient[57].

MIs have shown promise in promoting cognitive and motor functions, whether through playing musical instruments, cued motor imagery, or therapeutic instrumental music performance[42,43]. Mindful music listening, a unique approach, has also been introduced, where patients listen to music daily to assess its impact on cognitive function and overall quality of life[43]. The Therapeutic Instrument Music Performance and MI intervention improved cognitive adaptability, potentially through MI reinforcement, although the exact mechanism is still being explored[42]. Numerous studies have utilized music therapy to aid in stroke rehabilitation, with favorable outcomes[58]. It is safe, efficacious, and inexpensive, and patients accept it readily. The use of music therapy in rehabilitation following a stroke has begun to gain more traction[58].

Exercise-based cognitive rehabilitation combines physical exercise with cognitive tasks to enhance attention, memory, and cognitive functioning in patients who experienced a stroke[25,44]. Neurobics exercises, which merge physical senses and brain exercises, have been explored for their potential in post-stroke cognitive recovery[44]. Furthermore, eccentric-overload flywheel resistance training has shown remarkable improvements in cognitive function, muscle adaptations, balance, gait performance, and quality of life, suggesting a promising avenue for post-stroke cognitive recovery[21].

Memory skills training and cognitive training have remained at the forefront, featuring interventions such as group compensatory memory skills training, CCT, memory self-efficacy training, and home-based rehabilitation programs. These interventions collectively aim to enhance cognitive ability and improve overall quality of life[25,45,46]. Memory skills training has proven highly effective in achieving memory-related goals and internal memory strategy utilization, surpassing CCT in this regard. The utilization of programs such as "Making the Most of your Memory: An Everyday Memory Skills Program" exemplifies structured approaches to memory enhancement[45]. A systematic review conducted by das Nair *et al*[10] highlighted the same findings that memory rehabilitation was found to be effective for patients who experienced a stroke and reported fewer memory problems.

Moreover, the combination of CMDT with action observation-based motor simulation training is more effective than CMDT alone in enhancing cognitive function, attention, and memory in patients who experienced a stroke[29]. Fishman *et al*[48] introduced a straightforward yet effective intervention targeting goal setting to enhance cognitive performance across various domains in the chronic phase of stroke. This approach encouraged patients to set targets for improvement in specific cognitive domains, which can lead to tangible cognitive enhancements. Goal setting, as a cognitive rehabilitation technique, enhances cognitive performance, particularly in executive function, attention/working memory, and learning[48].

Abd-Elaziz *et al*[47] developed a comprehensive cognitive rehabilitation program that combined theoretical sessions about health education with practical sessions focused on spatial memory, attention, concentration, and visual concentration. This holistic approach aims to improve cognitive abilities while addressing the broader health and lifestyle aspects of patients who experienced a stroke. Tailored training programs for elderly patients who experienced a stroke have demonstrated therapeutic effects on cognitive function and daily living activities, emphasizing the importance of age-appropriate interventions[47].

NVR-based cognitive rehabilitation interventions offer versatile and personalized solutions for patients who experienced a stroke, leading to improvements in cognitive function, memory, attention, and overall quality of life. These diverse approaches underscore the importance of personalization, offering a multifaceted landscape for post-stroke cognitive recovery.

Limitations

This review provided a comprehensive exploration of cognitive rehabilitation interventions for memory enhancement in patients who experienced a stroke; however, it omits specific memory subtypes, such as working memory and semantic memory. The strict inclusion criteria, which focused solely on interventions explicitly labeled cognitive rehabilitation, may have excluded relevant studies using alternate terminology. Methodological challenges, including heterogeneity in study design and outcome measures, impact the reliability and comparability of the findings. Furthermore, the homogeneity of the sample, which was primarily recruited from specialized rehabilitation centers, limits the generalizability of the results to broader stroke populations with diverse demographics and varying degrees of cognitive impairment severity. The applicability of these interventions to community or home-based settings remains uncertain.

Additionally, many studies lacked control groups or direct comparisons with traditional rehabilitation methods, which may affect the robustness of the conclusions regarding intervention efficacy. Future research should prioritize controlled designs to strengthen the evidence base and enable more definitive comparisons.

CONCLUSION

VR-based approaches provide immersive experiences that enhance cognitive function, memory, and daily activities. NVR strategies encompass cognitive training, brain stimulation, music, exercise, and goal setting, all of which contribute to improved cognitive performance. The choice between VR and NVR depends on patient preferences and individual needs, highlighting the value of personalized approaches in post-stroke cognitive recovery. Our findings undemphasized the transformative potential of technology-driven and innovative strategies in post-stroke rehabilitation, offering a versatile landscape to support patients in their journey to recovery and an improved quality of life.

FOOTNOTES

Author contributions: Mathew R, Sapru K, Gandhi DN, Surve TAN, Pande D, Parikh A, Sharma RB, and Kaur R contributed to the administration, methodology, and original draft preparation; Hasibuzzaman MA participated in the conceptualization, methodology, and supervision of this study.

Conflict-of-interest statement: All authors report no relevant conflicts of interest for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Country of origin: India

ORCID number: Md Al Hasibuzzaman 0000-0003-0548-4836.

S-Editor: Wang JJ
L-Editor: Filipodia
P-Editor: Guo X

REFERENCES

1 **Al-Qazzaz NK**, Ali SH, Ahmad SA, Islam S, Mohamad K. Cognitive impairment and memory dysfunction after a stroke diagnosis: a post-stroke memory assessment. *Neuropsychiatr Dis Treat* 2014; **10**: 1677-1691 [PMID: 25228808 DOI: 10.2147/NDT.S67184]
2 **Donkor ES**. Stroke in the 21st Century: A Snapshot of the Burden, Epidemiology, and Quality of Life. *Stroke Res Treat* 2018; **2018**: 3238165 [PMID: 30598741 DOI: 10.1155/2018/3238165]
3 **GBD 2019 Stroke Collaborators**. Global, regional, and national burden of stroke and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol* 2021; **20**: 795-820 [PMID: 34487721 DOI: 10.1016/S1474-4422(21)00252-0]
4 **He A**, Wang Z, Wu X, Sun W, Yang K, Feng W, Wang Y, Song H. Incidence of post-stroke cognitive impairment in patients with first-ever ischemic stroke: a multicenter cross-sectional study in China. *Lancet Reg Health West Pac* 2023; **33**: 100687 [PMID: 37181529 DOI: 10.1016/j.lanwpc.2023.100687]
5 **Maier M**, Ballester BR, Verschure PFMJ. Principles of Neurorehabilitation After Stroke Based on Motor Learning and Brain Plasticity Mechanisms. *Front Syst Neurosci* 2019; **13**: 74 [PMID: 31920570 DOI: 10.3389/fnsys.2019.00074]
6 **Elendu C**, Amaechi DC, Elendu TC, Ibhiedu JO, Egbunu EO, Ndam AR, Ogala F, Ologunde T, Peterson JC, Boluwatife AI, Okongko AO, Fatoye JO, Akpovona OL, Onyekweli SO, Temitope AY, Achimugu AO, Temilade AV. Stroke and cognitive impairment: understanding the connection and managing symptoms. *Ann Med Surg (Lond)* 2023; **85**: 6057-6066 [PMID: 38098605 DOI: 10.1097/MS9.0000000000001441]
7 **Leys D**, Hénon H, Mackowiak-Cordoliani MA, Pasquier F. Poststroke dementia. *Lancet Neurol* 2005; **4**: 752-759 [PMID: 16239182 DOI: 10.1016/S1474-4422(05)70221-0]
8 **Ogourtsova T**, Kagan A, Henderson A, Korner-Bitensky N. Cognitive Rehabilitation - Strokengine. [cited 10 August 2024]. Available from: https://strokengine.ca/en/interventions/cognitive-rehabilitation/
9 **National Clinical Guideline Centre (UK)**. Stroke Rehabilitation: Long Term Rehabilitation After Stroke [Internet]. London: Royal College of Physicians (UK), 2013 [PMID: 25340225]
10 **das Nair R**, Cogger H, Worthington E, Lincoln NB. Cognitive rehabilitation for memory deficits after stroke. *Cochrane Database Syst Rev* 2016; **9**: CD002293 [PMID: 27581994 DOI: 10.1002/14651858.CD002293.pub3]
11 **Mulhern M**. Cognitive Rehabilitation Interventions for Post-Stroke Populations. *Dela J Public Health* 2023; **9**: 70-74 [PMID: 37701470 DOI: 10.32481/djph.2023.08.012]

12 **Cumming TB**, Marshall RS, Lazar RM. Stroke, cognitive deficits, and rehabilitation: still an incomplete picture. *Int J Stroke* 2013; **8**: 38-45 [PMID: 23280268 DOI: 10.1111/j.1747-4949.2012.00972.x]

13 **Tang EYH**, Price C, Stephan BCM, Robinson L, Exley C. Impact of Memory Problems Post-stroke on Patients and Their Family Carers: A Qualitative Study. *Front Med (Lausanne)* 2020; **7**: 267 [PMID: 32637417 DOI: 10.3389/fmed.2020.00267]

14 **Catania V**, Rundo F, Panerai S, Ferri R. Virtual Reality for the Rehabilitation of Acquired Cognitive Disorders: A Narrative Review. *Bioengineering (Basel)* 2023; **11** [PMID: 38247912 DOI: 10.3390/bioengineering11010035]

15 **Borgnis F**, Baglio F, Pedroli E, Rossetto F, Uccellatore L, Oliveira JAG, Riva G, Cipresso P. Available Virtual Reality-Based Tools for Executive Functions: A Systematic Review. *Front Psychol* 2022; **13**: 833136 [PMID: 35478738 DOI: 10.3389/fpsyg.2022.833136]

16 **Aderinto N**, Olatunji G, Abdulbasit MO, Edun M, Aboderin G, Egbunu E. Exploring the efficacy of virtual reality-based rehabilitation in stroke: a narrative review of current evidence. *Ann Med* 2023; **55**: 2285907 [PMID: 38010358 DOI: 10.1080/07853890.2023.2285907]

17 **Moher D**, Liberati A, Tetzlaff J, Altman DG; The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement (Chinese edition). *J Chin Integr Med* 2009; **7**: 889-896 [DOI: 10.3736/jcim20090918]

18 **Sterne JAC**, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, Cates CJ, Cheng HY, Corbett MS, Eldridge SM, Emberson JR, Hernán MA, Hopewell S, Hróbjartsson A, Junqueira DR, Jüni P, Kirkham JJ, Lasserson T, Li T, McAleenan A, Reeves BC, Shepperd S, Shrier I, Stewart LA, Tilling K, White IR, Whiting PF, Higgins JPT. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* 2019; **366**: l4898 [PMID: 31462531 DOI: 10.1136/bmj.l4898]

19 **Sterne JA**, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, Henry D, Altman DG, Ansari MT, Boutron I, Carpenter JR, Chan AW, Churchill R, Deeks JJ, Hróbjartsson A, Kirkham J, Jüni P, Loke YK, Pigott TD, Ramsay CR, Regidor D, Rothstein HR, Sandhu L, Santaguida PL, Schünemann HJ, Shea B, Shrier I, Tugwell P, Turner L, Valentine JC, Waddington H, Waters E, Wells GA, Whiting PF, Higgins JP. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* 2016; **355**: i4919 [PMID: 27733354 DOI: 10.1136/bmj.i4919]

20 **Maier M**, Ballester BR, Leiva Bañuelos N, Duarte Oller E, Verschure PFMJ. Adaptive conjunctive cognitive training (ACCT) in virtual reality for chronic stroke patients: a randomized controlled pilot trial. *J Neuroeng Rehabil* 2020; **17**: 42 [PMID: 32143674 DOI: 10.1186/s12984-020-0652-3]

21 **Fernandez-Gonzalo R**, Fernandez-Gonzalo S, Turon M, Prieto C, Tesch PA, García-Carreira Mdel C. Muscle, functional and cognitive adaptations after flywheel resistance training in stroke patients: a pilot randomized controlled trial. *J Neuroeng Rehabil* 2016; **13**: 37 [PMID: 27052303 DOI: 10.1186/s12984-016-0144-7]

22 **Faria AL**, Andrade A, Soares L, I Badia SB. Benefits of virtual reality based cognitive rehabilitation through simulated activities of daily living: a randomized controlled trial with stroke patients. *J Neuroeng Rehabil* 2016; **13**: 96 [PMID: 27806718 DOI: 10.1186/s12984-016-0204-z]

23 **Faria AL**, Pinho MS, Bermúdez I Badia S. A comparison of two personalization and adaptive cognitive rehabilitation approaches: a randomized controlled trial with chronic stroke patients. *J Neuroeng Rehabil* 2020; **17**: 78 [PMID: 32546251 DOI: 10.1186/s12984-020-00691-5]

24 **Liu Z**, He Z, Yuan J, Lin H, Fu C, Zhang Y, Wang N, Li G, Bu J, Chen M, Jia J. Application of Immersive Virtual-Reality-Based Puzzle Games in Elderly Patients with Post-Stroke Cognitive Impairment: A Pilot Study. *Brain Sci* 2022; **13**: 79 [PMID: 36672060 DOI: 10.3390/brainsci13010079]

25 **Aben L**, Heijenbrok-Kal MH, Ponds RW, Busschbach JJ, Ribbers GM. Long-lasting effects of a new memory self-efficacy training for stroke patients: a randomized controlled trial. *Neurorehabil Neural Repair* 2014; **28**: 199-206 [PMID: 24300949 DOI: 10.1177/1545968313478487]

26 **Adomavičienė A**, Daunoravičienė K, Kubilius R, Varžaitytė L, Raistenskis J. Influence of New Technologies on Post-Stroke Rehabilitation: A Comparison of Armeo Spring to the Kinect System. *Medicina (Kaunas)* 2019; **55**: 98 [PMID: 30970655 DOI: 10.3390/medicina55040098]

27 **Yun GJ**, Chun MH, Kim BR. The Effects of Transcranial Direct-Current Stimulation on Cognition in Stroke Patients. *J Stroke* 2015; **17**: 354-358 [PMID: 26438001 DOI: 10.5853/jos.2015.17.3.354]

28 **Hellgren L**, Samuelsson K, Lundqvist A, Börsbo B. Computerized Training of Working Memory for Patients with Acquired Brain Injury. *Open J Ther Rehabilit* 2015; **3**: 46-55 [DOI: 10.4236/ojtr.2015.32007]

29 **Park MO**, Lee SH. Effects of cognitive-motor dual-task training combined with auditory motor synchronization training on cognitive functioning in individuals with chronic stroke: A pilot randomized controlled trial. *Medicine (Baltimore)* 2018; **97**: e10910 [PMID: 29851819 DOI: 10.1097/MD.0000000000010910]

30 **Park JH**, Park JH. The effects of a Korean computer-based cognitive rehabilitation program on cognitive function and visual perception ability of patients with acute stroke. *J Phys Ther Sci* 2015; **27**: 2577-2579 [PMID: 26356152 DOI: 10.1589/jpts.27.2577]

31 **Prokopenko SV**, Mozheyko EY, Petrova MM, Koryagina TD, Kaskaeva DS, Chernykh TV, Shvetzova IN, Bezdenezhnih AF. Correction of post-stroke cognitive impairments using computer programs. *J Neurol Sci* 2013; **325**: 148-153 [PMID: 23312291 DOI: 10.1016/j.jns.2012.12.024]

32 **Wilson PH**, Rogers JM, Vogel K, Steenbergen B, McGuckian TB, Duckworth J. Home-based (virtual) rehabilitation improves motor and cognitive function for stroke patients: a randomized controlled trial of the Elements (EDNA-22) system. *J Neuroeng Rehabil* 2021; **18**: 165 [PMID: 34823545 DOI: 10.1186/s12984-021-00956-7]

33 **Oliveira J**, Gamito P, Lopes B, Silva AR, Galhordas J, Pereira E, Ramos E, Silva AP, Jorge Á, Fantasia A. Computerized cognitive training using virtual reality on everyday life activities for patients recovering from stroke. *Disabil Rehabil Assist Technol* 2022; **17**: 298-303 [PMID: 32255695 DOI: 10.1080/17483107.2020.1749891]

34 **Studer B**, Timm A, Sahakian BJ, Kalenscher T, Knecht S. A decision-neuroscientific intervention to improve cognitive recovery after stroke. *Brain* 2021; **144**: 1764-1773 [PMID: 33742664 DOI: 10.1093/brain/awab128]

35 **Jaywant A**, Mautner L, Waldman R, O'Dell MW, Gunning FM, Toglia J. Feasibility and Acceptability of a Remotely Delivered Executive Function Intervention That Combines Computerized Cognitive Training and Metacognitive Strategy Training in Chronic Stroke. *Int J Environ Res Public Health* 2023; **20**: 5714 [PMID: 37174232 DOI: 10.3390/ijerph20095714]

36 **Jung H**, Jeong JG, Cheong YS, Nam TW, Kim JH, Park CH, Park E, Jung TD. The Effectiveness of Computer-Assisted Cognitive Rehabilitation and the Degree of Recovery in Patients with Traumatic Brain Injury and Stroke. *J Clin Med* 2021; **10**: 5728 [PMID: 34945019 DOI: 10.3390/jcm10245728]

37 **Kober SE**, Schweiger D, Witte M, Reichert JL, Grieshofer P, Neuper C, Wood G. Specific effects of EEG based neurofeedback training on memory functions in post-stroke victims. *J Neuroeng Rehabil* 2015; **12**: 107 [PMID: 26625906 DOI: 10.1186/s12984-015-0105-6]

38 **Li W**, Wen Q, Xie YH, Hu AL, Wu Q, Wang YX. Improvement of poststroke cognitive impairment by intermittent theta bursts: A double-

blind randomized controlled trial. *Brain Behav* 2022; **12**: e2569 [PMID: 35484991 DOI: 10.1002/brb3.2569]

39 **Tsai PY**, Lin WS, Tsai KT, Kuo CY, Lin PH. High-frequency versus theta burst transcranial magnetic stimulation for the treatment of poststroke cognitive impairment in humans. *J Psychiatry Neurosci* 2020; **45**: 262-270 [PMID: 32159313 DOI: 10.1503/jpn.190060]

40 **Yin M**, Liu Y, Zhang L, Zheng H, Peng L, Ai Y, Luo J, Hu X. Effects of rTMS Treatment on Cognitive Impairment and Resting-State Brain Activity in Stroke Patients: A Randomized Clinical Trial. *Front Neural Circuits* 2020; **14**: 563777 [PMID: 33117131 DOI: 10.3389/fncir.2020.563777]

41 **Marangolo P**, Fiori V, Caltagirone C, Pisano F, Priori A. Transcranial Cerebellar Direct Current Stimulation Enhances Verb Generation but Not Verb Naming in Poststroke Aphasia. *J Cogn Neurosci* 2018; **30**: 188-199 [PMID: 29064340 DOI: 10.1162/jocn_a_01201]

42 **Haire CM**, Vuong V, Tremblay L, Patterson KK, Chen JL, Thaut MH. Effects of therapeutic instrumental music performance and motor imagery on chronic post-stroke cognition and affect: A randomized controlled trial. *NeuroRehabilitation* 2021; **48**: 195-208 [PMID: 33664157 DOI: 10.3233/NRE-208014]

43 **Baylan S**, Haig C, MacDonald M, Stiles C, Easto J, Thomson M, Cullen B, Quinn TJ, Stott D, Mercer SW, Broomfield NM, Murray H, Evans JJ. Measuring the effects of listening for leisure on outcome after stroke (MELLO): A pilot randomized controlled trial of mindful music listening. *Int J Stroke* 2020; **15**: 149-158 [PMID: 30940047 DOI: 10.1177/1747493019841250]

44 **Patani KA**. Effect of Neurobic exercises on cognitive function related to Post–Stroke. *J Appl Dent Med Sci* 2020; **6**

45 **Withiel TD**, Wong D, Ponsford JL, Cadilhac DA, New P, Mihaljcic T, Stolwyk RJ. Comparing memory group training and computerized cognitive training for improving memory function following stroke: A phase II randomized controlled trial. *J Rehabil Med* 2019; **51**: 343-351 [PMID: 30815708 DOI: 10.2340/16501977-2540]

46 **Chiu EC**, Chi FC, Chen PT. Investigation of the home-reablement program on rehabilitation outcomes for people with stroke: A pilot study. *Medicine (Baltimore)* 2021; **100**: e26515 [PMID: 34190182 DOI: 10.1097/MD.0000000000026515]

47 **Abd-Elaziz SAE**, Khedr EM, Ahmed HAE, Ibrahim HDF. Effect of Cognitive Rehabilitation on Improving Cognitive Function and Activities of Daily Living among Elderly Patients with Stroke at Assiut University Hospital. *J Educ Pract* 2015; **6**: 44-56

48 **Fishman KN**, Ashbaugh AR, Swartz RH. Goal Setting Improves Cognitive Performance in a Randomized Trial of Chronic Stroke Survivors. *Stroke* 2021; **52**: 458-470 [PMID: 33467876 DOI: 10.1161/STROKEAHA.120.032131]

49 **Chen J**, Or CK, Chen T. Effectiveness of Using Virtual Reality-Supported Exercise Therapy for Upper Extremity Motor Rehabilitation in Patients With Stroke: Systematic Review and Meta-analysis of Randomized Controlled Trials. *J Med Internet Res* 2022; **24**: e24111 [PMID: 35723907 DOI: 10.2196/24111]

50 **Parisi A**, Bellinzona F, Di Lernia D, Repetto C, De Gaspari S, Brizzi G, Riva G, Tuena C. Efficacy of Multisensory Technology in Post-Stroke Cognitive Rehabilitation: A Systematic Review. *J Clin Med* 2022; **11**: 6324 [PMID: 36362551 DOI: 10.3390/jcm11216324]

51 **Zhang B**, Li D, Liu Y, Wang J, Xiao Q. Virtual reality for limb motor function, balance, gait, cognition and daily function of stroke patients: A systematic review and meta-analysis. *J Adv Nurs* 2021; **77**: 3255-3273 [PMID: 33675076 DOI: 10.1111/jan.14800]

52 **He D**, Cao S, Le Y, Wang M, Chen Y, Qian B. Virtual Reality Technology in Cognitive Rehabilitation Application: Bibliometric Analysis. *JMIR Serious Games* 2022; **10**: e38315 [PMID: 36260388 DOI: 10.2196/38315]

53 **Despoti A**, Karatzanos E, Patsaki I, Tzoumi D, Roussou G, Leventakis N, Papathanasiou A, Nanas S, Dimitriadi N. Immersive Virtual Reality in Cognitive Rehabilitation: A systematic Review. *Health Res J* 2022; **8**: 225-241 [DOI: 10.12681/healthresj.28872]

54 **Maggio MG**, De Bartolo D, Calabrò RS, Ciancarelli I, Cerasa A, Tonin P, Di Iulio F, Paolucci S, Antonucci G, Morone G, Iosa M. Computer-assisted cognitive rehabilitation in neurological patients: state-of-art and future perspectives. *Front Neurol* 2023; **14**: 1255319 [PMID: 37854065 DOI: 10.3389/fneur.2023.1255319]

55 **Shetty S**, Riyas Basheer KB. A Scoping Literature Analysis on the Effect of Cognitive Rehabilitation in Improving Higher Mental Functions Following Stroke. *Int J Health Sci Res* 2023; **13**: 129-137 [DOI: 10.52403/ijhsr.20230417]

56 **Nie P**, Liu F, Lin S, Guo J, Chen X, Chen S, Yu L, Lin R. The effects of computer-assisted cognitive rehabilitation on cognitive impairment after stroke: A systematic review and meta-analysis. *J Clin Nurs* 2022; **31**: 1136-1148 [PMID: 34459041 DOI: 10.1111/jocn.16030]

57 **Gong C**, Hu H, Peng XM, Li H, Xiao L, Liu Z, Zhong YB, Wang MY, Luo Y. Therapeutic effects of repetitive transcranial magnetic stimulation on cognitive impairment in stroke patients: a systematic review and meta-analysis. *Front Hum Neurosci* 2023; **17**: 1177594 [PMID: 37250691 DOI: 10.3389/fnhum.2023.1177594]

58 **Xu C**, He Z, Shen Z, Huang F. Potential Benefits of Music Therapy on Stroke Rehabilitation. *Oxid Med Cell Longev* 2022; **2022**: 9386095 [PMID: 35757506 DOI: 10.1155/2022/9386095]

59 **Song X**, Fu Q. Effect of rehabilitation therapy for cognitive impairment in elderly stroke on cognitive function score. *Front Med Sci Res* 2022; **4**: 10-13 [DOI: 10.25236/FMSR.2022.040502]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 100459

DOI: 10.5662/wjm.v15.i3.100459

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Visual avatar to increase situational awareness in anaesthesia: Systematic review of recent evidence

Alessio Tramontana, Massimiliano Rulli, Andrea Falegnami, Federico Bilotta

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B

Novelty: Grade B

Creativity or Innovation: Grade B

Scientific Significance: Grade B

P-Reviewer: Morya AK

Received: August 16, 2024

Revised: January 13, 2025

Accepted: January 23, 2025

Published online: September 20, 2025

Processing time: 201 Days and 12.9 Hours

Alessio Tramontana, Massimiliano Rulli, Federico Bilotta, Department of Anesthesiology, Critical Care and Pain Medicine, Policlinico Umberto I, Sapienza University of Rome, Rome 00185, Lazio, Italy

Andrea Falegnami, Department of Mechanical and Aerospace, International University "Uninettuno", Corso Vittorio Emanuele II, Rome 00186, Lazio, Italy

Corresponding author: Federico Bilotta, MD, PhD, Professor, Department of Anesthesiology, Critical Care and Pain Medicine, Policlinico Umberto I, Sapienza University of Rome, Viale del Policlinico 37, Rome 00185, Lazio, Italy. bilotta@tiscali.it

Abstract

BACKGROUND

Systematic review focuses on the visual patient avatar (VPA) technology, a tool designed to enhance situational awareness in anesthesia by transforming traditional numerical data into intuitive visual displays.

AIM

To explore how VPA can improve perceptual performance, reduce cognitive load, and increase user acceptance, potentially leading to better patient outcomes.

METHODS

The review is based on 14 studies conducted between 2018 and 2023 in five different hospitals across Europe.

RESULTS

These studies demonstrate that VPA allows clinicians to perceive and recall vital signs more efficiently than conventional monitoring methods. The technology's intuitive design helps reduce cognitive workload, indicating less mental effort required for patient monitoring. Users' feedback on VPA was generally positive, highlighting its potential to enhance monitoring and decision-making in high-stress environments. However, some users noted the need for further development, particularly in visualization design and data integration.

CONCLUSION

Review concludes that VPA technology represents a significant advancement in patient monitoring, promoting better situational awareness and potentially improving safety in perioperative care.

Key Words: Situational; Situation; Awareness; Anesthesia; Visual patient avatar

Core Tip: This systematic review examines the use of visual patient avatar technology to enhance perception, integration, and interpretation of medical data. Grounded in psychological and neuroscientific principles, the technology transforms information into intuitive shapes, colors, and animations, providing a clear advantage over traditional numerical formats. By simplifying complex data, it reduces cognitive workload, improves diagnostic accuracy, and bolsters clinical confidence. This innovative approach underscores the potential of visual representations in medical practice, fostering more efficient and effective decision-making processes for healthcare professionals.

Citation: Tramontana A, Rulli M, Falegnami A, Bilotta F. Visual avatar to increase situational awareness in anaesthesia: Systematic review of recent evidence. *World J Methodol* 2025; 15(3): 100459
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/100459.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.100459

INTRODUCTION

Visual patient avatar (VPA) is a computer derived representation of physiological variables that transforms traditional numerical data from patient monitors into intuitive visual displays using colors, shapes, and animations[1]. This tool can enhance situational awareness (SA), which is defined as the capacity to maintain an accurate internal representation of events in the environment and is considered an essential prerequisite for effective decision-making[2]. Innovation could allow anesthesiologist to quickly grasp patient conditions at a glance, improving, promoting and implementing their ability to monitor and respond to changes without constantly focusing on numerical displays and ultimately contribute to improve SA[1,2]. Over 80% of adverse events in anaesthesia stem from a deficiency in SA, with the first level of SA, as defined by Endsley, representing the largest proportion (42%). For instance, in the context of anaesthesia, this level involves recognizing the status and dynamics of the patient's vital signs on the monitor[3]. Improving safety in anaesthesia is challenging due to the highly complex environment and the infrequent use of a systematic approach to errors, which leads to a low rate of error reduction[4]. Despite the continuing advancement in the use of VPA analog in anaesthesia, there are not comprehensive studies dedicated to summarizing the related available literature evidence on the improving of perceptual performances, the reducing of cognitive load and the users feedback and acceptance. Aim of this Systematic review is to summarize recent evidence related to the use of VPA in anaesthesia to promote SA.

MATERIALS AND METHODS

This systematic review is based on recent studies extracted by literature search in PubMed and retrieving selected evidence from Google scholar and Scopus. The PubMed for all types of articles using Medical Subject Headings terms, Boolean tools, and keywords for the topic of interest, such as "situational," "situation", "awareness," "anesthesia" and "anaesthesia", separately and in combination. All data were analyzed using a PRISMA 2009 checklist statement protocol as a guarantee of a complete and transparent data's report. Full-text peer-reviewed randomized controlled trials, case-control, cohort, and cross-sectional studies and reviews that were published from January 1, 1975 to August 1, 2024, which investigated the updates of the VPA to improving, promoting and implementing their ability to monitor and respond to changes without constantly focusing on numerical displays were considered eligible. Articles published in languages other than English, animal studies, grey literature, and articles which do not include anaesthesia environment or adult patients were excluded. Literature search led to retrieve 362 articles screened by title, by abstract and by the full text (Figure 1). Out of these 12 articles were selected as eligible for this systematic review. At the end of this screening, also the literature on Scopus and Google scholar was searched to find related articles that were not spotted in PubMed.

RESULTS

A total of 14 articles were selected as appropriate for the present systematic review (Table 1). These studies address 3 principal aspects of the role of VPA in clinical practice: Perceptual performance, cognitive load, and user acceptance and integration. These prospective studies were conducted between December 2018 and December 2023 in five different hospitals across Switzerland[5-18], Germany[6,7,9], and Spain[6,7,9]. The participants consisted of volunteer medical staff, including anesthesiologists and nurses. Approximately 50% of the participants were women, with ages ranging from 30 years to 37 years and work experience spanning approximately 4 years to 8 years[5-18]. As this was the first time

Table 1 Summary of findings

Characteristics	Improved perceptual performance	Reduce cognitive load	User acceptance and integration	Total
Articles	[5-9]	[5-9]	[8,11,13]	14
	[10,12,13]	[10,12,13]	[14,16,17]	
	[14,15,16]	[14,15,16]	[18]	
	[17,18]	[17,18]	-	
Total	13	13	7	-

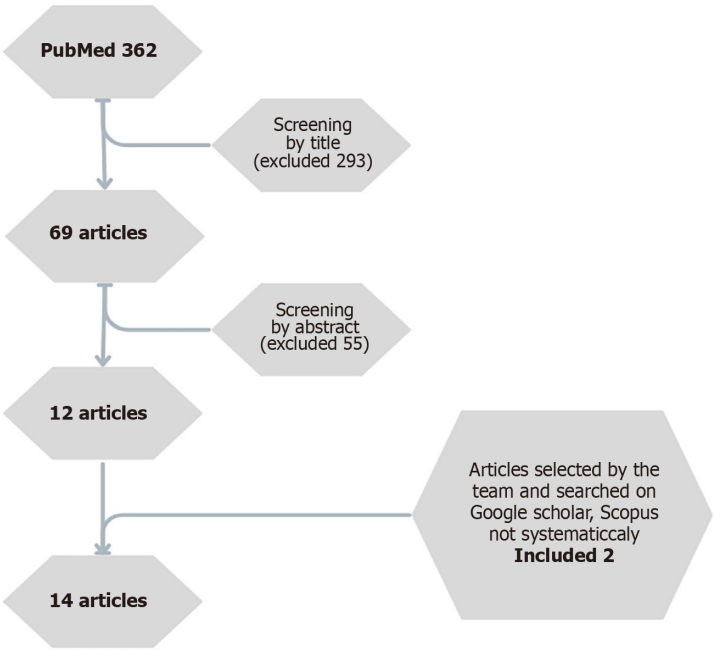


Figure 1 Flow chart.

implementing this VPA in simulated practice using various technologies, the researchers deemed it necessary to conduct preliminary educational sessions, followed by off-site training. Furthermore, a video and reference materials were provided to each physician *via* the hospital's intranet[5-7,9,15-18]. VPA technology is capable of displaying various parameters, including pulse rate, blood pressure, oxygen saturation, electrocardiogram ST-segment, central venous pressure, respiratory rate, tidal volume, expiratory carbon dioxide concentration, body temperature, brain activity, and neuromuscular relaxation[5].

Data were collected using an open questionnaire and a quiz administered *via* iSurvey on an iPad (Apple Inc., Cupertino, CA, United Sates). The data obtained were analyzed using a six-phase thematic analysis for the open-ended responses, employing the Harvest Your Data tool (Wellington, New Zealand)[5,6]. The quiz and demographic data were processed using Microsoft Excel (Microsoft Corporation), and translations were facilitated by DeepL (DeepL SE, Cologne, Germany)[5,6,15]. Some studies also utilized eye-tracking technology, such as the Gazepoint GP3 (Gazept, Vancouver, BC, Canada)[10,14,18] or the Pupil Invisible mobile eye-tracking device (Pupil Labs GmbH)[10], to assess clinicians' ability to retrieve and remember data. The resulting data provided enhanced insights into the underlying analytical pathways by offering information on spatial and temporal measurements, gaze coordinates, dwell times on areas of interest (AOI), as well as saccades and fixations. These data were analyzed using Gazepoint Professional Analysis software (Gazept, Vancouver, BC, Canada) or Pupil Player for eye-tracking data analysis (Pupil Labs GmbH)[6,10,14,18]. Additionally, some tests were conducted using a Google Glass headset (Alphabet Inc.)[18]. In all the referenced papers, tests were conducted to evaluate the participants' ability to rapidly comprehend situations and process additional information presented on a visual avatar monitor compared to a standard monitor[5-18]. The results demonstrated statistical significance, with a P value of < 0.05[6,9].

Perceptual performance

In 13 of 14 articles are reported evidenced related to the impact of implementing in clinical practice VPA on Perceptual Performance[5-10,12-18]. The thirteen articles reviewed claim that the VPA exhibits favorable usability characteristics, including rapid identification of underlying problems, excellent visibility of vital parameters from a distance, and valuable additional support, especially for beginners, compared to traditional displays. Participants also appreciated the

appealing graphic design of the VPA and emphasized its intuitive nature[5-10,12-18]. Participants were required to categorize data as "too low", "too high", or "normal" and remember the placement and location of installations in a patient[5,6]. The distribution of fixations and dwell time did not show statistically significant differences between the screen modes (avatar-only *vs* conventional monitor and split-screen). The monitoring modes also did not reveal significant differences in fixation and dwell time concerning variables such as profession, work experience, and scenario sequence.

The scenarios significantly influenced fixation and dwell time on the monitor, with participants showing greater attention during the myocardial infarction scenario. Better task performance was associated with a reduction in fixations and dwell time on the monitor, suggesting an inverse relationship between performance efficiency and the visual attention required. Participants had fewer fixations and spent less time on the avatar portion of the screen compared to the conventional part, indicating a difference in the mode of visualization[10]. In conventional monitoring, the time spent on AOIs for installations ranged from 2.13 seconds to 2.51 seconds, while for VPA-intensive care unit, the time distribution was more balanced, with no AOI viewed for an excessively long time[6].

Analysis of variance tests for time spent on alarms across cases indicated no statistically significant differences (P = 0.193). However, a greater amount of time was spent on alarms in instances involving yellow and red alerts (P = 0.018, moderate evidence). In conventional monitoring, more time was spent on installation areas, while the VPA exhibited a more balanced distribution of time across AOIs[6]. Other studies emphasize the need for further development of the VPA monitor into a subsequent model, known as VPA-intensive care unit, incorporating features identified by users in previous studies as essential[9]. Among the most notable features that need further improvements are: Enhancements in graphic design, improved accuracy in presenting displayed information, refinements in system-user interface[9].

The typically displayed information includes the following parameters: Pulse contour cardiac output catheter, intracranial pressur sensor, neuromuscular relaxation, central venous line, ST segment, arterial line, urinary catheter, peripheral venous line, tube functionality, brain activity, etCO_2, brain activity sensor, central venous pressure, temperature, peak inspiratory pressure, SpO_2, FiO_2, respiratory rate, cardiac index, electrocardiogram/pulse rate, tidal volume, and mean arterial blood pressure[6,9]. Controlled studies show that anesthesia providers using VPA technology were able to perceive and recall more vital signs correctly compared to traditional monitoring methods. For instance, in 10-second scenarios, providers identified a median of 11 vital signs using the avatar, compared to 7 with conventional monitors, indicating that the avatar-based system allows for more efficient information processing in high-pressure situations[5].

The study established the hypothesis that split-screen monitoring is non-inferior to conventional monitoring in terms of performance on critical tasks during anesthesia emergency scenarios. Furthermore, the use of the avatar enhanced the likelihood of verbalizing the cause of the emergency compared to conventional monitoring. The results indicate that this technology can serve as a safe and situational-awareness-focused supplement to conventional monitoring. However, the user's greater familiarity with conventional monitoring, as opposed to their limited experience with the avatar, may act as a significant confounding factor, potentially underestimating the true impact of the avatar[9,10].

In one study was used the Paced Auditory Serial Addition test, the test, which involves arithmetic tasks, simulates distraction by requiring participants to listen to and sum up numbers presented every 2 seconds[16]. This task necessitates various cognitive resources and ensures identical test conditions for all participants, evidenced no significant difference between the two types of monitoring and viewing times[16].

Cognitive load

In 13 of 14 articles are reported evidenced related to the impact of implementing in clinical practice VPA on cognitive load[5-10,12-18]. The second chapter delves into the capacity of this technology to reduce cognitive load, a topic of significant interest within the scientific community. Notably, this technology has demonstrated a reduction in perceived workload among anesthesia providers. In one study, the National Aeronautics and Space Administration Task Load Index, a recognized measure of cognitive workload, was significantly lower when using avatar-based monitoring compared to traditional methods. This finding suggests that the VPA can help alleviate the mental effort required to monitor patients, potentially leading to fewer errors and faster decision-making[5-10,12-18]. It is important to note that, with the advancement of technology, the increasing volume of data necessitates complex systems for integration and visualization to effectively reduce cognitive load[5]. In fact, 80% of errors during perioperative treatment are attributed to reduced SA caused by cognitive overload[5]. Avatar-based monitoring reduces perceived workload, particularly when the duration of monitor observation is brief[15,16].

User acceptance and integration:

In 7 of 14 articles are reported evidenced related to the impact of implementing in clinical practice VPA on perceptual performance[8,11,13,14,16-18]. Following the implementation of the VPA in clinical settings, anesthesia providers reported that it significantly enhanced their ability to monitor patients and make timely decisions, thanks to the quick overview and rapid problem identification facilitated by the avatar technology. Despite some initial resistance and the need for adjustments to accommodate individual preferences, the overall reception was positive, suggesting a high potential for broader adoption in clinical practice[8,11,13,14,16-18].

The limitations of the VPA include the need for further development to enhance visualization designs, particularly by implementing user-customizable thresholds or aligning them with predefined audible alarm limits. The potential solutions suggested by study participants, such as improvements in visualization design, should be further explored and refined to enhance the effectiveness and usability of the VPA[11,13,14]. Additionally, due to the data integration involved, there are instances where accurately conveying the situation can be challenging because of data volume reduction. These efforts have sparked a new interest not only in advancing scientific knowledge but also in integrating these advancements

into clinical practice. To monitor user acceptance, significant attention has been devoted to reflexivity, which in these studies is categorized into personal, methodological, and contextual reflexivity[8,18].

Positive feedback focused on the design, intuitiveness, time-saving features, and clarity in visualizing patient devices. Negative feedback included concerns about sensory overload, the need for more familiarity, and incomplete information. Key areas for improvement included usability, visibility, and the need for customizable alarm thresholds. Further development is necessary to enhance the effectiveness and usability of the VPA, particularly in its visualization designs and data integration[8,16].

DISCUSSION

This systematic review originally summarizes recent evidence related to simulate training of VPA in anesthesia to promote SA, which demonstrates that implementing VPA technology in anesthesia can enhance perceptual performance, reduce cognitive load, and increase user acceptance, thereby improving the safety and effectiveness of anaesthetic practices. As a matter of fact, 80% of participants reported that distractions occur frequently during their daily work in the operating theatre[8,11,13,14,16,18].

SA consists of three stages: Perception, comprehension, and projection, and is crucial for informed decision-making[14, 18]. Many technological advances use and implement the SA's stages to significantly improve patient care in perioperative and critical settings[5]. According to cognitive load theory, humans are incapable of processing massive amounts of data for long periods of time due to the limited capacity of working memory. Working memory can usually manage 5 items to 9 items at a time. Typically manages between 5 items and 9 items at a time. When working memory is overloaded, it leads to diminished information processing, comprehension, and retention[8,14]. Furthermore, the emotional burden experienced by care providers in life-critical situations can impair mental acuity. The interplay of cognitive and emotional load may contribute to occupational distress, fatigue, and burnout[8,14]. Drawing on the principles of cognitive psychology and neuroscience, humans are better able to perceive, understand, and process an integrated, user-centered visual language than a technology-centered approach that measures specific parameters and displays them as isolated numbers and waveforms. The VPAs tested in these studies were designed according to these principles[5]. Additionally, the VPA incorporated the Gestalt principles of perception, including similarity, enclosure, closure, continuity, and connection, which emerged in the early twentieth century and continue to serve as accurate descriptions of human visual behavior. Objects are perceived as a group when they are in close proximity, share similar attributes, appear to have a boundary around them, or are connected[8].

Dual-processing theory established that human thinking and visual information processing are divided into two complementary systems: The associative system (System 1) and the reasoning system (System 2). System 1 enables rapid, instinctive decision-making that does not rely on working memory and is primarily influenced by emotions and intuitive judgments. In contrast, System 2 governs slower, more deliberate, and rational decision, predominantly regulated by the frontal cortex. These two systems operate concurrently, integrating to form a cohesive perception of visual information. However, current state-of-the-art monitors that rely on waveforms are not optimized to support quick and confident interpretation with minimal cognitive effort[9].

So, human brain is capable of rapidly detecting color, motion, and shape, seamlessly integrating this information to form associations. These principles can provide a foundation for designing user-centered patient monitoring technologies that enhance SA and optimize sensory perception. In contrast, the traditional single-sensor, single-indicator model, which presents information in isolation, may impose a greater cognitive burden on the user[8].

Several distinctive features of conventional representation contribute to this issue: Individuals can only process numbers sequentially; the displayed numbers represent low-level data that is only indirectly relevant to the task; many of the numbers shown fall within similar ranges (*e.g.*, pulse rate, blood pressure, oxygen saturation); and individuals can retain only 7 digits, plus or minus 2, in their short-term memory at a time[18]. Conversely, the human brain can rapidly detect color, motion, and shape, integrating these cues to form associations. These principles can inform the design of user-centered patient monitoring technologies that enhance SA and optimize sensory perception, such as the VPA evaluated in these studies[8]. Comparing the results of this review on Philips VPA with previous research is possible to demonstrate that visualization technologies improve clinicians' SA, diagnostic confidence, and reduce workload, but is possible to demonstrate, also, the limitations of these studies. In fact, there are other examples of technologies of visualization in the literature that are not included in these studies (Table 2).

Literature search did not include databases other than PubMed, Scopus, and Google Scholar, which might have excluded relevant studies published elsewhere. However, the final article selection was carefully made, offering a balanced representation of the evidence. Most studies were conducted in controlled simulated environments, which may limit the applicability of the results to real clinical settings or other specialties. The participant group was voluntary and non-randomized, and some studies had a significant amount of missing data. Even though, the implications for clinical practice are significant. VPA technology represents a major advancement in patient monitoring, providing a more intuitive and effective way for clinicians to maintain SA and manage cognitive load during critical procedures, but not one of these studies has been done during clinical practice. Even though, the evidence suggests that adopting this technology can improve diagnostic accuracy, reduce clinical errors, and increase user satisfaction, indicating a high potential for broader integration into clinical practice. Maybe for now, as a safe addition to patient monitoring could be consider implementing a combination of avatar and conventional monitoring in a split-screen layout.

The chapter on integration and user acceptance was, in our opinion, the most interesting and original, as it has sparked less debate within the scientific community. We place significant importance on this chapter due to our team's

Table 2 Other technologies

Name	Company	Characteristics
Alert Watch	AlertWatch, Inc., Ann Arbor, MI, United States	Multifunction decision - supporting systems for intraoperative anesthetic care, obstetric care, and remote monitoring
Dynamic lung	Hamilton Medical AG, Bonaduz, Switzerland	Respiratory monitoring data from ventilators in an animated anatomical lung image
Hemo-sight	Mindray Medical International Limited, Shenzhen, Guangdong Province, China	Advanced hemodynamic monitoring
Pulmo-sight	Mindray Medical International Limited, Shenzhen, Guangdong Province, China	Anatomical lung image with a bronchial tree and trachea to visualize respiratory parameters
Physiology screen	Edwards Lifesciences Corp., Irvine, CA, United States	Advanced hemodynamic monitoring
Alarm status visualizer	Masimo Corp., Irvine, CA, United States	Colored-visual alarm indicators on a three - dimensional anatomical image
ROTEM sigma	Werfen Inc., Barcelona, Spain	Graphical and visual interpretation of rotational thromboelastometry
Visual blood	University of Zurich, Switzerland	Visualization of arterial blood gas analyses

longstanding interest in and sensitivity to the ergonomics and clinical integration of new technologies in anesthesia. Additionally, as highlighted in studies on the "cockpit" environment, the rapid integration and acceptance by clinical operators underscore the importance of ergonomics and usability of new technologies in healthcare settings. Future studies, appropriately design, should evaluate the impact in clinical practice. The present review can design the future research protocol.

CONCLUSION

This systematic review on the VPA technology facilitates perception, integration, and interpretation, according to psychological and neuroscientific foundations, integrated the information in shapes, colors, and animations, compared to traditional formats like numbers, reducing workload and enhancing diagnostic confidence. The technology is not only straightforward to learn but also proves particularly valuable in high-cognitive load environments, where it enhances SA and supports swift, accurate decision-making. In fact, Eye-tracking studies affirm that the design of visual patient enhances visual perception, enabling users to process vital information more effectively across their entire visual field. This comprehensive approach ensures that critical data is conveyed with clarity and speed, making it an invaluable tool in clinical settings where time and precision are of the essence. In conclusion, presented results suggest a promising role of VPA in improving SA ultimately leading to increased perioperative safety.

FOOTNOTES

Author contributions: Tramontana A contributed to the visualization of the manuscript; Falegnami A validated the manuscript; Bilotta F contributed to the supervision, project management of the manuscript; Tramontana A and Bilotta F contributed to the conceptualization, writing, reviewing and editing, methodology of the manuscript; Tramontana A and Rulli M wrote the original manuscript and contributed to the formal analysis, research, and resources to the manuscript; Falegnami A and Bilotta F performed data organization; and all authors thoroughly reviewed and endorsed the final manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Country of origin: Italy

ORCID number: Alessio Tramontana 0000-0001-7146-7044; Federico Bilotta 0000-0003-2496-6646.

S-Editor: Bai Y
L-Editor: A

P-Editor: Yu HG

REFERENCES

1 **Gaba DM**, Howard SK, Small SD. Situation awareness in anesthesiology. *Hum Factors* 1995; **37**: 20-31 [PMID: 7790008 DOI: 10.1518/001872095779049435]

2 **Weller JM**, Mahajan R, Fahey-Williams K, Webster CS. Teamwork matters: team situation awareness to build high-performing healthcare teams, a narrative review. *Br J Anaesth* 2024; **132**: 771-778 [PMID: 38310070 DOI: 10.1016/j.bja.2023.12.035]

3 **Endsley MR**. Designing for Situation Awareness. 2nd ed. Boca Raton: CRC Press, 2004

4 **Neves S**, Soto RG. Distraction in the OR: Bells and Whistles on Silent Mode. *Int Anesthesiol Clin* 2019; **57**: 62-67 [PMID: 31577238 DOI: 10.1097/AIA.0000000000000236]

5 **Hunn CA**, Lunkiewicz J, Noethiger CB, Tscholl DW, Gasciauskaite G. Qualitative Exploration of Anesthesia Providers' Perceptions Regarding Philips Visual Patient Avatar in Clinical Practice. *Bioengineering (Basel)* 2024; **11**: 323 [PMID: 38671745 DOI: 10.3390/bioengineering11040323]

6 **Viautour J**, Naegeli L, Braun J, Bergauer L, Roche TR, Tscholl DW, Akbas S. The Visual Patient Avatar ICU Facilitates Information Transfer of Written Information by Visualization: A Multicenter Comparative Eye-Tracking Study. *Diagnostics (Basel)* 2023; **13**: 3432 [PMID: 37998568 DOI: 10.3390/diagnostics13223432]

7 **Lunkiewicz J**, Gasciauskaite G, Roche TR, Akbas S, Nöthiger CB, Ganter MT, Meybohm P, Hottenrott S, Zacharowski K, Raimann FJ, Rivas E, López-Baamonde M, Beller EA, Tscholl DW, Bergauer L. User Perceptions of Avatar-Based Patient Monitoring for Intensive Care Units: An International Exploratory Sequential Mixed-Methods Study. *Diagnostics (Basel)* 2023; **13**: 3391 [PMID: 37958287 DOI: 10.3390/diagnostics13213391]

8 **Gasciauskaite G**, Lunkiewicz J, Roche TR, Spahn DR, Nöthiger CB, Tscholl DW. Human-centered visualization technologies for patient monitoring are the future: a narrative review. *Crit Care* 2023; **27**: 254 [PMID: 37381008 DOI: 10.1186/s13054-023-04544-0]

9 **Bergauer L**, Braun J, Roche TR, Meybohm P, Hottenrott S, Zacharowski K, Raimann FJ, Rivas E, López-Baamonde M, Ganter MT, Nöthiger CB, Spahn DR, Tscholl DW, Akbas S. Avatar-based patient monitoring improves information transfer, diagnostic confidence and reduces perceived workload in intensive care units: computer-based, multicentre comparison study. *Sci Rep* 2023; **13**: 5908 [PMID: 37041316 DOI: 10.1038/s41598-023-33027-z]

10 **Ljubenovic A**, Said S, Braun J, Grande B, Kolbe M, Spahn DR, Nöthiger CB, Tscholl DW, Roche TR. Visual Attention of Anesthesia Providers in Simulated Anesthesia Emergencies Using Conventional Number-Based and Avatar-Based Patient Monitoring: Prospective Eye-Tracking Study. *JMIR Serious Games* 2022; **10**: e35642 [PMID: 35172958 DOI: 10.2196/35642]

11 **Wetli DJ**, Bergauer L, Nöthiger CB, Roche TR, Spahn DR, Tscholl DW, Said S. Improving Visual-Patient-Avatar Design Prior to Its Clinical Release: A Mixed Qualitative and Quantitative Study. *Diagnostics (Basel)* 2022; **12**: 555 [PMID: 35204644 DOI: 10.3390/diagnostics12020555]

12 **Roche TR**, Said S, Braun J, Maas EJC, Machado C, Grande B, Kolbe M, Spahn DR, Nöthiger CB, Tscholl DW. Avatar-based patient monitoring in critical anaesthesia events: a randomised high-fidelity simulation study. *Br J Anaesth* 2021; **126**: 1046-1054 [PMID: 33879327 DOI: 10.1016/j.bja.2021.01.015]

13 **Tscholl DW**, Rössler J, Said S, Kaserer A, Spahn DR, Nöthiger CB. Situation Awareness-Oriented Patient Monitoring with Visual Patient Technology: A Qualitative Review of the Primary Research. *Sensors (Basel)* 2020; **20**: 2112 [PMID: 32283625 DOI: 10.3390/s20072112]

14 **Tscholl DW**, Rössler J, Handschin L, Seifert B, Spahn DR, Nöthiger CB. The Mechanisms Responsible for Improved Information Transfer in Avatar-Based Patient Monitoring: Multicenter Comparative Eye-Tracking Study. *J Med Internet Res* 2020; **22**: e15070 [PMID: 32175913 DOI: 10.2196/15070]

15 **Garot O**, Rössler J, Pfarr J, Ganter MT, Spahn DR, Nöthiger CB, Tscholl DW. Avatar-based versus conventional vital sign display in a central monitor for monitoring multiple patients: a multicenter computer-based laboratory study. *BMC Med Inform Decis Mak* 2020; **20**: 26 [PMID: 32041584 DOI: 10.1186/s12911-020-1032-4]

16 **Pfarr J**, Ganter MT, Spahn DR, Noethiger CB, Tscholl DW. Effects of a standardized distraction on caregivers' perceptive performance with avatar-based and conventional patient monitoring: a multicenter comparative study. *J Clin Monit Comput* 2020; **34**: 1369-1378 [PMID: 31768924 DOI: 10.1007/s10877-019-00429-2]

17 **Tscholl DW**, Handschin L, Neubauer P, Weiss M, Seifert B, Spahn DR, Noethiger CB. Using an animated patient avatar to improve perception of vital sign information by anaesthesia professionals. *Br J Anaesth* 2018; **121**: 662-671 [PMID: 30115265 DOI: 10.1016/j.bja.2018.04.024]

18 **Pfarr J**, Ganter MT, Spahn DR, Noethiger CB, Tscholl DW. Avatar-Based Patient Monitoring With Peripheral Vision: A Multicenter Comparative Eye-Tracking Study. *J Med Internet Res* 2019; **21**: e13041 [PMID: 31317870 DOI: 10.2196/13041]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 102477

DOI: 10.5662/wjm.v15.i3.102477

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Seeing the unseen: The low treatment rate of eye emergencies in Africa

Babatunde Ismail Bale, Marco Zeppieri, Obehi Suzan Idogen, Clinton Ifeanyi Okechukwu, Onakhe Emmanuel Ojo, Daniel Ayodele Femi, Abiola Afeez Lawal, Shalom Jesufunminiye Adedeji, Pirakalai Manikavasagar, Adewunmi Akingbola, Abdullahi Tunde Aborode, Mutali Musa

Specialty type: Ophthalmology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade C, Grade D

Novelty: Grade B, Grade B, Grade C

Creativity or Innovation: Grade B, Grade B, Grade C

Scientific Significance: Grade A, Grade B, Grade C

P-Reviewer: Han JM; Lopes LCP; Zheng P

Received: October 21, 2024

Revised: January 11, 2025

Accepted: January 21, 2025

Published online: September 20, 2025

Processing time: 138 Days and 18.3 Hours

Babatunde Ismail Bale, Obehi Suzan Idogen, Clinton Ifeanyi Okechukwu, Onakhe Emmanuel Ojo, Daniel Ayodele Femi, Mutali Musa, Department of Optometry, University of Benin, Benin 300283, Nigeria

Marco Zeppieri, Department of Ophthalmology, University Hospital of Udine, Udine 33100, Italy

Abiola Afeez Lawal, Faculty of Pharmacy, University of Benin, Benin 300283, Nigeria

Shalom Jesufunminiye Adedeji, Department of Nursing, Isolo General Hospital, Lagos 300283, Nigeria

Pirakalai Manikavasagar, Public Health for Eye Care, London School for Hygiene and Tropical Medicine, London CB21TN, United Kingdom

Adewunmi Akingbola, Department of Public Health, University of Cambridge, Cambridge CB2 1TN, Cambridgeshire, United Kingdom

Abdullahi Tunde Aborode, Department of Research and Development, Healthy Africans Platform, Ibadan, Nigeria

Corresponding author: Marco Zeppieri, MD, PhD, Department of Ophthalmology, University Hospital of Udine, p.le S. Maria della Misericordia 15, Udine 33100, Italy.
mark.zeppieri@asufc.sanita.fvg.it

Abstract

BACKGROUND

Emergency medical care is essential in preventing morbidity and mortality, especially when interventions are time-sensitive and require immediate access to supplies and trained personnel.

AIM

To assess the treatment rates of eye emergencies in Africa. Ocular emergencies are particularly delicate due to the eye's intricate structure and the necessity for its refractive components to remain transparent.

METHODS

This review examines the low treatment rates of eye emergencies in Africa, drawing on 96 records extracted from the PubMed database using predetermined search criteria.

RESULTS

The epidemiology of ocular injuries, as detailed in the studies, reveals significant relationships between the incidence and prevalence of eye injuries and factors such as age, gender, and occupation. The causes of eye emergencies range from accidents to gender-based violence and insect or animal attacks. Management approaches reported in the review include both surgical and non-surgical interventions, from medication to evisceration or enucleation of the eye. Preventive measures emphasize eye health education and the use of protective eyewear and facial protection. However, inadequate healthcare infrastructure and personnel, cultural and geographical barriers, and socioeconomic and behavioral factors hinder the effective prevention, service uptake, and management of eye emergencies.

CONCLUSION

The authors recommend developing eye health policies, enhancing community engagement, improving healthcare personnel training and retention, and increasing funding for eye care programs as solutions to address the low treatment rate of eye emergencies in Africa.

Key Words: Eye emergency; Ocular injury; Epidemiology; Treatment; Africa

Core Tip: Eye emergencies in Africa are severely under-treated due to a combination of factors including inadequate healthcare infrastructure, shortage of trained personnel, and socio-economic and cultural barriers. This results in a high prevalence of preventable blindness. This study emphasizes the need for urgent policy reforms, increased funding, and community engagement to improve access to timely and effective eye care across the continent.

Citation: Bale BI, Zeppieri M, Idogen OS, Okechukwu CI, Ojo OE, Femi DA, Lawal AA, Adedeji SJ, Manikavasagar P, Akingbola A, Aborode AT, Musa M. Seeing the unseen: The low treatment rate of eye emergencies in Africa. *World J Methodol* 2025; 15(3): 102477

URL: https://www.wjgnet.com/2222-0682/full/v15/i3/102477.htm

DOI: https://dx.doi.org/10.5662/wjm.v15.i3.102477

INTRODUCTION

Eye emergencies encompass a wide range of conditions that require immediate medical attention to prevent severe consequences, such as permanent vision loss[1]. These include acute infections like endophthalmitis and corneal ulcers, traumatic injuries such as open globe injuries and chemical burns, and sudden exacerbations of conditions like acute angle-closure glaucoma[1,2]. Timely and effective medical response is critical for these conditions, as they can rapidly worsen if not treated promptly. While eye emergencies are a global public health issue, their impact is particularly pronounced in low-resource settings, such as many regions in Africa[1].

In Africa, socio-economic barriers further exacerbate the challenge, making it difficult for many individuals, especially in rural areas, to access facilities capable of handling ocular emergencies[3]. The shortage of trained ophthalmologists and emergency eye care services worsens the situation, leading to low treatment rates for these critical conditions[4]. This inadequate response infrastructure contributes to a high prevalence of preventable blindness and underscores significant disparities in healthcare access and quality across the continent[5].

Addressing these challenges requires urgent investment in healthcare infrastructure, enhanced training for eye care professionals, and the establishment of surveillance systems to monitor the health situation[7,8]. Public health initiatives to raise awareness about the importance of eye care are also essential[9]. Therefore, this paper critically examines the current state of eye emergencies in Africa, exploring the causes, consequences, challenges, and potential solutions to improve outcomes in this critical area of health.

MATERIALS AND METHODS

This scoping review was conducted to investigate the epidemiology, causes, and management strategies for eye emergencies in Africa, while also identifying barriers to effective treatment. The methodology was carefully designed to ensure a thorough and systematic approach to the selection of literature, extraction of data, and synthesis of findings.

Search strategy

A comprehensive search was performed using the PubMed database to identify studies relevant to ocular emergencies in Africa. The search strategy, summarized in Table 1 below involved using a combination of keywords, including "ocular emergency", "eye injury", "Africa" and related terms. The search method utilized Boolean operators and a variety of keywords, including 'ocular emergency', 'eye damage', 'Africa' and associated terms. Search phrases were amalgamated utilizing Boolean operators (*e.g.*, 'AND', 'OR') to optimize the retrieval of pertinent articles. The search was confined to papers published between January 2014 and August 2024 in English, with full-text access. Inclusion criteria specifically targeted research that addressed the epidemiology, etiology, management approaches, and obstacles to the treatment of ocular emergencies in Africa. The search was deliberately limited to studies published between January 2014 and August 2024, ensuring that only the most recent and pertinent literature was considered for inclusion.

The data extraction form comprised fields for study characteristics (author, year, country, design), sample characteristics (size, demographics), types of ocular emergencies, causes, management options, and outcomes. Discrepancies among reviewers during research selection and data extraction were reconciled through consensus or by consulting a third reviewer. This method guaranteed impartiality and uniformity during the evaluation process. The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Inclusion and exclusion criteria

The selection of studies was guided by clearly defined inclusion and exclusion criteria:

Inclusion criteria: (1) Studies that specifically focus on ocular emergencies within African countries; (2) Articles published in the English language; (3) Studies that report on the epidemiology, causes, management strategies, and barriers to the treatment of eye emergencies; and (4) Studies with full-text availability.

Exclusion criteria: (1) Studies published prior to 2014; (2) Articles that are not directly relevant to the topic, including those not conducted within the African context; (3) Non-English language studies and studies without accessible full texts; and (4) Editorials, commentaries, and letters were excluded to maintain a focus on original research contributions.

Study selection

The initial search of the PubMed database yielded 136 records, encompassing a broad range of studies related to ocular emergencies across various African countries. Following this, a multi-stage filtering process was applied to ensure that only the most relevant and high-quality studies were included in the final review.

First, the records were filtered according to the predefined inclusion and exclusion criteria, which resulted in the exclusion of studies published before 2014, non-English language articles, and those that did not focus on the African context or eye emergencies. This initial filtering narrowed the list down to 96 records.

The remaining 96 studies were subjected to a rigorous screening process conducted independently by two reviewers. During this phase, the titles and abstracts of each study were meticulously reviewed to assess their relevance to the research objectives. Studies that appeared to meet the inclusion criteria were retained for full-text review. In cases where there was uncertainty or disagreement between the two reviewers regarding the inclusion of a study, the issue was discussed, and a third reviewer was consulted to reach a consensus. This collaborative approach ensured that the selection process was thorough and unbiased, ultimately leading to a final selection of studies that were deemed highly relevant and aligned with the research objectives. Although full-text availability was a criterion for inclusion to guarantee thorough data extraction, this requirement may have omitted significant studies accessible solely in abstract form.

Data extraction and synthesis

Data from the selected studies were extracted using a standardized data extraction form. The form captured details such as study characteristics (author, year of publication, country, study design), population characteristics (sample size, demographics), types of eye emergencies reported, causes, management approaches, outcomes, and barriers to treatment. Two reviewers (Bale BI and Idogen OS) independently performed data extraction to ensure accuracy and consistency. Disagreements were resolved by consensus or with the involvement of a third reviewer (Musa M). Given the heterogeneity in study designs, populations, and outcomes reported, a narrative synthesis was conducted. The synthesis focused on summarizing the prevalence and types of eye emergencies, the causes identified, management approaches, and barriers to effective treatment. Findings were grouped by themes related to epidemiology, causes, management strategies, and factors contributing to low treatment rates.

RESULTS

A total of 136 records were extracted using the search algorithm from the PubMed database as shown in Figure 1 below. After limiting the publication spread to 10 years spanning 2014 to 2024, a total of 96 records were obtained. Two authors then rigorously examined each record for relevance, language and completeness. Two records were excluded as they were an erratum to published studies which were also not included while another four were excluded because they were not in the English language. A further 53 records were out of the scope of this paper, and so they were also excluded. The references in 37 studies obtained were examined for relevant records; and a total of 21 relevant studies were further obtained, amounting to the usage of a total of 58 studies in this review.

Table 1 Summary of search strategy including search component and keywords used

Search component	Keywords/terms used
Ocular	"ocular"[All Fields] OR "oculars"[All Fields]
Emergency	"emerge"[All Fields] OR "emerged"[All Fields] OR "emergence"[All Fields] OR "emergences"[All Fields] OR "emergencies"[MeSH Terms] OR "emergencies"[All Fields] OR "emergency"[All Fields] OR "emergent"[All Fields] OR "emergently"[All Fields] OR "emergents"[All Fields] OR "emerges"[All Fields] OR "emerging"[All Fields]
Africa	"africa"[MeSH Terms] OR "africa"[All Fields] OR "africa s"[All Fields] OR "africas"[All Fields]
Date range	2014: 2024[pdat]
Language, full text, relevance	Records filtered to exclude non-English language, no full text, and non-relevant articles.

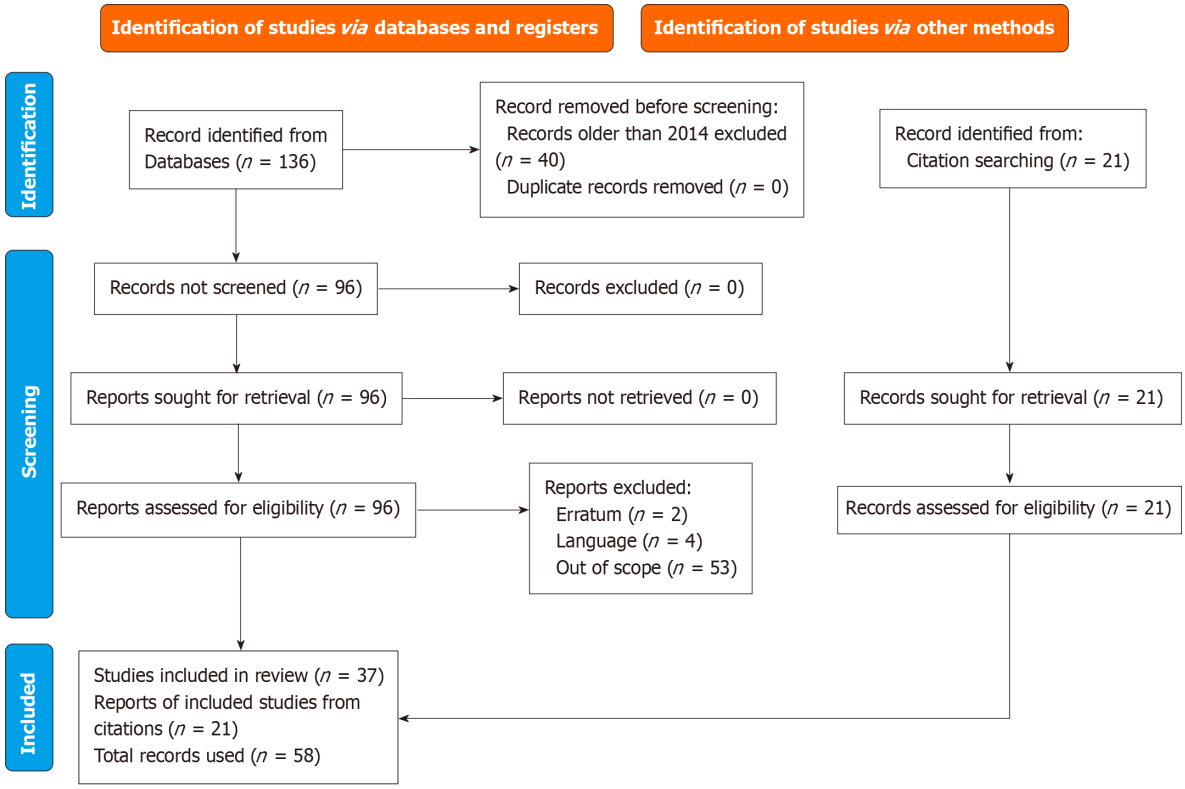


Figure 1 A PRISMA diagram showing the search stratification used in this study.

DISCUSSION

The factors affecting treatment rates for disease emergencies, such as maternal health and infectious diseases, and those for eye emergencies share common barriers but also exhibit unique challenges. Both categories are significantly hindered by inadequate healthcare infrastructure and a shortage of trained personnel. For instance, in Africa, maternal health and eye emergencies alike are often delayed due to insufficient emergency care services, limited availability of supplies, and long travel times to healthcare facilities in rural areas[10-12]. Additionally, socio-economic factors, such as poverty and cultural norms, play a pivotal role in reducing treatment uptake for both[13]. Gender-specific barriers, including women needing permission from male family members to seek care, exacerbate delays for maternal health emergencies and eye injuries in women[14].

Epidemiological characteristics of eye emergencies in Africa

Untreated ocular emergencies pose a significant threat to vision, with global estimates indicating that approximately 2.5 million people are affected by ocular injuries annually[13]. In affluent nations, ocular emergencies are generally addressed *via* robust healthcare systems featuring sufficient infrastructure, a high concentration of trained professionals, and readily available emergency services. In contrast, resource-constrained environments in Africa encounter substantial obstacles, such as insufficient healthcare facilities, restricted staff capacity, and socio-economic limitations. This significant differential highlights the necessity of resolving these gaps to enhance outcomes for ocular emergencies in

Africa.

Men and young individuals represent a significant proportion of those experiencing eye injuries, with one-fifth of adults reported to be affected[14]. In Nigeria, ocular injuries are a significant concern, particularly in Southeastern regions where there is a 3.5% incidence rate of ocular trauma, primarily involving closed globe injuries (76%) caused by blunt objects (57%), affecting mainly young people aged 10 to 19 years[15]. Children are often affected by eye emergencies, with a prevalence rate of 7.93% for eye injuries, commonly presenting as eyelid scars (5.34%), eyebrow scars (2.10%), and canthal scars (0.32%)[16].

Certain professions also face higher risks of eye emergencies due to occupational hazards. For instance, a study by Douglas and Koroye-Egbe[17] found a high prevalence of ocular injuries among welders, with 43.4% experiencing injuries such as burns, foreign bodies, and cuts. Similarly, among carpenters, work-related eye injuries and complaints were reported at rates of 30.7% and 32.5%, respectively, often due to inadequate use of protective eyewear, highlighting a significant occupational health issue[18].

In other parts of West Africa, such as Ghana, the occurrence of ocular injury among cocoa farmers was found to be 11.3 per 1000 worker-years, with lost work time at a rate of 37.3 per 1000 worker-years, indicating a substantial occupational risk for this group[19]. In Côte d'Ivoire, epidemiological data indicates that ocular burns, a form of ocular trauma, constitute 11% of ocular injury cases, with chemical agents being the primary cause in 54% of these incidents[20]. This highlights significant regional risks associated with chemical exposures.

In Southern Africa, particularly in Zimbabwe, there was a high prevalence of open-globe injuries (71.2%) in Zimbabwe with blunt trauma (90%) being the most significant cause[21]. In Northern Cape South Africa, it was reported that 3.2% of acute ocular trauma in which mechanical trauma (blunt, sharp and extraocular foreign body) accounted for over 90% that primarily affect young men (86.3%), with injuries mostly occurring at home (47.9%)[22].

In Ethiopia, ocular trauma predominantly affects males (71.0%) and children (62.87%), with nearly equal prevalence of open globe injuries (47.07%) and closed globe injuries (47.74%). Corneal tears are the most frequent type of injury, accounting for 39.33% of cases[23]. Similarly, in Uganda, eye emergencies are most common among males aged 10 to 20 years, with open globe injuries comprising 72% of cases. The most frequent specific incident is the presence of corneal foreign bodies, occurring in 42% of cases[24].

Other significant eye emergencies in the region include retinal detachment and retinal vessel occlusion. A 2023 prospective study across multiple centers in Nigeria found 237 cases of retinal detachment, with tractional retinal detachment accounting for 25.7% of these cases[25]. Additionally, retinal vascular occlusion was identified in 0.9% of patients in Nigeria[26]. The prevalence of other eye emergencies is listed in Table 2[27-34].

The epidemiology of ocular emergencies in Africa indicates a significant prevalence among males, younger populations, and individuals employed in hazardous professions. Principal findings reveal considerable disparities in prevalence rates among areas

Causes of eye emergencies in Africa

Accidents are a significant cause of eye emergencies in the African population, often occurring during social activities. Male children, in particular, are at higher risk of ocular trauma during play, with most incidents happening at home and commonly involving closed globe injuries from impacts with various objects such as canes, stones, broomsticks, wood, and fists[35]. A study in Cameroon identified fights as the most frequent cause of ocular trauma, accounting for nearly one-third of all cases, with punches being the predominant mechanism in 21.39% of these cases[36]. In Senegal, physical violence against women also contributes to ocular injuries[37]. Additionally, accidental events such as car crashes and gunshot wounds lead to ocular conditions requiring immediate medical attention[38].

Another form of environmental accident, though rare, is ocular Hymenoptera stings, which are considered an eye emergency due to the severe ocular complications they can cause when the eye is stung or comes into contact with venom from insects of the Hymenoptera order, such as bees, wasps, and ants[39]. Similarly, although rare, snake envenomation, a common public health concern in the savanna regions of West Africa, can cause immediate severe eye damage, inflammation, necrosis, and vision loss if not treated promptly, with potential systemic toxicity and infections necessitating emergency care[40].

Furthermore, ocular infections are a significant cause of eye emergencies, demanding immediate medical intervention to avert severe complications and potential vision loss. Bacteria are one of the causes of most ocular infections such as keratitis, corneal ulcer and endophthalmitis that are ocular emergencies[41]. Several significant factors have been identified as being associated with the prevalence of bacterial ocular infections. These factors include age, farming activities, a history of previous eye surgeries, and poor facial hygiene habits[42]. Similarly, open-globe injuries have been found to potentially result in endophthalmitis[44]. Corneal ulcers also often result from ocular trauma and lead to severe pain, potential corneal scarring, and risk of permanent vision loss if not treated promptly[45]. Moreover, fungal keratitis is one of the most challenging forms of infectious keratitis and is considered an eye emergency[46]. Like corneal ulcer, fungal keratitis can also be caused by ocular trauma[47]. A study by Fekih *et al*[48] reported that in 78.8% of fungal keratitis cases in Tunisia, fungal filaments were identified as the cause of infection, with Fusarium species being the most frequently isolated, found in 39.4% of the patients, especially those who had experienced ocular trauma. Similarly, parasitic keratitis, particularly caused by Acanthamoeba is another serious, though rare, corneal infection that can lead to emergency ocular injury, particularly among contact lens wearers in rural areas with low hygiene practices[49].

Occupational risks: Occupational hazards constitute a primary source of ocular emergencies, especially among welders, carpenters, and farmers, where insufficient utilization of protective eyewear intensifies the risk

Table 2 Summary of the prevalence and incidence rates of eye emergencies across various countries in Africa

Ref.	Type of eye emergency (prevalence)	Study period	Sample size	Data sources	Study design	Country in Africa
Kyei *et al*[21]	Open globe injuries (71.2%). Blunt trauma causing open-globe injuries (90%). Penetrating Intraocular, and perforating injuries causing open-globe injuries (10%)	January 2017 to December 2021 (4 years)	863	Patients records	Retrospective cross-sectional	Zimbabwe
Bert *et al*[19]	Ocular trauma (25.7%)	2 days	556	Questionnaire and ocular examination	Cross-sectional survey	Ghana
Douglas and Koroye-Egbe[17]	Ocular burns (42%). Foreign body injury to the eye (32%). Injuries caused by cuts to the eye (4%)	-	212	Ocular examination	Cross-sectional descriptive	Nigeria
Onyekwelu *et al* [18]	Superficial foreign body to the eye (88.6%). Chemical injury (8.6%). Nail injury to the eye (5.7%)	April 7, 2017 to May 15, 2017 (1 month)	114	Questionnaire, ocular examination and interview	Descriptive cross-sectional	Nigeria
Daoudi *et al*[27]	Preseptal cellulitis (85%). Orbital cellulitis (15%)	2008 to 2014 (6 years)	28	Patient records	Retrospective cohort	Morocco
Ajayi *et al*[28]	Neovascular glaucoma (0.05%)	January 2015 to December 2019 (4 years)	566	Patient records	Retrospective cohort	Nigeria
Koki *et al*[29]	Ocular trauma (16.92%)	January 2008 to December 2014 (6 years)	591	Patient records	Retrospective cohort	Cameroon
Kibret and Bitew [30]	Fungal keratitis (45.1%)	September 2014 to August 2015 (11 months)	153	Clinical examination	Cross-sectional	Ethiopia
Haingomalala *et al*[31]	Serious ocular trauma (5.75%)	January 1, 2009 to December 31, 2011 (2 years)	1267	Patient records	Retrospective cohort	Madagascar
Damtie and Siraj [32]	Eye injury (7.7%)	2019	300	Questionnaire	Cross-sectional	Ethiopia
Baba *et al*[33]	Penetrating ocular injury (65.7%)	January 2006 to November 2013 (7 years)	100	Patient records	Retrospective cohort	Tunisia
Bastola *et al*[34]	Ocular trauma (1.94%)	September to November 2018 (3 months)	280	Clinical examination	Prospective observational	Eritrea

Failure to use protective eyewear and inadequate ocular health and safety training are major contributors to the reported cases of ocular emergencies in Africa[50]. This issue is particularly pronounced among farmers and individuals involved in agricultural activities, who are frequently exposed to hazards such as trauma from farming equipment, contact with vegetable material, eye injuries from animal attacks, and spillage of sand into the eye[50]. While welders in sub-Saharan Africa generally exhibit good ocular protection practices, linked to on-the-job training, work experience, and a history of previous ocular injuries[51], mechanics often suffer from prevalent eye injuries due to not using protective devices, exposing them to hazards such as dust, engine oil, fire sparks, metal crusting, and battery acid[52]. A study in Ethiopia showed that the risk of occupational ocular injuries was seven times higher for workers who did not use protective eyewear and 2.22 times higher for those without health and safety training compared to those who received such training[53]. Furthermore, within work environments involving Africans, the most common ocular injuries included blunt trauma and incidents involving foreign bodies[54].

Obstacles to treatment

Healthcare infrastructure, human resources and policy: Government policies are essential in determining how resources are allocated to healthcare infrastructure, directly influencing the availability and quality of care. In African countries, healthcare is delivered across primary, secondary, and tertiary levels, each offering varying degrees of service. Primary eye care services are particularly important, as they enable trained mid-level personnel to manage common eye emergencies, thereby reducing the burden on secondary and tertiary hospitals, as noted by Patel *et al*[6]. However, these primary services are often underdeveloped in many African nations, leaving gaps in early intervention for eye conditions. The lack of critical supplies, such as surgical instruments and medications, further hampers the effectiveness of treatment, as documented by research[55]. In response to these challenges, the World Health Organization (WHO) has made strides by introducing a "Primary Eye Care Training Manual" aimed at equipping healthcare workers with the skills needed to handle common eye emergencies. Furthermore, collaborative efforts between the International Agency for the Prevention of Blindness and nursing colleges in East and South Africa have led to the expansion of primary eye care services across

12 additional countries, enhancing access to essential eye care at the primary health care level[56,57].

In addition, sub-Saharan Africa faces a shortage of eye health professionals, including ophthalmologists, optometrists, ophthalmic nurses, and allied personnel, with a particularly uneven distribution, leaving rural areas significantly underserved compared to urban areas[58]. Current challenges include inadequate working conditions (structural issues) and a lack of security prompting trained eye care professionals to work in the city or emigrate internationally[59].

In Africa, the density of ophthalmologists is alarmingly low, with roughly 2.5 ophthalmologists per million individuals, in contrast to over 60 per million in high-income nations. Emergency eye care services are equally limited and frequently concentrated in urban regions, hence restricting access for rural communities. This imbalance is reflected in other emerging areas, including South Asia and Latin America. However, the exact figures change due to variations in healthcare infrastructure and personnel capability.

Also, although many African countries have developed national eye health plans and fostered collaborations between non-governmental organizations (NGOs) and the private sector, there remains a significant disconnect at regional and district levels[60,61]. This lack of local engagement and representation results in insufficient healthcare infrastructure and human resources at the grassroots level, undermining the effectiveness of national strategies[62]. Without the active involvement of local entities in decision-making processes, there is a risk of misaligned priorities and inefficient use of resources, ultimately hampering efforts to improve eye health outcomes and address the needs of communities effectively [63].

Geographical and Socio-economic factors

People from poorer backgrounds rely on government services for eye care, and when these are not available, affordable and accessible this delays timely care, leading to long-term vision complications[64]. In remote regions, patients often face long travel times to reach adequately equipped facilities, leading to delays in treatment[65]. Moreover, high costs associated with medical consultations, treatments, and surgeries may prevent many individuals from seeking timely care for eye emergencies[66]. Even when services are subsidized, the cost of transportation to healthcare facilities can be prohibitive for low-income families[67].

Cultural and behavioral factors

Cultural and behavioral factors play a significant role in the low treatment rates of eye emergencies in Africa. One such factor is the additional barriers faced by women. In many African societies, women may lack financial autonomy, limiting their ability to pay for medical services[68]. Furthermore, cultural norms often require women to obtain permission from male family members before seeking medical care, which can delay or prevent access to timely treatment for eye emergencies[69]. These gender-specific barriers compound the overall challenges in accessing healthcare, contributing to the high prevalence of untreated eye conditions and resulting in greater risk of severe vision loss and other complications [70].

Consequences of untreated eye emergencies

Individual impact: A person's ability to carry out daily tasks is significantly impaired by vision loss, which results in a loss of independence[71]. Simple tasks such as reading, driving, and face recognition become challenging, drastically reducing the quality of life[72]. Untreated eye emergencies can have serious consequences on a person's quality of life that can be severe and permanent[73]. Some of these emergencies include acute glaucoma, retinal detachment, severe eye infections, and traumatic eye injuries[74].

In Sub-Saharan Africa, trauma-induced orbito-oculoplastics diseases, if untreated, significantly harm individuals' psycho-social well-being, economic stability, educational achievements, quality of life, and pose a substantial threat to vision[75]. In cases of traumatic cataract it can lead to partial or total loss of vision[76]. Additionally, ocular trauma can result in the development of superficial corneal scars, which significantly impair vision by causing visual blur, glare, and reduced visual acuity, thereby affecting daily activities and overall quality of life[77].

In addition, untreated eye emergencies may result in persistent pain and discomfort. Acute angle-closure glaucoma, an eye emergency, is linked to excruciating eye discomfort and nausea[78]. Moreover, untreated infections can cause chronic inflammation and possibly spread to other body regions[79]. Untreated ocular syphilis, particularly prevalent among HIV-positive individuals, can cause severe eye conditions such as uveitis, retinitis, optic neuritis, and panuveitis, leading to sudden vision loss if not promptly addressed[80]. Psychological discomfort is frequently present alongside these physical symptoms. Anxiety and sadness might result from the pain and discomfort as well as the fear of permanent vision loss[81,82].

Public health and economic impact: On a larger scale, untreated eye emergencies represent a significant public health concern[83]. Ocular injury is a significant public health concern, particularly in low-resource cultures, as it is a primary cause of ocular morbidity and unilateral vision impairment[84]. Vision impairment and blindness substantially burden healthcare systems, increasing the need for specialized care, prolonged treatment, and potential complications, increasing the demand for healthcare professionals and facilities[85,86]. Individuals with untreated eye conditions often require more frequent medical visits and interventions, which puts additional strain on already limited healthcare resources, especially in low-income and underserved areas[87].

Vision impairment reduces workforce participation and productivity, impacting national economies[88]. Individuals who suffer from vision loss may find themselves unemployed or unable to make as much money. Good vision is necessary for many occupations, thus persons who lose their vision might face career limitations[89]. In addition to impacting the individual, this loss of income strains their families financially and makes them more dependent on welfare assistance[90].

Interventions and programs rendered for the management of eye emergencies in Africa

In many regions of Africa, healthcare systems face numerous challenges, including limited resources, infrastructure, and access to specialized medical care. Access to treatment for eye emergencies varies significantly, with some ophthalmological services available, particularly in urban areas.

Over the past decade (2014–2024), trends in eye emergencies have shown significant strides in certain areas alongside persistent challenges. Awareness campaigns, particularly in urban centers, have improved due to enhanced public health initiatives and collaborations with NGOs, leading to greater recognition of eye health issues[91]. Urban areas have seen better access to basic care and advanced treatments, as highlighted by the establishment of primary eye care facilities and integration into primary health care systems in some African regions. However, rural areas still face barriers contributing to delayed treatment and higher rates of preventable blindness.

Surgical procedures, including corneal transplants and cataract surgeries, are essential in mitigating impaired vision resulting from ocular crises. Enhancing accessibility to these procedures, especially in rural regions, is crucial for alleviating the impact of vision impairment**.** Corneal transplantation is a surgical procedure that generally yields positive results. Unfortunately, in regions where subspecialty treatments like corneal transplants are not readily available, patients can develop serious complications which may result in blindness. A study in Malawi revealed that while many ocular trauma patients did not require surgical intervention, about one-third needed procedures such as corneal repair and cataract surgery[92]. Also, in Lubumbashi, Democratic Republic of Congo, the management of ocular foreign bodies included either straightforward removal or removal with suturing for deeper foreign bodies, which successfully preserved visual acuity in the majority of patients[93].

In Lagos, Nigeria, a study found that ocular trauma was the most common indication for destructive eye surgeries, with evisceration being frequently performed due to trauma or infection, highlighting the significant impact of ocular trauma and the urgent need for advanced surgical options to prevent severe outcomes[94]. For the management of ocular or peri-ocular trauma, the majority of patients requiring urgent surgery to the peri-ocular region, the treatment landscape in South Africa involves a high demand for immediate and multi-disciplinary surgical care, encompassing specialties like ophthalmology, maxillofacial, plastic, otorhinolaryngology, and neurosurgery[95,96]. This underscores the complexity of the injuries, highlights the extensive nature of trauma and the necessity for a coordinated and comprehensive approach to treatment (such as orthopedic operations, laparotomies, and vascular procedures).

Furthermore, due to the unavailability of some technological diagnostic testing in Africa, it may delay access to treatment of eye emergencies that can result in visual impairment or even blindness. A study by Miller *et al*[97] found that African ophthalmologists specialized in ophthalmic trauma adopt a more conservative approach to managing open globe injuries, using computed tomography (CT) imaging selectively for specific indications like suspected intraocular foreign bodies, unlike their counterparts in North and South America who routinely obtain CT imaging for all suspected cases. In Liberia, the lack of magnetic resonance imaging in a resource-limited setting necessitated the use of CT scans (coronal and sagittal cuts with variable window width) to detect intra-orbital wooden foreign bodies, facilitating their surgical removal to relieve pain, treat infection, and prevent complications[98]. This suggests that there may be fewer resources, such as availability of advanced imaging equipment, which can impact the thoroughness and immediacy of the diagnosis and treatment. This can potentially lead to differences in outcomes and quality of care for patients with eye emergencies in Africa. One such occurrence was reported in a case of retrobulbar hematoma reported in Uganda. Retrobulbar hematoma, an uncommon emergency that can cause blindness, requires prompt surgical intervention and may be delayed due to unavailability of radiological evaluation, particularly in rural areas where patients often present late and lack access to radiological services, thereby necessitating emergency surgical decompression of the orbit[99].

Certain ocular infections are considered eye emergencies and require immediate medical attention to prevent complications and preserve vision. In the case of ocular infections like endophthalmitis and bacterial keratitis, prompt and intensive treatment with topical antibiotics is crucial[100]. Despite this, according to some studies conducted in South Africa, there is still inadequate scientific evidence to support the effectiveness and safety of adjunctive steroid use (systemic or topical) compared to antibiotics alone in the treatment of these conditions[101,102]. Unlike fungal keratitis, which commonly occurs among people living in rural communities and often has worse outcomes than bacterial keratitis, its early management with drug-based medical treatments has achieved good outcomes in Egypt despite the shortage of medical resources[103].

Chemical injuries due to toxins and other harmful substances are significant causes of eye emergencies, requiring prompt and effective management to prevent long-term damage and vision loss. Venom ophthalmia, resulting from ocular contact with snake venom from various species of spitting cobras in Africa, is a severe ocular chemical injury that necessitates immediate medical attention, including thorough irrigation, analgesics, antibiotics, antihistamines, and anti-inflammatory topical drugs[104]. Similar to a reported case of venom ophthalmia in South Africa, the initial management involves extensive irrigation, followed by the application of topical cycloplegics and antibiotics to prevent secondary infection, without requiring topical steroids or antivenom (topical or intravenous)[105].

Optic neuritis, however, less commonly documented than other causes, constitutes a significant etiology of ocular emergencies in Africa. The prevalence may be undervalued due to restricted diagnostic skills. Timely diagnosis and intervention are essential to avert permanent vision impairment.

Challenges and barriers to effective interventions

Resource limitations: Despite a strong commitment to managing eye emergencies effectively, African countries frequently encounter significant challenges due to limited financial and technical resources[106,107]. These constraints are a major impediment to the provision of high-quality eye care and the timely treatment of eye emergencies. Figure 2 summarizes the challenges and most appropriate solutions.

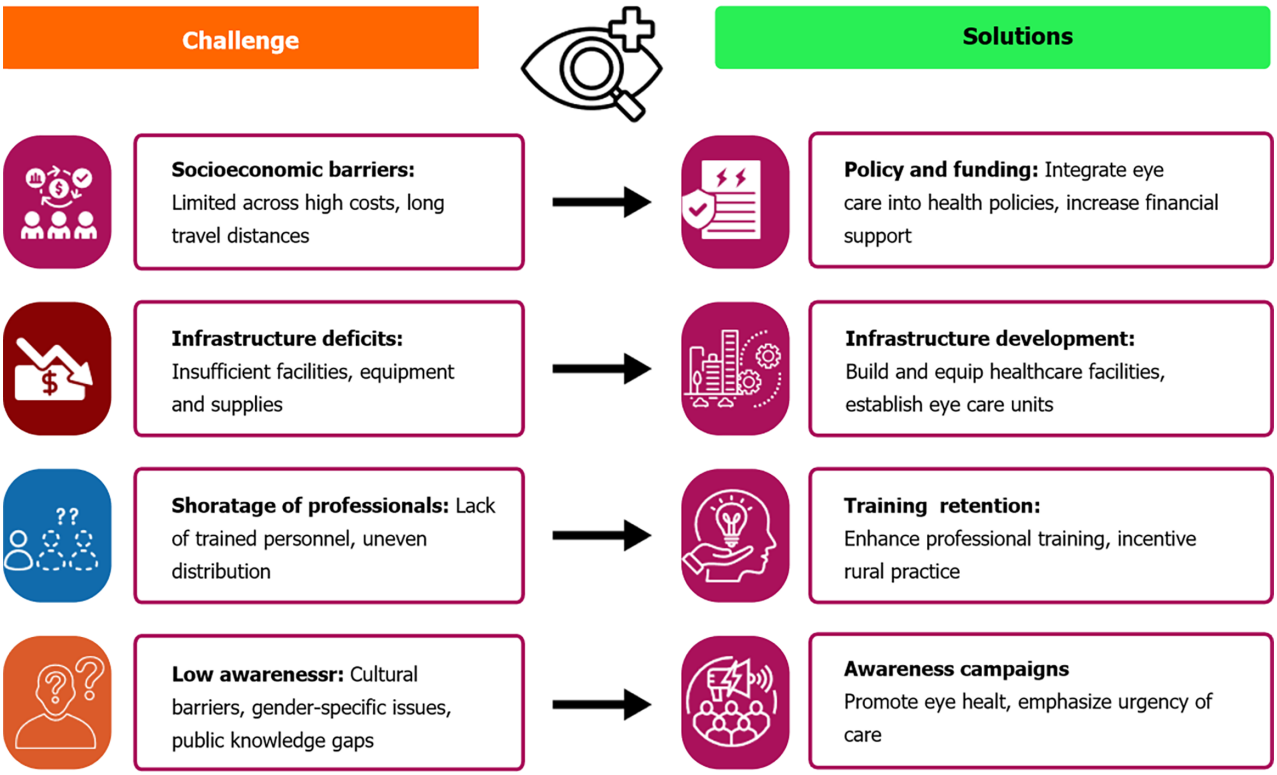


Figure 2 Challenges and solutions to eye emergencies in Africa.

The financial constraints faced by many African countries significantly impact their ability to manage eye emergencies. Health budgets are often limited and must be stretched to cover a wide range of medical needs, leaving insufficient funds for eye care[108,109]. Eye care services, including routine examinations, emergency treatments, and surgical interventions, require substantial investment in infrastructure, equipment, and supplies[110]. Many eye care facilities, especially in rural areas, are poorly equipped to handle complex eye emergencies[111]. Basic diagnostic equipment, surgical tools, and necessary medications are often in short supply. Additionally, the costs associated with consumables like intraocular lenses, medications, and diagnostic tools further strain limited budgets[112].

In addition to financial constraints, African countries face significant technical challenges in managing eye emergencies. There is a critical shortage of trained ophthalmologists, optometrists, and other eye care professionals[113]. For instance, in Nigeria, the availability and regional distribution of oculoplastic surgical services are inadequate, largely due to insufficient training[114]. In addition, the training and retention of these specialists are hampered by inadequate educational facilities and opportunities for professional development[115]. Consequently, the few available specialists are often concentrated in urban centers, leaving rural areas underserved[116].

Logistical challenges

Logistical challenges are a significant barrier to effective interventions for eye emergencies in Africa. One of the primary logistical issues is the scarcity of transportation funds, which can delay or completely hinder patients from accessing medical facilities for necessary interventions[117]. In addition, the distribution of eye care facilities is uneven, with most specialized centers situated in urban areas[118]. This urban-rural disparity contributes to the challenges of accessing eye care services in rural regions and people would often have to travel long distances to reach specialized eye care centers [119]. This can be particularly challenging for elderly patients, those with disabilities, and families with limited financial means.

Awareness and knowledge

Awareness and knowledge regarding eye health issues are essential for the timely treatment of eye emergencies. When individuals are unaware of their conditions, they are less likely to seek appropriate care[120]. Furthermore, the sources from which patients obtain information about their conditions can impact their response to emergency eye cases[121]. This is such that even when individuals actively seek information, they may encounter a lack of reliable sources for accurate details about their eye conditions. For instance, a study in South Africa found that limited education on eye issues and the necessity for regular eye screenings among patients in a diabetic outpatient clinic affected their diagnosis and treatment[122].

Awareness of the importance of adhering to treatment plans is crucial for achieving effective medical outcomes[123]. This is significantly true for eye emergency cases. This lack of awareness can lead to poor compliance[124], as patients who do not perceive the benefits of following recommended care may neglect or discontinue it altogether. Similarly, unawareness of available facilities for managing eye emergencies can cause patients to seek alternative, and often less

effective, treatments[125].

The demographic and geographic traits of the study population may restrict the applicability of the findings to wider populations." Although our results offer significant insights into low treatment rates of eye emergencies in Africa, one must exercise caution when generalizing these findings to communities with differing demographic, socioeconomic, or healthcare circumstances. This study's findings, especially within the African setting, highlight the necessity of customizing interventions to local conditions while evaluating their possible relevance to other worldwide locations.

Recommendations and future directions

Community engagement and education: Effective community engagement and education are essential components in addressing the low treatment rates of eye emergencies in Africa. Preventive strategies should prioritize promoting and teaching the use of eye protective gear, particularly for individuals involved in high-risk activities such as operating machinery, agriculture and participating in certain sports[126]. Educational campaigns can highlight the importance of protective eyewear in preventing eye injuries and related emergencies, thus reducing the incidence of such cases.

Moreover, there is a crucial need to emphasize the importance of seeking prompt medical attention in the event of eye emergencies[127]. Public health initiatives should focus on raising awareness about the symptoms and dangers of delayed treatment for eye injuries and infections[128]. By educating communities about the urgency of timely medical intervention, it is possible to mitigate the risk of severe complications and improve overall eye health outcomes[129].

To address the difficulties of ocular emergencies in Africa, it is imperative to implement people-centered eye care systems. These encompass community-oriented screening initiatives, the incorporation of ocular health into primary healthcare services, and the formation of referral networks to enhance access to specialized care. Furthermore, comprehensive monitoring frameworks, including real-time surveillance systems for ocular crises, can facilitate targeted treatments and resource distribution.

Healthcare infrastructure enhancement

To address the low treatment rate of eye emergencies in Africa, enhancing healthcare infrastructure is paramount. This involves substantial investment in the construction and refurbishment of healthcare facilities for emergency care services [130]. Upgrading existing hospitals and clinics with state-of-the-art ophthalmic equipment and ensuring a steady supply of essential medical materials are crucial steps[131]. Furthermore, establishing specialized eye care units within these facilities can significantly improve the accessibility and quality of emergency eye care[132]. Ensuring that these infrastructures are evenly distributed across rural and urban areas will help bridge the gap in eye care services and provide timely treatment to those in need[133].

Training and retention of eye care professionals

Training and retaining more eye care professionals, such as ophthalmologists, optometrists, and nurses, are also critical for improving the treatment rates of eye emergencies especially in rural areas[134,135]. Investment in professional development and continuing education programs can provide the necessary skills to manage eye emergencies effectively [136]. Furthermore, creating a robust referral system to ensure that patients can access specialized care promptly can significantly enhance the management of eye emergencies[137].

Strengthening local organizations

Partnerships with NGOs and the private sector can also play a crucial role in bridging resource gaps. Ensuring that local organizations are actively involved in planning and resource allocation can help bridge the gap between national strategies and grassroots needs[137]. The Gambia's model exemplifies how effective partnerships and health system frameworks can address vision care challenges and serves as a potential blueprint for other African countries seeking to enhance their vision care systems[138]. Therefore, not only empowering local organizations but also integrating them into the decision-making processes can ensure that resources are used effectively and that services are aligned with community needs[139].

Policy implementation and advocacy

The WHO's data indicating a reduction in vision loss in the African Region is a positive advancement. This trend illustrates the effects of enhanced healthcare delivery, heightened awareness, and focused efforts like the WHO's Vision 2020 campaign[57]. Yet, awareness of eye care is insufficient in numerous areas of the African Region, with misunderstandings regarding the urgency of ocular emergencies leading to procrastination in obtaining treatment. Augmenting financial support for eye care initiatives is warranted due to the considerable burden of avoidable visual impairment and its socioeconomic repercussions. Targeted expenditures in eye care do not exclude financing for other diseases but rather address a significant deficiency in healthcare services.

Comprehensive health policies and advocacy are vital for improving healthcare across Africa[140]. Developing standardized frameworks and guiding principles will help countries better incorporate ocular health into their healthcare systems[141]. Especially in the area of financial integration, these policies should be thorough, including budgets, plans, and guidelines to tackle the issue of inadequate eye care treatment in the region[142]. Integrating comprehensive eye care into Universal Health Coverage (UHC) is also vital for improving public health outcomes and ensuring equitable access to vision care[143]. However, many UHC programs currently lack comprehensive coverage for eye health care, resulting in significant out-of-pocket expenses and limited access to specialized care, such as in eye emergencies[144]. Innovative financing mechanisms, such as health insurance schemes and community health funds, can also be explored as has been done in Ghana and South Africa[145,146]. This approach will promote equitable access to eye care services and improve

overall health outcomes[137]. Therefore, the challenges and solutions to eye emergencies in Africa are summarized in Figure 2 below.

Limitations

The drawback of this study lies in the possibility of residual confounding, as not all variables that potentially affect the observed relationships may have been evaluated or considered. Lifestyle factors or genetic predispositions, which were outside the purview of this investigation, may have influenced the outcomes. Subsequent study should endeavor to incorporate a broader array of variables to mitigate this constraint.

Also, the dependence on self-reported and secondary data for certain variables creates the potential for recall and misclassification biases. Recall bias may arise if people wrongly recollect past events, whereas misclassification bias could stem from inaccuracies in data entry or categorization. These biases may compromise the validity of the findings. We therefore suggest utilizing objective metrics or primary data acquisition may alleviate these constraints.

While the longitudinal design of this study offers a more robust foundation for inferring temporal relationships than cross-sectional studies, it does not definitively establish causality. Unmeasured variables and other biases intrinsic to observational studies may continue to affect the outcomes. Consequently, prudence is necessary in interpreting the data as causal, and experimental research is required to validate these connections.

CONCLUSION

Summary of key points

This study discusses the critical and under-addressed issue of low treatment rates for eye emergencies in Africa, which significantly contributes to the high prevalence of preventable blindness across the continent. Eye emergencies require immediate medical attention to prevent severe outcomes, such as permanent vision loss. However, the ability to respond effectively to these emergencies is severely constrained by several factors unique to Africa. Socioeconomic barriers, particularly in rural regions, impede access to healthcare facilities equipped to handle ocular emergencies. The continent faces a significant shortage of trained eye care professionals, compounded by the uneven distribution of available specialists between the rural and urban regions, which further limits access to timely care. The epidemiology of eye emergencies in Africa reveals a worrying trend of increasing incidence, with young individuals, men, and those in high-risk occupations disproportionately affected. This study also notes that untreated eye emergencies can have devastating personal consequences, including a drastic reduction in quality of life, loss of independence, and long-term economic impacts. The challenges in managing these emergencies are exacerbated by inadequate healthcare infrastructure, lack of essential supplies, and insufficient training for healthcare workers at the primary care level. Our findings corroborate existing literature that underscores the significant deficiency of eye care experts in Africa and its effect on emergency care provision. This study emphasizes the necessity for policies that promote workforce development, encompassing the training and retention of eye care workers. The results underscore the necessity of incorporating eye care into primary healthcare systems to improve accessibility. These results ought to guide national health programs and international partnerships focused on mitigating preventable eyesight loss

Call to action

Effective initiatives to enhance eye emergency care in Africa encompass the establishment of mobile eye clinics for remote populations, the incorporation of eye care within existing primary healthcare systems, and the implementation of specialized training programs for primary healthcare practitioners. Public awareness efforts, customized to local contexts, should be executed to inform populations about the significance of prompt medical attention for ocular emergencies. These programs necessitate collaborative efforts among governments, NGOs, and international organizations to guarantee sustainability and efficacy. This study calls for urgent action to address the low treatment rates of eye emergencies in Africa. This includes enhancing healthcare infrastructure, especially in rural areas, and improving the training and retention of eye care professionals. It also advocates for stronger community engagement and education to raise awareness about the importance of eye care and the need for timely medical intervention. Furthermore, the study emphasizes the necessity of policy reforms and increased funding to ensure that eye health is integrated into broader public health strategies and that resources are allocated effectively to prevent avoidable vision loss across the continent.

FOOTNOTES

Author contributions: Bale BI and Zeppieri M conceptualized the study, developed the outline, oversaw the research process, drafted the initial manuscript, and provided the final approval of the article; Idogen OS, contributed to the discussion section, critically revised the manuscript, and gave final approval of the article; Musa M, Okechukwu CI, Femi DA, Manikavasagar P, Ojo OE, Lawal AA, and Adedeji SJ developed the methodology, conducted data collection and analysis, refined the manuscript, and provided final approval for publication; Okechukwu CI, Femi DA, Manikavasagar P, Ojo OE, Lawal AA, and Adedeji SJ, all provided assistance in writing various sections of the discussion; Akingbola A, assisted the editing process, focusing on both English and scientific editing, and played a key role in revising the manuscript, writing the conclusion, and approving the final version; Aborode AT, Okechukwu CI, Femi DA, Manikavasagar P, Ojo OE, Lawal AA, and Adedeji SJ contributed to the study's conception, assisted with the manuscript's revision, and was involved in writing the introduction, with final approval of the article for publication; Zeppieri M, facilitated the submission process, and coordinated revisions. All authors approved the final version of the manuscript for publication.

Conflict-of-interest statement: All authors declare no conflict of interest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Country of origin: Italy

ORCID number: Babatunde Ismail Bale 0000-0003-2028-3960; Marco Zeppieri 0000-0003-0999-5545; Mutali Musa 0000-0001-7486-8361.

S-Editor: Liu H
L-Editor: Webster JR
P-Editor: Yu HG

REFERENCES

1 **Jairath N**, Commiskey P, Kaplan A, Paulus YM. FLASH: A Novel Tool to Identify Vision-Threating Eye Emergencies. *Int J Ophthalmic Res* 2020; **6**: 336-343 [PMID: 34141947]
2 **Murray D**. Emergency management: angle-closure glaucoma. *Community Eye Health* 2018; **31**: 64 [PMID: 30487684]
3 **Oluyemi F**. Epidemiology of penetrating eye injury in ibadan: a 10-year hospital-based review. *Middle East Afr J Ophthalmol* 2011; **18**: 159-163 [PMID: 21731328 DOI: 10.4103/0974-9233.80706]
4 **Kayoma DH**, Oronsaye DA. Management of painful blind eye in Africa: A review. *J West Afr Coll Surg* 2024; **14**: 245-248 [PMID: 38988421 DOI: 10.4103/jwas.jwas_164_23]
5 **Babalola OE**. The peculiar challenges of blindness prevention in Nigeria: a review article. *Afr J Med Med Sci* 2011; **40**: 309-319 [PMID: 22783680]
6 **Patel D**, Gilbert S. Investment in human resources improves eye health for all. *Community Eye Health* 2018; **31**: 37-39 [PMID: 30220794]
7 **Jones I**. Delivering universal eye health coverage: a call for more and better eye health funding. *Int Health* 2022; **14**: i6-i8 [PMID: 35385866 DOI: 10.1093/inthealth/ihab073]
8 **Aborode AT**, Hasan MM, Jain S, Okereke M, Adedeji OJ, Karra-Aly A, Fasawe AS. Impact of poor disease surveillance system on COVID-19 response in africa: Time to rethink and rebuilt. *Clin Epidemiol Glob Health* 2021; **12**: 100841 [PMID: 34368503 DOI: 10.1016/j.cegh.2021.100841]
9 **Xulu-Kasaba Z**, Mashige K, Naidoo K. Knowledge, Attitudes and Practices of Eye Health among Public Sector Eye Health Workers in South Africa. *Int J Environ Res Public Health* 2021; **18** [PMID: 34886238 DOI: 10.3390/ijerph182312513]
10 **Page MJ**, Moher D, Brennan S, McKenzie JE. The PRISMATIC project: protocol for a research programme on novel methods to improve reporting and peer review of systematic reviews of health evidence. *Syst Rev* 2023; **12**: 196 [PMID: 37833767 DOI: 10.1186/s13643-023-02363-6]
11 **Parmar UPS**, Surico PL, Singh RB, Romano F, Salati C, Spadea L, Musa M, Gagliano C, Mori T, Zeppieri M. Artificial Intelligence (AI) for Early Diagnosis of Retinal Diseases. *Medicina (Kaunas)* 2024; **60** [PMID: 38674173 DOI: 10.3390/medicina60040527]
12 **Dotse-Gborgbortsi W**, Tatem AJ, Matthews Z, Alegana VA, Ofosu A, Wright JA. Quality of maternal healthcare and travel time influence birthing service utilisation in Ghanaian health facilities: a geographical analysis of routine health data. *BMJ Open* 2023; **13**: e066792 [PMID: 36657766 DOI: 10.1136/bmjopen-2022-066792]
13 **Opara UC**, Iheanacho PN, Li H, Petrucka P. Facilitating and limiting factors of cultural norms influencing use of maternal health services in primary health care facilities in Kogi State, Nigeria; a focused ethnographic research on Igala women. *BMC Pregnancy Childbirth* 2024; **24**: 555 [PMID: 39192210 DOI: 10.1186/s12884-024-06747-x]
14 **Greenspan JA**, Chebet JJ, Mpembeni R, Mosha I, Mpunga M, Winch PJ, Killewo J, Baqui AH, McMahon SA. Men's roles in care seeking for maternal and newborn health: a qualitative study applying the three delays model to male involvement in Morogoro Region, Tanzania. *BMC Pregnancy Childbirth* 2019; **19**: 293 [PMID: 31409278 DOI: 10.1186/s12884-019-2439-8]
15 **Jac-Okereke CC**, Jac-Okereke CA, Ezegwui IR, Umeh RE. Current pattern of ocular trauma as seen in tertiary institutions in south-eastern Nigeria. *BMC Ophthalmol* 2021; **21**: 420 [PMID: 34865621 DOI: 10.1186/s12886-021-02162-4]
16 **Okpala NE**, Umeh RE, Onwasigwe EN. Eye Injuries Among Primary School Children in Enugu, Nigeria: Rural vs Urban. *Ophthalmol Eye Dis* 2015; **7**: 13-19 [PMID: 26124686 DOI: 10.4137/OED.S18659]
17 **Douglas KE**, Koroye-Egbe A. Prevalence of Ocular Injuries among Welders in Yenagoa, Bayelsa State, Nigeria. *Nig Hosp Pract* 2018; **21**: 179044
18 **Onyekwelu OM**, Aribaba OT, Musa KO, Idowu OO, Salami MO, Odiaka YN. Ocular morbidity and utilisation of protective eyewear among carpenters in Mushin local government, Lagos, Nigeria. *Niger Postgrad Med J* 2019; **26**: 199-204 [PMID: 31621658 DOI: 10.4103/npmj.npmj_51_19]
19 **Bert BK**, Rekha H, Percy MK. Ocular injuries and eye care seeking patterns following injuries among cocoa farmers in Ghana. *Afr Health Sci* 2016; **16**: 255-265 [PMID: 27358640 DOI: 10.4314/ahs.v16i1.34]
20 **Ko Man CE**, Konan Manmi SMP, Agbohoun RP, Kouassi-Rebours C, Sowagnon YTC, N'da HC, Kouadio Kouao CR, N'guessan LC, Kouassi FX. [Ocular burns: epidemiological, clinical, therapeutic and evolutionary aspects at the Cocody University Hospital, Côte d'Ivoire]. *Med Trop Sante Int* 2024; **4** [PMID: 38846126 DOI: 10.48327/mtsi.v4i1.2024.486]

21 **Kyei S**, Kwarteng MA, Asare FA, Jemitara M, Mtuwa CN. Ocular trauma among patients attending a tertiary teaching hospital in Zimbabwe. *PLoS One* 2023; **18**: e0292392 [PMID: 37792744 DOI: 10.1371/journal.pone.0292392]

22 **Stuart KV**, Dold C, van der Westhuizen DP, de Vasconcelos S. The epidemiology of ocular trauma in the Northern Cape, South Africa. *African Vision and Eye Health* 2022; **81** [DOI: 10.4102/aveh.v81i1.710]

23 **Alem KD**, Arega DD, Weldegiorgis ST, Agaje BG, Tigneh EG. Profile of ocular trauma in patients presenting to the department of ophthalmology at Hawassa University: Retrospective study. *PLoS One* 2019; **14**: e0213893 [PMID: 30921358 DOI: 10.1371/journal.pone.0213893]

24 **Mudondo M**, Kitanda J. Prevalence, incidence and management practices of ophthalmic emergencies, A cross-sectional study in Jinja district referral hospitals. *SJ-Ophthalmology* 2024; **1**: 11 [DOI: 10.51168/hmveq993]

25 **Nkanga DG**, Agweye CT, Okonkwo ON, Ovienria W, Adenuga O, Akanbi T, Udoh ME, Oyekunle I, Ibanga AA; Collaborative Retina Research Network (CRRN), Study Report 1. Tractional Retinal Detachment: Prevalence and Causes in Nigerians. *J West Afr Coll Surg* 2023; **13**: 58-62 [PMID: 38449554 DOI: 10.4103/jwas.jwas_40_23]

26 **Okonkwo ON**, Adenuga OO, Nkanga D, Ovienria W, Ibanga A, Agweye CT, Oyekunle I, Akanbi T; Collaborative Retina Research Network Report II. Prevalence and systemic associations of retinal vascular occlusions in Sub-Saharan Africa. *Ann Afr Med* 2023; **22**: 279-285 [PMID: 37417014 DOI: 10.4103/aam.aam_44_22]

27 **Daoudi A**, Ajdakar S, Rada N, Draiss G, Hajji I, Bouskraoui M. [Orbital and periorbital cellulitis in children. Epidemiological, clinical, therapeutic aspects and course]. *J Fr Ophtalmol* 2016; **39**: 609-614 [PMID: 27587345 DOI: 10.1016/j.jfo.2016.05.008]

28 **Ajayi I**, Omotoye O, Ajite K, Abah E. Presentation, etiology and treatment outcome of neovascular glaucoma in Ekiti state, South Western Nigeria. *Afr Health Sci* 2021; **21**: 1266-1272 [PMID: 35222591 DOI: 10.4314/ahs.v21i3.37]

29 **Koki G**, Helles G, Bilong Y, Biangoup P, Aboubakar H, Epée E, Bella AL, Ebana Mvogo C. [Characteristics of post-traumatic blindness at the Yaoundé Army Training, Application and Referral Hospital]. *J Fr Ophtalmol* 2018; **41**: 540-545 [PMID: 29914763 DOI: 10.1016/j.jfo.2017.11.026]

30 **Kibret T**, Bitew A. Fungal keratitis in patients with corneal ulcer attending Minilik II Memorial Hospital, Addis Ababa, Ethiopia. *BMC Ophthalmol* 2016; **16**: 148 [PMID: 27576913 DOI: 10.1186/s12886-016-0330-1]

31 **Haingomalala Z**, Randrianarisoa HL, Volamarina RF, Raobela L, Bernardin P, Andriantsoa V. [Serious ocular trauma in children: Retrospective study of 74 cases]. *J Fr Ophtalmol* 2016; **39**: e81-e82 [PMID: 26826741 DOI: 10.1016/j.jfo.2015.07.014]

32 **Damtie D**, Siraj A. The Prevalence of Occupational Injuries and Associated Risk Factors among Workers in Bahir Dar Textile Share Company, Amhara Region, Northwest Ethiopia. *J Environ Public Health* 2020; **2020**: 2875297 [PMID: 32774391 DOI: 10.1155/2020/2875297]

33 **Baba A**, Zbiba W, Korbi M, Mrabet A. [Epidemiology of open globe injuries in the Tunisian region of Cap Bon: Retrospective study of 100 cases]. *J Fr Ophtalmol* 2015; **38**: 403-408 [PMID: 25913659 DOI: 10.1016/j.jfo.2014.11.011]

34 **Bastola P**, Ibrahim A, Michael D, Misghna H, Russom R, Polina Dahal, Ibrahim F. Patterns of ocular trauma and visual acuity outcomes in patients attending the National Eye Referral Hospital of Eritrea. *JCMC* 2021; **11**: 80-87 [DOI: 10.54530/jcmc.508]

35 **Ugalahi MO**, Adebusoye SO, Olusanya BA, Baiyeroju A. Ocular injuries in a paediatric population at a child eye health tertiary facility, Ibadan, Nigeria. *Injury* 2023; **54**: 917-923 [PMID: 36646534 DOI: 10.1016/j.injury.2023.01.014]

36 **Koki G**, Epée E, Omgbwa Eballe A, Ntyame E, Mbogos Nsoh C, Bella AL, Ebana Mvogo C. Ocular trauma in an urban Cameroonian setting: A study of 332 cases evaluated according to the Ocular Trauma Score. *J Fr Ophtalmol* 2015; **38**: 735-742 [PMID: 26386513 DOI: 10.1016/j.jfo.2015.03.009]

37 **Leye MMM**, Ndiaye P, Ndiaye D, Seck I, Faye A, Tal Dia A. [Epidemiological, clinical and forensic physical violence against women in Tambacounda (Senegal)]. *Rev Epidemiol Sante Publique* 2017; **65**: 189-196 [PMID: 28153645 DOI: 10.1016/j.respe.2016.10.061]

38 **Murray AD**. An Approach to Some Aspects of Strabismus from Ocular and Orbital Trauma. *Middle East Afr J Ophthalmol* 2015; **22**: 312-319 [PMID: 26180469 DOI: 10.4103/0974-9233.159732]

39 **Rouatbi A**, Chebbi A, Bouguila H. Hymenoptera insect stings: Ocular manifestations and management. *J Fr Ophtalmol* 2019; **42**: 37-43 [PMID: 30559016 DOI: 10.1016/j.jfo.2018.04.014]

40 **Katibi OS**, Adepoju FG, Olorunsola BO, Ernest SK, Monsudi KF. Blindness and scalp haematoma in a child following a snakebite. *Afr Health Sci* 2015; **15**: 1041-1044 [PMID: 26958000 DOI: 10.4314/ahs.v15i3.46]

41 **Watson S**, Cabrera-Aguas M, Khoo P. Common eye infections. *Aust Prescr* 2018; **41**: 67-72 [PMID: 29922000 DOI: 10.18773/austprescr.2018.016]

42 **Teweldemedhin M**, Saravanan M, Gebreyesus A, Gebreegziabiher D. Ocular bacterial infections at Quiha Ophthalmic Hospital, Northern Ethiopia: an evaluation according to the risk factors and the antimicrobial susceptibility of bacterial isolates. *BMC Infect Dis* 2017; **17**: 207 [PMID: 28292273 DOI: 10.1186/s12879-017-2304-1]

43 **Haile Z**, Mengist HM, Dilnessa T. Bacterial isolates, their antimicrobial susceptibility pattern, and associated factors of external ocular infections among patients attending eye clinic at Debre Markos Comprehensive Specialized Hospital, Northwest Ethiopia. *PLoS One* 2022; **17**: e0277230 [PMID: 36327266 DOI: 10.1371/journal.pone.0277230]

44 **Du Toit N**, Mustak S, Cook C. Randomised controlled trial of prophylactic antibiotic treatment for the prevention of endophthalmitis after open globe injury at Groote Schuur Hospital. *Br J Ophthalmol* 2017; **101**: 862-867 [PMID: 27793818 DOI: 10.1136/bjophthalmol-2016-309736]

45 **Abubakar UM**, Lawan A, Muhammad I. Clinical pattern and antibiotic sensitivity of bacterial corneal ulcers in Kano, Northern Nigeria. *Ann Afr Med* 2018; **17**: 151-155 [PMID: 30185685 DOI: 10.4103/aam.aam_59_17]

46 **Bisen AC**, Sanap SN, Agrawal S, Biswas A, Mishra A, Verma SK, Singh V, Bhatta RS. Etiopathology, Epidemiology, Diagnosis, and Treatment of Fungal Keratitis. *ACS Infect Dis* 2024; **10**: 2356-2380 [PMID: 38847789 DOI: 10.1021/acsinfecdis.4c00203]

47 **Zbiba W**, Baba A, Bouayed E, Abdessalem N, Daldoul A. A 5-year retrospective review of fungal keratitis in the region of Cap Bon. *J Fr Ophtalmol* 2016; **39**: 843-848 [PMID: 27839848 DOI: 10.1016/j.jfo.2016.09.006]

48 **Fekih O**, Haj Said O, Zgolli HM, Mabrouk S, Bakir K, Nacef L. Microbiologic profile of the mycosic abscess on a reference center in Tunisia. *Tunis Med* 2019; **97**: 644-649 [PMID: 31729735]

49 **Zbiba W**, Abdesslem NB. Acanthamoeba keratitis: An emerging disease among microbial keratitis in the Cap Bon region of Tunisia. *Exp Parasitol* 2018; **192**: 42-45 [PMID: 29859227 DOI: 10.1016/j.exppara.2018.05.005]

50 **Boadi-Kusi SB**, Hansraj R, Mashige KP, Ilechie AA. Factors associated with protective eyewear use among cocoa farmers in Ghana. *Inj Prev* 2016; **22**: 365-369 [PMID: 26319689 DOI: 10.1136/injuryprev-2014-041531]

51 **Atalay YA**, Gebeyehu NA, Gelaw KA. Systematic review and meta-analysis on prevalence, pattern, and factors associated with ocular protection practices among welders in sub-Saharan Africa. *Front Public Health* 2024; **12**: 1397578 [PMID: 38952737 DOI: 10.3389/fpubh.2024.1397578]

52 **Abu EK**, Boadi-Kusi SB, Opuni PQ, Kyei S, Owusu-Ansah A, Darko-Takyi C. Ocular Health and Safety Assessment among Mechanics of the Cape Coast Metropolis, Ghana. *J Ophthalmic Vis Res* 2016; **11**: 78-83 [PMID: 27195090 DOI: 10.4103/2008-322X.158890]

53 **Mengistu HG**, Alemu DS, Alimaw YA, Yibekal BT. Prevalence of Occupational Ocular Injury and Associated Factors Among Small-Scale Industry Workers in Gondar Town, Northwest Ethiopia, 2019. *Clin Optom (Auckl)* 2021; **13**: 167-174 [PMID: 34079416 DOI: 10.2147/OPTO.S290257]

54 **Schwartz R**, Goldstein M, Loewenstein A, Barak A. [Presentation of ocular problems among displaced persons from Sudan and Eritrea at the Tel Aviv medical center]. *Harefuah* 2017; **156**: 19-21 [PMID: 28530314]

55 **Hsia RY**, Mbembati NA, Macfarlane S, Kruk ME. Access to emergency and surgical care in sub-Saharan Africa: the infrastructure gap. *Health Policy Plan* 2012; **27**: 234-244 [PMID: 21441566 DOI: 10.1093/heapol/czr023]

56 **Nyabera K**. Strengthening Eye Health in ECSA: Integrating Primary Eye Care into Primary Health Care. 2024. Available from: https://www.iapb.org/blog/strengthening-eye-health-in-ecsa-integrating-primary-eye-care-into-primary-health-care

57 **World Health Organization**. Primary Eye Care training manual. Available from: https://www.afro.who.int/publications/primary-eye-care-training-manual

58 **LeBrun DG**, Chackungal S, Chao TE, Knowlton LM, Linden AF, Notrica MR, Solis CV, McQueen KA. Prioritizing essential surgery and safe anesthesia for the Post-2015 Development Agenda: operative capacities of 78 district hospitals in 7 low- and middle-income countries. *Surgery* 2014; **155**: 365-373 [PMID: 24439745 DOI: 10.1016/j.surg.2013.10.008]

59 **Sharma D**, Cotton M. Overcoming the barriers between resource constraints and healthcare quality. *Trop Doct* 2023; **53**: 341-343 [PMID: 37366617 DOI: 10.1177/00494755231183784]

60 **Sukati VN**, Moodley VR, Mashige KP. A situational analysis of eye care services in Swaziland. *J Public Health Afr* 2018; **9**: 892 [PMID: 30854181 DOI: 10.4081/jphia.2018.892]

61 **Jolley E**, Mafwiri M, Hunter J, Schmidt E. Integration of eye health into primary care services in Tanzania: a qualitative investigation of experiences in two districts. *BMC Health Serv Res* 2017; **17**: 823 [PMID: 29237503 DOI: 10.1186/s12913-017-2787-x]

62 **Azevedo MJ**. The State of Health System(s) in Africa: Challenges and Opportunities. In: Historical Perspectives on the State of Health and Health Systems in Africa, Volume II. African Histories and Modernities. African Histories and Modernities. New York, United States: Palgrave Macmillan Cham, 2017 [DOI: 10.1007/978-3-319-32564-4_1]

63 **Buthelezi LM**, van Staden D. Integrating eye health into policy: Evidence for health systems strengthening in KwaZulu-Natal. *Afr Vision Eye Health* 2020; **79** [DOI: 10.4102/aveh.v79i1.549]

64 **Ntsoane MD**, Oduntan OA. A review of factors influencing the utilization of eye care services. *Afr Vision Eye Health* 2010; **69** [DOI: 10.4102/aveh.v69i4.143]

65 **Arunga S**, Kintoki GM, Gichuhi S, Onyango J, Newton R, Leck A, Macleod D, Hu VH, Burton MJ. Delay Along the Care Seeking Journey of Patients with Microbial Keratitis in Uganda. *Ophthalmic Epidemiol* 2019; **26**: 311-320 [PMID: 31088316 DOI: 10.1080/09286586.2019.1616775]

66 **Alrasheed SH**. A systemic review of barriers to accessing paediatric eye care services in African countries. *Afr Health Sci* 2021; **21**: 1887-1897 [PMID: 35283961 DOI: 10.4314/ahs.v21i4.47]

67 **Gai MJ**, Reddy V, Xu V, Noori NH, Demory Beckler M. Illuminating Perspectives: Navigating Eye Care Access in Sub-Saharan Africa Through the Social Determinants of Health. *Cureus* 2024; **16**: e61841 [PMID: 38975490 DOI: 10.7759/cureus.61841]

68 **Idris IB**, Hamis AA, Bukhori ABM, Hoong DCC, Yusop H, Shaharuddin MA, Fauzi NAFA, Kandayah T. Women's autonomy in healthcare decision making: a systematic review. *BMC Womens Health* 2023; **23**: 643 [PMID: 38042837 DOI: 10.1186/s12905-023-02792-4]

69 **Rono MMed HK**, Macleod D, Bastawrous A, Wanjala E, Gichangi M, Burton MJ. Utilization of Secondary Eye Care Services in Western Kenya. *Int J Environ Res Public Health* 2019; **16** [PMID: 31547252 DOI: 10.3390/ijerph16183371]

70 **Omar F**, McCluskey K, Mashayo E, Yong AC, Mulewa D, Graham C, Price-Sanchez C, Othman O, Graham R, Chan VF. Needs and views on eye health and women's empowerment and theory of change map: implication on the development of a women-targeted eyecare programme for older Zanzibari craftswomen. *BMJ Open Ophthalmol* 2024; **9** [PMID: 38395461 DOI: 10.1136/bmjophth-2023-001292]

71 **Stevenson MR**, Hart PM, Montgomery AM, McCulloch DW, Chakravarthy U. Reduced vision in older adults with age related macular degeneration interferes with ability to care for self and impairs role as carer. *Br J Ophthalmol* 2004; **88**: 1125-1130 [PMID: 15317701 DOI: 10.1136/bjo.2003.032383]

72 **Jammal HM**, Khader Y, Kanaan SF, Al-Dwairi R, Mohidat H, Al-Omari R, Alqudah N, Saleh OA, Alshorman H, Al Bdour M. The Effect of Visual Impairment and Its Severity on Vision-Related and Health-Related Quality of Life in Jordan: A Comparative Cross-Sectional Study. *J Multidiscip Healthc* 2023; **16**: 3043-3056 [PMID: 37873536 DOI: 10.2147/JMDH.S431159]

73 **Finger RP**, Fenwick E, Marella M, Dirani M, Holz FG, Chiang PP, Lamoureux EL. The impact of vision impairment on vision-specific quality of life in Germany. *Invest Ophthalmol Vis Sci* 2011; **52**: 3613-3619 [PMID: 21357395 DOI: 10.1167/iovs.10-7127]

74 **Emrani E**, Haritoglou C. Ocular emergencies. *MMW Fortschr Med* 2019; **161**: 60-67 [PMID: 31631282 DOI: 10.1007/s15006-019-0030-x]

75 **Balogun BG**, Adekoya BJ, Balogun MM, Ehikhamen OA. Orbito-oculoplastic diseases in lagos: a 4-year prospective study. *Middle East Afr J Ophthalmol* 2014; **21**: 236-239 [PMID: 25100908 DOI: 10.4103/0974-9233.134678]

76 **Saa KB**, Maneh N, Vonor K, Banla M, Sounouvou I, Alaglo K, Balo KP. Management and functional results of traumatic cataract in the central region of Togo. *Pan Afr Med J* 2016; **25**: 107 [PMID: 28292070 DOI: 10.11604/pamj.2016.25.107.7422]

77 **Maghsoudlou P**, Sood G, Gurnani B, Akhondi H. Cornea Transplantation. 2024 Feb 24. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2025 Jan- [PMID: 30969512]

78 **Kahraman N**, Durmaz O, Durna MM. Mirtazapine-induced acute angle closure. *Indian J Ophthalmol* 2015; **63**: 539-540 [PMID: 26265648 DOI: 10.4103/0301-4738.162612]

79 **Teweldemedhin M**, Gebreyesus H, Atsbaha AH, Asgedom SW, Saravanan M. Bacterial profile of ocular infections: a systematic review. *BMC Ophthalmol* 2017; **17**: 212 [PMID: 29178851 DOI: 10.1186/s12886-017-0612-2]

80 **Mathew D**, Smit D. Clinical and laboratory characteristics of ocular syphilis and neurosyphilis among individuals with and without HIV infection. *Br J Ophthalmol* 2021; **105**: 70-74 [PMID: 32220852 DOI: 10.1136/bjophthalmol-2019-315699]

81 **Virgili G**, Parravano M, Petri D, Maurutto E, Menchini F, Lanzetta P, Varano M, Mariotti SP, Cherubini A, Lucenteforte E. The Association

between Vision Impairment and Depression: A Systematic Review of Population-Based Studies. *J Clin Med* 2022; **11** [PMID: 35566537 DOI: 10.3390/jcm11092412]

82 **Binder KW**, Wrzesińska MA, Kocur J. Anxiety in persons with visual impairment. *Psychiatr Pol* 2020; **54**: 279-288 [PMID: 32772060 DOI: 10.12740/PP/OnlineFirst/85408]

83 **Ezinne NE**, Shittu O, Ekemiri KK, Kwarteng MA, Tagoh S, Ogbonna G, Mashige KP. Visual Impairment and Blindness among Patients at Nigeria Army Eye Centre, Bonny Cantonment Lagos, Nigeria. *Healthcare (Basel)* 2022; **10** [PMID: 36421637 DOI: 10.3390/healthcare10112312]

84 **Abu EK**, Ocansey S, Gyamfi JA, Ntodie M, Morny EK. Epidemiology and visual outcomes of ocular injuries in a low resource country. *Afr Health Sci* 2020; **20**: 779-788 [PMID: 33163044 DOI: 10.4314/ahs.v20i2.31]

85 **Soboka JG**, Teshome TT, Salamanca O, Calise A. Evaluating eye health care services progress towards VISION 2020 goals in Gurage Zone, Ethiopia. *BMC Health Serv Res* 2022; **22**: 768 [PMID: 35689276 DOI: 10.1186/s12913-022-08144-6]

86 **Jeon B**, Koo H, Choi HK, Han E. The Impact of Visual Impairment on Healthcare Use among Four Medical Institution Types: A Nationwide Retrospective Cohort Study in Korea. *Yonsei Med J* 2023; **64**: 455-462 [PMID: 37365740 DOI: 10.3349/ymj.2022.0610]

87 **Hashemi H**, Yekta A, Jafarzadehpur E, Doostdar A, Ostadimoghaddam H, Khabazkhoob M. The prevalence of visual impairment and blindness in underserved rural areas: a crucial issue for future. *Eye (Lond)* 2017; **31**: 1221-1228 [PMID: 28430177 DOI: 10.1038/eye.2017.68]

88 **Frick KD**, Foster A. The magnitude and cost of global blindness: an increasing problem that can be alleviated. *Am J Ophthalmol* 2003; **135**: 471-476 [PMID: 12654362 DOI: 10.1016/s0002-9394(02)02110-4]

89 **Chakravarthy U**, Biundo E, Saka RO, Fasser C, Bourne R, Little JA. The Economic Impact of Blindness in Europe. *Ophthalmic Epidemiol* 2017; **24**: 239-247 [PMID: 28665742 DOI: 10.1080/09286586.2017.1281426]

90 **Varma R**, Wu J, Chong K, Azen SP, Hays RD; Los Angeles Latino Eye Study Group. Impact of severity and bilaterality of visual impairment on health-related quality of life. *Ophthalmology* 2006; **113**: 1846-1853 [PMID: 16889831 DOI: 10.1016/j.ophtha.2006.04.028]

91 **Kiva Z**, Wolvaardt JE. Assessing awareness and treatment knowledge of preventable blindness in rural and urban South African communities. *S Afr Med J* 2024; **114**: e1309 [PMID: 39041534 DOI: 10.7196/SAMJ.2024.v114i16b.1309]

92 **Zungu T**, Mdala S, Manda C, Twabi HS, Kayange P. Characteristics and visual outcome of ocular trauma patients at Queen Elizabeth Central Hospital in Malawi. *PLoS One* 2021; **16**: e0246155 [PMID: 33780448 DOI: 10.1371/journal.pone.0246155]

93 **Maloba V**, Nday F, Mwamba B, Tambwe H, Senda F, Ktanga L, Borasisi G. [Ocular foreign bodies: Epidemiological, clinical and therapeutic aspects in Lubumbashi: About 98 cases]. *J Fr Ophtalmol* 2020; **43**: 704-709 [PMID: 32636035 DOI: 10.1016/j.jfo.2020.03.003]

94 **Musa KO**, Aribaba OT, Onakoya AO, Rotimi-Samuel A, Akinsola FB. Indications for destructive eye surgeries at a Nigerian tertiary eye care centre: A ten-year review. *Niger Postgrad Med J* 2016; **23**: 12-16 [PMID: 27098943 DOI: 10.4103/1117-1936.180119]

95 **Dai D**, Meyer S, Kaltheuner LC, Plani F. On a knife-edge: clinical uncertainty with an extensive knife blade in situ in the craniofacial region. *BMJ Case Rep* 2018; **2018** [PMID: 30249732 DOI: 10.1136/bcr-2018-226054]

96 **Kruse C**, Bruce JL, Bekker W, Clarke DL. The management of ocular and peri-ocular trauma needs to be co-ordinated according to ATLS principles and requires multi-disciplinary collaboration. *Injury* 2021; **52**: 2606-2610 [PMID: 33593527 DOI: 10.1016/j.injury.2021.02.010]

97 **Miller SC**, Fliotsos MJ, Justin GA, Yonekawa Y, Chen A, Hoskin AK, Blanch RJ, Cavuoto K, Meeralakshmi P, Low R, Gardiner M, Liu TYA, Agrawal R, Woreta FA; International Globe and Adnexal Trauma Epidemiology Study (IGATES). Global Current Practice Patterns for the Management of Open Globe Injuries. *Am J Ophthalmol* 2022; **234**: 259-273 [PMID: 34416182 DOI: 10.1016/j.ajo.2021.08.003]

98 **Pehere NK**, Dokie UF, Bornguoi GT, Gofer K, Ganguly Kapoor A, Naik M. Management of Intra-orbital Wooden Foreign Bodies at a Resource-limited Setting in Sub-Saharan Africa. *J West Afr Coll Surg* 2020; **10**: 36-41 [PMID: 35558572 DOI: 10.4103/jwas.jwas_16_21]

99 **Mugarura R**, Katushabe M, Kisakye NI, Jonathan NA. Emergency surgical decompression of the orbit may rescue impaired vision even in a patient presenting late with a retrobulbar haematoma: A case report from rural southwestern Uganda. *Trop Doct* 2024; **54**: 287-289 [PMID: 38646713 DOI: 10.1177/00494755241244546]

100 **Bale BI**, Elebesunu EE, Manikavasagar P, Agwuna FO, Ogunkola IO, Sow AU, Lucero-Prisno DE 3rd. Antibiotic resistance in ocular bacterial infections: an integrative review of ophthalmic chloramphenicol. *Trop Med Health* 2023; **51**: 15 [PMID: 36895063 DOI: 10.1186/s41182-023-00496-x]

101 **Emami S**, Kitayama K, Coleman AL. Adjunctive steroid therapy versus antibiotics alone for acute endophthalmitis after intraocular procedure. *Cochrane Database Syst Rev* 2022; **6**: CD012131 [PMID: 35665485 DOI: 10.1002/14651858.CD012131.pub3]

102 **Herretes S**, Wang X, Reyes JM. Topical corticosteroids as adjunctive therapy for bacterial keratitis. *Cochrane Database Syst Rev* 2014; **10**: CD005430 [PMID: 25321340 DOI: 10.1002/14651858.CD005430.pub3]

103 **Sadik N**, Elzeiny SM, Ali YE, Sobeih D. Fungal Keratitis in the Egyptian Delta: Epidemiology, Risk Factors, and Microbiological Diagnosis. *Ophthalmic Epidemiol* 2022; **29**: 198-205 [PMID: 33853473 DOI: 10.1080/09286586.2021.1914667]

104 **Chang KC**, Huang YK, Chen YW, Chen MH, Tu AT, Chen YC. Venom Ophthalmia and Ocular Complications Caused by Snake Venom. *Toxins (Basel)* 2020; **12** [PMID: 32911777 DOI: 10.3390/toxins12090576]

105 **Hoffman J**. Venom ophthalmia from Naja mossambica in KwaZulu Natal, South Africa: a reminder to all that for ocular chemical injury, dilution is the solution. *Trop Doct* 2015; **45**: 250-251 [PMID: 25614535 DOI: 10.1177/0049475514564695]

106 Oyediji FJ, Alfin RJ, Bupwatda NG. Evaluation of Impact of Ophthalmology Rotation on Family Medicine Practice in Northern Nigeria: A Multicenter Study. *West Afr J Med* 2023; **40**: S28-S29 [PMID: 37978914]

107 **Gebrezgabiher G**, Mekonnen Z, Yewhalaw D, Hailu A. Reaching the last mile: main challenges relating to and recommendations to accelerate onchocerciasis elimination in Africa. *Infect Dis Poverty* 2019; **8**: 60 [PMID: 31269966 DOI: 10.1186/s40249-019-0567-z]

108 **Ogundo CLA**, Bascaran C, Habtamu E, Buchan J, Mwangi N. Eye Health Integration in Southern and Eastern Africa: A Scoping Review. *Middle East Afr J Ophthalmol* 2023; **30**: 44-50 [PMID: 38435102 DOI: 10.4103/meajo.meajo_320_21]

109 **Aghaji AE**, Gilbert C, Ihebuzor N, Faal H. Strengths, challenges and opportunities of implementing primary eye care in Nigeria. *BMJ Glob Health* 2018; **3**: e000846 [PMID: 30613423 DOI: 10.1136/bmjgh-2018-000846]

110 **du Toit R**, Faal HB, Etya'ale D, Wiafe B, Mason I, Graham R, Bush S, Mathenge W, Courtright P. Evidence for integrating eye health into primary health care in Africa: a health systems strengthening approach. *BMC Health Serv Res* 2013; **13**: 102 [PMID: 23506686 DOI: 10.1186/1472-6963-13-102]

111 **Atipo-Tsiba PW**. Traumatic ocular emergencies: the difficulties associated with their care at the university hospital of Brazzaville. *East Afr Med J* 2015; **92**: 90-92

112 **Marques AP**, Ramke J, Cairns J, Butt T, Zhang JH, Jones I, Jovic M, Nandakumar A, Faal H, Taylor H, Bastawrous A, Braithwaite T,

Resnikoff S, Khaw PT, Bourne R, Gordon I, Frick K, Burton MJ. The economics of vision impairment and its leading causes: A systematic review. *EClinicalMedicine* 2022; **46**: 101354 [PMID: 35340626 DOI: 10.1016/j.eclinm.2022.101354]

113 **Gilbert SS**, Courtright P, Ramasamy D. Expanding and Optimizing Human Resources for Eye Care. In: Khanna, R., Rao, G., Marmamula, S. (eds) Innovative Approaches in the Delivery of Primary and Secondary Eye Care. Essentials in Ophthalmology. Berlin, Germany: Springer, 2019 [DOI: 10.1007/978-3-319-98014-0_4]

114 **Idowu OO**, Oldenburg CE, Vagefi MR. Oculoplastic surgical services in Nigeria: status and challenges. *Int Ophthalmol* 2020; **40**: 109-116 [PMID: 31440936 DOI: 10.1007/s10792-019-01163-z]

115 **Oduntan OA**, Mashige KP, Kio FE, Boadi-Kusi SB. Optometric education in Africa: historical perspectives and challenges. *Optom Vis Sci* 2014; **91**: 359-365 [PMID: 24374636 DOI: 10.1097/OPX.0000000000000153]

116 **Alrasheed SH**, Mohamed ZD, Alluwimi MS. Childhood visual impairment causes and barriers to accessing eye care: A suggested approach for Africa. *Afr J Prim Health Care Fam Med* 2024; **16**: e1-e7 [PMID: 39099279 DOI: 10.4102/phcfm.v16i1.4556]

117 **Schulze Schwering M**, Gandiwa M, Msukwa G, Spitzer M, Kalua K, Molyneux EM. [Retinoblastoma in Malawi: why are admissions too late?]. *Ophthalmologe* 2014; **111**: 1189-1193 [PMID: 25278348 DOI: 10.1007/s00347-014-3117-x]

118 **Eze BI**, Maduka-Okafor FC. An assessment of the eye care workforce in Enugu State, south-eastern Nigeria. *Hum Resour Health* 2009; **7**: 38 [PMID: 19435503 DOI: 10.1186/1478-4491-7-38]

119 **Sengo DB**, Marraca NA, Muaprato AM, García-Sanjuan S, Caballero P, López-Izquierdo I. Barriers to Accessing Eye Health Services in Suburban Communities in Nampula, Mozambique. *Int J Environ Res Public Health* 2022; **19** [PMID: 35409600 DOI: 10.3390/ijerph19073916]

120 **Gilmour-White JA**, Shah P, Cross V, Makupa W, Philippin H. Glaucoma awareness and access to healthcare: perceptions among glaucoma patients in Tanzania. *Postgrad Med J* 2015; **91**: 373-378 [PMID: 26069217 DOI: 10.1136/postgradmedj-2014-133094]

121 **Kyei S**, Tettey B, Asiedu K, Awuah A. Knowledge and awareness of ocular allergy among undergraduate students of public universities in Ghana. *BMC Ophthalmol* 2016; **16**: 190 [PMID: 27793114 DOI: 10.1186/s12886-016-0366-2]

122 **Cairncross JP**, Steinberg WJ, Labuschagne MJ. Prevalence of eye pathology in a group of diabetic patients at National District Hospital Outpatient Department in Bloemfontein, South Africa. *Afr J Prim Health Care Fam Med* 2017; **9**: e1-e7 [PMID: 29041796 DOI: 10.4102/phcfm.v9i1.1440]

123 **Jimmy B**, Jose J. Patient medication adherence: measures in daily practice. *Oman Med J* 2011; **26**: 155-159 [PMID: 22043406 DOI: 10.5001/omj.2011.38]

124 **Abdull MM**, Chandler C, Gilbert C. Glaucoma, "the silent thief of sight": patients' perspectives and health seeking behaviour in Bauchi, northern Nigeria. *BMC Ophthalmol* 2016; **16**: 44 [PMID: 27102524 DOI: 10.1186/s12886-016-0220-6]

125 **Sukati VN**, Moodley VR, Mashige KP. Knowledge and practices of parents about child eye health care in the public sector in Swaziland. *Afr J Prim Health Care Fam Med* 2018; **10**: e1-e13 [PMID: 30456970 DOI: 10.4102/phcfm.v10i1.1808]

126 **Patel D**. Preventing eye injuries. *Community Eye Health* 2015; **28**: 51 [PMID: 26989313]

127 **Muth CC**. Eye Emergencies. *JAMA* 2017; **318**: 676 [PMID: 28810025 DOI: 10.1001/jama.2017.9899]

128 **Arunga S**, Asiimwe A, Apio Olet E, Kagoro-Rugunda G, Ayebazibwe B, Onyango J, Newton R, Leck A, Macleod D, Hu VH, Seeley J, Burton MJ. Traditional eye medicine use in microbial keratitis in Uganda: a mixed methods study. *Wellcome Open Res* 2019; **4**: 89 [PMID: 31633056 DOI: 10.12688/wellcomeopenres.15259.2]

129 **Capó H**, Edmond JC, Alabiad CR, Ross AG, Williams BK, Briceño CA. The Importance of Health Literacy in Addressing Eye Health and Eye Care Disparities. *Ophthalmology* 2022; **129**: e137-e145 [PMID: 36058736 DOI: 10.1016/j.ophtha.2022.06.034]

130 **Ouma PO**, Maina J, Thuranira PN, Macharia PM, Alegana VA, English M, Okiro EA, Snow RW. Access to emergency hospital care provided by the public sector in sub-Saharan Africa in 2015: a geocoded inventory and spatial analysis. *Lancet Glob Health* 2018; **6**: e342-e350 [PMID: 29396220 DOI: 10.1016/S2214-109X(17)30488-6]

131 **Aghaji A**, Burchett HED, Oguego N, Hameed S, Gilbert C. Primary health care facility readiness to implement primary eye care in Nigeria: equipment, infrastructure, service delivery and health management information systems. *BMC Health Serv Res* 2021; **21**: 1360 [PMID: 34930271 DOI: 10.1186/s12913-021-07359-3]

132 **Mohammed AK**, Munsamy AJ. Utilisation pattern of ophthalmic services in Ashanti Region, Ghana. *Afr J Prim Health Care Fam Med* 2024; **16**: e1-e8 [PMID: 38426774 DOI: 10.4102/phcfm.v16i1.4326]

133 **Senyonjo L**, Lindfield R, Mahmoud A, Kimani K, Sanda S, Schmidt E. Ocular morbidity and health seeking behaviour in Kwara state, Nigeria: implications for delivery of eye care services. *PLoS One* 2014; **9**: e104128 [PMID: 25165984 DOI: 10.1371/journal.pone.0104128]

134 **Oduntan OO**, Mashige KP, Hansraj R, Ovenseri-ogbomo G. Strategies for reducing visual impairment and blindness in rural and remote areas of Africa. *Afr Vision Eye Health* 2015; **74** [DOI: 10.4102/aveh.v74i1.25]

135 **Monsudi K**, Ademola-popoola D, Ayodapo A. Ophthalmology in Nigeria: Challenges and Success. *Niger J Ophthalmol* 2019; **27**: 100 [DOI: 10.4103/njo.njo_14_18]

136 Senjam SS. Public-Private Partnership for Eye Health Care Delivery in Developing Nations. *BAOJ Ophthalmol* 2017; **1**: 2

137 Making Eye Health a Population Health Imperative: Vision for Tomorrow. Washington (DC): National Academies Press (US); 2016-Sep-15 [PMID: 27656731]

138 **Bowser D**, Landey N, Njie MA, Dabideen R, Gianfagna M. Health system strengthening for vision care in The Gambia. *Rural Remote Health* 2021; **21**: 6245 [PMID: 33822638 DOI: 10.22605/RRH6245]

139 **MacFarlane AE**. Optimising individual and community involvement in health decision-making in general practice consultations and primary care settings: A way forward. *Eur J Gen Pract* 2020; **26**: 196-201 [PMID: 33337921 DOI: 10.1080/13814788.2020.1861245]

140 **Lane J**, Andrews G, Orange E, Brezak A, Tanna G, Lebese L, Carter T, Naidoo E, Levendal E, Katz A. Strengthening health policy development and management systems in low- and middle- income countries: South Africa's approach. *Health Policy Open* 2020; **1**: 100010 [PMID: 37383321 DOI: 10.1016/j.hpopen.2020.100010]

141 **Yu M**, Keel S, Mariotti S, Mills JA, Müller A. Development of the WHO eye care competency framework. *Hum Resour Health* 2023; **21**: 46 [PMID: 37337207 DOI: 10.1186/s12960-023-00834-4]

142 **Osborne SR**, Alston LV, Bolton KA, Whelan J, Reeve E, Wong Shee A, Browne J, Walker T, Versace VL, Allender S, Nichols M, Backholer K, Goodwin N, Lewis S, Dalton H, Prael G, Curtin M, Brooks R, Verdon S, Crockett J, Hodgins G, Walsh S, Lyle DM, Thompson SC, Browne LJ, Knight S, Pit SW, Jones M, Gillam MH, Leach MJ, Gonzalez-Chica DA, Muyambi K, Eshetie T, Tran K, May E, Lieschke G, Parker V, Smith A, Hayes C, Dunlop AJ, Rajappa H, White R, Oakley P, Holliday S. Beyond the black stump: rapid reviews of health research issues

affecting regional, rural and remote Australia. *Med J Aust* 2020; **213** Suppl 11: S3-S32.e1 [PMID: 33314144 DOI: 10.5694/mja2.50881]

143 **Stenberg K**, Hanssen O, Bertram M, Brindley C, Meshreky A, Barkley S, Tan-Torres Edejer T. Guide posts for investment in primary health care and projected resource needs in 67 low-income and middle-income countries: a modelling study. *Lancet Glob Health* 2019; **7**: e1500-e1510 [PMID: 31564629 DOI: 10.1016/S2214-109X(19)30416-4]

144 **Nelissen HE**, Brals D, Ameen HA, van der List M, Kramer B, Akande TM, Janssens W, Van't Hoog AH. The prominent role of informal medicine vendors despite health insurance: a weekly diaries study in rural Nigeria. *Health Policy Plan* 2020; **35**: 354-363 [PMID: 31965167 DOI: 10.1093/heapol/czz172]

145 **Blanchet K**, Gilbert C, de Savigny D. Rethinking eye health systems to achieve universal coverage: the role of research. *Br J Ophthalmol* 2014; **98**: 1325-1328 [PMID: 24990874 DOI: 10.1136/bjophthalmol-2013-303905]

146 **Sithole HL**. An overview of the National Health Insurance and its possible impact on eye healthcare services in South Africa. *Afr Vision Eye Health* 2015; **74** [DOI: 10.4102/aveh.v74i1.18]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 97415

DOI: 10.5662/wjm.v15.i3.97415

ISSN 2222-0682 (online)

META-ANALYSIS

Comparison of standard *vs* auxiliary (contrast or elastography) endoscopic ultrasound-guided fine needle aspiration/biopsy in solid pancreatic lesions: A meta-analysis

Mitali Madhumita Rath, Prajna Anirvan, Jijo Varghese, Tara Prasad Tripathy, Ranjan K Patel, Manas Kumar Panigrahi, Suprabhat Giri

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade D
Novelty: Grade C
Creativity or Innovation: Grade C
Scientific Significance: Grade C

P-Reviewer: Niu C

Received: May 29, 2024
Revised: November 3, 2024
Accepted: December 2, 2024
Published online: September 20, 2025
Processing time: 280 Days and 17.4 Hours

Mitali Madhumita Rath, Department of Pathology, IMS and SUM Hospital Campus-2, Bhubaneshwar 754001, Odisha, India

Prajna Anirvan, Department of Gastroenterology, Kalinga Gastroenterology Foundation, Cuttack 753001, Odisha, India

Jijo Varghese, Department of Gastroenterology, NS Memorial Institute of Medical Sciences, Kollam 691020, Kerala, India

Tara Prasad Tripathy, Ranjan K Patel, Department of Radiodiagnosis, All India Institute of Medical Sciences, Bhubaneshwar 751019, Odisha, India

Manas Kumar Panigrahi, Department of Gastroenterology, All India Institute of Medical Sciences, Bhubaneshwar 751019, Odisha, India

Suprabhat Giri, Department of Gastroenterology and Hepatology, Kalinga Institute of Medical Sciences, Bhubaneswar 751024, Odisha, India

Co-first authors: Mitali Madhumita Rath and Prajna Anirvan.

Corresponding author: Suprabhat Giri, DM, Assistant Professor, Department of Gastroenterology and Hepatology, Kalinga Institute of Medical Sciences, Kushabhadra Campus, 5 KIIT Rd, Patia, Bhubaneswar 751024, Odisha, India. supg19167@gmail.com

Abstract

BACKGROUND

Endoscopic ultrasound-guided fine-needle aspiration/biopsy (EUS-FNA/B) is the most common modality for tissue acquisition from pancreatic masses. Despite high specificity, sensitivity remains less than 90%. Auxiliary techniques like elastography and contrast-enhanced EUS may guide tissue acquisition from viable tumor tissue and improve the diagnostic outcomes theoretically. However, data regarding the same have shown conflicting results.

AIM

To compare the diagnostic outcomes of auxiliary-EUS-FNA/B to standard EUS-FNA/B for pancreatic lesions.

METHODS
The electronic databases of MEDLINE, EMBASE, and Scopus were searched from inception to February 2024 for all relevant studies comparing diagnostic outcomes of auxiliary-EUS-FNA/B to standard EUS-FNA/B for pancreatic lesions. A bivariate hierarchical model was used to perform the meta-analysis.

RESULTS
A total of 10 studies were identified. The pooled sensitivity, specificity, and area under the receiver-operated curve (AUROC) for standard EUS-FNA/B were 0.82 (95%CI: 0.79-0.85), 1.00 (95%CI: 0.96-1.00), and 0.97 (95%CI: 0.95-0.98), respectively. The pooled sensitivity, specificity, and AUROC for EUS-FNA/B with auxiliary techniques were 0.86 (95%CI: 0.83-0.89), 1.00 (95%CI: 0.94-1.00), and 0.96 (95%CI: 0.94-0.98), respectively. Comparing the two diagnostic modalities, sensitivity [Risk ratio (RR): 1.04, 95%CI: 0.99-1.09], specificity (RR: 1.00, 95%CI: 0.99-1.01), and diagnostic accuracy (RR: 1.03, 95%CI: 0.98-1.09) were comparable.

CONCLUSION
Analysis of the currently available literature did not show any additional advantage of EUS-FNA/B with auxiliary techniques for pancreatic solid lesions over standard EUS-FNA/B. Further randomized studies are required to demonstrate the benefit of auxiliary techniques before they can be recommended for routine practice.

Key Words: Endoscopic ultrasonography; Fine-needle aspiration Biopsies; Pancreatic ductal carcinomas; Contrast media; Elastography

Core Tip: Auxiliary techniques like elastography and contrast enhancement are being used during endoscopic ultrasound-guided fine-needle aspiration/biopsy (EUS-FNA/B) to guide tissue acquisition from viable tumor tissue and improve diagnostic outcomes. However, the present meta-analysis reported comparable sensitivity, specificity, and diagnostic accuracy between EUS-FNA/B with and without auxiliary techniques. Subgroup analysis of studies exclusively using contrast-enhanced harmonic-EUS-FNA/B, randomized studies, and studies reporting diagnostic outcomes after the first pass reported no difference between both modalities. Thus, using EUS-FNA/B with auxiliary techniques for pancreatic solid lesions does not provide any additional advantage over standard EUS-FNA/B.

Citation: Rath MM, Anirvan P, Varghese J, Tripathy TP, Patel RK, Panigrahi MK, Giri S. Comparison of standard *vs* auxiliary (contrast or elastography) endoscopic ultrasound-guided fine needle aspiration/biopsy in solid pancreatic lesions: A meta-analysis. *World J Methodol* 2025; 15(3): 97415
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/97415.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.97415

INTRODUCTION

The assessment of pancreatic head lesions has been greatly aided by the development of endoscopic ultrasound-guided fine-needle aspiration/biopsy (EUS-FNA/B). EUS-FNA/B has a sensitivity, specificity, and accuracy of 84%–92%, 96%–98%, and 86%–91%, respectively, in diagnosing malignant pancreatic masses[1]. The sensitivity depends upon several factors – the type of needle used, use of suction, stylet, use of rapid on-site evaluation (ROSE) or macroscopic on-site evaluation, and indwelling biliary stent[2-6]. The median survival in patients of pancreatic cancer who don't receive a diagnosis on time and present with distant metastases is 8–12 months and 3–6 months, respectively[7]. On the flip side, the diagnosis of mass-forming chronic pancreatitis as pancreatic cancer, often to the tune of 5% to 35%[8], leads to unnecessary psychological trauma and needless surgeries. Auxiliary techniques that have been developed to increase the sensitivity and accuracy of EUS-FNA/B include real-time elastography (RTE)-EUS-FNA/B and contrast-enhanced harmonic (CEH)-EUS-FNA/B[9].

CEH-EUS-FNA/B uses a contrast agent with harmonic imaging to characterize tissue blood flow within the pancreatic masses and differentiate benign from malignant masses. The technique relies on generating a time-intensity curve (TIC) based on the temporal change in intensity of echo enhancement. CEH-EUS-FNA/B can differentiate benign from malignant pancreatic lesions[10]. For malignant lesions, the TIC is flat and low compared to benign lesions since the desmoplastic nature of the lesion limits blood supply[10]. The pooled sensitivity and specificity of CEH-EUS-FNA/B for diagnosis of pancreatic cancer are 91% and 86%, respectively[11]. However, there is a lack of uniformity concerning the standard cut-off values of TIC. CEH-EUS-FNA/B is also operator-dependent, and the endoscopist's experience may affect the results[12].

There have been conflicting results in studies comparing CEH-EUS-FNA/B to standard EUS-FNA/B. The aim of this meta-analysis was to compare the diagnostic outcome of auxiliary-EUS-FNA/B compared to standard EUS-FNA/B in the

diagnosis of pancreatic solid lesions.

MATERIALS AND METHODS

The present meta-analysis was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[13].

Information sources and search strategy

The electronic databases of MEDLINE, EMBASE, and Scopus were searched from their inception to February 2024 for all relevant studies. The following keywords were used for the search: (Endoscopic ultrasound OR EUS) AND (FNA OR Fine needle aspiration OR FNB OR Fine needle biopsy) AND (Elastography OR Contrast) AND (Pancreas OR Pancreatic). Furthermore, relevant studies were searched from the reference lists of all retrieved studies, guidelines, and reviews.

Study selection

Two reviewers independently reviewed the obtained search records for inclusion and exclusion criteria from the titles and abstracts. The same independent reviewers went through the full text of the shortlisted articles. Any dispute was settled by a third reviewer. Comparative prospective and retrospective studies (parallel as well as cross-over) fulfilling the following criteria were included in this meta-analysis: (1) Participants-patients with a solid pancreatic lesion; (2) Index test-RTE-EUS-FNA/B or CEH-EUS-FNA/B; (3) Comparator test-Standard EUS-FNA/B; and (4) Reference standards-surgical pathology for patients who underwent surgery or clinical and radiological follow-up of 12 months in unresectable cases. Conference abstracts, non-comparative single-arm studies, and case reports were excluded from the analysis.

Data extraction and risk of bias assessment

Two reviewers worked independently to retrieve the data. A third reviewer arbitrated any disputes. The following categories were utilized to gather data: Author and publication year, number of patients, age distribution, location and size of the lesion, type of needle and technique used, and the diagnostic outcomes.

The risk of bias and applicability in the included studies were evaluated using the QUADAS-2 tool[14]. Patient selection, index test, reference standard, and patient flow during the research and timing of the index tests are the four domains that make up this tool.

Statistical analysis

The sensitivity, specificity, likelihood ratio (LR), and diagnostic odds ratio (DOR) were determined using the true positive, false positive, false negative, and true negative data. The receiver operating characteristic curve (sROC) was summarized, and the area under the ROC (AUROC) was computed. The bivariate hierarchical model was used for meta-analysis of the summary estimate for sensitivity and specificity together, whereas the hierarchical summary ROC model was used to model the parameters for the sROC curve. The bivariate model models the sensitivity and specificity more directly, assuming that their logit (log-odds) transforms have a bivariate normal distribution between studies[15]. In random-effects meta-analysis, the extent of variation among the effects observed in different studies is referred to as tau-squared (τ^2), which is a measure of heterogeneity. A sensitivity analysis was conducted to evaluate the reliability of the study's findings. Based on the LR of the diagnostic test, a Fagan nomogram was utilized to calculate the post-test probability. A P value of < 0.10 for the slope coefficient in Deek's plot indicated publication bias[16]. Data analysis was performed using Meta-DiSc version 1.4 (Madrid, Spain), RevMan version 5.4, and STATA version 17 (College Station, Tex).

RESULTS

Study characteristics and risk of bias assessment

Ten studies out of the 2698 retrieved records were included in the meta-analysis. The PRISMA flowchart for the inclusion and selection of studies is displayed in Figure 1. The baseline characteristics of the studies that were included in the meta-analysis are summarized in Table 1[17-26]. Only one study used RTE-EUS-FNA/B[24], while the other nine used CEH-EUS FNA/B. Five of these nine studies on CEH-EUS FNA/B used SonoVue[17,18,20,22,23], and four used Sonazoid[19,21,25,26]. Four studies were randomized trials[19,22,23,26], three were prospective[18,21,24], and three were retrospective [17,20,25]. The mean size of the lesion varied from 25 mm to 39.5 mm. Except for two studies[19,23], the rest all used a 22-G size needle. ROSE was available in only two studies. The study by Lai *et al*[25] reported data from only malignant lesions[25] and, hence, was not included in the calculation of sensitivity and specificity. Concerning study quality, three studies had low[22,24,26], four had intermediate[18,19,21,23], and three had high risk of bias[17,20,25] (Figure 2).

Diagnostic utility of standard EUS-FNA/B

The pooled sensitivity estimates of standard EUS-FNA/B was 0.82 (95%CI: 0.79-0.85) (I^2 = 89.8%), and the pooled specificity estimate was 1.00 (95%CI: 0.96-1.00) (I^2 = 0.0%) (Figure 3A). Additionally, the pooled positive LR, negative LR,

Table 1 Baseline characteristics of the studies included in the meta-analysis, mean ± SD/median (range/25th-75th percentiles)

Ref.	Country	Study design	Arm	Number of patients	Age	Lesion size, in mm	Lesion location (H/U-B/T)	Contrast	Needle	Suction	Number of passes	ROSE
Hou *et al*[17]	China	Retrospective	CEH-EUS FNA	58	55.1 ± 11.7	38 ± 12	35/23	SonoVue	22-G	-	3.7 ± 0.9	No
			EUS-FNA	105	56.2 ± 12.5	39 ± 8.0	65/40	-			3.6 ± 0.8	
Seicean *et al*[18]	Romania	Prospective, cross-over	CEH-EUS FNA	51	64 (39-83)	35	31/10	SonoVue	22-G	-	2	No
			EUS-FNA					-			2	
Sugimoto *et al*[19]	Japan	RCT	CEH-EUS FNA	20	69.5 ± 10.5	25.0 ± 8.0	13/7	Sonazoid	22-G	DS	1-5	Yes
			EUS-FNA	20	67.1 ± 9.9	26.5 ± 9.2	13/7	-	22-G or 25-G		1-5	
Facciorusso *et al*[20]	Italy	Retrospective	CEH-EUS FNA	103	66 ± 6	32 ± 11	71/32	SonoVue	22-G	DS	2.4 ± 0.6	No
			EUS-FNA	103	66 ± 8	32 ± 10	71/32	-			2.7 ± 0.8	
Itonaga *et al*[21]	Japan	Prospective, cross-over	CEH-EUS FNA	93	72.5 (34–89)	25.2 (12-56)	55/38	Sonazoid	22-G	DS	2.6 (2-5)	No
			EUS-FNA					-				
Seicean *et al*[22]	Romania	RCT, cross-over	CEH-EUS FNA	148	64.5 (62.6 – 66.3)	30 (20.8- 35)	103/45	SonoVue	22-G	-	1	No
			EUS-FNA					-			1	
Cho *et al*[23]	South Korea	RCT	CEH-EUS FNA/B	120	66.3 ± 11.8	30.9 ± 2.1	54/66	SonoVue	19- to 25-G	DS	1-5	No
			EUS-FNA/B	120	68.3 ± 11.9	33.1 ± 16.4	60/60	-			1-5	
Gheorghiu *et al*[24]	Romania	Prospective, cross-over	RTE-EUS FNA	60	66.4 ± 10.0	30 (29.5 -35)	44/16	-	22-G	No	1	No
			EUS-FNA					-			1	
Lai *et al*[25]	Taiwan	Retrospective	CEH-EUS FNB	48	63.6 ± 12.6	29.5 ± 11.5	29/11/8	Sonazoid	22-G	SSP	2.2 ± 0.7	No
			EUS-FNB	85		34.8 ± 18.2	39/36/10	-			3.6 ± 1.2	
Kuo *et al*[26]	Taiwan	RCT	CEH-EUS FNB	59	64.7 ± 11.6	37.5 (28.8-45.9)	30/29	Sonazoid	22-G	No	1-6	Yes
			EUS-FNB	59	64.1 ± 12.6	37.5 (30.6-46.2)	34/25	-			1-6	

EUS-FNA/B: Endoscopic ultrasound-guided fine-needle aspiration/biopsy; CEH-EUS-FNA/B: Contrast-enhanced harmonic-EUS-FNA/B; RTE-EUS-FNA/B: Real-time elastography EUS-FNA/B; Lesion location (H/U-B/T): Head or uncinate process-body or tail; RCT: Randomized controlled trial; ROSE: Rapid on-site evaluation; DS: Dry suction; SSP: Slow stylet pull.

and DOR were 14.42 (95%CI: 5.64-36.90), 0.19 (95%CI: 0.13-0.30), and 105.51 (95%CI: 36.38-305.99), respectively (Table 2). Using the Fagan nomogram, a positive result raised the pre-test likelihood of diagnosis from 50% to 99%, whereas a negative result lowered the pre-test probability from 50% to 13% (Figure 4A). An sROC curve was plotted, and the

Table 2 Summary of results of diagnostic test accuracy meta-analysis

Parameters	Standard EUS-FNA/B		EUS-FNA/B with auxiliary techniques	
	Values with 95%CI	Heterogeneity (I^2)	Values with 95%CI	Heterogeneity (I^2)
Sensitivity	0.82 (0.79-0.85)	89.8%	0.86 (0.83-0.89)	73.6%
Specificity	1.00 (0.96-1.00)	0.0%	1.00 (0.94-1.00)	2.7%
Positive LR	14.42 (5.64-36.90)	0.0%	11.11 (4.30-28.71)	21.6%
Negative LR	0.19 (0.13-0.30)	82.5%	0.17 (0.13-0.22)	40.4%
DOR	105.51 (36.38-305.99)	0.0%	126.87 (44.43-362.28)	0.0%
AUROC	0.97 (0.95-0.98)	-	0.96 (0.94-0.98)	-

LR: Likelihood ratio; DOR: Diagnostic odds ratio; AUROC: Area under the receiver operating characteristic curve; EUS-FNA/B: Endoscopic ultrasound-guided fine-needle aspiration/biopsy.

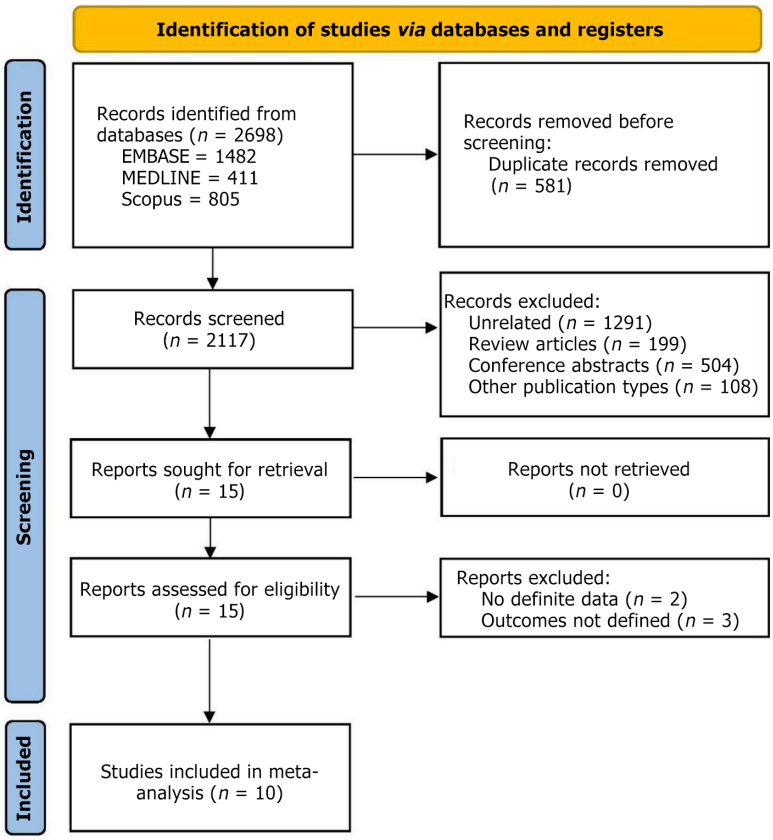


Figure 1 PRISMA flowchart for study identification, selection, and inclusion process.

AUROC with 95%CI was 0.97 (95%CI: 0.95-0.98) (Figure 3C).

Diagnostic utility of EUS-FNA/B with auxiliary technique

The pooled sensitivity estimate was 0.86 (95%CI: 0.83-0.89) (I^2 = 73.6%), and the pooled specificity estimate was 1.00 (95%CI: 0.94-1.00) with evidence of statistical heterogeneity (I^2 = 2.7%) (Figure 3B). The pooled positive LR, negative LR, and DOR for EUS-FNA/B with auxiliary techniques were 11.11 (95%CI: 4.30-28.71), 0.17 (95%CI: 0.13-0.22), and 126.87 (95%CI: 44.43-362.28), respectively (Table 2). Using the Fagan nomogram, a positive result raised the pre-test likelihood of diagnosis from 50% to 100%, whereas a negative result reduced the pre-test probability from 50% to 11% (Figure 4C). An sROC curve was plotted, and the AUROC with 95%CI was 0.96 (95%CI: 0.94-0.98) (Figure 3D).

Comparison of standard vs auxiliary EUS-FNA/B

On comparison of standard *vs* auxiliary EUS-FNA/B, both sensitivity [Risk ratio (RR): 1.04, 95%CI: 0.99-1.09, P = 0.0828; τ^2

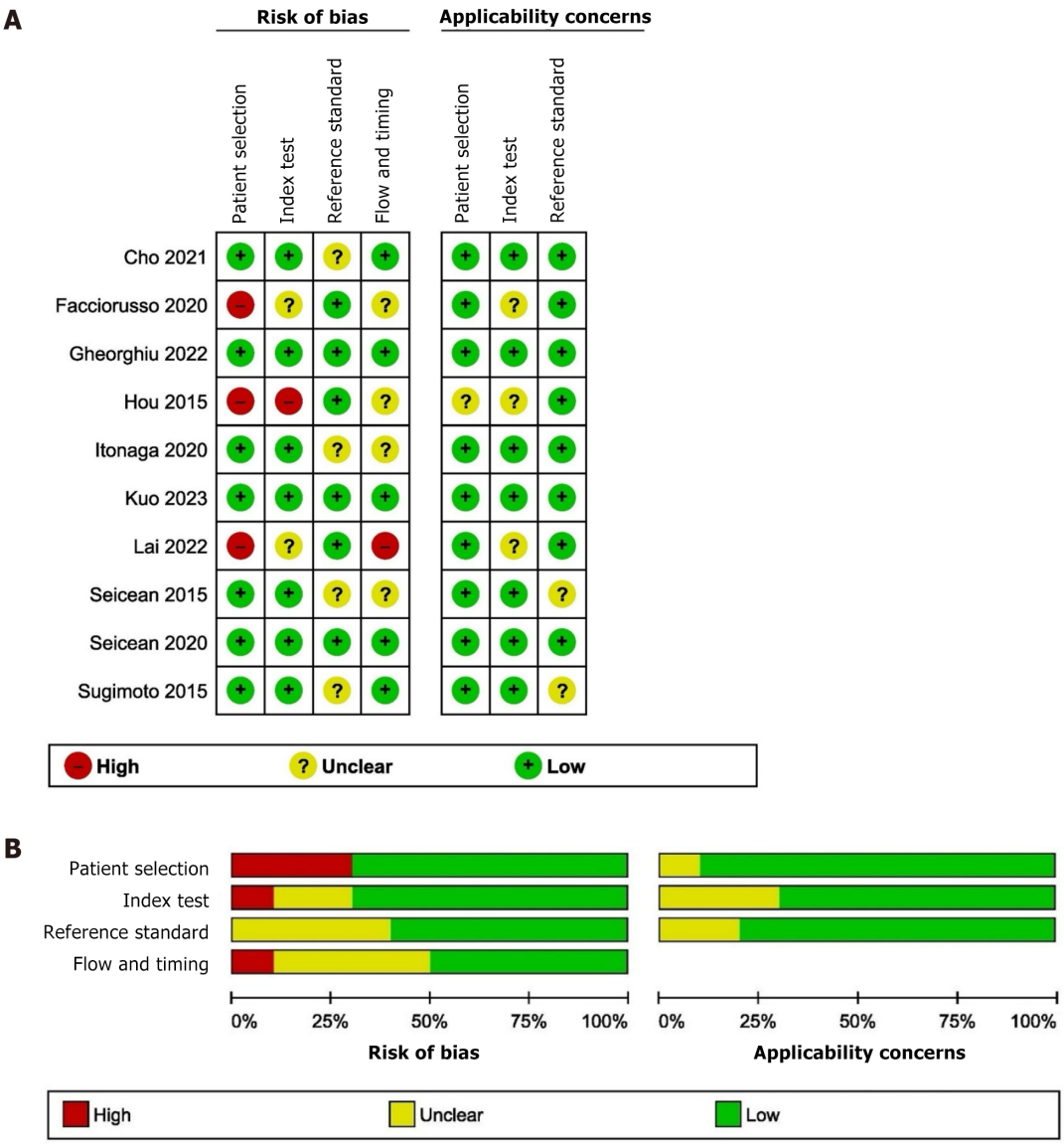


Figure 2 Risk of bias assessment using QUADAS-2 tool. A: Methodological quality for individual studies; B: Methodological quality summary graph.

= 0.91] and specificity (RR: 1.00, 95%CI: 0.99-1.01, P = 0.8123; τ2 = 8.41) were comparable (Figure 5). The diagnostic accuracy between the two modalities (RR: 1.02, 95%CI: 0.98-1.07; I^2 = 50%, P = 0.04) was also comparable, with evidence of statistical heterogeneity (Figure 6).

Publication bias, sensitivity analysis, and certainty of evidence

Assessment of Deeks' plot did not show evidence of publication bias for standard EUS-FNA/B (Figure 4B) but for EUS-FNA/B with auxiliary technique (P = 0.054) (Figure 4D). A sensitivity analysis was conducted using studies on CEH-EUS-FNA/B, randomized studies, and first-pass data. Sensitivity analysis showed comparable sensitivity, specificity, and diagnostic accuracy between both groups (Table 3). Table 4 shows the certainty of evidence for the analyzed outcomes.

DISCUSSION

EUS-guided advanced imaging techniques, such as elastography and contrast enhancement, offer the advantage of distinguishing between fibrotic/inflammatory tissues and malignant pancreatic lesions. These auxiliary techniques have been touted to improve the diagnostic performance of EUS in solid pancreatic lesions, and a recent guideline recommends using contrast guidance for EUS-guided tissue acquisition from solid lesions with avascular areas[27]. However, there is a paucity of data to support this argument fully. The present meta-analysis reported pooled sensitivity estimates of standard EUS-FNA/B and EUS-FNA/B with auxiliary technique as 82% (79%-85%) and 86% (83%-89%), without any significant difference (1.04, 95%CI: 0.99-1.09). Similarly, the pooled specificity estimates of standard EUS-FNA/B and EUS-FNA/B with the auxiliary technique were 100% (96%-100%) and 100% (94%-100%) with relative specificity of 1.00 (95%CI: 1.00-1.01). The odds of diagnostic accuracy were comparable also comparable between both groups (1.03, 95%CI:

Table 3 Comparative analysis of endoscopic ultrasound-guided fine-needle aspiration/biopsy with auxiliary *vs* standard technique with sensitivity analysis

Comparison of EUS-FNA/B with auxiliary *vs* standard techniques	No. of studies	Relative risk	*P* value	Tau^2
Relative sensitivity				
Overall	9	1.04 (0.99-1.09)	0.0828	0.91
Studies with CEH-EUS-FNA/B	8	1.05 (1.00-1.10)	0.0729	1.05
Randomized studies	4	1.00 (0.97-1.03)	0.9006	1.41
Relative specificity				
Overall	9	1.00 (1.00-1.01)	0.8123	0.41
Studies with CEH-EUS-FNA/B	8	1.00 (1.00-1.01)	0.8096	8.42
Randomized studies	4	1.01 (0.96-1.06)	0.7840	6.31
				I^2
Diagnostic accuracy				
Overall	10	1.02 (0.98-1.07)	0.33	50%
Studies with CEH-EUS-FNA/B	9	1.03 (0.98-1.09)	0.23	55%
After single pass	5	1.01 (0.93-1.10)	0.19	35%
Randomized studies	4	0.99 (0.95-1.02)	0.45	0%

EUS-FNA/B: Endoscopic ultrasound-guided fine-needle aspiration/biopsy; CEH-EUS-FNA/B: Contrast-enhanced harmonic-EUS-FNA/B.

Table 4 Grade of evidence table[1]

Outcomes	Anticipated absolute effects (95%CI)		Relative effect (95%CI)	No. of patients (studies)	Certainty assessment				Overall certainty of evidence
	With standard EUS-FNA/B	With EUS-FNA/B and auxiliary techniques			Risk of bias	Inconsistency	Indirectness	Imprecision	
Sensitivity	0.82 (0.79-0.85)	0.86 (0.83-0.89)	RR: 1.04 (0.99-1.09)	1471 (9 studies)	+	+	-	-	Low
Specificity	1.00 (0.96-1.00)	1.00 (0.94-1.00)	RR: 1.00 (0.99-1.01)	1471 (9 studies)	+	+	-	-	Low
Diagnostic accuracy	846 per 1000	17 higher per 1000 (17 lower to 59 more)	RR: 1.02 (0.98-1.07)	1604 (10 studies)	+	+	-	-	Low

[1]Population: Patients with a solid pancreatic lesion. Index test: Real-time elastography-endoscopic ultrasound-guided fine-needle aspiration/biopsy/B or Contrast-enhanced harmonic-endoscopic ultrasound-guided fine-needle aspiration/biopsy. Comparator test: Standard Endoscopic ultrasound-guided fine-needle aspiration/biopsy.
EUS-FNA/B: Endoscopic ultrasound-guided fine-needle aspiration/biopsy.

0.98-1.09). These findings suggest that the auxiliary techniques may not improve the diagnostic outcome of EUS-FNA/B.

Other factors that may affect diagnostic outcomes of EUS-FNA/B include the size and location of the lesion. Itonaga *et al*[21] reported higher adequacy and sensitivity with CEH-EUS-FNA in lesion size > 15 mm and lesions located in the body/tail[21]. Kuo *et al*[26] reported reduced cytologic and histologic accuracy in lesions larger than 40 mm[26]. This may be due to the increased proportion of necrotic material in larger lesions. CEH-EUS may help target viable tissue in such lesions. However, Facciorusso *et al*[20] and Seicean *et al*[22] reported no difference with respect to size[20,22], and Cho *et al*[23] reported no difference with respect to the location of the lesion[23]. Hence, further studies are required to demonstrate the benefit of CEH-EUS-FNA/B in larger lesions.

Another benefit of the auxiliary technique, which has been reported in some studies, is the higher diagnostic yield in a single pass. Sugimoto *et al*[19] reported that a sufficient biopsy sample after a single needle pass was obtained in 60% of the CEH-EUS-FNA group compared with 25% of the standard EUS-FNA group[19]. Similarly, the rate of adequate

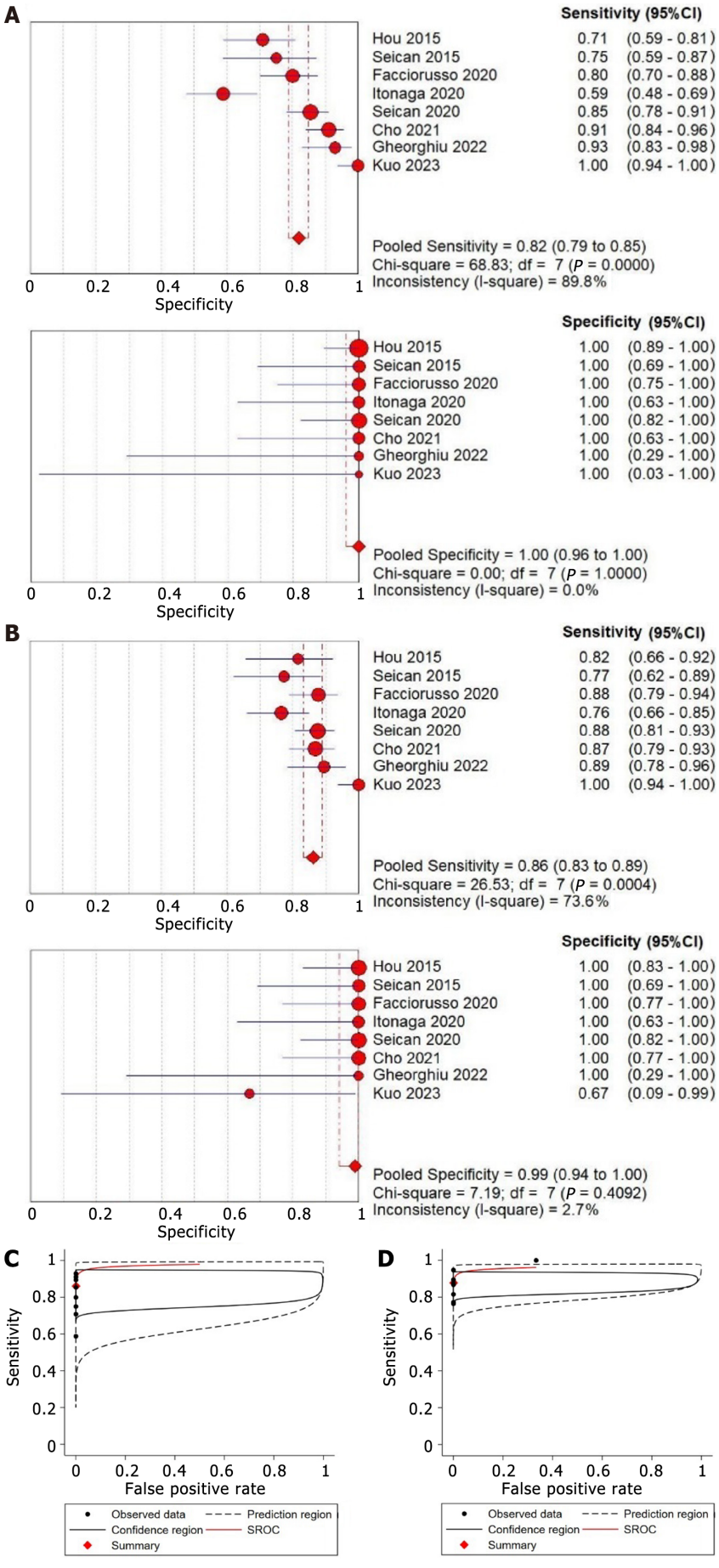


Figure 3 Sensitivity and specificity for diagnosis of malignancy from pancreatic solid lesion with endoscopic ultrasound-guided fine

needle aspiration/biopsy using. A and C: Standard; B: Auxiliary technique. The summary receiver operating characteristic curves for sensitivity and specificity of endoscopic ultrasound-guided fine needle aspiration/biopsy using; D: Auxiliary technique with their 95%CI and 95% prediction interval.

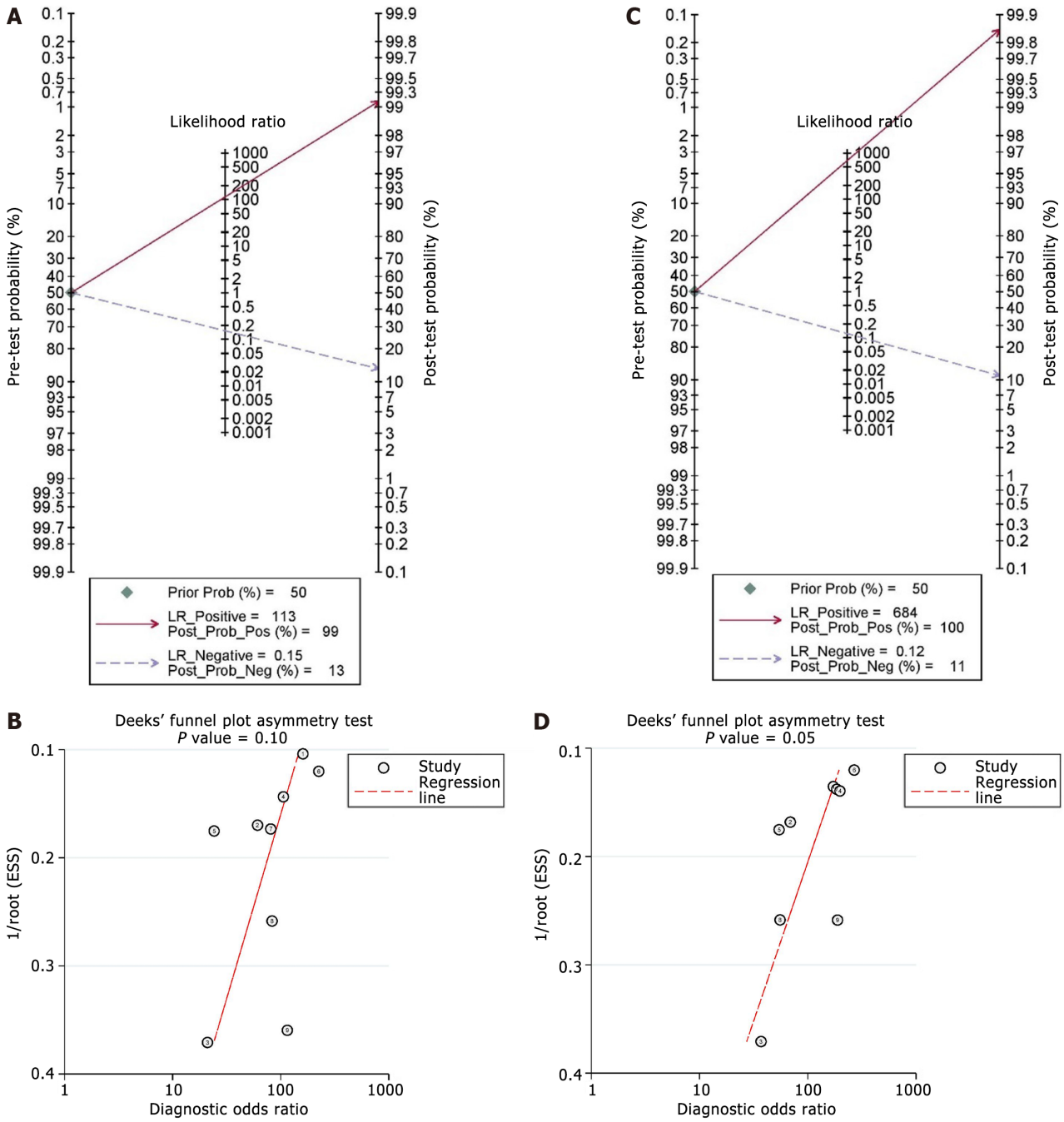


Figure 4 Fagan plot for endoscopic ultrasound-guided fine needle aspiration/biopsy using. A and B: Standard; C: Auxiliary technique. Deeks' plot for endoscopic ultrasound-guided fine needle aspiration/biopsy using; D: Auxiliary technique.

sampling and sensitivity of one pass with EUS-FNA-CHI was higher in the study by Itonaga *et al*[21]. However, the studies by Cho *et al*[23] and Kuo *et al*[26] reported no difference in the diagnostic sensitivity after the first needle pass between the two techniques[23,25]. One reason for this difference between the studies may be that Sugimoto *et al*[19] and Itonaga *et al*[21] used FNA needles, while Cho *et al*[23] and Kuo *et al*[26] used FNB needles predominantly. However, a recent RCT using an FNA needle reported no significant difference between conventional and CEH-EUS-FNA in terms of diagnostic accuracy, sensitivity, specificity and positive and negative predictive values[28]. Also, a meta-analysis using the data on first-pass from four studies showed no difference in the diagnostic accuracy. Thus, with the increasing use of newer-generation FNB needles, auxiliary techniques may not be useful as a routine procedure.

Despite some studies highlighting the utility of CH-EUS-FNA, only one study has shown that CH-EUS-FNA enhances diagnostic sensitivity[21]. With the advent of precision medicine, particularly in the realm of oncogene panel testing on

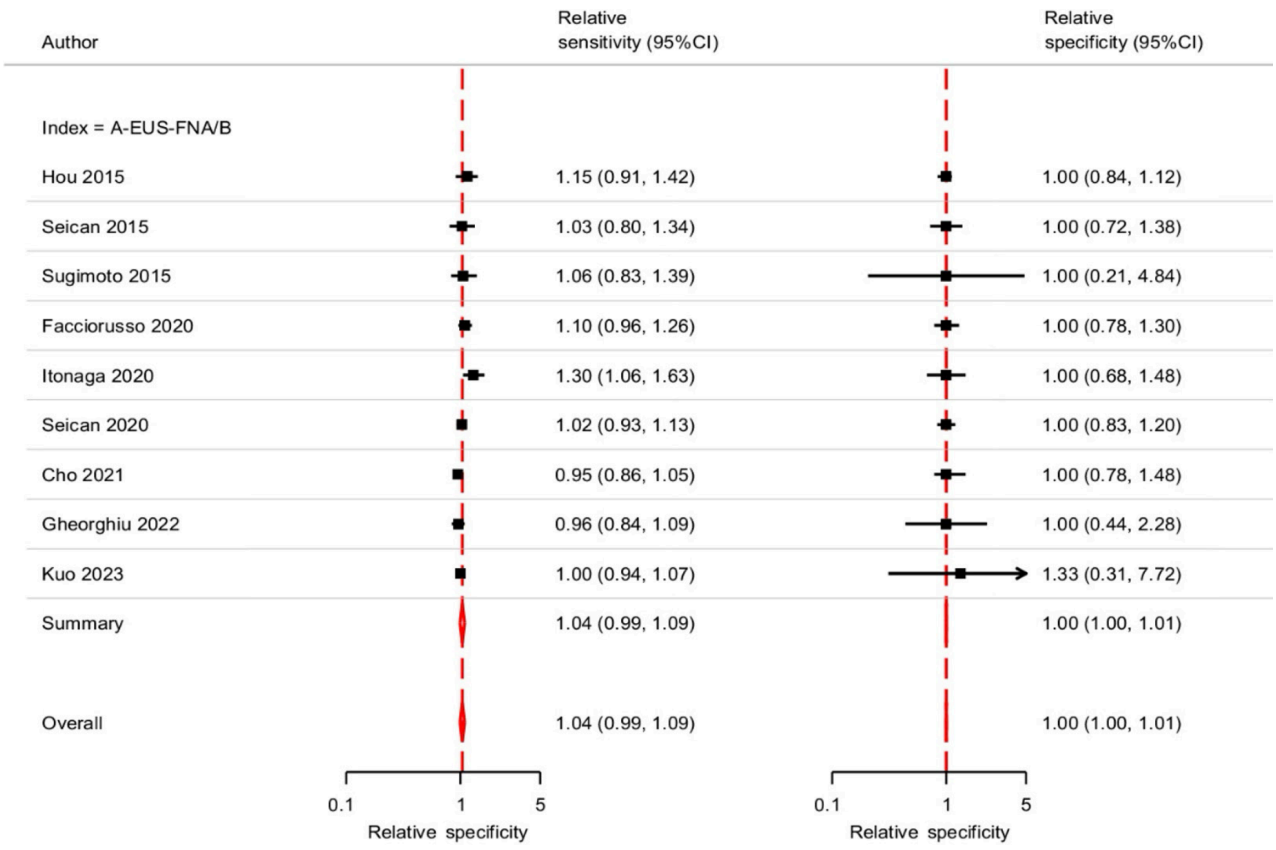


Figure 5 Relative sensitivity and specificity of endoscopic ultrasound-guided fine needle aspiration/biopsy using auxiliary technique, compared to standard technique.

EUS-FNA specimens, it will be crucial to obtain cancer tissues from sites with a high cellular yield[29]. In this context, CH-EUS-FNA could be a valuable technique, especially for collecting tissue samples from solid pancreatic neoplasms. The ability of CH-EUS-FNA to provide high-quality, cell-rich samples can make it an indispensable tool in the advancement of personalized treatment strategies for pancreatic cancer patients, underscoring its significance in the diagnostic and therapeutic landscape of pancreatic cancer[30].

EUS elastography is being promoted because it offers important information complementary to B-mode imaging. However, elastography cannot be considered a required addition to EUS because most endosonographers achieve excellent EUS outcomes without it. No evidence indicates that elastography enhances clinical outcomes compared to traditional EUS, whether or not EUS-guided tissue acquisition is used. Specifically, no studies demonstrate that elastography is superior in targeting cancer within suspicious lesions. Despite its high sensitivity for diagnosing malignancy, the clinical utility of EUS elastography is limited by its low specificity. In one large prospective study, the specificity for characterizing solid pancreatic lesions was as low as 22%, undermining its overall effectiveness in clinical practice[31]. In addition, it has been observed that the accuracy of EUS-FNA largely depends on the expertise of the endosonographers and similarly, the evaluation of pancreatic lesions using CH-EUS or elastography is also reliant on their examination skills[32].

The other disadvantage of the contrast agent is that it is expensive, costing approximately $250 *per* bottle, and requires an additional 3 to 5 minutes to prepare the contrast injection and observe the enhancement pattern[26]. This information is valuable for healthcare providers and policy-makers aiming to optimize resource allocation. However, none of the studies compared the overall difference in cost between the two modalities.

There are a few limitations to the present analysis warranting discussion. First, not all studies were randomized, increasing the risk of selection and reporting bias. However, a subgroup analysis of randomized studies did not report any difference between both modalities. Second, an assessment of publication bias, using the Deeks plot, revealed the presence of publication bias for the auxiliary techniques. This suggests that the studies included in the analysis may not fully represent the entire spectrum of clinical scenarios, and the results should be interpreted cautiously. Third, we could not perform a meta-analysis to assess the impact of size on diagnostic outcomes due to limited data from the included studies. Fourth, there was heterogeneity concerning the type of needle, type of suction method, and use of ROSE. Fifth, we did not compare the occurrence of adverse effects between the two modalities. However, the existing literature does not suggest any increase in adverse events using auxiliary techniques. The reported adverse effects related to the use of ultrasound contrast medium do not pose any clinical problems[33].

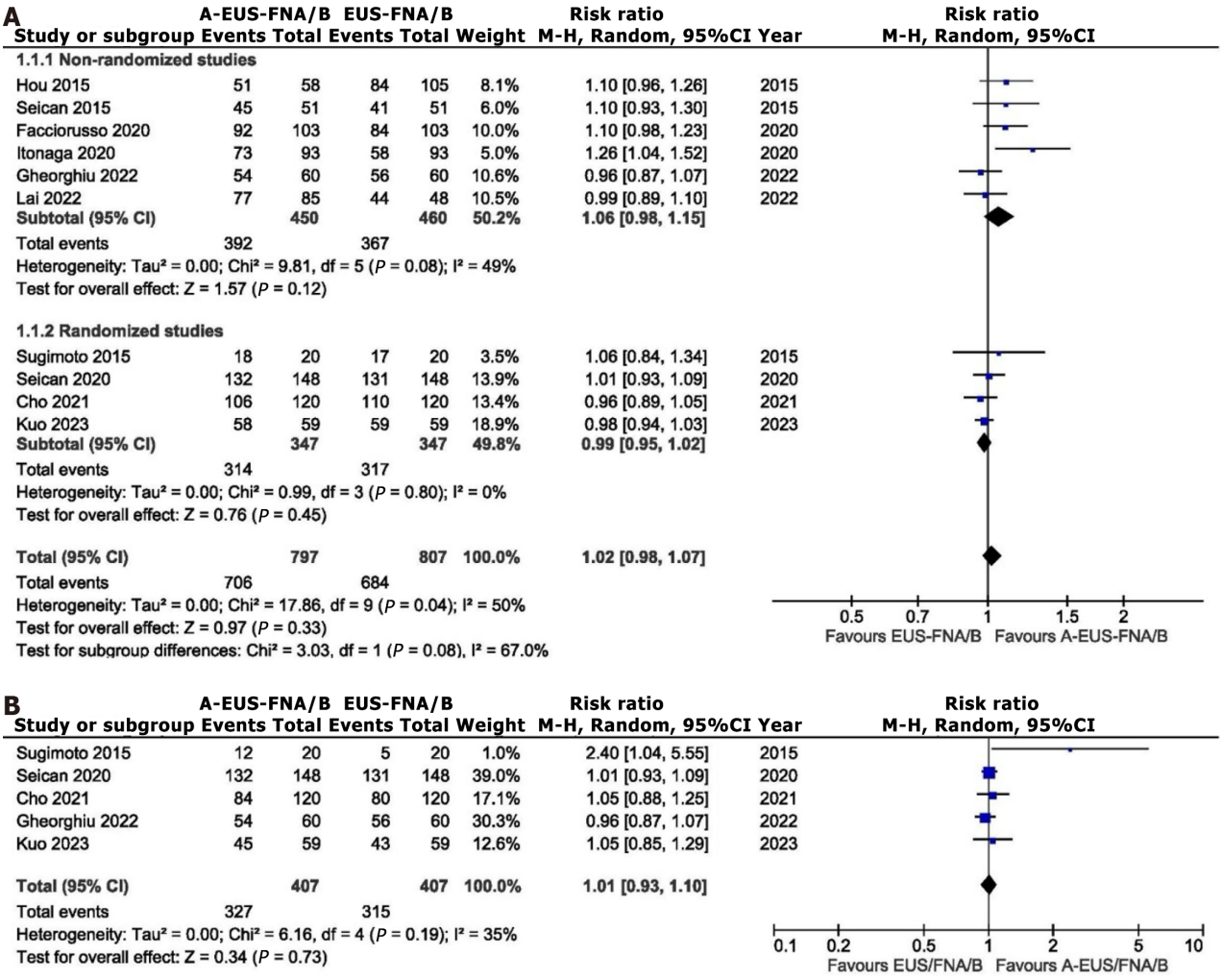


Figure 6 Forest plot for comparison of diagnostic accuracy between endoscopic ultrasound-guided fine needle aspiration/biopsy using auxiliary technique and standard technique. A: All studies; B: Studies reporting data after the first pass.

CONCLUSION

In conclusion, EUS-FNA/B, both standard and with auxiliary techniques, demonstrates high diagnostic utility with excellent specificity. Comparable sensitivity, specificity, and diagnostic accuracy were reported in the current meta-analysis comparing EUS-FNA/B with and without auxiliary techniques. Subgroup analysis of studies exclusively using CEH-EUS-FNA/B, randomized studies, and studies reporting diagnostic outcomes after the first pass reported no difference between both modalities. Thus, the currently available literature for pancreatic solid lesions does not show any additional benefit from utilizing auxiliary techniques in conjunction with standard EUS-FNA/B. Further randomized studies are required before auxiliary techniques can be recommended for routine practice.

FOOTNOTES

Author contributions: Panigrahi MK and Giri S contributed to the conception and design of the manuscript; Rath MM, Anirvan P, Varghese J, Tripathy TP, Patel RK, and Giri S contributed to the literature review, analysis, data collection, and interpretation; Rath MM, Anirvan P, and Giri S drafted the initial manuscript; Rath MM, Panigrahi MK and Giri S contributed to the critical revision of the initial manuscript; All the authors approved the final version of the manuscript.

Conflict-of-interest statement: All the authors declare no conflict of interest for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2021 Checklist, and the manuscript was prepared and revised according to the PRISMA 2021 Checklist.

Country of origin: India

ORCID number: Mitali Madhumita Rath 0000-0001-6167-0778; Prajna Anirvan 0000-0003-4494-0865; Jijo Varghese 0000-0002-9794-9188; Tara Prasad Tripathy 0000-0003-3763-9089; Ranjan K Patel 0000-0003-4780-5810; Manas Kumar Panigrahi 0000-0001-9540-3686; Suprabhat Giri 0000-0002-9626-5243.

S-Editor: Liu H
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Yang Y**, Li L, Qu C, Liang S, Zeng B, Luo Z. Endoscopic ultrasound-guided fine needle core biopsy for the diagnosis of pancreatic malignant lesions: a systematic review and Meta-Analysis. *Sci Rep* 2016; **6**: 22978 [PMID: 26960914 DOI: 10.1038/srep22978]

2 **Yao DW**, Qin MZ, Jiang HX, Qin SY. Comparison of EUS-FNA and EUS-FNB for diagnosis of solid pancreatic mass lesions: a meta-analysis of prospective studies. *Scand J Gastroenterol* 2024; **59**: 972-979 [PMID: 38769625 DOI: 10.1080/00365521.2024.2354908]

3 **Giri S**, Afzalpurkar S, Angadi S, Marikanty A, Sundaram S. Comparison of suction techniques for EUS-guided tissue acquisition: Systematic review and network meta-analysis of randomized controlled trials. *Endosc Int Open* 2023; **11**: E703-E711 [PMID: 37564335 DOI: 10.1055/a-2085-3674]

4 **Sundaram S**, Chhanchure U, Patil P, Seth V, Mahajan A, Bal M, Kaushal RK, Ramadwar M, Prabhudesai N, Bhandare M, Shrikhande SV, Mehta S. Rapid on-site evaluation (ROSE) versus macroscopic on-site evaluation (MOSE) for endoscopic ultrasound-guided sampling of solid pancreatic lesions: a paired comparative analysis using newer-generation fine needle biopsy needles. *Ann Gastroenterol* 2023; **36**: 340-346 [PMID: 37144017 DOI: 10.20524/aog.2023.0790]

5 **Giri S**, Uppin MS, Kumar L, Uppin S, Pamu PK, Angadi S, Bhrugumalla S. Impact of macroscopic on-site evaluation on the diagnostic outcomes of endoscopic ultrasound-guided fine-needle aspiration. *Diagn Cytopathol* 2023; **51**: 569-574 [PMID: 37260107 DOI: 10.1002/dc.25175]

6 **Giri S**, Afzalpurkar S, Angadi S, Varghese J, Sundaram S. Influence of biliary stents on the diagnostic outcome of endoscopic ultrasound-guided tissue acquisition from solid pancreatic lesions: a systematic review and meta-analysis. *Clin Endosc* 2023; **56**: 169-179 [PMID: 37013391 DOI: 10.5946/ce.2022.282]

7 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

8 **Wolske KM**, Ponnatapura J, Kolokythas O, Burke LMB, Tappouni R, Lalwani N. Chronic Pancreatitis or Pancreatic Tumor? A Problem-solving Approach. *Radiographics* 2019; **39**: 1965-1982 [PMID: 31584860 DOI: 10.1148/rg.2019190011]

9 **Costache MI**, Cazacu IM, Dietrich CF, Petrone MC, Arcidiacono PG, Giovannini M, Bories E, Garcia JI, Siyu S, Santo E, Popescu CF, Constantin A, Bhutani MS, Saftoiu A. Clinical impact of strain histogram EUS elastography and contrast-enhanced EUS for the differential diagnosis of focal pancreatic masses: A prospective multicentric study. *Endosc Ultrasound* 2020; **9**: 116-121 [PMID: 32295969 DOI: 10.4103/eus.eus_69_19]

10 **Spadaccini M**, Koleth G, Emmanuel J, Khalaf K, Facciorusso A, Grizzi F, Hassan C, Colombo M, Mangiavillano B, Fugazza A, Anderloni A, Carrara S, Repici A. Enhanced endoscopic ultrasound imaging for pancreatic lesions: The road to artificial intelligence. *World J Gastroenterol* 2022; **28**: 3814-3824 [PMID: 36157539 DOI: 10.3748/wjg.v28.i29.3814]

11 **Mei S**, Wang M, Sun L. Contrast-Enhanced EUS for Differential Diagnosis of Pancreatic Masses: A Meta-Analysis. *Gastroenterol Res Pract* 2019; **2019**: 1670183 [PMID: 30962802 DOI: 10.1155/2019/1670183]

12 **Zhang J**, Zhu L, Yao L, Ding X, Chen D, Wu H, Lu Z, Zhou W, Zhang L, An P, Xu B, Tan W, Hu S, Cheng F, Yu H. Deep learning-based pancreas segmentation and station recognition system in EUS: development and validation of a useful training tool (with video). *Gastrointest Endosc* 2020; **92**: 874-885.e3 [PMID: 32387499 DOI: 10.1016/j.gie.2020.04.071]

13 **Page MJ**, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021; **372**: n71 [PMID: 33782057 DOI: 10.1136/bmj.n71]

14 **Whiting PF**, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM; QUADAS-2 Group. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011; **155**: 529-536 [PMID: 22007046 DOI: 10.7326/0003-4819-155-8-201110180-00009]

15 **Leeflang MM**, Deeks JJ, Takwoingi Y, Macaskill P. Cochrane diagnostic test accuracy reviews. *Syst Rev* 2013; **2**: 82 [PMID: 24099098 DOI: 10.1186/2046-4053-2-82]

16 **Deeks JJ**, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. *J Clin Epidemiol* 2005; **58**: 882-893 [PMID: 16085191 DOI: 10.1016/j.jclinepi.2005.01.016]

17 **Hou X**, Jin Z, Xu C, Zhang M, Zhu J, Jiang F, Li Z. Contrast-enhanced harmonic endoscopic ultrasound-guided fine-needle aspiration in the diagnosis of solid pancreatic lesions: a retrospective study. *PLoS One* 2015; **10**: e0121236 [PMID: 25793739 DOI: 10.1371/journal.pone.0121236]

18 **Seicean A**, Badea R, Moldovan-Pop A, Vultur S, Botan EC, Zaharie T, Săftoiu A, Mocan T, Iancu C, Graur F, Sparchez Z, Seicean R. Harmonic Contrast-Enhanced Endoscopic Ultrasonography for the Guidance of Fine-Needle Aspiration in Solid Pancreatic Masses. *Ultraschall Med* 2017; **38**: 174-182 [PMID: 26274382 DOI: 10.1055/s-0035-1553496]

19 **Sugimoto M**, Takagi T, Hikichi T, Suzuki R, Watanabe K, Nakamura J, Kikuchi H, Konno N, Waragai Y, Watanabe H, Obara K, Ohira H. Conventional versus contrast-enhanced harmonic endoscopic ultrasonography-guided fine-needle aspiration for diagnosis of solid pancreatic

lesions: A prospective randomized trial. *Pancreatology* 2015; **15**: 538-541 [PMID: 26145837 DOI: 10.1016/j.pan.2015.06.005]

20 **Facciorusso A**, Cotsoglou C, Chierici A, Mare R, Crinò SF, Muscatiello N. Contrast-Enhanced Harmonic Endoscopic Ultrasound-Guided Fine-Needle Aspiration versus Standard Fine-Needle Aspiration in Pancreatic Masses: A Propensity Score Analysis. *Diagnostics (Basel)* 2020; **10** [PMID: 33036222 DOI: 10.3390/diagnostics10100792]

21 **Itonaga M**, Kitano M, Kojima F, Hatamaru K, Yamashita Y, Tamura T, Nuta J, Kawaji Y, Shimokawa T, Tanioka K, Murata SI. The usefulness of EUS-FNA with contrast-enhanced harmonic imaging of solid pancreatic lesions: A prospective study. *J Gastroenterol Hepatol* 2020; **35**: 2273-2280 [PMID: 32529685 DOI: 10.1111/jgh.15144]

22 **Seicean A**, Samarghitan A, Bolboacă SD, Pojoga C, Rusu I, Rusu D, Sparchez Z, Gheorghiu M, Al Hajjar N, Seicean R. Contrast-enhanced harmonic versus standard endoscopic ultrasound-guided fine-needle aspiration in solid pancreatic lesions: a single-center prospective randomized trial. *Endoscopy* 2020; **52**: 1084-1090 [PMID: 32650346 DOI: 10.1055/a-1193-4954]

23 **Cho IR**, Jeong SH, Kang H, Kim EJ, Kim YS, Cho JH. Comparison of contrast-enhanced versus conventional EUS-guided FNA/fine-needle biopsy in diagnosis of solid pancreatic lesions: a randomized controlled trial. *Gastrointest Endosc* 2021; **94**: 303-310 [PMID: 33497643 DOI: 10.1016/j.gie.2021.01.018]

24 **Gheorghiu M**, Sparchez Z, Rusu I, Bolboacă SD, Seicean R, Pojoga C, Seicean A. Direct Comparison of Elastography Endoscopic Ultrasound Fine-Needle Aspiration and B-Mode Endoscopic Ultrasound Fine-Needle Aspiration in Diagnosing Solid Pancreatic Lesions. *Int J Environ Res Public Health* 2022; **19** [PMID: 35162325 DOI: 10.3390/ijerph19031302]

25 **Lai JH**, Lin CC, Lin HH, Chen MJ. Is contrast-enhanced endoscopic ultrasound-guided fine needle biopsy better than conventional fine needle biopsy? A retrospective study in a medical center. *Surg Endosc* 2022; **36**: 6138-6143 [PMID: 35484412 DOI: 10.1007/s00464-022-09253-3]

26 **Kuo YT**, Chu YL, Wong WF, Han ML, Chen CC, Jan IS, Cheng WC, Shun CT, Tsai MC, Cheng TY, Wang HP. Randomized trial of contrast-enhanced harmonic guidance versus fanning technique for EUS-guided fine-needle biopsy sampling of solid pancreatic lesions. *Gastrointest Endosc* 2023; **97**: 732-740 [PMID: 36509113 DOI: 10.1016/j.gie.2022.12.004]

27 **Kitano M**, Yamashita Y, Kamata K, Ang TL, Imazu H, Ohno E, Hirooka Y, Fusaroli P, Seo DW, Napoléon B, Teoh AYB, Kim TH, Dietrich CF, Wang HP, Kudo M; Working group for the International Consensus Guidelines for Contrast-Enhanced Harmonic Endoscopic Ultrasound. The Asian Federation of Societies for Ultrasound in Medicine and Biology (AFSUMB) Guidelines for Contrast-Enhanced Endoscopic Ultrasound. *Ultrasound Med Biol* 2021; **47**: 1433-1447 [PMID: 33653627 DOI: 10.1016/j.ultrasmedbio.2021.01.030]

28 **Nayak HK**, Rai A, Gupta S, Prakash JH, Patra S, Panigrahi C, Patel RK, Pattnaik B, Kar M, Panigrahi MK, Samal SC. Endoscopic ultrasound (EUS) elastography-guided fine-needle aspiration cytology (FNAC) versus conventional EUS FNAC for solid pancreatic lesions: A pilot randomized trial. *Indian J Gastroenterol* 2024 [PMID: 39230660 DOI: 10.1007/s12664-024-01673-4]

29 **Manrai M**, Tilak TVSVGK, Dawra S, Srivastava S, Singh A. Current and emerging therapeutic strategies in pancreatic cancer: Challenges and opportunities. *World J Gastroenterol* 2021; **27**: 6572-6589 [PMID: 34754153 DOI: 10.3748/wjg.v27.i39.6572]

30 **Ozono Y**, Kawakami H, Uchiyama N, Hatada H, Ogawa S. Current status and issues in genomic analysis using EUS-FNA/FNB specimens in hepatobiliary-pancreatic cancers. *J Gastroenterol* 2023; **58**: 1081-1093 [PMID: 37698719 DOI: 10.1007/s00535-023-02037-z]

31 **Dawwas MF**, Taha H, Leeds JS, Nayar MK, Oppong KW. Diagnostic accuracy of quantitative EUS elastography for discriminating malignant from benign solid pancreatic masses: a prospective, single-center study. *Gastrointest Endosc* 2012; **76**: 953-961 [PMID: 22854060 DOI: 10.1016/j.gie.2012.05.034]

32 **Otsuka Y**, Kamata K, Kudo M. Contrast-Enhanced Harmonic Endoscopic Ultrasound-Guided Puncture for the Patients with Pancreatic Masses. *Diagnostics (Basel)* 2023; **13** [PMID: 36980346 DOI: 10.3390/diagnostics13061039]

33 **Chou YH**, Liang JD, Wang SY, Hsu SJ, Hu JT, Yang SS, Wang HK, Lee TY, Tiu CM. Safety of Perfluorobutane (Sonazoid) in Characterizing Focal Liver Lesions. *J Med Ultrasound* 2019; **27**: 81-85 [PMID: 31316217 DOI: 10.4103/JMU.JMU_44_19]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 99080

DOI: 10.5662/wjm.v15.i3.99080

ISSN 2222-0682 (online)

META-ANALYSIS

Evidence-based approach for intraabdominal drainage in pancreatic surgery: A systematic review and meta-analysis

Rohith Kodali, Kunal Parasar, Utpal Anand, Basant Narayan Singh, Kislay Kant, Abhishek Arora, Venkatesh Karthikeyan, Saad Anwar, Bijit Saha, Siddhali Wadaskar

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade C, Grade C

Novelty: Grade B, Grade C

Creativity or Innovation: Grade B, Grade C

Scientific Significance: Grade B, Grade B

P-Reviewer: Ulasoglu C

Received: July 13, 2024

Revised: November 9, 2024

Accepted: December 5, 2024

Published online: September 20, 2025

Processing time: 236 Days and 4.6 Hours

Rohith Kodali, Kunal Parasar, Utpal Anand, Basant Narayan Singh, Kislay Kant, Abhishek Arora, Saad Anwar, Bijit Saha, Siddhali Wadaskar, Department of Surgical Gastroenterology, All India Institute of Medical Sciences, Patna 801507, Bihar, India

Venkatesh Karthikeyan, Department of Community Medicine, All India Institute of Medical Sciences, Patna 801507, Bihar, India

Corresponding author: Kunal Parasar, Associate Professor, Department of Surgical Gastroenterology, All India Institute of Medical Science, Phulwarisharif, Patna 801507, Bihar, India. kunal.parasar@gmail.com

Abstract

BACKGROUND

Historically intraoperative drains were employed after pancreatic surgery but over the last decade, there has been debate over the routine usage of drains.

AIM

To assess the necessity of intra-abdominal drain placement, identify the most effective drain type, and determine the optimal timing for drain removal.

METHODS

A systematic review of electronic databases, including PubMed, MEDLINE, PubMed Central, and Google Scholar, was conducted using Medical Subject Headings and keywords until December 2023. From an initial pool of 1910 articles, 48 were included after exclusion and screening. The primary outcomes analyzed were clinically relevant postoperative pancreatic fistula (CR-POPF), delayed gastric emptying (DGE), overall morbidity, and mortality. Subgroup analyses were performed for pancreaticoduodenectomy and distal pancreatectomy.

RESULTS

Routine use of drains is associated with a statistically significant increase in the risk of CR-POPF and DGE. Conversely, patients who did not have drains placed experienced a significant reduction in morbidity, readmission rates, and reoperations. No significant differences were observed between active and passive drain types. Early drain removal (< 3 days) yielded favorable outcomes compared to delayed removal.

CONCLUSION
Analysis of randomized controlled trials and cohort studies did not demonstrate an advantage of routine drain placement following pancreatic resection, potentially contributing to increased morbidity and mortality. The decision to use drains should be left to the discretion of the operating surgeon. However, early drain removal can substantially reduce morbidity.

Key Words: Intraabdominal drain; Pancreatic resection; Post-operative pancreatic fistula; Delayed gastric emptying; Early drain removal; Drainage duration; Post pancreatectomy drainage

Core Tip: Routine intraoperative drain placement after pancreatic surgery increases the risk of clinically relevant postoperative pancreatic fistula (CR-POPF) and delayed gastric emptying. Patients without drains had lower morbidity, readmission rates, and reoperations. The necessity of routine drain placement is questionable. No clear recommendation can be made between active suction and passive gravity drainage methods. Early drain removal is supported to reduce the occurrence of CR-POPF and associated morbidity and mortality.

Citation: Kodali R, Parasar K, Anand U, Singh BN, Kant K, Arora A, Karthikeyan V, Anwar S, Saha B, Wadaskar S. Evidence-based approach for intraabdominal drainage in pancreatic surgery: A systematic review and meta-analysis. *World J Methodol* 2025; 15(3): 99080
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/99080.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.99080

INTRODUCTION

Pancreatic resection, encompassing procedures like pancreaticoduodenectomy (PD) and distal pancreatectomy (DP), has evolved to become safer especially with advancements in surgical techniques and perioperative care[1,2]. Despite a mortality rate of less than 5%, pancreatic surgery still presents a considerable challenge due to high morbidity rates ranging from 30% to 60%, primarily attributed to complications such as postoperative pancreatic fistula (POPF)[3]. Given the significant impact on patient's quality of life and healthcare costs, there is a need to focus on strategies to mitigate POPF occurrence and improve postoperative outcomes[4,5].

The routine placement and management of intra-abdominal drains after pancreatic resection have garnered significant attention and discussion in recent years[6-10]. The practice of inserting prophylactic intra-abdominal drains has roots dating back to the 19th century, driven by the belief that these drains could evacuate fluids such as blood, bile, and pancreatic juice that might accumulate post-surgery[11]. In modern pancreatic surgery, the management of intra-abdominal drains has become a vital consideration, as indicated by earlier research highlighting its substantial impact on the frequency of postoperative complications[12]. Additionally, drains play a role in mitigating complications related to POPF and aid in the detection of other intra-abdominal hemorrhage. However, the contrarian view of routine use of abdominal drains can lead to retrograde infection, discomfort, foreign body reactions, and prolonged hospital stays[13]. Furthermore drains, which generate substantial negative pressure may contribute to the development of POPFs.

One of the pivotal questions concerning the necessity of placing drains after pancreatic resection was addressed by Conlon *et al*[7] which marked the initial evidence-based approach, reinforcing the idea that intra-abdominal drains should not be deemed mandatory. However, subsequent randomized controlled trials (RCTs) yielded conflicting results on the efficacy of drains[6,10]. Several systematic reviews and meta-analyses have since been conducted on the role of routine drainage after pancreatic resection. Unfortunately, the available evidence remains limited, underscoring the need for establishing evidence-based guidelines for prophylactic intraabdominal drainage in pancreatic surgery[13,14]. Thus, this systematic review and meta-analysis were aimed to analyse the most recent evidence based data and to answer the following questions: (1) Is the drainage at the operative site more beneficial after pancreatectomy compared to no drain; and (2) If drainage is used, how long should it remain, and what type of drainage should be preferred.

MATERIALS AND METHODS

Search strategy

A comprehensive literature search was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines[15]. PubMed, MEDLINE, PubMed Central, and Google Scholar databases were systematically reviewed using Medical Subject Headings terms and keywords up to December 2023. The search strategy outlined in (Table 1) aimed to identify the relevant studies. After applying inclusion criteria and removing duplicates, abstracts of shortlisted articles were screened, followed by independent full-text review by two authors.

Table 1 Search strategy for electronic databases

Search strategy	Database
(("Drainage" [Mesh] OR "Drainage, Surgical" [Mesh] OR "Suction" [Mesh] OR "Drainage, Closed" [Mesh]) AND ("Pancreatectomy" [Mesh] OR "Pancreatic resection" [Mesh] OR "Distal pancreatectomy" [Mesh] OR "Pancreatic tail resection" [Mesh] OR "Pancreaticoduodenectomy" [Mesh] OR "Whipple procedure" [Mesh] OR "Duodenopancreatectomy" [Mesh])	PubMed, PubMed Central, and MEDLINE
"Intraabdominal drainage", "Pancreatic resection", "Distal pancreatectomy", "Pancreaticoduodenectomy" separately and in combination	Google Scholar

Mesh: Medical Subject Headings.

Inclusion criteria

Studies comparing the following parameters were included: (1) Intrabdominal drainage *vs* no drainage; (2) Active (suction) *vs* passive (gravity) drainage; (3) Early *vs* late (traditional) drain removal; and (4) Selective drain usage. Studies in which the full text is available in English, and studies that were conducted on humans.

Exclusion criteria

Review articles, letters to the editor, case reports, case series, systematic reviews, meta-analyses, and animal studies were excluded.

Data extraction and quality assessment

Data extraction was carried out by two researchers independently using standardized forms. The quality of the RCTs was evaluated using the Cochrane risk of bias tool while the quality of the non-RCTs was evaluated using the Newcastle-Ottawa scale.

Outcome of interest

The main outcomes considered were the clinically relevant POPF (CR-POPF), delayed gastric emptying (DGE), and overall morbidity rate. The outcome was measured in terms of the reoperation, readmission, length of hospital stay, and overall mortality rate.

Statistical analysis

For continuous variables, we utilized the inverse variance method to determine the standardized mean differences and 95%CI. Dichotomous outcomes were assessed using risk ratios and 95%CI were calculated through the Mantel-Haenszel model. The results were visually displayed in forest plots, and a random effects model was applied to estimate pooled odds ratios (OR) for postoperative complications. Heterogeneity was assessed using the χ^2 test and I^2 statistic, while publication bias was examined through Egger's test and funnel plots. The analysis was conducted using R programming version 4.2.

RESULTS

Study selection

Initially, 1910 studies were identified from major databases (Figure 1). After removing 40 duplicates and excluding 1749 studies not meeting inclusion criteria, full texts of 121 remaining studies were retrieved. Following further screening, 73 studies were eliminated for inconsistency with inclusion criteria, and two inaccessible full-text studies were removed. Ultimately, 48 articles were included, focusing on comparisons between presence or absence of drainage, active (closed suction) *vs* passive (gravity) drainage, and early *vs* traditional removal of drainage.

Intra-abdominal drain and without drain

In the current systematic review and meta-analysis, we meticulously examined a total of 22 studies, comprising five RCTs, and the remaining were retrospective, or cohort studies as shown in Supplementary Table 1[4,6-10,16-31]. These studies collectively encompassed a broad population, involving a total of 30029 participants.

CR-POPF

Twelve studies, comprising 16754 patients in the drain group and 3402 patients in the no-drain group, examined CR-POPF outcomes in PD[4,6,8-10,18-27,29-31]. Substantial heterogeneity was observed (I^2 = 90%, P < 0.01) (Figure 2A)[4,8,10,18,20-22,24-27,30]. The pooled analysis revealed a significantly higher CR-POPF rate in the drain group (OR = 1.58, 95%CI: 1.04-2.38), supported by a symmetrical funnel plot and non-significant Egger test (P = 0.7613) (Figure 3A). Additionally, seven studies involving 2329 patients in the drain group and 899 in the no-drain group examined CR-POPF outcomes in DP[6,8,9,19,29-31]. Significant heterogeneity was noted (I^2 = 55%, P = 0.04), with the drain group exhibiting a significantly higher CR-POPF rate (OR = 2.71, 95%CI: 1.73-4.24), supported by a symmetrical funnel plot (Figure 2B,

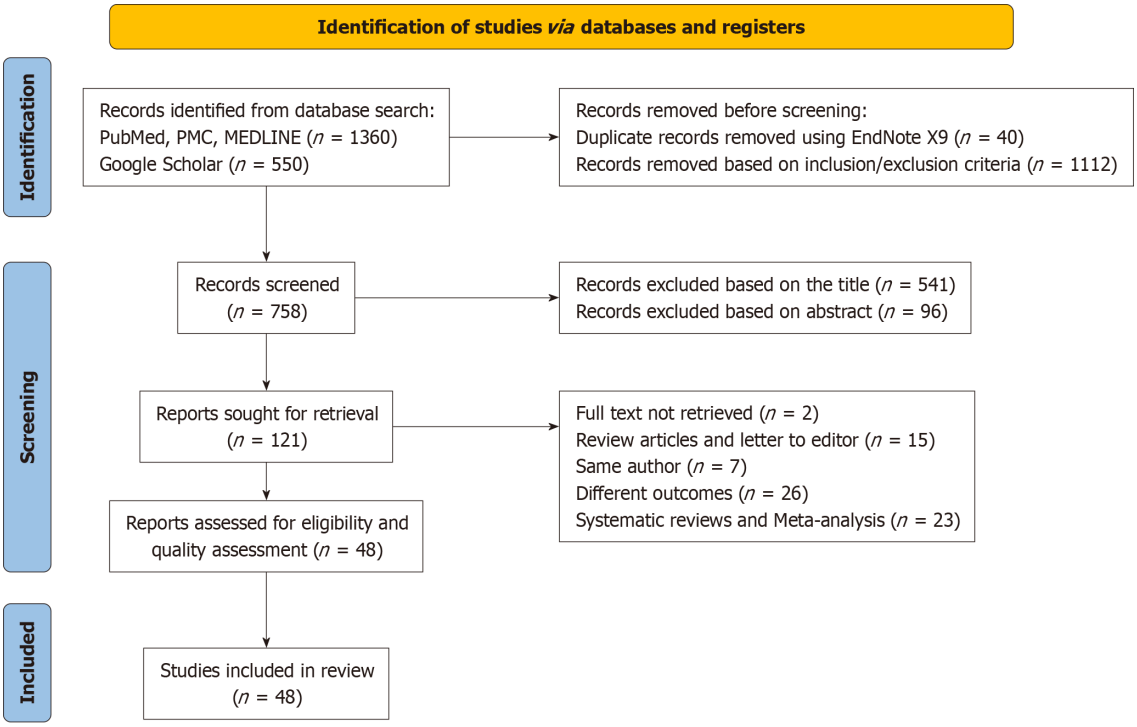


Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses Flowchart.

Figure 3B).

DGE

In the pooled analysis of eight studies focusing on DGE post-PD, the drain group (n = 13276) exhibited a significantly higher incidence compared to the no-drain group (n = 2365), with OR of 1.36 (95%CI: 1.18-1.55)[4,10,20,21,23,26,27,30]. Moderate heterogeneity was observed (I^2 = 33%, P = 0.16), and the funnel plot was symmetrical (Figure 2C, Figure 3C). Conversely, pooled analysis of two studies on DGE post-DP showed no significant correlation between the drain (n = 861) and no-drain (n = 214) groups, with a pooled OR of 3.79 (95%CI: 0.6–23.8). No heterogeneity was present among the included studies (I^2 = 0%, P = 0.44) (Figure 2D)[30,31].

Morbidity

Pooled analysis of nine studies reporting the outcome of overall morbidity in PD showed no significant difference between the drain group (n = 16167) and the no-drain group (n = 2847) with OR of 1.18 (95%CI: 0.82-1.7) (Figure 2E)[4,10,20-22,24-27]. There was considerable heterogeneity present among the included studies (I^2 = 90%, P < 0.01). The funnel plot was symmetrical (Figure 3D).

Pooled analysis of four studies reporting the outcome of overall morbidity in DP showed no significant difference between the drain group (n = 1314) and the no-drain group (n = 489) with OR of 0.95 (95%CI: 0.45-2.02) (Figure 2H)[6,9,19,29]. There was substantial heterogeneity present among the included studies (I^2 = 83%, P < 0.01) (Figure 2F).

Mortality

Pooled analysis with seven studies reporting mortality post-PD showed a significantly lower incidence of mortality among the drain group (n = 13623) than in the no-drain group (n = 2904), with an OR of 0.59 (95%CI: 0.44–0.79) (Figure 2G)[8,19-21,25-27]. No heterogeneity was present among the included studies (I^2 = 0%, P = 0.63). The funnel plot was symmetrical (Figure 3E).

Pooled analysis of four studies reporting the outcome of mortality in DP showed no significant difference between the drain group (n = 2118) and the no-drain group (n = 697) with OR of 1.99 (95%CI: 0.51-7.87) (Figure 2H)[8,19,29,31]. No heterogeneity was present among the included studies (I^2 = 0%, P = 0.62).

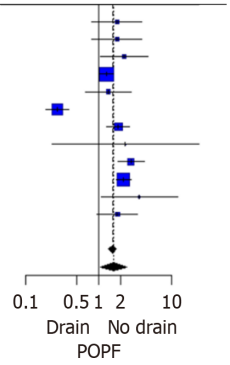
Active closed suction and passive gravity drainage

A comprehensive analysis of ten studies, including three RCTs and seven retrospective or cohort studies, involving 24475 patients as shown in Supplementary Table 2[32–41]. While four studies reported a higher incidence of CR-POPF with closed suction drainage[35,37,39,41], others employing gravity drainage showed no significant difference in complication rates. Secondary outcomes indicated slightly more adverse events with active closed suction drainage but no notable impact on 30-day postoperative mortality, overall morbidity, or re-intervention rates. These findings suggest that the choice of drainage method has limited effect on specific outcomes in pancreatic surgery.

A

Ref.	Drain Events	Drain Total	No drain Events	No drain Total	Weight (common)	Weight (random)	Odds ratio MH, Fixed + Random, 95%CI
Van Buren *et al* [10]	21	68	14	69	2.0%	7.9%	1.76 (0.8-3.83)
Mc Millan *et al* [18]	21	68	14	69	2.0%	7.9%	1.76 (0.8-3.83)
Witzigmann *et al* [20]	24	202	11	193	2.1%	8.1%	2.23 (1.06-4.69)
Addison *et al* [21]	837	6666	93	917	30.0%	10.6%	1.27 (1.01-1.60)
Adham *et al* [4]	21	130	14	112	2.7%	8.2%	1.35 (0.65-2.80)
Brubaker *et al* [22]	328	2749	49	146	17.2%	10.1%	0.27 (0.19-0.39)
Correa-Gallego *et al* [8]	104	386	59	353	9.5%	10.1%	1.84 (1.28-2.63)
Heslin *et al* [24]	3	51	1	38	0.2%	2.5%	2.31 (0.23-23.14)
Mehta *et al* [25]	61	251	48	458	5.4%	9.9%	2.74 (1.81-4.15)
Zaghal *et al* [26]	1151	5997	85	861	25.2%	10.6%	2.17 (1.72-2.74)
Kunstman *et al* [27]	12	53	4	53	0.7%	5.7%	3.59 (1.07-11.97)
Nickel *et al* [30]	29	133	18	133	3.0%	8.6%	1.78 (0.93-3.40)
Total (common effect, 95%CI)		**16754**		**3402**	**100.0%**	**--**	**1.53 (1.36-1.73)**
Total (random effect, 95%CI)					**--**	**100.0%**	**1.58 (1.04-2.38)**

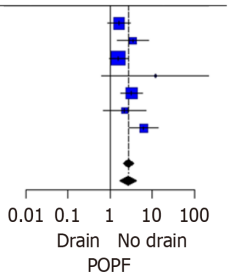
Heterogeneity: $Tau^2 = 0.4044$; $Chi^2 = 111.29$, df = 11 (***P < 0.01***); I^2 = ***90%***



B

Ref.	Drain Events	Drain Total	No drain Events	No drain Total	Weight (common)	Weight (random)	Odds ratio MH, Fixed + Random, 95%CI
Van Buren *et al* [19]	31	174	20	170	21.5%	18.7%	1.63 (0.89-2.98)
Behman *et al* [6]	24	116	8	116	8.2%	14.1%	3.52 (1.51-8.22)
Correa-Gallego *et al* [8]	42	154	38	196	31.4%	21.0%	1.56 (0.94-2.57)
Paulus *et al* [9]	6	39	0	30	0.6%	2.2%	11.84 (0.64-219.01)
Mangieri *et al* [29]	191	985	12	173	21.3%	18.7%	3.23 (1.76-5.92)
Nickel *et al* [30]	10	56	5	56	5.3%	9.9%	2.22 (0.71-6.97)
Van Bodegraven *et al* [31]	182	805	7	158	11.7%	15.4%	6.30 (2.90-13.69)
Total (common effect, 95%CI)	**2329**		**899**		**100.0%**	**--**	**2.74 (2.10-3.58)**
Total (random effect, 95%CI)					**--**	**100.0%**	**2.71 (1.73-4.24)**

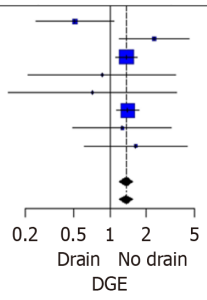
Heterogeneity: $Tau^2 = 0.0.1821$; $Chi^2 = 13.22$, df = 6 (***P < 0.04***); I^2 = ***55%***



C

Ref.	Drain Event	Drain Total	No drain Events	No drain Total	Weight (common)	Weight (random)	Odds ratio MH, Fixed + Random, 95%CI
Van Buren *et al* [10]	16	68	26	69	5.0%	3.3%	0.51 (0.24-1.07)
Witzigmann *et al* [20]	31	202	14	193	3.0%	4.1%	2.32 (1.19-4.51)
Addison *et al* [21]	1173	6666	124	917	45.2%	45.4%	1.37 (1.12-1.67)
Adham *et al* [4]	4	130	4	112	1.0%	0.9%	0.86 (0.21-3.51)
Chetana lim *et al* [23]	3	27	4	27	0.9%	0.7%	0.72 (0.14-3.57)
Zaghal *et al* [26]	1055	5997	114	861	41.3%	41.7%	1.40 (1.14-1.72)
Kunstman *et al* [27]	12	53	10	53	1.9%	2.0%	1.26 (0.49-3.23)
Nickel *et al* [30]	11	133	7	133	1.6 %	1.9%	1.62 (0.61-4.32)
Total (common effect, 95%CI)		**13276**		**2365**	**100.0%**	**--**	**1.36 (1.19-1.55)**
Total (random effect, 95%CI)					**--**	**100.0%**	**1.35 (1.18-1.55)**

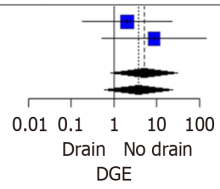
Heterogeneity: $Tau^2 = 0$; $Chi^2 = 10.45$, df = 7 (***P = 0.16***); I^2 = ***33%***



D

Ref.	Drain Events	Drain Total	No drain Events	No drain Total	Weight (common)	Weight (random)	Odds ratio MH, Fixed + Random, 95%CI
Nickel *et al* [30]	2	56	1	56	54.3%	57.2%	2.04 (0.18-23.13)
Van Bodegraven *et al* [31]	21	805	0	158	45.7%	42.8%	8.69 (0.52-144.16)
Total (common effect, 95%CI)		**861**		**214**	**100.0%**	**--**	**5.08 (0.84-30.81)**
Total (random effect, 95%CI)					**--**	**100.0%**	**3.79 (0.60-23.80)**

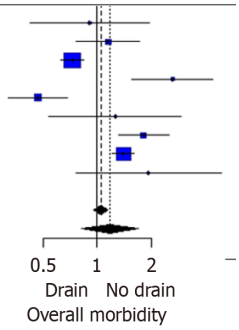
Heterogeneity: $Tau^2 = 0$; $Chi^2 = 0.59$, df = 1 (***P = 0.44***); I^2 = ***0%***



E

Ref.	Drain Events	Drain Total	No drain Events	No drain Total	Weight (common)	Weight (random)	Odds ratio MH, Fixed + Random, 95%CI
Van Buren *et al* [10]	50	68	52	69	1.4%	8.7%	0.91 (0.42-1.96)
Witzigmann *et al* [20]	127	202	115	193	4.5%	12.1%	1.15 (0.77-1.72)
Addison *et al* [21]	2199	6666	358	917	43.9%	13.9%	0.73 (0.63-0.84)
Adham *et al* [4]	83	130	45	112	1.8%	11.0%	2.63(1.56-4.42)
Brubaker *et al* [22]	1568	2749	108	146	9.0%	12.3%	0.47 (0.32-0.68)
Heslin *et al* [24]	23	51	15	38	1.0%	8.0%	1.26 (0.54-2.96)
Mehta *et al* [25]	171	251	248	458	5.7%	12.8%	1.81 (1.31-2.50)
Zaghal *et al* [26]	2969	5997	355	861	32.1%	13.9%	1.40 (1.21-1.62)
Kunstman *et al* [27]	44	53	38	53	0.7%	7.4%	1.93 (0.76-4.91)
Total (common effect, 95%CI)		**16167**		**2847**	**100.0%**	**--**	**1.05 (0.96-1.15)**
Total (random effect, 95%CI)					**--**	**100.0%**	**1.18 (0.82-1.70)**

Heterogeneity: $Tau^2 = 0.2451$; $Chi^2 = 83.18$, df = 8 (***P < 0.01***); I^2 = ***90%***



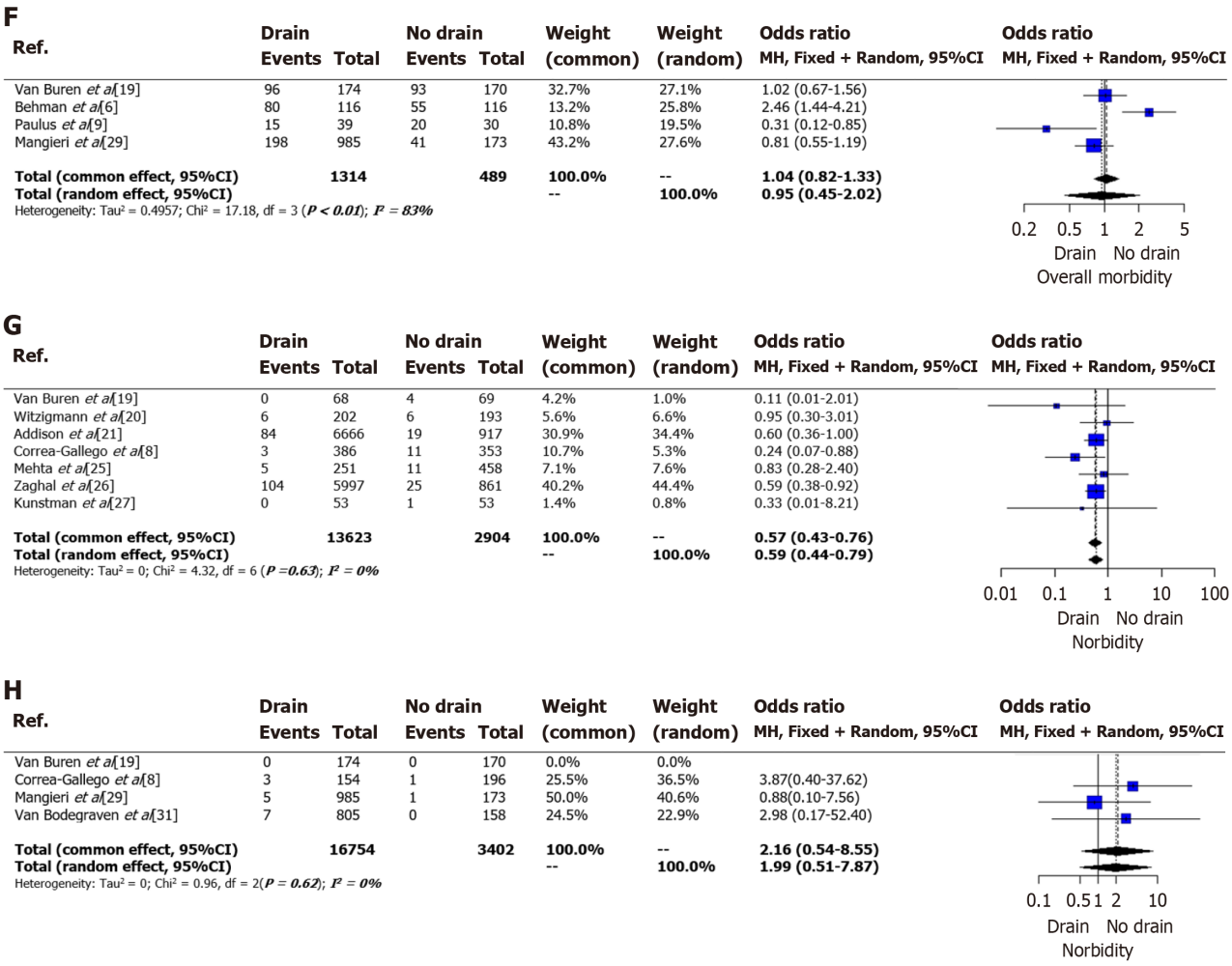


Figure 2 Forest plot. A: Forest plot showing the association of clinically relevant postoperative pancreatic fistula (CR-POPF) incidence between the drain and no drain groups among patients who underwent pancreaticoduodenectomy (PD); B: Forest plot showing the association of CR-POPF between the drain and no drain groups among patients who underwent DP; C: Forest plot showing the association of delayed gastric emptying (DGE) between the drain and no drain groups among patients who underwent PD; D: Forest plot showing the association of DGE between the drain and no drain groups among patients who underwent DP; E: Forest plot showing the association of overall morbidity between the drain and no drain groups among patients who underwent PD; F: Forest plot showing the association of overall morbidity between the drain and no drain groups among patients who underwent DP; G: Forest plot shows the association of mortality between the drain and no drain groups among patients who underwent PD; H: Forest plot shows the association of mortality between the drain and no drain groups among patients who underwent DP. POPF: Postoperative pancreatic fistula; DGE: Delayed gastric emptying.

Early and late drain removal

A review of sixteen studies, including five RCTs and eleven retrospective cohort studies, focused on the timing of drain removal following pancreatic surgery as shown in Supplementary Table 3[13,42–56]. Definitions of "early removal" varied, with most studies considering removal within 3 days. Late removal (> 5 days) was associated with higher surgical complication rates, though not statistically significant. Delayed removal correlated with increased rates of CR-POPF, bile leak, Clavien Dindo grade ≥ 3 complications, reoperations, readmissions, and 30-day mortality. These findings highlight the importance of timely drain removal for better postoperative outcomes in pancreatic surgery.

DISCUSSION

The historical tradition of employing prophylactic peritoneal drainage following gastrointestinal surgery, epitomized by the adage "when in doubt, drain", lacks compelling contemporary data validating its efficacy[57]. While drains placed near anastomoses serve to remove pancreatic juice or bile and can indicate complications, they come with associated risks such as infections, abdominal pain, and prolonged hospital stays. Despite their routine use for preventing POPFs, the clinical impact can still be significant, and timely identification is crucial for mitigating severe complications[33]. Thus, the ongoing debate within the surgical community questions the necessity of prophylactic drain placement and emphasizes the need for evidence-based practices in this regard.

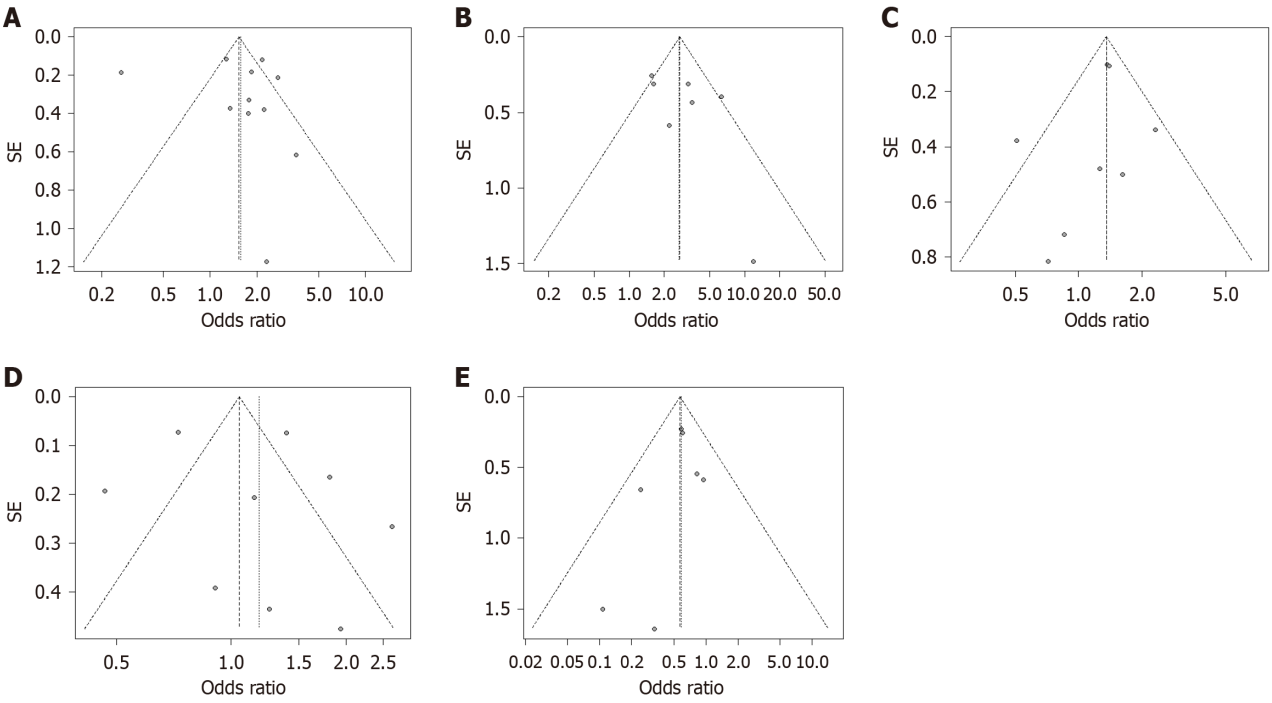


Figure 3 Funnel plot. A: Funnel plot of clinically relevant postoperative pancreatic fistula (CR-POPF) between the drain and no-drain groups among patients who underwent pancreaticoduodenectomy (PD). Egger test result: T = 0.31, df = 10, *P* value = 0.7657; B: Funnel plot of CR-POPF between the drain and no-drain groups among patients who underwent DP; C: Funnel plot of delayed gastric emptying between the drain and no drain groups among patients who underwent PD; D: Funnel plot of overall morbidity between the drain and no drain groups among patients who underwent PD; E: Funnel plot of mortality between the drain and no-drain groups among those who have undergone PD.

Intra-abdominal drain and without drain

In our comprehensive review, comparing outcomes between patients with and without drainage after pancreatic resection revealed significant differences. The drainage cohort exhibited higher rates of CR-POPFs and DGE, along with prolonged hospital stays and increased intervention requirements. Despite higher rates of CR-POPFs and DGE in the drainage cohort, this group exhibited a lower mortality rate. The presence of a drain allows for early detection and effective management of complications, preventing severe outcomes like sepsis and organ failure. Consequently, the benefits of early intervention in the drainage group have likely contributed to the observed reduction in mortality. Subgroup analysis of PD and DP revealed consistent findings indicating increased risks of CR-POPFs in both PD and DP groups, and increased DGE specifically in the PD group. These findings underscore the potential disadvantages of routine drainage in pancreatic surgery.

A meta-analysis of 15290 patients from ten studies found a higher incidence of CR-POPF in the drainage group compared to the no-drainage group[58]. However, there was no substantial correlation between drainage and DGE. Subgroup analyses for PD and DP showed comparable outcomes. Additionally, comprehensive analysis of eleven studies on postoperative complications, including PPH, intra-abdominal abscess, wound infection, and reoperation, revealed no notable differences between the groups[4,7,8,16,19,20,24,27,29,31,32]. However, the group with drains exhibited a significantly higher rate of readmission compared to the group without drain[58].

Conflicting findings from recent studies suggest varied impacts of drainage in pancreatic surgery. Brubaker *et al*[22] found higher rates of POPF, reoperation, and serious morbidity in patients without drainage, while Fisher *et al*[16] reported increased incidences of POPF, DGE, and readmission with drain usage. Other studies, such as those by Paulus *et al*[9] and Van Buren *et al*[10], found no significant differences in outcomes with or without drainage. Behrman *et al*[6] and Mangieri *et al*[29] observed increased morbidity and readmission rates associated with drain placement, and Liu *et al*[58] suggested a potential elevation in POPF incidence with use of drainage. These findings suggest caution in routine drainage employment during pancreatic resection.

Active and passive drainage

Patients undergoing surgical drain placement commonly experience two main drainage methods: (1) Closed-suction drains (CSDs); and (2) Passive gravity drains. CSDs function by generating a negative pressure gradient, ensuring continuous suction within the abdominal cavity irrespective of the patient's position. In contrast, gravity drainage devices establish a route for fluid extraction by leveraging the pressure difference between intra-abdominal and atmospheric pressure. In our review, we found significant differences between CSDs, and passive gravity drains for patients undergoing pancreatic resection. CSDs were associated with higher rates of CR-POPF and DGE, while passive gravity drains led to longer hospital stays and a greater need for intervention but lower mortality. Čečka *et al*[34] found no significant differences in rates of CR-POPF or overall morbidity between groups using different drainage methods after

PD. Additionally, there were no notable variations in reoperation rate, readmission rate, length of hospital stay, or incidence of post-pancreatectomy hemorrhage. Lee *et al*[32] demonstrated that utilizing a closed-suction drainage system with an external pancreatic duct stent led to a significant reduction in CR-POPF following PD. The system's negative pressure effectively diverted pancreatic juice away from the anastomotic site, potentially enhancing healing and long-term patency of the pancreatic duct.

Veziant *et al*[3] found no significant differences in outcomes between active and passive drainage methods, including rates of CR-POPF, overall morbidity, and length of hospital stay. Similarly, Aumont *et al*[35] reported that gravity drainage was independently associated with lower rates of CR-POPF, DGE , and readmission following PD. Overall, the literature suggests that the choice of drainage method does not significantly impact postoperative outcomes, particularly after PD, and may be left to surgeon preference.

Early and late drain removal

The absence of a universally agreed-upon definition for early removal of abdominal drainage following pancreatic surgery has led to a typical interval ranging from 3 days to 5 days. Late removal is generally considered when drains are in place for at least five days. In our systematic review, we found significant differences between early and late removal of abdominal drainage following pancreatic surgery. Late removal (≥ 5 days) was associated with higher rates of complications such as CR-POPF, DGE, severe morbidity, prolonged hospital stays, and increased need for intervention and readmission. However, mortality rates did not differ notably. Therefore, if drainage is necessary, early removal within three days seems to offer more benefits.

Xourafas *et al*[50] found that early drain removal in patients undergoing PD, especially those with POD1 amylase levels of 5000, was associated with improved perioperative outcomes regardless of the Fistula Risk Score (FRS). Both high-risk and low-risk modified FRS patients showed reduced rates of CR-POPF, shorter hospital stays, and overall morbidity with early drain removal. In the RCT by Bassi *et al*[42] which included both PD and DP, the results posed some interpretative challenges, despite the notable reduction in POPF and overall morbidity associated with early drain removal. Conversely, Dembinski *et al*[44] focusing on PD patients did not show statistically significant differences between groups, although there was a trend toward reduced rates of complications and shorter hospital stays with early drain removal, potentially limited by statistical power of the study. Overall, while the definitive benefits of early drain removal remain uncertain, evidence suggests it may facilitate earlier recovery without increasing complications.

The meta-analysis faced limitations due to predominantly nonrandomized studies, potentially biasing results. Several studies lacked comprehensive data on secondary outcomes. High heterogeneity, indicated by I^2 statistic and low *P*-value, urges cautious interpretation. Further well-designed randomized trials with robust data collection are crucial to enhance understanding of intra-abdominal drains in pancreatic surgery.

CONCLUSION

After a comprehensive review of 48 articles encompassing patients with PD and DP, our study refrained from making specific recommendations regarding the routine use of drainage. However, an increasing body of evidence suggests that the routine placement of primary drains is not mandatory. Our systematic review does not yield a definitive recommendation concerning the choice between active suction or passive gravity drainage following pancreatic resection. The choice of the drainage method can be at the discretion of the surgeon. Furthermore, our findings support the safe practice of early drain removal for patients undergoing pancreatic surgery which may decrease the occurrence of post operative pancreatic fistula and mitigate the associated morbidity and mortality. Recognizing the potential contribution of intra-abdominal drainage to increased morbidity, further research should be done in this area. This approach will aid in refining the guidelines to optimize drainage practices, balancing the benefits and risks, and promoting evidence-based decision-making in pancreatic surgery.

ACKNOWLEDGEMENTS

We extend our sincere gratitude to Naga KG, John AG, Anto JV and Yadav S for their invaluable contributions and support in this study. Their expertise and dedication have been instrumental in shaping our research, and we are deeply appreciative of their guidance and collaboration throughout the project.

FOOTNOTES

Author contributions: Kodali R contributed to the design and implementation of the study and the writing of the manuscript; Parasar K contributed to the design of the study and the revision of the manuscript; Anand U and Singh NB contributed to the performance of the research; Kant K and Anwar S contributed to the quality and professional revision; Karthikeyan V contributed to the statistical analyses; Arora A contributed to the writing of the manuscript; Saha B and Wadaskar S contributed to the quality and professional revision and the writing of the manuscript; all of the authors read and approved the final version of the manuscript to be published.

Conflict-of-interest statement: All authors declare no conflicts of interest regarding this article/research publication. No financial, personal, or professional relationships with organizations or entities that could influence or appear to influence the content presented.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Country of origin: India

ORCID number: Rohith Kodali 0000-0003-3202-1896; Kunal Parasar 0000-0002-9189-2539; Utpal Anand 0000-0003-0653-4129; Basant Narayan Singh 0000-0003-2966-2211; Kislay Kant 0000-0001-8406-2134; Abhishek Arora 0000-0002-0956-4887; Saad Anwar 0000-0002-3803-0155; Bijit Saha 0009-0003-2810-1786; Siddhali Wadaskar 0009-0004-6424-2145.

S-Editor: Luo ML
L-Editor: A
P-Editor: Zheng XM

REFERENCES

1 **Pedrazzoli S**. Pancreatoduodenectomy (PD) and postoperative pancreatic fistula (POPF): A systematic review and analysis of the POPF-related mortality rate in 60,739 patients retrieved from the English literature published between 1990 and 2015. *Medicine (Baltimore)* 2017; **96**: e6858 [PMID: 28489778 DOI: 10.1097/MD.0000000000006858]
2 **Büchler MW**, Wagner M, Schmied BM, Uhl W, Friess H, Z'graggen K. Changes in morbidity after pancreatic resection: toward the end of completion pancreatectomy. *Arch Surg* 2003; **138**: 1310-1314; discussion 1315 [PMID: 14662530 DOI: 10.1001/archsurg.138.12.1310]
3 **Veziant J**, Selvy M, Buc E, Slim K. Evidence-based evaluation of abdominal drainage in pancreatic surgery. *J Visc Surg* 2021; **158**: 220-230 [PMID: 33358121 DOI: 10.1016/j.jviscsurg.2020.11.001]
4 **Adham M**, Chopin-Laly X, Lepilliez V, Gincul R, Valette PJ, Ponchon T. Pancreatic resection: drain or no drain? *Surgery* 2013; **154**: 1069-1077 [PMID: 23876363 DOI: 10.1016/j.surg.2013.04.017]
5 **Čečka F**, Loveček M, Jon B, Skalický P, Šubrt Z, Neoral Č, Ferko A. Intra-abdominal drainage following pancreatic resection: A systematic review. *World J Gastroenterol* 2015; **21**: 11458-11468 [PMID: 26523110 DOI: 10.3748/wjg.v21.i40.11458]
6 **Behrman SW**, Zarzaur BL, Parmar A, Riall TS, Hall BL, Pitt HA. Routine drainage of the operative bed following elective distal pancreatectomy does not reduce the occurrence of complications. *J Gastrointest Surg* 2015; **19**: 72-79; discussion 79 [PMID: 25115324 DOI: 10.1007/s11605-014-2608-z]
7 **Conlon KC**, Labow D, Leung D, Smith A, Jarnagin W, Coit DG, Merchant N, Brennan MF. Prospective randomized clinical trial of the value of intraperitoneal drainage after pancreatic resection. *Ann Surg* 2001; **234**: 487-493; discussion 493 [PMID: 11573042 DOI: 10.1097/00000658-200110000-00008]
8 **Correa-Gallego C**, Brennan MF, D'angelica M, Fong Y, Dematteo RP, Kingham TP, Jarnagin WR, Allen PJ. Operative drainage following pancreatic resection: analysis of 1122 patients resected over 5 years at a single institution. *Ann Surg* 2013; **258**: 1051-1058 [PMID: 23360918 DOI: 10.1097/SLA.0b013e3182813806]
9 **Paulus EM**, Zarzaur BL, Behrman SW. Routine peritoneal drainage of the surgical bed after elective distal pancreatectomy: is it necessary? *Am J Surg* 2012; **204**: 422-427 [PMID: 22579230 DOI: 10.1016/j.amjsurg.2012.02.005]
10 **Van Buren G 2nd**, Bloomston M, Hughes SJ, Winter J, Behrman SW, Zyromski NJ, Vollmer C, Velanovich V, Riall T, Muscarella P, Trevino J, Nakeeb A, Schmidt CM, Behrns K, Ellison EC, Barakat O, Perry KA, Drebin J, House M, Abdel-Misih S, Silberfein EJ, Goldin S, Brown K, Mohammed S, Hodges SE, McElhany A, Issazadeh M, Jo E, Mo Q, Fisher WE. A randomized prospective multicenter trial of pancreaticoduodenectomy with and without routine intraperitoneal drainage. *Ann Surg* 2014; **259**: 605-612 [PMID: 24374513 DOI: 10.1097/SLA.0000000000000460]
11 **Nitsche U**, Müller TC, Späth C, Cresswell L, Wilhelm D, Friess H, Michalski CW, Kleeff J. The evidence based dilemma of intraperitoneal drainage for pancreatic resection - a systematic review and meta-analysis. *BMC Surg* 2014; **14**: 76 [PMID: 25291982 DOI: 10.1186/1471-2482-14-76]
12 **Cyr DP**, Truong JL, Lam-McCulloch J, Cleary SP, Karanicolas PJ. Canadian practice patterns for pancreaticoduodenectomy. *Can J Surg* 2015; **58**: 121-127 [PMID: 25799248 DOI: 10.1503/cjs.011714]
13 **Villafane-Ferriol N**, Baugh KA, McElhany AL, Van Buren G 2nd, Fang A, Tashakori EK, Reyes JEM, Cao HST, Silberfein EJ, Massarweh N, Hsu C, Barakat O, Schmidt C, Zyromski NJ, Dillhoff M, Villarreal JA, Fisher WE. Evidence Versus Practice in Early Drain Removal After Pancreatectomy. *J Surg Res* 2019; **236**: 332-339 [PMID: 30694774 DOI: 10.1016/j.jss.2018.11.048]
14 **Zhang W**, He S, Cheng Y, Xia J, Lai M, Cheng N, Liu Z. Prophylactic abdominal drainage for pancreatic surgery. *Cochrane Database Syst Rev* 2018; **6**: CD010583 [PMID: 29928755 DOI: 10.1002/14651858.CD010583.pub4]
15 **Page MJ**, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021; **372**: n71 [PMID: 33782057 DOI: 10.1136/bmj.n71]
16 **Fisher WE**, Hodges SE, Silberfein EJ, Artinyan A, Ahern CH, Jo E, Brunicardi FC. Pancreatic resection without routine intraperitoneal drainage. *HPB (Oxford)* 2011; **13**: 503-510 [PMID: 21689234 DOI: 10.1111/j.1477-2574.2011.00331.x]
17 **Jeekel J**. No abdominal drainage after Whipple's procedure. *Br J Surg* 1992; **79**: 182 [PMID: 1348202 DOI: 10.1002/bjs.1800790237]
18 **McMillan MT**, Fisher WE, Van Buren G 2nd, McElhany A, Bloomston M, Hughes SJ, Winter J, Behrman SW, Zyromski NJ, Velanovich V,

Brown K, Morgan KA, Vollmer C. The value of drains as a fistula mitigation strategy for pancreatoduodenectomy: something for everyone? Results of a randomized prospective multi-institutional study. *J Gastrointest Surg* 2015; **19**: 21-30; discussion 30 [PMID: 25183409 DOI: 10.1007/s11605-014-2640-z]

19 **Van Buren G 2nd**, Bloomston M, Schmidt CR, Behrman SW, Zyromski NJ, Ball CG, Morgan KA, Hughes SJ, Karanicolas PJ, Allendorf JD, Vollmer CM Jr, Ly Q, Brown KM, Velanovich V, Winter JM, McElhany AL, Muscarella P 2nd, Schmidt CM, House MG, Dixon E, Dillhoff ME, Trevino JG, Hallet J, Coburn NSG, Nakeeb A, Behrns KE, Sasson AR, Ceppa EP, Abdel-Misih SRZ, Riall TS, Silberfein EJ, Ellison EC, Adams DB, Hsu C, Tran Cao HS, Mohammed S, Villafañe-Ferriol N, Barakat O, Massarweh NN, Chai C, Mendez-Reyes JE, Fang A, Jo E, Mo Q, Fisher WE. A Prospective Randomized Multicenter Trial of Distal Pancreatectomy With and Without Routine Intraperitoneal Drainage. *Ann Surg* 2017; **266**: 421-431 [PMID: 28692468 DOI: 10.1097/SLA.0000000000002375]

20 **Witzigmann H**, Diener MK, Kienkötter S, Rossion I, Bruckner T, Bärbel Werner, Pridöhl O, Radulova-Mauersberger O, Lauer H, Knebel P, Ulrich A, Strobel O, Hackert T, Büchler MW. No Need for Routine Drainage After Pancreatic Head Resection: The Dual-Center, Randomized, Controlled PANDRA Trial (ISRCTN04937707). *Ann Surg* 2016; **264**: 528-537 [PMID: 27513157 DOI: 10.1097/SLA.0000000000001859]

21 **Addison P**, Nauka PC, Fatakhova K, Amodu L, Kohn N, Rodriguez Rilo HL. Impact of Drain Placement and Duration on Outcomes After Pancreaticoduodenectomy: A National Surgical Quality Improvement Program Analysis. *J Surg Res* 2019; **243**: 100-107 [PMID: 31170551 DOI: 10.1016/j.jss.2019.04.071]

22 **Brubaker LS**, Casciani F, Fisher WE, Wood AL, Cagigas MN, Trudeau MT, Parikh VJ, Baugh KA, Asbun HJ, Ball CG, Behrman SW, Berger AC, Bloomston MP, Callery MP, Christein JD, Fernandez-Del Castillo C, Dillhoff ME, Dixon E, House MG, Hughes SJ, Kent TS, Kunstman JW, Wolfgang CL, Zureikat AH, Vollmer CM Jr, Van Buren G 2nd. A risk-adjusted analysis of drain use in pancreaticoduodenectomy: Some is good, but more may not be better. *Surgery* 2022; **171**: 1058-1066 [PMID: 34433515 DOI: 10.1016/j.surg.2021.07.026]

23 **Lim C**, Dokmak S, Cauchy F, Aussilhou B, Belghiti J, Sauvanet A. Selective policy of no drain after pancreaticoduodenectomy is a valid option in patients at low risk of pancreatic fistula: a case-control analysis. *World J Surg* 2013; **37**: 1021-1027 [PMID: 23412469 DOI: 10.1007/s00268-013-1947-3]

24 **Heslin MJ**, Harrison LE, Brooks AD, Hochwald SN, Coit DG, Brennan MF. Is intra-abdominal drainage necessary after pancreaticoduodenectomy? *J Gastrointest Surg* 1998; **2**: 373-378 [PMID: 9841995 DOI: 10.1016/s1091-255x(98)80077-2]

25 **Mehta VV**, Fisher SB, Maithel SK, Sarmiento JM, Staley CA, Kooby DA. Is it time to abandon routine operative drain use? A single institution assessment of 709 consecutive pancreaticoduodenectomies. *J Am Coll Surg* 2013; **216**: 635-642; discussion 642 [PMID: 23521944 DOI: 10.1016/j.jamcollsurg.2012.12.040]

26 **Zaghal A**, Tamim H, Habib S, Jaafar R, Mukherji D, Khalife M, Mailhac A, Faraj W. Drain or No Drain Following Pancreaticoduodenectomy: The Unsolved Dilemma. *Scand J Surg* 2020; **109**: 228-237 [PMID: 30931801 DOI: 10.1177/1457496919840960]

27 **Kunstman JW**, Starker LF, Healy JM, Salem RR. Pancreaticoduodenectomy Can Be Performed Safely with Rare Employment of Surgical Drains. *Am Surg* 2017; **83**: 265-273 [PMID: 28316311]

28 **El Khoury R**, Kabir C, Maker VK, Banulescu M, Wasserman M, Maker AV. Do Drains Contribute to Pancreatic Fistulae? Analysis of over 5000 Pancreatectomy Patients. *J Gastrointest Surg* 2018; **22**: 1007-1015 [PMID: 29435899 DOI: 10.1007/s11605-018-3702-4]

29 **Mangieri CW**, Kuncewitch M, Fowler B, Erali RA, Moaven O, Shen P, Clark CJ. Surgical drain placement in distal pancreatectomy is associated with an increased incidence of postoperative pancreatic fistula and higher readmission rates. *J Surg Oncol* 2020; **122**: 723-728 [PMID: 32614999 DOI: 10.1002/jso.26072]

30 **Nickel F**, Lang F, Kowalewski KF, Haney CM, Menrath M, Berchtold C, Hoffmann K, Loos M, Mehrabi A, Probst P, Schmidt T, Schneider M, Diener MK, Strobel O, Müller-Stich BP, Hackert T. Pancreatic surgery with or without drainage: propensity score-matched study. *Br J Surg* 2022; **109**: 739-745 [PMID: 35578893 DOI: 10.1093/bjs/znac123]

31 **van Bodegraven EA**, De Pastena M, Vissers FL, Balduzzi A, Stauffer J, Esposito A, Malleo G, Marchegiani G, Busch OR, Salvia R, van Hilst J, Bassi C, Besselink MG, Asbun HJ. Routine prophylactic abdominal drainage versus no-drain strategy after distal pancreatectomy: A multicenter propensity score matched analysis. *Pancreatology* 2022; **22**: 797-802 [PMID: 35690539 DOI: 10.1016/j.pan.2022.06.002]

32 **Lee SE**, Ahn YJ, Jang JY, Kim SW. Prospective randomized pilot trial comparing closed suction drainage and gravity drainage of the pancreatic duct in pancreaticojejunostomy. *J Hepatobiliary Pancreat Surg* 2009; **16**: 837-843 [PMID: 19730769 DOI: 10.1007/s00534-009-0171-x]

33 **Jiang H**, Liu N, Zhang M, Lu L, Dou R, Qu L. A Randomized Trial on the Efficacy of Prophylactic Active Drainage in Prevention of Complications after Pancreaticoduodenectomy. *Scand J Surg* 2016; **105**: 215-222 [PMID: 27528694 DOI: 10.1177/1457496916665543]

34 **Čečka F**, Jon B, Skalický P, Čermáková E, Neoral Č, Loveček M. Results of a randomized controlled trial comparing closed-suction drains versus passive gravity drains after pancreatic resection. *Surgery* 2018; **164**: 1057-1063 [PMID: 30082139 DOI: 10.1016/j.surg.2018.05.030]

35 **Aumont O**, Dupré A, Abjean A, Pereira B, Veziant J, Le Roy B, Pezet D, Buc E, Gagnière J. Does intraoperative closed-suction drainage influence the rate of pancreatic fistula after pancreaticoduodenectomy? *BMC Surg* 2017; **17**: 58 [PMID: 28511699 DOI: 10.1186/s12893-017-0257-3]

36 **Marchegiani G**, Perri G, Pulvirenti A, Sereni E, Azzini AM, Malleo G, Salvia R, Bassi C. Non-inferiority of open passive drains compared with closed suction drains in pancreatic surgery outcomes: A prospective observational study. *Surgery* 2018; **164**: 443-449 [PMID: 29903511 DOI: 10.1016/j.surg.2018.04.025]

37 **Lemke M**, Park L, Balaa FK, Martel G, Khalil JA, Bertens KA. Passive Versus Active Intra-Abdominal Drainage Following Pancreaticoduodenectomy: A Retrospective Study Using The American College of Surgeons NSQIP Database. *World J Surg* 2021; **45**: 554-561 [PMID: 33078216 DOI: 10.1007/s00268-020-05823-5]

38 **O'Grady J**, Sutton TL, Potter KC, Gilbert E, Pommier R, Mayo SC, Sheppard BC. The power of suction: Theory and practice in closed suction vs gravity drains and postoperative pancreatic fistulas. *Am J Surg* 2022; **224**: 737-741 [PMID: 35248372 DOI: 10.1016/j.amjsurg.2022.02.063]

39 **Hall BR**, Egr ZH, Krell RW, Padussis JC, Shostrom VK, Are C, Reames BN. Association of gravity drainage and complications following Whipple: an analysis of the ACS-NSQIP targeted database. *World J Surg Oncol* 2021; **19**: 118 [PMID: 33853623 DOI: 10.1186/s12957-021-02227-0]

40 **Kone LB**, Maker VK, Banulescu M, Maker AV. Should Drains Suck? A Propensity Score Analysis of Closed-Suction Versus Closed-Gravity Drainage After Pancreatectomy. *J Gastrointest Surg* 2021; **25**: 1224-1232 [PMID: 32394123 DOI: 10.1007/s11605-020-04613-7]

41 **Schmidt CM**, Choi J, Powell ES, Yiannoutsos CT, Zyromski NJ, Nakeeb A, Pitt HA, Wiebke EA, Madura JA, Lillemoe KD. Pancreatic fistula following pancreaticoduodenectomy: clinical predictors and patient outcomes. *HPB Surg* 2009; **2009**: 404520 [PMID: 19461951 DOI: 10.1155/2009/404520]

42 **Bassi C**, Molinari E, Malleo G, Crippa S, Butturini G, Salvia R, Talamini G, Pederzoli P. Early versus late drain removal after standard pancreatic resections: results of a prospective randomized trial. *Ann Surg* 2010; **252**: 207-214 [PMID: 20622661 DOI: 10.1097/SLA.0b013e3181e61e88]

43 **McMillan MT**, Malleo G, Bassi C, Butturini G, Salvia R, Roses RE, Lee MK, Fraker DL, Drebin JA, Vollmer CM Jr. Drain Management after Pancreatoduodenectomy: Reappraisal of a Prospective Randomized Trial Using Risk Stratification. *J Am Coll Surg* 2015; **221**: 798-809 [PMID: 26278037 DOI: 10.1016/j.jamcollsurg.2015.07.005]

44 **Dembinski J**, Mariette C, Tuech JJ, Mauvais F, Piessen G, Fuks D, Schwarz L, Truant S, Cosse C, Pruvot FR, Regimbeau JM. Early removal of intraperitoneal drainage after pancreatoduodenectomy in patients without postoperative fistula at POD3: Results of a randomized clinical trial. *J Visc Surg* 2019; **156**: 103-112 [PMID: 30713100 DOI: 10.1016/j.jviscsurg.2018.06.006]

45 **Dai M**, Liu Q, Xing C, Kleeff J, Liao Q, Guo J, Han X, Xu Q, Wang S. [Early drain removal after major pancreatectomy reduces postoperative complications: a single-center, randomized, controlled trial]. *Yixianbingxue Zazhi* 2020; **3**: 93-100 [DOI: 10.1097/jp9.0000000000000049]

46 **Dai M**, Liu Q, Xing C, Tian X, Cao F, Tang W, Lv S, Ma Y, Zhang D, Kleeff J, Yang Y, Liu R, He Q, Li F, Li G, Guo J, Liao Q, Zhao Y. Early Drain Removal is Safe in Patients With Low or Intermediate Risk of Pancreatic Fistula After Pancreaticoduodenectomy: A Multicenter, Randomized Controlled Trial. *Ann Surg* 2022; **275**: e307-e314 [PMID: 34117153 DOI: 10.1097/SLA.0000000000004992]

47 **Balzano G**, Zerbi A, Cristallo M, Di Carlo V. The unsolved problem of fistula after left pancreatectomy: the benefit of cautious drain management. *J Gastrointest Surg* 2005; **9**: 837-842 [PMID: 15985241 DOI: 10.1016/j.gassur.2005.01.287]

48 **Beane JD**, House MG, Ceppa EP, Dolejs SC, Pitt HA. Variation in Drain Management After Pancreatoduodenectomy: Early Versus Delayed Removal. *Ann Surg* 2019; **269**: 718-724 [PMID: 29064899 DOI: 10.1097/SLA.0000000000002570]

49 **Ven Fong Z**, Correa-Gallego C, Ferrone CR, Veillette GR, Warshaw AL, Lillemoe KD, Fernández-del Castillo C. Early Drain Removal--The Middle Ground Between the Drain Versus No Drain Debate in Patients Undergoing Pancreaticoduodenectomy: A Prospective Validation Study. *Ann Surg* 2015; **262**: 378-383 [PMID: 25563864 DOI: 10.1097/SLA.0000000000001038]

50 **Xourafas D**, Ejaz A, Tsung A, Dillhoff M, Pawlik TM, Cloyd JM. Population-Based Assessment of Selective Drain Placement During Pancreatoduodenectomy Using the Modified Fistula Risk Score. *J Am Coll Surg* 2019; **228**: 583-591 [PMID: 30586644 DOI: 10.1016/j.jamcollsurg.2018.12.007]

51 **Kawai M**, Tani M, Terasawa H, Ina S, Hirono S, Nishioka R, Miyazawa M, Uchiyama K, Yamaue H. Early removal of prophylactic drains reduces the risk of intra-abdominal infections in patients with pancreatic head resection: prospective study for 104 consecutive patients. *Ann Surg* 2006; **244**: 1-7 [PMID: 16794381 DOI: 10.1097/01.sla.0000218077.14035.a6]

52 **Seykora TF**, Liu JB, Maggino L, Pitt HA, Vollmer CM Jr. Drain Management Following Distal Pancreatectomy: Characterization of Contemporary Practice and Impact of Early Removal. *Ann Surg* 2020; **272**: 1110-1117 [PMID: 30943185 DOI: 10.1097/SLA.0000000000003205]

53 **Adachi T**, Kuroki T, Kitasato A, Hirabaru M, Matsushima H, Soyama A, Hidaka M, Takatsuki M, Eguchi S. Safety and efficacy of early drain removal and triple-drug therapy to prevent pancreatic fistula after distal pancreatectomy. *Pancreatology* 2015; **15**: 411-416 [PMID: 26073457 DOI: 10.1016/j.pan.2015.05.468]

54 **Linnemann RJA**, Patijn GA, van Rijssen LB, Besselink MG, Mungroop TH, de Hingh IH, Kazemier G, Festen S, de Jong KP, van Eijck CHJ, Scheepers JJG, van der Kolk M, Dulk MD, Bosscha K, Busch OR, Boerma D, van der Harst E, Nieuwenhuijs VB; Dutch Pancreatic Cancer Group. The role of abdominal drainage in pancreatic resection - A multicenter validation study for early drain removal. *Pancreatology* 2019; **19**: 888-896 [PMID: 31378583 DOI: 10.1016/j.pan.2019.07.041]

55 **Sakamoto T**, Yagyu Y, Uchinaka EI, Hanaki T, Miyatani K, Kihara K, Yamamoto M, Matsunaga T, Tokuyasu N, Honjo S, Fujiwara Y. Surgical Outcomes Following Early Drain Removal After Distal Pancreatectomy in Elderly Patients. *In Vivo* 2020; **34**: 2837-2843 [PMID: 32871822 DOI: 10.21873/invivo.12110]

56 **Yoon SJ**, Yoon SK, Jung JH, Han IW, Choi DW, Heo JS, Shin SH. Realistic Advantages of Early Surgical Drain Removal after Pancreatoduodenectomy: A Single-Institution Retrospective Study. *J Clin Med* 2021; **10**: 2716 [PMID: 34205447 DOI: 10.3390/jcm10122716]

57 **Pai D**, Sharma A, Kanungo R, Jagdish S, Gupta A. Role of abdominal drains in perforated duodenal ulcer patients: a prospective controlled study. *Aust N Z J Surg* 1999; **69**: 210-213 [PMID: 10075361 DOI: 10.1046/j.1440-1622.1999.01524.x]

58 **Liu X**, Chen K, Chu X, Liu G, Yang Y, Tian X. Prophylactic Intra-Peritoneal Drainage After Pancreatic Resection: An Updated Meta-Analysis. *Front Oncol* 2021; **11**: 658829 [PMID: 34094952 DOI: 10.3389/fonc.2021.658829]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 99403

DOI: 10.5662/wjm.v15.i3.99403

ISSN 2222-0682 (online)

SCIENTOMETRICS

Use of artificial intelligence in neurological disorders diagnosis: A scientometric study

Alaa Tarazi, Ahmad Aburrub, Mohammad Hijah

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B

Novelty: Grade B

Creativity or Innovation: Grade B

Scientific Significance: Grade B

P-Reviewer: Liu Y

Received: July 21, 2024

Revised: December 3, 2024

Accepted: December 23, 2024

Published online: September 20, 2025

Processing time: 227 Days and 10.1 Hours

Alaa Tarazi, Ahmad Aburrub, Mohammad Hijah, School of Medicine, University of Jordan, Amman 11942, Jordan

Corresponding author: Alaa Tarazi, MD, Doctor, School of Medicine, University of Jordan, Queen Rania St, Amman 11942, Jordan. alaatarazi11@gmail.com

Abstract

BACKGROUND

Artificial intelligence (AI) has become significantly integrated into healthcare, particularly in the diag-nosing of neurological disorders. This advancement has enabled neurologists and physicians to diagnose conditions more quickly and effectively, ultimately benefiting patients.

AIM

To explore the current status and key highlights of AI-related articles in dia-gnosing of neurological disorders.

METHODS

A systematic literature review was conducted in the Web of Science Core Collection database using the following strategy: TS = ("Artificial Intelligence" OR "Computational Intelligence" OR "Machine Learning" OR "AI") AND TS = ("Neurological disorders" OR "CNS disorder" AND "diagnosis"). The search was limited to articles and reviews. Microsoft Excel 2019 and VOSviewer were utilized to identify major contributors, including authors, institutions, countries, and journals. Additionally, VOSviewer was employed to analyze and visualize current trends and hot topics through network visualization maps.

RESULTS

A total of 276 publications from 2000 to 2024 were retrieved. The United States, India, and China emerged as the top contributors in this field. Major institutions included Johns Hopkins University, King's College London, and Harvard Medical School. The most prolific author was U. Rajendra Acharya from the University of Southern Queensland (Australia). Among journals, *IEEE Access*, *Scientific Reports*, and *Sensors* were the most productive, while *Frontiers in Neuroscience* led in total citations. Central topics in AI-related articles on neurological disorders diagnosis included Alzheimer's disease, Parkinson's disease, dementia, epilepsy, autism, attention deficit hyperactivity disorder, and their intersections with deep learning and AI.

CONCLUSION
Research on AI's role in diagnosing neurological disorders is becoming widely recognized for its growing importance. AI shows promise in diagnosing various neurological disorders, yet requires further improvement and extensive future research.

Key Words: Artificial intelligence; Machine learning; Neurological disorders; Diagnosis; Bibliometric analysis

Core Tip: Artificial intelligence's (AI) role in diagnosing neurological disorders has been increasingly recognized. We conducted a scientometric analysis to explore the current status of articles in this field and identify the most prolific contributors from various perspectives. Johns Hopkins University in the United States emerged as the leading institution, with the United States also leading overall productivity in this field. *IEEE Access* was noted as the top journal. Research highlights AI's effectiveness in diagnosing diverse neurological disorders, offering significant benefits for patients and healthcare providers. Continued advancements are expected in AI's role in neurological disorder diagnosis.

Citation: Tarazi A, Aburrub A, Hijah M. Use of artificial intelligence in neurological disorders diagnosis: A scientometric study. *World J Methodol* 2025; 15(3): 99403
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/99403.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.99403

INTRODUCTION

Artificial intelligence (AI) is the capability of computers to perform tasks typically requiring human intelligence[1]. AI and its various branches are being increasingly integrated into many aspects of business and society, including healthcare settings[2]. In healthcare, AI has generated a large amount of enthusiasm, especially through the development of precise machine-learning models[3]. Common applications of AI in healthcare include diagnosis, drug discovery, treatment, and enhancing doctor-patient communication[4]. Since AI is a relatively new technology, it is a vibrant area of research across all domains including medicine.

There is a growing interest in using AI to improve disease diagnosis[5]. Improvement in AI and computer vision show a good potential to significantly contribute to diagnostic specialties including radiology and pathology[6]. One of the most compelling applications of AI in healthcare is its ability to improve the accuracy of diagnosis and treatment. It can help healthcare providers in detecting symptoms earlier and more swiftly than many healthcare experts[7]. By using advanced branches of AI like machine learning and deep learning, AI can provide a more accurate understanding and predictions of disease behavior patterns; this would support clinical decision-making, enhance diagnostic accuracy, and reduce the physician's workload[8].

AI has earned significant interest over the past decade, leading to increased research in the field. The AI application in diagnosis has proven to be an impressive novel factor in the diagnosis of critical illnesses and the prediction of disease prognosis[9]. For instance, AI can assist physicians in distinguishing between common and rare neurological disorders [10], such as Pompe disease, which is treatable if diagnosed early despite its bad progressive nature[11]. This development has been driven by an extensive collaboration between neuroscientists and AI experts, aiming to push the boundaries of neurological diagnosis to achieve early detection, a critical factor for successful treatment or disease progression prevention. Targeted treatments resulting from early diagnosis contribute to improving the quality of life for individuals affected by various neurological conditions.

Furthermore, AI is also capable of continuous analysis of diverse information coming promptly from various data sources like wearable devices, facilitating early diagnosis and treatment recommendations based on real-time information and current guidelines[12]. Most studies use machine learning for prognosis or diagnosis, although its application in treatment improvement remains limited[13]. Notably, some neurological machine learning algorithms have achieved impressive accuracy and precision rates, such as the VGG-19 model achieving 99.48% accuracy in magnetic resonance imaging (MRI) image classification, which offers a great classification of raw images without the need for manual extraction[14]. Another remarkable achievement includes the support vector machine (SVM), accurately predicting the progression of Alzheimer's disease over a 4-year period with F1 scores of 88% for binary tasks and 72.8% for multitask scenarios[15]. These advancements underscore AI's potential to revolutionize neurological diagnosis and treatment, offering promising avenues for enhancing patient care and outcomes.

The main objective of this study is to conduct a comprehensive bibliometric analysis of AI applications in diagnosing neurological diseases, aiming to benefit both patients and healthcare providers. This review aims to serve as a foundational resource for anyone interested in the use of AI in neurological disease diagnosis, identifying key trends, top researchers, leading institutions, prominent countries, and frequently used keywords in this domain. By addressing these aspects, the study seeks to alleviate the challenges researchers face in navigating the literature on this topic. Ultimately, the insights gathered can advance the understanding, development, and dissemination of innovative diagnostic

approaches, thereby improving patient outcomes. Furthermore, this study intends to highlight areas requiring further attention and research efforts within the field.

MATERIALS AND METHODS

Data collection

Data were retrieved from the Web of Science Core Collection (WoSCC) database on June 12, 2024, using the search criteria "full record and cited references" and "plain text". This database was selected due to its curation of high-quality, peer-reviewed literature from around the world[16]. The search strategy used the formula TS = ("Artificial Intelligence" OR "Computational Intelligence" OR "Machine Learning" OR "AI") AND TS = ("Neurological disorders" OR "CNS disorders" AND "diagnosis"). Papers considered were limited to articles and reviews. To ensure the accurate inclusion of papers on the use of AI in diagnosing neurological disorders, all retrieved literature underwent screening based on their titles and abstracts. Any discrepancies were resolved through discussion until a consensus was reached.

The initial search yielded 471 articles, and after excluding entries outside the articles and reviews category, 381 articles remained. Subsequently, 276 articles were analyzed after excluding non-English articles and those unrelated to AI use in the diagnosis of neurological disorders. Detailed information about the screening process is illustrated in Figure 1.

Statistical analysis

Microsoft Excel 2019 and VOSviewer (Centre for Science and Technology Studies, Leiden University, The Netherlands) were used to analyze all 276 publications. Full records for all publications were systematically extracted from WoSCC, including bibliometric parameters such as title, keywords, authors, countries, institutions, journals, citations, and publication year.

VOSviewer (1.6.20) was used to identify the primary contributors such as prolific authors, countries, and institutions. Furthermore, it was utilized to conduct keyword co-occurrence analysis, offering a comprehensive exploration of the scholarly landscape in the field. VOSviewer is a bibliometric software known for creating visualization maps, which display clusters and density colors[17]. Its algorithm ensures that frequently occurring terms are represented by larger bubbles, while terms with high similarity are positioned close to each other[18]. After extracting the data from the database, it was saved in .txt format and imported into VOSviewer. Next, we selected the type of analysis (co-authorship, co-occurrence, citation, or bibliographic coupling) and determined the unit of analysis, which varies depending on the chosen method (such as authors, countries, institutions, keywords, or sources). Finally, the data was visualized and further processed.

Microsoft Excel was used to organize the articles, prepare tables, and create a trend chart showing annual publications using its charting tools. It was also employed for data screening after extracting the data from the WoSCC database. Additionally, Excel helped present information about countries, institutions, authors, and journals in an organized manner. The data analysis results from VOSviewer were exported to Excel, where they were organized and structured to create the final tables.

RESULTS

Publications among years

Over the past few years, there has been an increase in the number of studies investigating the role of AI in diagnosing neurological disorders. This upward trend was particularly noticeable between 2021 and 2024 (Figure 2), reaching its peak in 2023 with 65 publications.

Distribution of authors

A total of 1611 authors contributed to articles exploring AI's role in diagnosing neurological disorders. The most prolific author, U Rajendra Acharya, has published 5 documents and is affiliated with the University of Southern Queensland, Australia, boasting the highest H-index among the top ten most productive authors. Following closely, the next eight authors each have three publications related to AI in neurological disorder diagnosis, representing diverse affiliations. Norlinah Mohamed Ibrahim and Khairiyah Mohamad are affiliated with the University Kebangsaan Malaysia, while the remaining authors hail from various institutions across Malaysia, Kuwait, the United Kingdom, and the United States, as detailed in Table 1. Figure 3A, generated using VOSviewer, illustrates a map where authors are depicted as nodes; larger nodes indicate higher publication counts. It also visualizes collaboration networks among authors studying AI's role in diagnosing neurological disorders. For instance, Mohammad Iqbal Omar is prominently connected to other authors such as Khairiyah Mohamad and Norlinah Mohamed Ibrahim, reflecting active collaboration in this field.

Distribution of institutions

Table 2 presents the top ten institutions contributing to AI articles on diagnosing neurological disorders. Johns Hopkins University (United States) and King's College London (United Kingdom) led with 7 articles each (2.54%), achieving the highest citations: 244 and 419 respectively. Following closely is Harvard Medical School (United States) with 6 articles (2.17%). Among these top ten institutions, four are in the United States: Johns Hopkins University, Harvard Medical School, University of Pennsylvania, and Boston Children's Hospital. Figure 3B illustrates the network of collaborations

Table 1 Top ten authors of artificial intelligence articles in neurology disorders diagnosis

Rank	Authors	Documents	Country	H-index	Institute
1	U Rajendra Acharya	5	Australia	146	University of Southern Queensland
2	Norlinah Mohamed Ibrahim	3	Malaysia	39	University Kebangsaan Malaysia
3	Khairiyah Mohamad	3	Malaysia	-	University Kebangsaan Malaysia
4	M Murugappan	3	Kuwait	43	Kuwait College of Science and Technology
5	Mohammad Iqbal Omar	3	Malaysia	19	MERCY Malaysia
6	Ramaswamy Palaniappan	3	United Kingdom	39	University of Kent
7	Kenneth Sundaraj	3	Malaysia	30	Technical University of Malaysia, Malacca
8	Rjamanickam Yuvaraj	3	Malaysia	23	University Malaysia Perlis
9	Islem Rekik	3	United Kingdom	30	Imperial College London
10	Hojjat Adeli	2	United States	136	The Ohio State University

Table 2 Top ten institutions of artificial intelligence articles in neurology disorders diagnosis

Rank	Institute	Country	Documents	Percentage (*n* = 276)	Citations
1	Johns Hopkins University	United States	7	2.54%	244
2	King's College London	United Kingdom	7	2.54%	419
3	Harvard Medical School	United States	6	2.17%	34
4	Chinese Academy of Sciences	China	5	1.81%	131
5	University of Oxford	United Kingdom	5	1.81%	73
6	King Saudi University	Saudi Arabia	4	1.45%	40
7	University of Pennsylvania	United States	4	1.45%	132
8	University of Sao Paulo	Brazil	4	1.45%	37
9	Zhejiang University	China	4	1.45%	23
10	Boston Children's Hospital	United States	3	1.09%	4

between institutions. Collaborations occur more frequently between geographically closer institutions, such as Harvard Medical School and Boston Children's Hospital, or between Johns Hopkins University and the University of California San Francisco.

Distribution of countries

Researchers from 62 countries participated in studies within this field, as detailed in Table 3. The United States ranked first as the most contributing country with 77 documents (27.9% of the total), followed by India with 42 documents (15.22%), and China with 36 documents (13%). Figure 3C displays the top 10 contributing countries. Many collaborations are evident between countries such as the United States, England, India, and Germany.

Analysis of journals

Table 4 lists the top 10 active journals publishing articles on AI in the diagnosis of neurological disorders. The top 10 journals contributed 23.9% of the articles in the field. The three most prolific journals are *IEEE Access* with 12 articles, *Scientific Reports* with 10 articles, and *Sensors* with 8 articles. Regarding impact, *Frontiers in Neuroscience* ranks first with a total of 151 citations, followed by *Plos One* with 137 citations, and *Sensors* with 100 citations. Most journals are classified as Q1 or Q2, except for two journals-*Frontiers in Computational Neuroscience* and *Frontiers in Neurology*-which are classified as Q3 journals.

Analysis of hotspots

The keyword map is derived from the frequency of keyword occurrences in the literature. Table 5 displays the top 20 keywords with high occurrence frequencies in AI-related articles on neurological disorders diagnosis. The most frequently occurring keyword is "machine learning" with 91 occurrences, followed by "classification" with 63 occurrences, and 'EEG' with 47 occurrences, highlighting the significant role of AI in diagnosing neurological disorders. Figure 3D illustrates the keyword occurrence network map, with each cluster representing a distinct research hotspot. In this study, six clusters were identified. Keywords such as "Alzheimer's disease", "Machine learning", and "Artificial

Table 3 Top ten countries of artificial intelligence articles in neurology disorders diagnosis

Rank	Country	Documents	Percentages (*n*= 276)
1	United States	77	27.90%
2	India	42	15.22%
3	China	36	13.00%
4	United Kingdom	31	11.23%
5	Germany	22	7.97%
6	Italy	22	7.97%
7	Canada	15	5.43%
8	Australia	13	4.71%
9	Brazil	12	4.35%
10	Saudi Arabia	11	3.99%

Table 4 Top ten journals of artificial intelligence articles in neurology disorders diagnosis

Rank	Journal	Documents	Total citations	IF (JCR, 2024)	Quartile in category (JCR, 2024)
1	*IEEE Access*	12	78	3.4	Q2
2	*Scientific Reports*	10	96	3.8	Q1
3	*Sensors*	8	100	3.4	Q2
4	*Applied Sciences-Basel*	7	64	2.5	Q2
5	*Frontiers in Neuroscience*	7	151	3.2	Q2
6	*Biomedical Signal Processing and Control*	5	62	4.9	Q1
7	*Plos One*	5	137	2.9	Q1
8	*Diagnostics*	4	20	3.0	Q1
9	*Frontiers in Computational Neuroscience*	4	14	2.1	Q3
10	*Frontiers in Neurology*	4	13	2.7	Q3

IF: Impact factor; JCR: Journal Citation Reports.

intelligence" formed one cluster (red). Another cluster (green) included "Parkinson's disease", "Classification", and "Speech". Additionally, "EEG", "Epilepsy", and "Seizure detection" keywords were grouped in the blue cluster.

DISCUSSION

General

With the rapid evolution of AI and its use in healthcare, its application has significantly increased in recent years, particularly in diagnosing neurological disorders. Our study reveals that the number of published articles on this topic was relatively low between 2000 and 2019, but there has been a substantial increase in recent years. Articles published after 2019 constitute 81.2% of the total publications, indicating a surge in research during this period to explore advanced AI methods for supporting the diagnosis of neurological disorders[19]. AI has become increasingly crucial for neurologists as it enables the prediction of disease progression, facilitates adjustment of treatment plans, and ensures more accurate prognoses for patients[20].

Analysis on authors

The top three authors in this field were from Australia and Malaysia. Half of the top ten leading authors were from Malaysia, which can be attributed to the country's increased investment in education and research[21]. Malaysia's government and academic institutions have significantly bolstered research infrastructure and funding[22,23]. However, Malaysia was not among the top ten countries listed, and none of its institutions were among the top ten productive institutions. Regarding the H-index-a metric that assesses the quantity and quality of an author's publications-the most prolific author, U Rajendra Acharya (from Australia), had the highest H-index. Following him, Hojjat Adeli from the

Table 5 Top 20 keywords of artificial intelligence articles in neurology disorders diagnosis

Rank	Keyword	Occurrence
1	Machine learning	91
2	Classification	63
3	EEG	47
4	Alzheimer's-disease	46
5	Deep learning	44
6	Parkinsons-disease	33
7	Diagnosis	32
8	Artificial intelligence	27
9	Epilepsy	26
10	Neurological disorders	23
11	Brain	16
12	Disease	16
13	Children	15
14	Features	15
15	Functional connectivity	15
16	MRI	15
17	Prediction	14
18	Dementia	12
19	Feature extraction	12
20	Performance	11

MRI: Magnetic resonance imaging; EEG: Electroencephalogram.

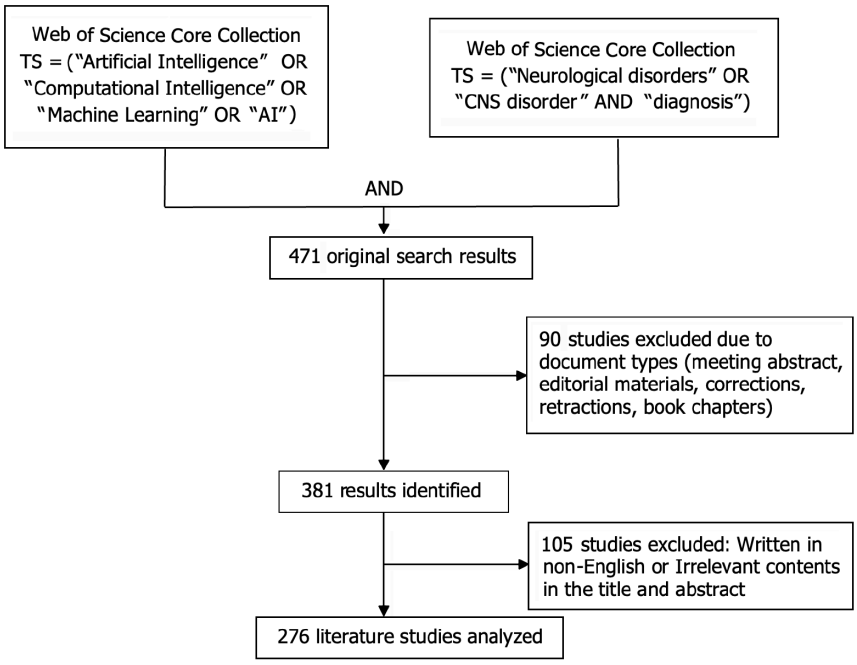


Figure 1 Flowchart of data screening. AI: Artificial Intelligence; CNS: Central nervous system.

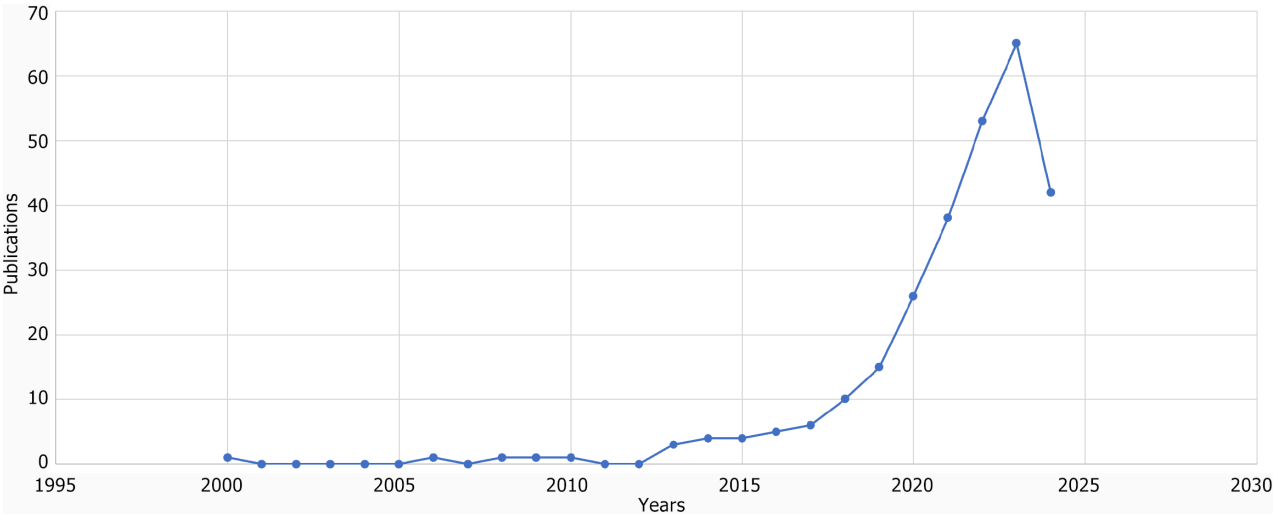


Figure 2 Number of publications among the years (2000-2024).

United States, despite being the least prolific among the top ten authors, had an H-index of 136, indicating his potentially higher impact and productivity compared to other authors.

Analysis of institutions & countries

Among the top ten productive institutions, four are located in the United States: Johns Hopkins University, Harvard Medical School, University of Pennsylvania, and Boston Children's Hospital. Together, these institutions contributed 7.2% of the total published literature. The United States has been identified as the most prolific country in articles on the role of AI in diagnosing neurological disorders. This observation is consistent with numerous bibliometric studies in healthcare and diagnostics, which consistently highlight the United States as a leading contributor in the field[7,9,24]. One of these studies also noted a significant presence of top institutions from the United States, corroborating our findings. This trend can be attributed to the United States's status as one of the wealthiest nations, with substantial investments and initiatives in advancing AI technologies[25]. Most of the top ten institutions and countries involved in such AI research in neurological disorders diagnosis are classified as high-income countries, which likely explains their extensive contributions, except for Brazil, classified as an upper-middle-income country, and India, classified as a lower-middle-income country according to the latest World Bank report (2024). The United States and the United Kingdom, which rank among the top five most productive countries in AI neurological research, have the most productive institutions in this field. This can largely be attributed to their substantial investments in research funding, their robust policy support for scientific innovation, and their commitment to fostering academic excellence. Both countries allocate significant budgets to support AI research, which enables them to attract top-tier researchers and facilitate high-impact collaborations. Moreover, their policies incentivize the growth of cutting-edge research in AI, creating a dynamic environment for innovation. Additionally, the ability of these countries to attract researchers from around the world for collaborative projects further enhances their research output. The research strength of the United States and United Kingdom in AI healthcare applications, in particular, is likely a result of these factors, which provide valuable insights for other countries seeking to develop their own research capacities and infrastructure[26].

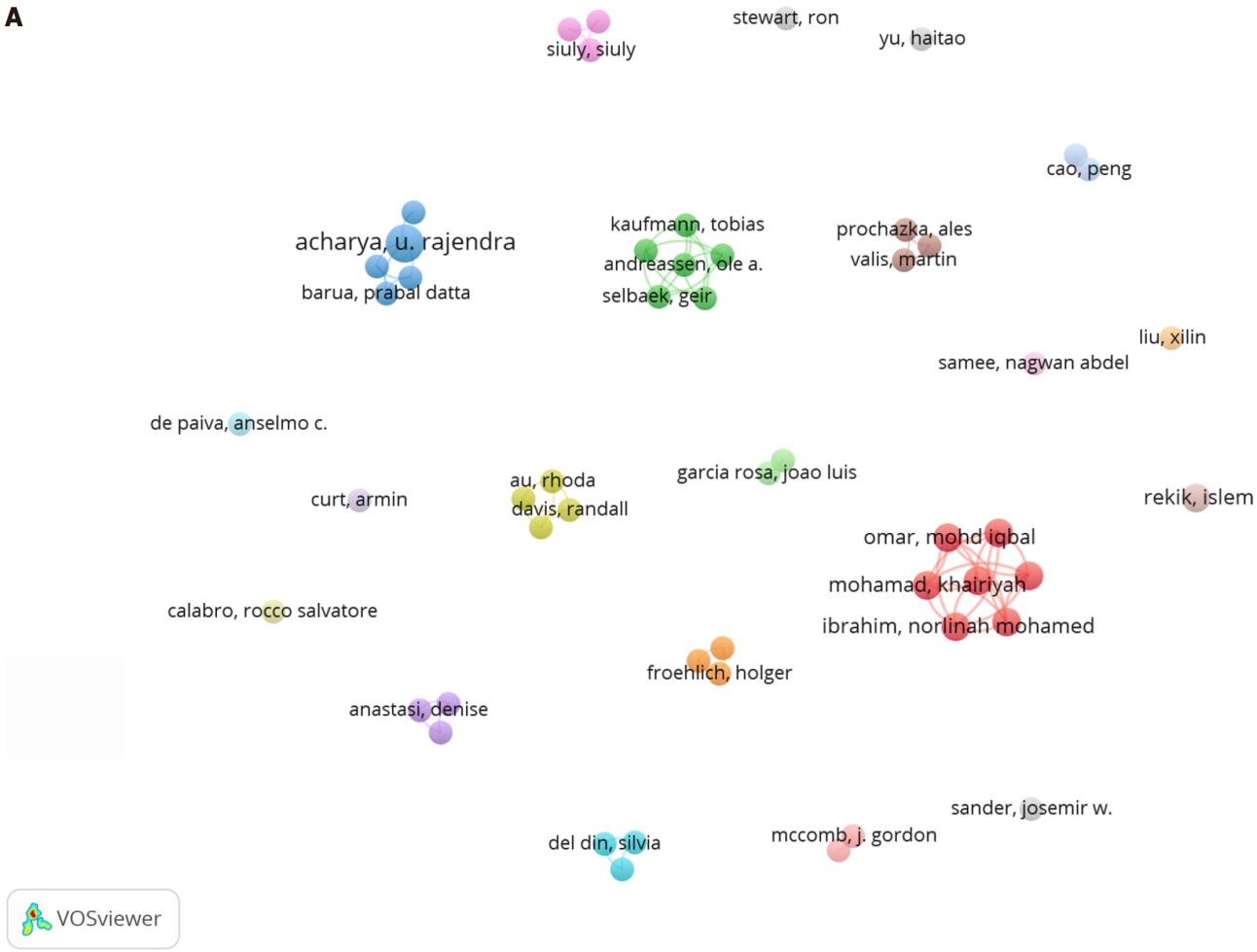
Analysis on journals

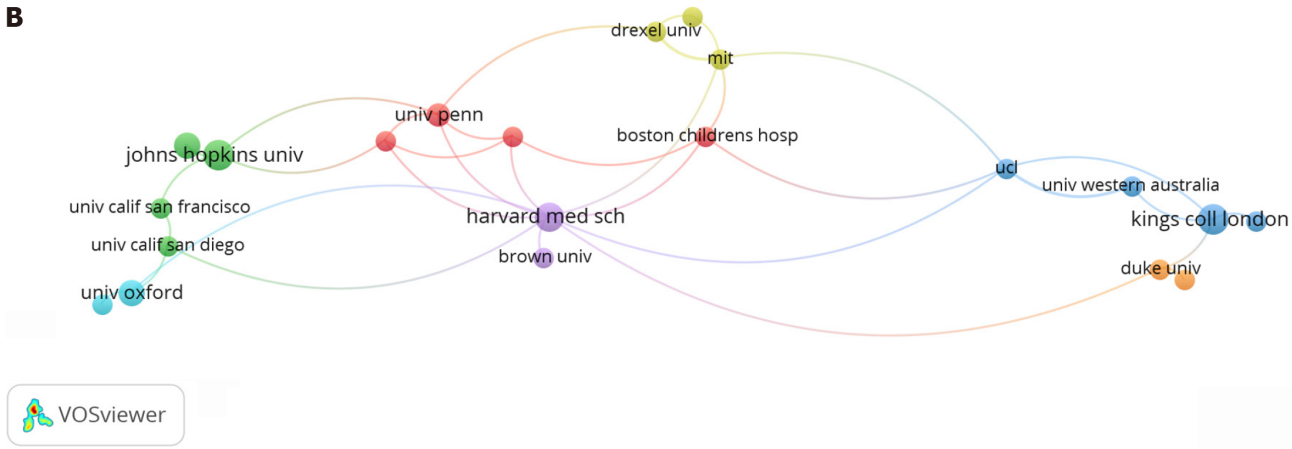
IEEE Access and *Scientific Reports* journals were ranked as the top two most prolific journals listed as Q2 and Q1, respectively, by the Journal Citation Reports. This finding is consistent with another bibliometric study focused on AI in medicine, which identified *IEEE Access* as the most productive journal in the field[27]. This underscores the journal's significant impact and influence on AI within medical and neurological domains, as indicated by our study. Regarding impact, *Frontiers in Neuroscience* had the highest number of citations, highlighting its substantial contributions to the field. Researchers are encouraged to explore articles on AI in neurological diagnosis from these leading journals to establish a foundational knowledge base and stay abreast of the latest developments.

Research trends and frontiers

This study presented the key developments and roles of AI in diagnosing neurological disorders by analyzing a list of keywords that highlight current hotspots in the field.

The first cluster of keywords (red): Includes Alzheimer's disease, artificial intelligence, machine learning, and stroke. AI, particularly machine learning, has significantly contributed to Alzheimer's imaging, aiding early diagnosis and guiding treatment efficacy assessments and strategies[28,29]. Such contribution includes: Utilizing fundamental machine learning architectures such as SVM, decision trees, and ensemble models[30]. Additionally, AI has been involved in stroke diagnosis, rehabilitation, and recommending optimal therapies[31].


A
siuly, siuly
stewart, ron
yu, haitao
cao, peng
acharya, u. rajendra
barua, prabal datta
kaufmann, tobias
andreassen, ole a.
selbaek, geir
prochazka, ales
valis, martin
liu, xilin
samee, nagwan abdel
de paiva, anselmo c.
curt, armin
au, rhoda
davis, randall
garcia rosa, joao luis
rekik, islem
omar, mohd iqbal
mohamad, khairiyah
ibrahim, norlinah mohamed
calabro, rocco salvatore
froehlich, holger
anastasi, denise
sander, josemir w.
del din, silvia
mccomb, j. gordon
VOSviewer


B
drexel univ
mit
univ penn
johns hopkins univ
boston childrens hosp
ucl
univ western australia
univ calif san francisco
harvard med sch
kings coll london
univ calif san diego
brown univ
duke univ
univ oxford
VOSviewer


C
italy
sweden
germany
england
usa
india
peoples r china
canada
australia
saudi arabia
turkey
VOSviewer

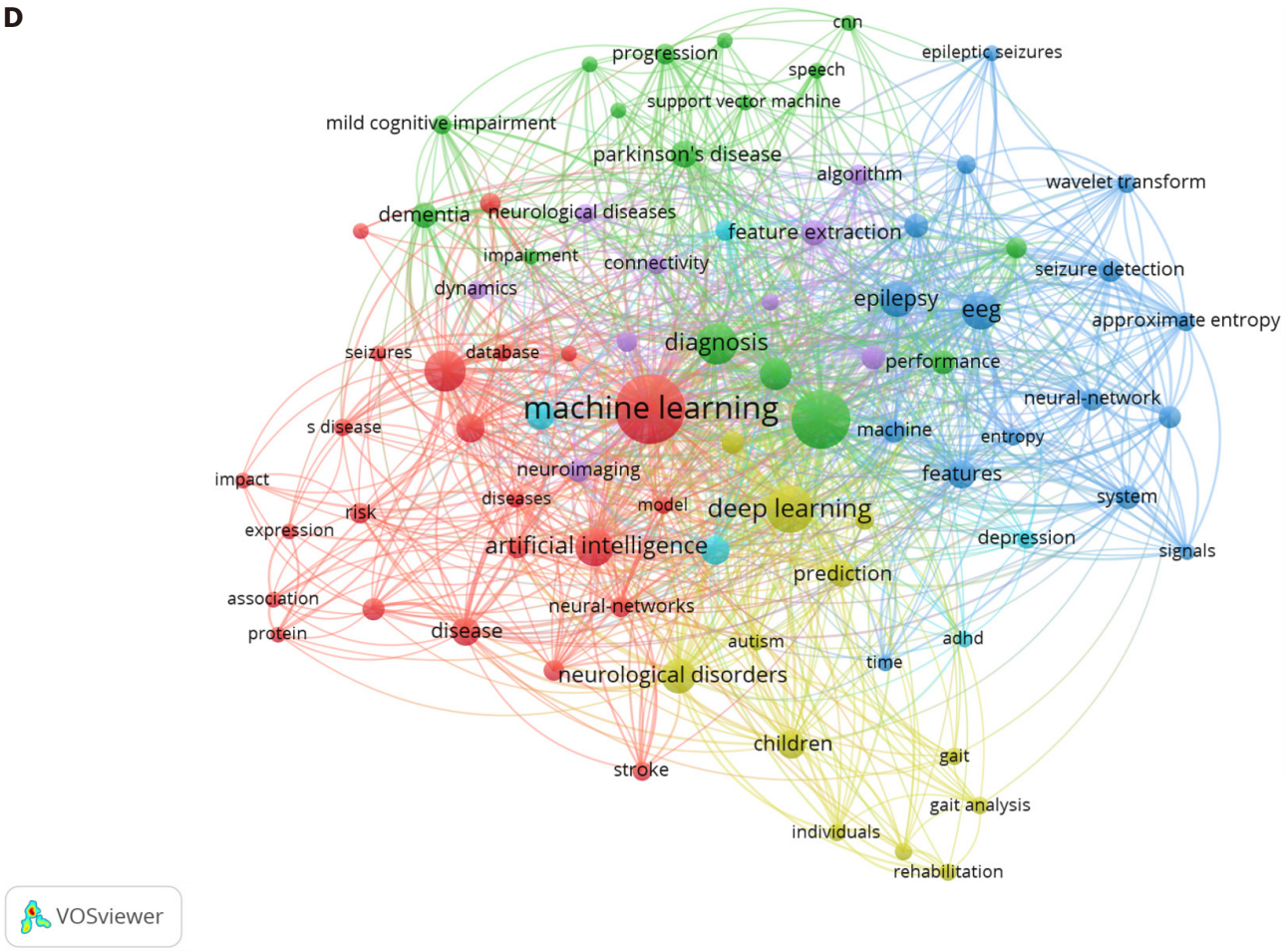


Figure 3 Visualization map of artificial intelligence articles in neurology disorders diagnosis. A: Visualization map of productive authors of artificial intelligence (AI) articles in neurology disorders diagnosis; B: Visualization map of productive institutions of AI articles in neurology disorders diagnosis; C: Visualization map of productive countries of AI articles in neurology disorders diagnosis; D: Network Visualization analysis of the keywords of AI articles in neurology disorders diagnosis.

The second cluster (green): Includes the following keywords: Parkinson's disease, computer-aided diagnosis, and dementia. Computer-aided diagnosis systems are recognized as AI tools that have witnessed growing use in recent years. They have made significant contributions, particularly in the detection and classification of Parkinson's disease[32,33]. One example includes a study that used biomedical sound measurements obtained from continuous phonation samples which were used as attributes in the diagnosis of Parkinson disease[34]. Various AI methods have also shown promise in early screening and detection of dementia, which can improve diagnosis and management of the disease's complications [35].

The third cluster (blue): Encompasses the following keywords: Electroencephalogram (EEG), epilepsy, seizure detection, and neural network. A neural network is a type of machine learning model. Several studies have employed neural networks to extract spatial characteristics from EEG data, leading to increased accuracy in epilepsy and seizure detection [36-38]. These studies consistently demonstrate improved epilepsy detection using this approach, which holds promise for enhancing the future management of seizures by neurologists. Another study utilized neural network systems, including 2D-CNN and LSTM, to detect and classify epilepsy seizures, and found high accuracy in both seizure detection and classification[39].

The fourth cluster (yellow): Encompasses the keywords: Autism, deep learning, and children. AI, particularly deep learning, has been utilized to assist in screening and diagnosing autism in children. Two studies examined the application of these AI models for early autism diagnosis using facial expressions of children[40,41]. One study employed five models to evaluate the accuracy of autism detection[40], while another utilized three models for diagnosis[41]. Both studies demonstrated more effective autism diagnosis, offering potential benefits to neurologists for early detection and management of the condition.

The fifth cluster (violet): Includes the keywords: Feature extraction, algorithm, and neurological diseases. Feature extraction is recognized as a method utilized in machine learning and AI tools. It has been increasingly employed alongside algorithms in neural network devices to aid in the diagnosis and treatment of brain and neurological diseases such as dementia, epilepsy, migraine, and autism[42].

The last cluster (light blue): Includes the keywords: Attention deficit hyperactivity disorder (ADHD), MRI, network, and depression. Adults with ADHD are three times more likely to develop depression than those without ADHD[43]. A study investigated the relationship between hippocampal function and levels of depressive symptoms in ADHD using MRI to assess resting-state functional connectivity of the hippocampus. The findings revealed that hippocampal abnormalities are linked to depressive symptoms, highlighting the need for further research in this area[44].

CONCLUSION

To our knowledge, this is the first scientometric paper focusing on the role of AI in diagnosing neurological disorders. This study offers valuable insights into research trends, potential collaborations, and cutting-edge topics in AI-related research on neurological disorders diagnosis. Over the past decade, interest in this field has significantly increased. According to our findings, U Rajendra Acharya, affiliated with an institution in Australia, emerged as the most productive author. Johns Hopkins University in the United States was identified as the most prolific institution. The United States also led in terms of overall productivity in this field. Among journals, *IEEE Access* stood out as the most productive journal. As AI technology continues to evolve, its applications are likely to expand to include more personalized diagnostic tools and predictive models for disease progression. The integration of AI with emerging technologies, such as personalized medicine and computer-aided diagnosis for movement disorders like Parkinson's disease and dementia, will likely open new frontiers in neurological healthcare. Future research should focus on exploring these opportunities, as they have the potential to revolutionize how we diagnose, treat, and manage neurological diseases.

FOOTNOTES

Author contributions: Tarazi A had the idea of the article and its design, collected the data, contributed to the study conception and its design, conducted the data analysis, investigation, writing original draft, editing, and review; Aburrub A contributed to the study conception and design, did the data curation, investigation, writing of original draft, editing, and review; Hijah M contributed to the study conception and design, did the data curation, investigation, writing of original draft, editing, and review.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

PRISMA 2009 Checklist statement: The PRISMA checklist file was provided in the first submission and will be provided again this time.

Country of origin: Jordan

ORCID number: Alaa Tarazi 0009-0002-8483-483X.

S-Editor: Li L
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Richardson JP**, Smith C, Curtis S, Watson S, Zhu X, Barry B, Sharp RR. Patient apprehensions about the use of artificial intelligence in healthcare. *NPJ Digit Med* 2021; **4**: 140 [PMID: 34548621 DOI: 10.1038/s41746-021-00509-1]
2 **Davenport T**, Kalakota R. The potential for artificial intelligence in healthcare. *Future Healthc J* 2019; **6**: 94-98 [PMID: 31363513 DOI: 10.7861/futurehosp.6-2-94]
3 **Li RC**, Asch SM, Shah NH. Developing a delivery science for artificial intelligence in healthcare. *NPJ Digit Med* 2020; **3**: 107 [PMID: 32885053 DOI: 10.1038/s41746-020-00318-y]
4 **Basu K**, Sinha R, Ong A, Basu T. Artificial Intelligence: How is It Changing Medical Sciences and Its Future? *Indian J Dermatol* 2020; **65**: 365-370 [PMID: 33165420 DOI: 10.4103/ijd.IJD_421_20]
5 **Summerton N**, Cansdale M. Artificial intelligence and diagnosis in general practice. *Br J Gen Pract* 2019; **69**: 324-325 [PMID: 31249070 DOI: 10.3399/bjgp19X704165]
6 **Sarwar S**, Dent A, Faust K, Richer M, Djuric U, Van Ommeren R, Diamandis P. Physician perspectives on integration of artificial intelligence into diagnostic pathology. *NPJ Digit Med* 2019; **2**: 28 [PMID: 31304375 DOI: 10.1038/s41746-019-0106-0]
7 **Jimma BL**. Artificial intelligence in healthcare: A bibliometric analysis. *Telemat Inform* 2023; **9**: 100041 [DOI: 10.1016/j.teler.2023.100041]
8 **Tran AQ**, Nguyen LH, Nguyen HSA, Nguyen CT, Vu LG, Zhang M, Vu TMT, Nguyen SH, Tran BX, Latkin CA, Ho RCM, Ho CSH. Determinants of Intention to Use Artificial Intelligence-Based Diagnosis Support System Among Prospective Physicians. *Front Public Health* 2021; **9**: 755644 [PMID: 34900904 DOI: 10.3389/fpubh.2021.755644]

9 **Liu YX**, Zhu C, Wu ZX, Lu LJ, Yu YT. A bibliometric analysis of the application of artificial intelligence to advance individualized diagnosis and treatment of critical illness. *Ann Transl Med* 2022; **10**: 854 [PMID: 36111047 DOI: 10.21037/atm-22-913]

10 **Molnar MJ**, Molnar V. AI-based tools for the diagnosis and treatment of rare neurological disorders. *Nat Rev Neurol* 2023; **19**: 455-456 [PMID: 37400549 DOI: 10.1038/s41582-023-00841-y]

11 **Khushi Jha**, Kumar A. Role of Artificial Intelligence in Detecting Neurological Disorders. *Int Res J Adv Engg Hub* 2024; **2**: 73-79 [DOI: 10.47392/irjaeh.2024.0015]

12 **Schaefer J**, Lehne M, Schepers J, Prasser F, Thun S. The use of machine learning in rare diseases: a scoping review. *Orphanet J Rare Dis* 2020; **15**: 145 [PMID: 32517778 DOI: 10.1186/s13023-020-01424-6]

13 **Lin S**, Nateqi J, Weingartner-Ortner R, Gruarin S, Marling H, Pilgram V, Lagler FB, Aigner E, Martin AG. An artificial intelligence-based approach for identifying rare disease patients using retrospective electronic health records applied for Pompe disease. *Front Neurol* 2023; **14**: 1108222 [PMID: 37153672 DOI: 10.3389/fneur.2023.1108222]

14 **Krishnapriya S**, Karuna Y. Pre-trained deep learning models for brain MRI image classification. *Front Hum Neurosci* 2023; **17**: 1150120 [PMID: 37151901 DOI: 10.3389/fnhum.2023.1150120]

15 **Alatrany AS**, Khan W, Hussain A, Kolivand H, Al-Jumeily D. An explainable machine learning approach for Alzheimer's disease classification. *Sci Rep* 2024; **14**: 2637 [PMID: 38302557 DOI: 10.1038/s41598-024-51985-w]

16 **Zhu X**, Hu J, Deng S, Tan Y, Qiu C, Zhang M, Ni X, Lu H, Wang Z, Li L, Chen H, Huang S, Xiao T, Shang D, Wen Y. Bibliometric and Visual Analysis of Research on the Links Between the Gut Microbiota and Depression From 1999 to 2019. *Front Psychiatry* 2020; **11**: 587670 [PMID: 33488420 DOI: 10.3389/fpsyt.2020.587670]

17 **Li C**, Wu K, Wu J. A bibliometric analysis of research on haze during 2000-2016. *Environ Sci Pollut Res Int* 2017; **24**: 24733-24742 [PMID: 29034422 DOI: 10.1007/s11356-017-0440-1]

18 **Chen C**. Searching for intellectual turning points: progressive knowledge domain visualization. *Proc Natl Acad Sci U S A* 2004; **101** Suppl 1: 5303-5310 [PMID: 14724295 DOI: 10.1073/pnas.0307513100]

19 **Au Yeung J**, Wang YY, Kraljevic Z, Teo JTH. Artificial intelligence (AI) for neurologists: do digital neurones dream of electric sheep? *Pract Neurol* 2023; **23**: 476-488 [PMID: 37977806 DOI: 10.1136/pn-2023-003757]

20 **Kalani M**, Anjankar A. Revolutionizing Neurology: The Role of Artificial Intelligence in Advancing Diagnosis and Treatment. *Cureus* 2024; **16**: e61706 [PMID: 38975469 DOI: 10.7759/cureus.61706]

21 **Taskinsoy J**. The Return of Investment on Tertiary Education in Malaysia. *J Educ Vocat Res* 2012; **3**: 183-192 [DOI: 10.22610/jevr.v3i6.67]

22 **Knight J**, Morshidi S. The complexities and challenges of regional education hubs: focus on Malaysia. *High Educ* 2011; **62**: 593-606 [DOI: 10.1007/s10734-011-9467-2]

23 **Mazzarol T**, Norman Soutar G, Sim Yaw Seng M. The third wave: future trends in international education. *Int J Educ Manag* 2003; **17**: 90-99 [DOI: 10.1108/09513540310467778]

24 **Guo Y**, Hao Z, Zhao S, Gong J, Yang F. Artificial Intelligence in Health Care: Bibliometric Analysis. *J Med Internet Res* 2020; **22**: e18228 [PMID: 32723713 DOI: 10.2196/18228]

25 **Nahar S**. Modeling the effects of artificial intelligence (AI)-based innovation on sustainable development goals (SDGs): Applying a system dynamics perspective in a cross-country setting. *Technol Forecast Soc Change* 2024; **201**: 123203 [DOI: 10.1016/j.techfore.2023.123203]

26 **Bohr A**, Memarzadeh K. The rise of artificial intelligence in healthcare applications. In: Bohr A, Memarzadeh K, editors. Artificial Intelligence in Healthcare. NY: Academic Press, 2020: 25-60 [DOI: 10.1016/b978-0-12-818438-7.00002-2]

27 **Andrade-arenas L**, Yactayo-arias C. A bibliometric analysis of the advance of artificial intelligence in medicine. *Int J Electr Comput Eng* 2024; **14**: 3350 [DOI: 10.11591/ijece.v14i3.pp3350-3361]

28 **Zhang W**, Li Y, Ren W, Liu B. Artificial intelligence technology in Alzheimer's disease research. *Intractable Rare Dis Res* 2023; **12**: 208-212 [PMID: 38024585 DOI: 10.5582/irdr.2023.01091]

29 **Mirkin S**, Albensi BC. Should artificial intelligence be used in conjunction with Neuroimaging in the diagnosis of Alzheimer's disease? *Front Aging Neurosci* 2023; **15**: 1094233 [PMID: 37187577 DOI: 10.3389/fnagi.2023.1094233]

30 **Dara OA**, Lopez-guede JM, Raheem HI, Rahebi J, Zulueta E, Fernandez-gamiz U. Alzheimer's Disease Diagnosis Using Machine Learning: A Survey. *Appl Sci* 2023; **13**: 8298 [DOI: 10.3390/app13148298]

31 **Dresser LP**, Kohn MA. Artificial Intelligence and the Evaluation and Treatment of Stroke. *Dela J Public Health* 2023; **9**: 82-84 [PMID: 37701474 DOI: 10.32481/djph.2023.08.014]

32 **Reddy A**, Reddy RP, Roghani AK, Garcia RI, Khemka S, Pattoor V, Jacob M, Reddy PH, Sehar U. Artificial intelligence in Parkinson's disease: Early detection and diagnostic advancements. *Ageing Res Rev* 2024; **99**: 102410 [PMID: 38972602 DOI: 10.1016/j.arr.2024.102410]

33 **Brahim A**, Khedher L, Gorriz JM, Ramirez J, Toumi H, Lespessailles E, Jennane R, Hassouni ME. A proposed computer-aided diagnosis system for Parkinson's disease classification using123I-FP-CIT imaging. 2017 International Conference on Advanced Technologies for Signal and Image Processing (ATSIP); 2017 May 22-24; Fez, Morocco. IEEE, 2017: 1-6 [DOI: 10.1109/atsip.2017.8075510]

34 **Peker M**, Sen B, Delen D. Computer-Aided Diagnosis of Parkinson's Disease Using Complex-Valued Neural Networks and mRMR Feature Selection Algorithm. *J Healthc Eng* 2015; **6**: 281-302 [PMID: 26753436 DOI: 10.1260/2040-2295.6.3.281]

35 **Li R**, Wang X, Lawler K, Garg S, Bai Q, Alty J. Applications of artificial intelligence to aid early detection of dementia: A scoping review on current capabilities and future directions. *J Biomed Inform* 2022; **127**: 104030 [PMID: 35183766 DOI: 10.1016/j.jbi.2022.104030]

36 **Wang B**, Xu Y, Peng S, Wang H, Li F. Detection Method of Epileptic Seizures Using a Neural Network Model Based on Multimodal Dual-Stream Networks. *Sensors (Basel)* 2024; **24**: 3360 [PMID: 38894151 DOI: 10.3390/s24113360]

37 **Shah SY**, Larijani H, Gibson RM, Liarokapis D. Epileptic Seizure Classification Based on Random Neural Networks Using Discrete Wavelet Transform for Electroencephalogram Signal Decomposition. *Appl Sci* 2024; **14**: 599 [DOI: 10.3390/app14020599]

38 **Yogarajan G**, Alsubaie N, Rajasekaran G, Revathi T, Alqahtani MS, Abbas M, Alshahrani MM, Soufiene BO. EEG-based epileptic seizure detection using binary dragonfly algorithm and deep neural network. *Sci Rep* 2023; **13**: 17710 [PMID: 37853025 DOI: 10.1038/s41598-023-44318-w]

39 **Liu YH**, Chen L, Li XW, Wu YC, Liu S, Wang JJ, Hu SG, Yu Q, Chen TP, Liu Y. Epilepsy detection with artificial neural network based on as-fabricated neuromorphic chip platform. *AIP Advances* 2022; **12**: 035106 [DOI: 10.1063/5.0075761]

40 **Khan B**, Bhatti SM, Akram A. Autism Spectrum Disorder Detection in Children Via Deep Learning Models Based on Facial Images. *B Bus Econ* 2024; **13** [DOI: 10.61506/01.00241]

41 **Reddy P**, Andrew J. Diagnosis of Autism in Children Using Deep Learning Techniques by Analyzing Facial Features. *Eng Proc* 2023; **59**: 198

[DOI: 10.3390/engproc2023059198]

42 **Stacey WC**, Litt B. Technology insight: neuroengineering and epilepsy-designing devices for seizure control. *Nat Clin Pract Neurol* 2008; **4**: 190-201 [PMID: 18301414 DOI: 10.1038/ncpneuro0750]

43 **Babcock T**, Ornstein CS. Comorbidity and its impact in adult patients with attention-deficit/hyperactivity disorder: a primary care perspective. *Postgrad Med* 2009; **121**: 73-82 [PMID: 19491543 DOI: 10.3810/pgm.2009.05.2005]

44 **Posner J**, Siciliano F, Wang Z, Liu J, Sonuga-Barke E, Greenhill L. A multimodal MRI study of the hippocampus in medication-naive children with ADHD: what connects ADHD and depression? *Psychiatry Res* 2014; **224**: 112-118 [PMID: 25220159 DOI: 10.1016/j.pscychresns.2014.08.006]

World Journal of Methodology

Submit a Manuscript: https://www.f6publishing.com

World J Methodol 2025 September 20; 15(3): 100840

DOI: 10.5662/wjm.v15.i3.100840

ISSN 2222-0682 (online)

CASE REPORT

The remarkable effects of the ionized medical water Asea® in 3 boys with Duchenne dystrophy: Three case reports

Andrei-Lucian Drăgoi, Roxana-Maria Nemeș

Specialty type: Medical laboratory technology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade D
Novelty: Grade C
Creativity or Innovation: Grade C
Scientific Significance: Grade C

P-Reviewer: Jiang Y

Received: August 28, 2024
Revised: October 26, 2024
Accepted: December 11, 2024
Published online: September 20, 2025
Processing time: 190 Days and 10.2 Hours

Andrei-Lucian Drăgoi, Roxana-Maria Nemeș, Medical Doctoral School, University of "Titu Maiorescu", Targoviste 130056, Romania

Corresponding author: Andrei-Lucian Drăgoi, MD, Doctor, Researcher, Medical Doctoral School of University "Titu Maiorescu", Str. General I. E. Florescu nr. 9, Targoviste 130056, Romania. dr.dragoi@yahoo.com

Abstract

BACKGROUND

Duchenne muscular dystrophy (DMD) is a severe lethal X-linked monogenic recessive congenital muscular dystrophy caused by various types of mutations in the dystrophin gene (DG). It is one of the most common human genetic diseases and the most common type of muscular dystrophy, in part because DG is one of the largest protein-coding genes in the human genome with a relatively high risk of being affected by a large palette of mutations. Long-term corticosteroid therapy (LTCT) with *deflazacort* started at age 4 is the most accessible and used pharmacological therapy for DMD in Romania. "*Asea® redox supplement*" (ARS) is an approved dietary supplement in the European Union. Several studies have shown that it is a very potent selective NRF2 activator, and thus a very potent, albeit indirect, antioxidant, with no toxicity up to high doses, in contrast to LTCT.

CASE SUMMARY

This paper presents a 3-case series on the effects of ARS in a 4-year-old, 5-year-old and 3-year-old boy all with DMD from Bucharest or Slobozia (Romania). This is the first report of this type worldwide. The parents of these boys had refused LTCT. They were treated with relatively high doses of ARS (3-7 mL/kg/day). For two patients, ARS was administered in combination with medium doses of L-carnitine and omega-3 fatty acids for various intellectual disabilities. Periodic consults and assessments for rhabdomyolysis, medullar and liver toxicity markers (blood count, gamma-glutamyl transferase, aspartate aminotransferase, alanine transaminase, lactate dehydrogenase, creatine kinase, creatine kinase-MB and serum myoglobin) were performed. *In vitro* studies showed that ARS is a very potent and selective NRF2 activator, and thus a very potent indirect antioxidant. The *in vivo* studies also support this main pharmacological mechanism of ARS, with no toxicity at high doses, in contrast with much more toxic corticosteroids which are often refused by parents for their children with DMD. Although they were three distinct ages and carried three distinct DG mutations, from the first months of ARS-based treatment, the children responded similarly to ARS. The

rhabdomyolysis markers, which were initially very high, significantly dropped, and there was no evidence for medullar and/or hepatic toxicity in any of the 3 patients.

CONCLUSIONS

ARS has significant indirect antioxidant effects *via* NRF2 and deserves extensive trials in children with DMD, as an adjuvant to corticoids or as a substitute in DMD patients who refuse corticoids. Future trials should also focus on ARS as an adjuvant in many types of acute/chronic infectious/non-infectious diseases where cellular oxidative stress is involved.

Key Words: Asea redox supplement oral solution; Duchenne muscular dystrophy; Corticosteroids; NRF2 and NF-kB nuclear transcription factors; NRF2 selective activation; Case report

Core Tip: Asea redox supplement has significant indirect antioxidant effects *via* NRF2. Based on the case studies here, extensive trials should be initiated in children with Duchenne muscular dystrophy (DMD) as an adjuvant to corticoids or as a substitute in DMD patients who refuse corticoids. Because of its antioxidant effects, it should also be studied as an adjuvant in many types of acute/chronic infectious/non-infectious diseases in which cellular oxidative stress is involved.

Citation: Drăgoi AL, Nemeș RM. The remarkable effects of the ionized medical water Asea® in 3 boys with Duchenne dystrophy: Three case reports. *World J Methodol* 2025; 15(3): 100840
URL: https://www.wjgnet.com/2222-0682/full/v15/i3/100840.htm
DOI: https://dx.doi.org/10.5662/wjm.v15.i3.100840

INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked monogenic muscular dystrophy primarily affecting boys. It is caused by various types of mutations in the dystrophin (DG) gene, one of the longest human genes. It is the most common type of muscular dystrophy, affecting approximately 1/5000 males at birth. Approximately 1/3 of the DMD cases are caused by spontaneous mutations in DG after fertilization, with no family history of DMD. DMD is usually clinically asymptomatic in the first 1-3 years of life. However, routine rhabdomyolysis screening at birth may show increased transaminases [aspartate aminotransferase (AST) or alanine aminotransferase (ALT)] and creatine kinase (CK) serum levels, as in the case of one of the patients presented in this paper. He presented with increased serum CK levels at birth, though the neonatologist did not refer the parents for additional investigations in a pediatric or neurological service. Therefore, DMD was detected and diagnosed much later for this patient, at age 2.

Various muscular deficits may appear in DMD patients between 3-4 years of age. They typically first occur in the femoral and pelvic muscular groups, and then in the axial muscular groups and may significantly worsen in the next years, progressively affecting the capacity of standing and walking. Most patients with DMD lose ambulation at around 12 years of age. The average life span of DMD patients is approximately 26 years. Some DMD patients are also mentally affected, demonstrating language development delay, an intelligence quotient below average, *etc*. The exact mechanism by which DG mutations affect neurons is unknown.

Long-term corticosteroid therapy (LTCT) with *deflazacort* is the first line treatment for DMD. LTCT is started at age 4, as it was clearly demonstrated to decrease chronic muscular inflammation (CMI) and slow the progressive muscular fibrosis in DMD patients. In addition, LTCT induces the expression of *utrophin*, a cytoskeletal protein homologous to dystrophin that can partially compensate for the lack of normal dystrophin in the affected muscular fibers of DMD patients. There have been calls to start LTCT earlier. However, many Romanian parents refuse LTCT for their DMD children because of the large spectrum of adverse effects of LTCT. The genetic therapies of DMD attempt to sidestep the DG mutation by exon skipping or to correct the DG mutation by CRISPR: While they have shown some promise, they are still under investigations and inaccessible in Romania.

Nuclear factor (Erythroid-derived 2)-like 2 (NFE2 L2 or NRF2) is a transcription factor that governs phase II of the cellular stress response by activating over 150 genes coding various proteins, especially endogenous antioxidant enzymes (EAEs) like glutathione synthase, glutathione peroxidases, superoxide dismutase, and catalase. EAEs are mainly activated by reactive oxygen species (ROS) and neutralize those ROS. Physical effort constantly generates ROS, primarily superoxides, which then stimulate the production of EAEs *via* NRF2 activation. This is a well-known essential process for cell survival and viability called "oxidative eustress", which also explains why physical therapy significantly helps DMD patients. By also activating tissular lipases, NRF2 partially switches glucose metabolism to lipid metabolism, increasing cellular energy production. NRF2 has higher cellular cytoplasmatic concentrations in the kidneys, muscles and lungs, compared to lower cytoplasmatic concentrations in the heart, liver and brain.

There are many known NRF2 activators, both allopathic (monomethyl and dimethyl fumarate, hyperbaric oxygen, some NSAIDs)[1] and natural (sulforaphane, alpha-lipoic acid, curcumin, polyphenols like quercetin and resveratrol, ginseng plant extract, ginkgo biloba tree extract, oxidative eustress *via* physical effort, caloric restriction, sleep, *etc*).

Asea redox supplement (ARS) is a superoxide-based ionized medical water. Very low non-toxic concentrations of superoxides are the main ingredient of ARS and the most potent NRF2 activator from its composition, with sufficient oral bioavailability to produce notable biological effects measured both *in vitro*[2] and *in vivo*[3,4]. Studies on ARS so far, including studies on "MDI-P", the former name for ARS[2], are very encouraging and motivate in-depth future research on the biological and therapeutic effects of ARS in both healthy or ill adults and children with various pathologies. There was also a clinical trial on ARS organized in 2013 and called "*Effect of ASEA on Energy Expenditure and Fat Oxidation in Humans*", but the results of this trial have not yet been published online for unknown reasons[3].

ARS is at the bridge between allopathic and natural NRF2 activators because it contains many types of ROS (mainly superoxides) and reductive species artificially produced by patented multi-stage electrolysis and stabilized by a special method, which are also naturally produced by all animal cells, including human.

In vitro studies demonstrated that ARS is a dietary supplement (approved in both the United States and European Union) with strong selective NRF2-activating properties. It is significantly "stronger" than sulforaphane, which is the most potent natural NRF2-activator known until present. Therefore, it also acts as a very potent indirect antioxidant by increasing the cellular concentrations of EAEs. *In vivo* studies also support the non-toxic NRF2-activating properties of ARS.

An initial double-blind randomized trial of metabolomics from 2010 on the effects of ARS consumption in athletes [4] demonstrated a spectacular increase in the serum levels of many fatty acids compared to a placebo group. The fatty acids are mobilized from the body's own adipose tissue, most likely by activating tissular lipases *via* NRF2, leading to a higher lipidic catabolic rate, increasing the capacity for physical effort. ARS also induced a remarkable increase in serum vitamin C levels by mobilizing it from hepatic reserves and thus increasing the blood antioxidant capacity of those tested.

Another very interesting study demonstrated that ARS can activate several genes, most likely *via* NRF2. The target genes are involved in activation of some important physiological mechanisms, including: (1) Innate immune mechanisms; (2) Some vascular regeneration systems (also involved in maintaining vascular elasticity); (3) Digestive enzymatic systems (which increase digestion efficiency); (4) Hormonal pathways; and (5) Anti-inflammatory and immunomodulatory mechanisms, which are also involved in immune tolerance[5].

CASE PRESENTATION

Chief complaints

This paper presents a series of 3 pediatric clinical DMD cases treated by using a combination of ARS, L-carnitine and omega-3 fatty acids (eicosapentaenoic and docosahexaenoic acids) as an adjunctive treatment, mainly because the parents of these boys firmly refused LTCT for various reasons, although it was proposed to them by the attending neurologists, including the pediatrician author. The main clinical and paraclinical data of patients are summarized below.

Case 1: This 2-year-and-8-month-old boy (initial age when he entered this study) was diagnosed with DMD at 1-year-and-4-months. His parent refused kinesiotherapy, rejected LTCT in advance and requested for an adjuvant/LTCT replacement long before he reached 4 years of age.

Case 2: This 4-year-and-8-month-old boy (initial age when he entered this study) was diagnosed with DMD at 2-years-and-11-months, but his parents rejected LTCT and requested an adjuvant/LTCT replacement.

Case 3: This 3-year-old boy (initial age when he entered this study) was diagnosed with DMD at 2-years-and-9-months, but his parents rejected LTCT in advance and requested for an adjuvant/LTCT-replacement long before he reached 4 years of age.

History of present illness

The 3 boys reported here were diagnosed with DMD at relatively young ages between 1 and 3 years-old. All 3 cases were confirmed to have DMD by genetic tests.

Case 1: This patient had a duplication of the 7547th nucleotide in the 52nd exon of DG.

Case 2: This patient had a hemizygous duplication of a block of exons (from the 8th to the 43rd) of DG. This is a rare DG mutation present in only about 5% of all known DMD cases.

Case 3: This patient had a heterozygous complete deletion of the 49th and 50th exons of DG, which is the most frequent type of exon-deletion in DMD.

History of past illness

There were no significant past illness besides DMD, except one urinary tract infection (UTI) episode (case 1) and respiratory failure with marked cyanosis at birth associated with anemia and unrecognized rhabdomyolysis (case 3).

Case 1: The patient had an UTI episode at the age of 1-year with a slightly enlarged left kidney according to the renal ultrasound, which resulted in hospitalization. Suspicion of DMD was first raised due to his increased rhabdomyolysis

biomarkers.

Case 2: The patient had no significant history.

Case 3: The patient had a history of prematurity (33 weeks of gestation) caused by a severe episode of pyelonephritis of his mother, who had a history of unilateral kidney stones. His body mass (BM) at birth was 2.15 kg. His Apgar score was 6 due to marked respiratory insufficiency and secondary marked cyanosis and altered state, requiring oxygen therapy at birth. He had a systolic heart murmur (grade III-IV/VI) and anemia at birth (Hgb = 10.5 g/dL), for which he needed a blood transfusion with two units of blood. After transfusion, his hemoglobin increased to Hgb = 12.8 g/dL. He remained in the hospital for about 3 weeks after birth; CK = 2600 U/L (normal range, 0-370 U/L) and AST = 780 U/L at birth (normal range, 0-40 U/L). Despite these increased AST and CK serum levels, the patient was not referred to any neurological and/or infectious diseases consult, nor were CK-MB levels measured. On January 22, 2019, at the age of 2 years and 5 months, a dermatologist performed routine screening on the patient for allergy concerns. Blood tests were run by a private lab in Slobozia and indicated AST = 970 U/L, ALT = 844.5 U/L. He was redirected to the "Victor Babes" Infectious Diseases Hospital in Bucharest. Common hepatic infectious diseases were excluded, and the patient was transferred to the "Victor Gomoiu" Pediatric Hospital on February 27th, 2019 for muscular dystrophy genetic screening.

Personal and family history

The most significant data from the family history are two maternal uncles (case 1 and case 3) who were described by the mothers to have typical DMD phenotypes, although they were undiagnosed at that time. They both lost ambulation around age 7 and died around age 20.

Case 1: The patient had a maternal uncle "immobilized in bed" from the age of 7 and who died at the age of 18. This is a classical DMD phenotype, although the boy's mother said that "her uncle was not diagnosed with Duchenne".

Case 2: The patient had no significant family history.

Case 3: According to his mother, the patient had a maternal uncle who "walked on his toes until the age of 6-7 years old and lost his capacity to walk at the age of approximately 7 years old and died at the age of 20 years old", which is a classical DMD phenotype. The boy's mother had unilateral kidney stone disease complicated with acute pyelonephritis with high fever and severe renal pain in the 33rd week of gestation, which caused premature labor and birth.

Physical examination

All 3 boys from this case series had typical DMD phenotypes when examined physically, mainly with symmetric bilateral pseudohypertrophy of both calves and slight axial hypotonia.

Case 1: The patient had a normal BM of 14 kg (55th percentile), a normal body size of 91cm (30th percentile), a normal cranial circumference of 47cm (10th percentile), an incomplete extension of the right calf especially when walking and running, a symmetric bilateral pseudohypertrophy of both calves (maximum diameter of calves= 23/23 cm), a slight axial muscular tone deficit but absent Gowers's sign, a tendency for constipation, and a slight delay in language development. He used approximately 20 words, rarely combined into two-word sentences, and rarely used a verb.

Case 2: The patient had a normal BM of 15 kg (25th percentile), a loss of muscular strength predominantly in axial muscles and lower limbs with Gowers's sign present, pseudohypertrophy of both calves, a North Star Ambulatory Assessment score (January 2018) of 17/34 (half of the maximum score), a 6-minute walk test result (January 2018): 292 m, without any stops or falls and no need for any external physical support during testing, normal cranial nerves, and normal intellect.

Case 3: The patient had a low development quotient = 62% of the normal for age and sex according to the psychologist who evaluated the child at "Victor Gomoiu" Children's Hospital in Bucharest, a normal BM of 12.5 kg (10th percentile), walked and ran independently but with a slightly enlarged sustaining base, had a slight axial muscular hypotonia with mild kyphosis and lumbar hyperlordosis. He also had slight pseudohypertrophy of the calf muscles (both with 19.5 cm in circumference). The boy did not cooperate during assessment for the Gower's sign due to marked agitation. He did not have urethral or anal sphincter control, as he did not announce his imminent micturitions and defecations. He had normal cranial nerves and tight phimosis. His mental examination indicated the following. Language: Language development delay with predominant expressive language delay. He used only about five Romanian words; he only used two verbs "give me" (distorted) and "bye", both correctly used. He did not build simple sentences; he could not combine two or more words. He demonstrated inconsistent visual contact with the examiner and his parents when he was called by name. He could follow simple instructions (to stand on his potty or to take out his pampers by himself alone; he brought and offered various objects at request). He pointed to various objects with his index finger or hand at request; Social skills: He did not get closer to smaller children but he sometimes wanted to socialize with children older than his age; Play skills: He used toys in normal ways. He did not prefer atypical toys (like bottles or laces/cords/strings, leaf, *etc*); he preferred to play with balls, and also with water.

Laboratory examinations

All 3 DMD patients had significant rhabdomyolysis, which is part of a typical DMD phenotype and its main paraclinical feature.

Case 1: At the age of 2-years-and-8-months (January 16, 2018) he had the following labs before starting ARS (N = Normal/upper limit of the normal range): AST: 473 U/L (about 10 × N); ALT: 558 U/L (about 17 × N); gamma-glutamyl transferase (GGT): 10 U/mL (N); CK: 34453 U/L (about 201 × N); CK-MB: 1241 U/L (about 52 × N); myoglobin (MG): 2006 ng/mL (about 28 × N); C-reactive protein: 0.6 mg/L (N); erythrocyte sedimentation rate: 9 mm/h (N); complete blood count (CBC): N.

Case 2: At the age of 6-months-and-3-weeks (October 11, 2014), the patient had the following labs long before starting ARS: AST: 279 U/L (about 6 × N); ALT: 285 U/L (about 9 × N); GGT: 8 U/mL (N); CBC: N. At the age of 2-years-and-8-months (November 2, 2016) he had the following labs long before starting ARS: CK: 27609 U/L (about 161 × N); CK-MB: 704 U/L (about 29 × N); lactate dehydrogenase (LDH): 4572 U/L; GGT: 10 U/mL (N).

Case 3: At the age of 2-years-and-6-months (January 22, 2019), the patient had the following labs long before starting ARS: AST: 970.9 U/L (> 20 × N); ALT: 844.5 U/L (> 20 × N). On January 30, 2019, at his routine hepatitis screening conducted by an infectious disease specialist from the "Victor Babes" Infectious Diseases Hospital from Bucharest), his tests were as follows: Negative B and C hepatitis serologies; negative Toxocara serology; AST: 685 U/L (> 15 × N); ALT: 770 U/L (> 15 × N); GGT: 10 U/L (N); CK: 27 713 U/L (> 200 × N); LDH: 5317 U/L (a non-specific marker for tissular damage, including rhabdomyolysis, especially myocardium damage). On February 27, 2019, during routine DMD screening by a neurologist from the "Victor Gomoiu" Pediatric Hospital, before starting any therapy, his tests were as follows: AST: 860 U/L (> 20 × N); ALT: 770 U/L (> 15 × N); CK: 24000 U/L (> 200 × N); LDH: 3026 U/L. During a routine check with the same neurologist from the "Victor Gomoiu" Pediatric Hospital after the first 3 months of L-carnitine 1 g/day, his test results were as follows: AST: 311 U/L (> 7 × N); ALT: 356 U/L (> 8 × N); CK: 18350 U/L (> 200 × N); LDH: 2670 U/L.

Imaging examinations

Imaging studies of these 3 DMD-patients, primarily abdominal and cardiac ultrasound, did not provide additional significant insights.

Case 1: Abdominal ultrasound indicated slight hepatomegaly with otherwise normal ultrasound.

Case 2: Heart ultrasound at the age of 3 months indicated a large stenosis of the right pulmonary artery with no hemodynamic significance, and thus no clinical signs.

Case 3: Heart ultrasound (September 28, 2016; at the age of 7 months) indicated the following: Ventricular septal defect with diameters 3/3.6mm (with secondary left-to-right cardiac shunt). The defect spontaneously healed according to the next heart ultrasound performed at the age of 2 years and 7 months. His abdominal ultrasound from the age of 2 years and 5 months was normal.

MULTIDISCIPLINARY EXPERT CONSULTATION

All the 3 patients were already diagnosed with DMD before the pediatric consult performed by the author of this paper and long before starting the ARS treatment. The various multidisciplinary consults offered to the DMD-patients before starting ARS per oris (/by mouth) were accomplished by general practitioners, neurologists, geneticists, infectious disease specialists, nephrologists and kineto-therapists.

Case 1

None.

Case 2

None.

Case 3

On January 30, 2019, during routine hepatitis screening conducted by an infectious disease specialist from the "Victor Babes" Infectious Diseases Hospital from Bucharest, his test results were as follows: Negative B and C hepatitis serologies; negative Toxocara serology; AST: 685 U/L (> 15 × N); ALT: 770 U/L (> 15 × N); GGT: 10 U/L (N); CK: 27 713 U/L (> 200 × N); LDH: 5317 U/L (a non-specific marker for tissular damage, including rhabdomyolysis, especially myocardium damage). On February 27, 2019 during routine DMD screening conducted by a neurologist from the "Victor Gomoiu" Pediatric Hospital before starting any therapy, his levels were as follows: AST: 860 U/L (> 20 × N); ALT: 770 U/L (> 15 × N); CK: 24 000 U/L (> 200 × N); LDH: 3026 U/L. On a routine check after the first 3 months of treatment with L-carnitine 1 g/day for DMD, conducted by the same neurologist from the "Victor Gomoiu" Pediatric Hospital, his levels were as follows: AST: 311 U/L (> 7 × N); ALT: 356 U/L (> 8 × N); CK: 18 350 U/L (> 200 × N); LDH: 2670 U/L.

FINAL DIAGNOSIS

All the 3 patients were already diagnosed with DMD long before the pediatric consult by the author of this paper and before starting the ARS treatment.

Case 1

DMD with typical phenotype.

Case 2

DMD with typical phenotype.

Case 3

DMD with typical phenotype.

TREATMENT

All 3 DMD patients were prescribed a mix of ARS, L-carnitine and omega-3 fatty acids. Progressively higher doses of ARS were administered, and labs were performed after each increase of the ARS dose.

Case 1

The initial dose of ARS was 4 mL/kg/day and was progressively increased up to 7.3 mL/kg/day.

Case 2

The initial dose of ARS was 4 mL/kg/day and was maintained as such throughout the study.

Case 3

The initial dose of ARS was 2.3 mL/kg/day and was then progressively increased up to 4.6 mL/kg/day.

OUTCOME AND FOLLOW-UP

In the next graphs, we present the significant decrease in rhabdomyolysis markers after the introduction of ARS (associated with L-carnitine and omega-3 fatty acids) as an adjuvant treatment of these 3 patients with DMD.

Case 1

After 6 months of ARS treatment with dose 4-6.5 mL/kg/day, the patient's results were as follows: Normal BM of 14.2 kg (50th percentile), normal body size of 96.5 cm (50th percentile), a relatively N axial muscular tonus (absent Gowers's sign), no deficit of right calf extension, no tendency of constipation, clear improvements in language development (he uses approx. 30-40 words; he could imitate over 100 words; he used simple propositions and even short phrases). Notably, the boy's parents were not compliant in starting physiotherapy for the child. After 11 months of ARS treatment with doses of 4-6.5 mL/kg/day, his results were as follows: A relatively normal-but-under-average BM of 14.2 kg (30th percentile), normal body size of 98 cm (40th percentile), N axial muscular tonus (absent Gowers's sign), no deficit of right calf extension, a North Star score of 34 (maximum), no tendency for constipation, clear improvements in language development (he used approx. 50-100 words; he could imitate over 150 words; he used propositions and phrases). He also had two successive UTI episodes (empirically treated by the family doctor of the boy, without renal ultrasound, without urine exam nor urinalysis before and/or after antibiotic therapy). Notably, the parents refused referral to nephrological consult for a voiding cystography to exclude vesicoureteral reflux. After 16 months of ARS treatment with doses of 4-6.5 mL/kg/day, his results were as follows: No UTI episodes from the previous consult; normal and above average BM of 15.5 kg (70th percentile), normal body size of 101 cm (50th percentile), N axial muscular tonus (absent Gowers's sign), no deficit of right calf extension, a North Star score of 34 (maximum), no tendency for constipation, clear improvement in language development (he uses approx. 150-200 words; he can imitate any word; he uses relatively complex propositions and phrases). After 19 months of ARS treatment with doses of 4-7.3 mL/kg/day, he had a 6-minute walking test result of 240 m in 4 minutes (> 50 m/min) because the boy did not cooperate for the full 6 min duration of this test. After 3 months of ARS treatment with doses of 4 mL/kg/day, his lab results were as follows: AST: 453 U/L (about 9 × N); ALT: 712 U/L (about 22 × N); CK: 25 426 U/L (about 149 × N); CK-MB: 632 U/L (about 26 × N). After 6 months of ARS treatment with doses of 6.5 mL/kg/day, his lab results were as follows: AST: 205 U/L (about 5 × N); ALT: 492 U/L (about 12 × N); CK: 13900 U/L (about 70 × N); CK-MB: 365 U/L (about 14 × N); MG: 886 ng/mL (about 12 × N). After 11 months of ARS treatment with doses of 4-6.5 mL/kg/day: AST: 262 U/L (about 6 × N); ALT: 461 U/L (about 12 × N); CK: 16271 U/L (about 81 × N); CK-MB: 437 U/L (about 17 × N); MG: 885 ng/mL (about 12 × N). After 16 months of ARS treatment with doses of 4-6.5 mL/kg/day he had: CK: 18537 U/L (about 92 × N); CK-MB: 486 U/L (about 19 × N); MG: 275 ng/mL (about 4 × N). After 30 months of ARS treatment with doses of 4-5 mL/kg/day he had: CK: 26330 U/L (about 153 × N); CK-MB: 626 U/L (about 26 × N); MG: 1980 ng/mL (about 28 × N). After 41 months of ARS treatment with doses of 4-5

mL/kg/day: CK: 15937 U/L (about 93 × N); CK-MB: 389 U/L (about 16 × N); MG: 1455 ng/mL (about 20 × N) (Figure 1A).

Case 2

After 7 months of ARS treatment with a dose of 4 mL/kg/day, his test results were as follows: A slight clinical improvement in muscle strength as primarily demonstrated by the 6-minute walk test; the boy had also followed other types of therapy partially initiated by his mother: Physical therapy, hydrotherapy, multivitamins and minerals, acupuncture, homeopathic remedies. The 6-minutes walking test result (July 27, 2019) (under the surveillance of his mother only) was: 359 m in 4 min (> 50 m/min) because the boy would not cooperate for the full 6 min duration of this test. The 6-minutes walking test result (July 29, 2019, under the surveillance of the pediatrician) was 320 m in 6 minutes and 20 seconds (given a fall and a short pause of 20 sec between min 4:18 and 4:38). After 3.5 months of 4 mL/kg/day of ARS treatment, he had: AST: 213 U/L (about 4 × N); ALT: 264 U/L (about 8 × N); CK: 8979 U/L (about 53 × N); CK-MB: 295 U/L (about 7 × N); LDH: 806 U/L; GGT: 10 U/mL (N); anti-streptolysin O: 317 IU/mL; Ferritin: 87 ng/mL. After 3 years and 3 months of 4 mL/kg/day of ARS treatment, he had: AST: 142 U/L (about 3 × N); ALT: 180 U/L (about 6 × N); CK: 5337 U/L (about 31 × N); Ferritin: 47.6 ng/mL (Figure 1B).

Case 3

After 4 months of ARS treatment at a dose of 2.3 mL/kg/day, he had an improved axial muscular tonus and strength. The boy had become more attentive and was more cooperative with his parents. However, he showed no significant language improvements. After 3 weeks of 2.3 mL/kg/day ARS treatment, he had: AST: 303 U/L (7 × N), ALT: 175 U/L (4 × N), CK: 21000 U/L (4 × N), LDH: 3448 U/L (10 × N). After 4 months of ARS treatment with dose 2.3 mL/kg/day, he had: AST: 241.98 U/L (5 × N), CK: 7885.7 U/L (46 × N), LDH: 1 318.65 U/L (3.8 × N) (Figure 1C).

DISCUSSION

In vitro studies[6] have clearly demonstrated that ARS is a strong selective NRF2 activator and consequently increases (up to 8-fold) the cellular concentrations of EAEs (like glutathione synthase, SOD *etc*). *In vivo* studies also support the NRF2-activating properties of ARS. Therefore, we hypothesize that the significant decrease in measured rhabdomyolysis markers (CK, CK-MB, LDH, AST, ALT) after ARS treatment is due to its potent NRF2-activating mechanism and subsequent activation of EAEs that neutralize a large quantity of ROS from muscle cells, including cardiomyocytes, to decrease the CMI and rhabdomyolysis rate.

Given that the rhabdomyolysis lab tests were all paid by the patients' parents, and also given the low average monthly income of Romanians, it would have been too expensive to cover the additional labs needed to clearly demonstrate the NRF2-activating effect of ARS *in vivo:* These tests would have included SOD plasma levels, the total antioxidant capacity of serum, malondialdehyde plasma levels, the intracellular level of the reduced glutathione, among others. The preliminary results on these 3 DMD cases deserve a future grant to extensively study more DMD patients and use an extensive panel of labs that includes antioxidant markers, as also discussed below.

Some rhabdomyolysis markers are missing from the monitoring of these 3 boys with DMD, because the parents did not always collaborate for a complete set of rhabdomyolysis markers, often for objective reasons related to familial financial resources. The previously described positive effects of ARS are plausibly determined *via* NRF2, by acting on the oxidative-stress link of the DMD pathogenic chain.

It would have been ideal to check both serum and urinary MG levels to exclude the possibility that increased MG excretion caused the observed decreased MG serum levels, rather than an actual decrease in MG production rate by decreased rhabdomyolysis. ARS may activate the cellular excretion pumps *via* NRF2, allowing for renal excretion of some cellular toxins, as NRF2 expression and activity are increased in kidneys. The concomitant determination of both serum and urinary MG is relatively expensive and inaccessible for the average Romanian patient.

The main explanation of the low number of DMD patients reported here (with no control group) is that DMD is relatively rare and DMD child-patients whose parents refuse corticosteroids but accept ARS as a compensatory adjuvant are very rare in Romania. Furthermore, very few Romanian medical doctors know about ARS and much fewer have experience in prescribing ARS as an adjuvant dietary supplement.

We have given many details and explanations of the adjuvant medication and other non-pharmacological therapies used in all 3 DMD cases. However, we had to synthesize a large quantity of data as not to overwhelm the potential readers with a very large article. Unfortunately, the cited papers on ARS were very difficult to find because many references did not have DOIs, as they were not published in peer-review journals.

Although this report contains only 3 cases, it is invaluable in that we followed these 3 patients for several years (4 years in average). This relatively long time-scale provides an additional argument for the long-term safety of ARS, which is very important for children in general. Furthermore, all 3 cases showed significant decreases in rhabdomyolysis markers, despite the patients having distinct genetic mutations in the DMD gene, indicating that chronic oxidative stress (from the muscular cells of patients with DMD phenotype) is a common physiopathogenic link on which ARS predominantly acts.

These promising preliminary results should be ideally followed by a rigorous randomized double-blind study in which a much larger number of DMD boys are divided into at least four or five groups: (1) A placebo group; (2) A group treated with ARS only; (3) A group treated with L-carnitine only; (4) A group treated with both ARS and L-carnitine simultaneously (given their plausible synergy, as shown in this case series); and (5) A group treated with ARS, L-carnitine and omega-3 fatty acids simultaneously. The initiation of such a large clinical trial would be very costly for Romanian patients

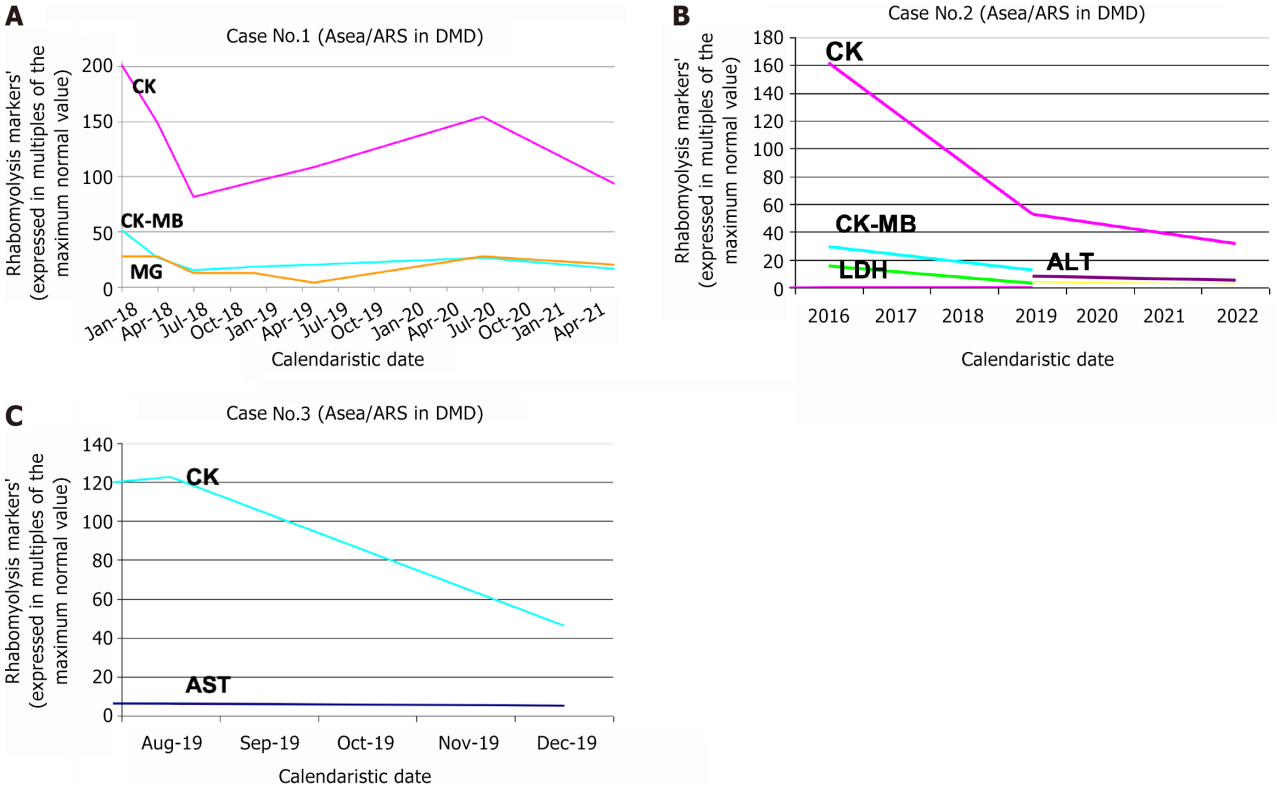


Figure 1 Changes in rhabdomyolysis markers after starting Asea redox supplement as adjuvant therapy. A: Case 1; B: Case 2; C: Case 3. ALT: Alanine aminotransferase; ARS: Asea redox supplement; AST: Aspartate aminotransferase; CK-MB: Creatine kinase-MB; DMD: Duchenne muscular dystrophy; LDH: Lactate dehydrogenase; MG: Myoglobin.

without a public or private grant.

Because ARS increases the rate of glutathione-synthesis *via* NRF2, it is possible that an additional association between ARS and N-acetylcysteine (NAC) would be synergistic, given that NAC is a glutathione precursor.

By reporting and discussing this case series, we re-emphasize the importance of physiopathological reasoning in medical research, as also implemented in the "Electronic pediatrician" software developed by the main author of this paper[7]. An important part of DMD physiopathogenesis is the chronic oxidative stress within skeletal and cardiac muscle fibers that leads to significant CMI and consequent progressive muscular fibrosis. Physiopathological reasoning centered around this chronic oxidative stress inspired the use of NRF2-activating ARS as an adjuvant treatment for these 3 boys with DMD, with encouraging results to date.

CONCLUSION

ARS has significant indirect antioxidant effects *via* NRF2 and deserves extensive trials in children with DMD, alone or in combination with L-carnitine and/or omega-3 fatty acids and/or NAC, as an adjuvant to corticoids or as a substitute in DMD patients who refuse corticoids. ARS also deserves future trials as an adjuvant in many types of acute/chronic infectious/non-infectious diseases in which cellular oxidative stress is involved.

FOOTNOTES

Author contributions: Drăgoi AL designed, analyzed, interpreted and prepared the manuscript; Nemeş RM initially reviewed the manuscript and provided essential feedback.

Informed consent statement: All parents of the 3 DMD boys provided their consent to publish these clinical cases without any personal identification details.

Conflict-of-interest statement: The authors of this paper declare no existing competing interests. The authors of this paper do not hold any positions in any commercial companies that sell Asea®.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Country of origin: Romania

ORCID number: Andrei-Lucian Drăgoi 0000-0002-2261-1350.

S-Editor: Liu H
L-Editor: Filipodia
P-Editor: Zhang XD

REFERENCES

1 **Eisenstein A**, Hilliard BK, Pope SD, Zhang C, Taskar P, Waizman DA, Israni-Winger K, Tian H, Luan HH, Wang A. Activation of the transcription factor NRF2 mediates the anti-inflammatory properties of a subset of over-the-counter and prescription NSAIDs. *Immunity* 2022; **55**: 1082-1095.e5 [PMID: 35588739 DOI: 10.1016/j.immuni.2022.04.015]

2 **Baltch AL**, Smith RP, Franke MA, Ritz WJ, Michelsen P, Bopp LH, Singh JK. Microbicidal activity of MDI-P against Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, and Legionella pneumophila. *Am J Infect Control* 2000; **28**: 251-257 [PMID: 10840346 DOI: 10.1067/mic.2000.105287]

3 Effect of ASEA on Energy Expenditure and Fat Oxidation in Humans. Available from: https://clinicaltrials.gov/study/NCT01884727

4 **Shanely RA**, Nieman DC, Henson DA, Knab AM, Cialdella-kam L, Meaney MP, Baxter S, Sha W. Influence of a redox-signaling supplement on biomarkers of physiological stress in athletes: a metabolomics approach. *FASEB J* 2012; **26** [DOI: 10.1096/fasebj.26.1_supplement.lb713]

5 Ward K. Initial Gene Study Showed ASEA REDOX Affected Important Signaling Pathway Gene. Available from: https://ugc.production.linktr.ee/3ea87dc0-3668-47af-b5c5-4a5fd83d193e_Gene-Study-Actual-Data.pdf

6 **Samuelson GL**. White Paper on In-Vitro Bioactivity of ASEA™ Related to Toxicity, Glutathione Peroxidase, Superoxide Dismutase Efficacy and Related Transcription Factors. Available from: https://www.amazingmolecules.com/pdf/Antioxidant-Efficacy-White-Paper.pdf

7 **Drăgoi AL**. The Remarkable Effects of "ASEA redox Supplement" In A Child with Duchenne Muscular Dystrophy (DMD)-A Case Report. *Can J Biomed Res Technol* 2019; **1**

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: office@baishideng.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com