ISSN 2222-0682 (online)

World Journal of *Methodology*

Quarterly Volume 14 Number 1 March 20, 2024

Published by Baishideng Publishing Group Inc

World Journal of Methodology

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ABOUT COVER

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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
March 20, 2024

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World Journal of Methodology

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World J Methodol 2024 March 20; 14(1): 90624

DOI: 10.5662/wjm.v14.i1.90624

ISSN 2222-0682 (online)

EDITORIAL

Pivotal role of exosomes in diagnosis and treatment of esophageal cancer in a new era of precision medicine

Grigorios Christodoulidis, Konstantinos Eleftherios Koumarelas, Marina Nektaria Kouliou

Specialty type: Oncology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Zhang X, China

Received: December 8, 2023
Peer-review started: December 8, 2023
First decision: December 21, 2023
Revised: December 23, 2023
Accepted: January 17, 2024
Article in press: January 17, 2024
Published online: March 20, 2024

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Abstract

In this editorial we comment on the article published by Ning *et al*, "Role of exosomes in metastasis and therapeutic resistance in esophageal cancer". Esophageal cancer (EC) represents a significant global health concern, being the seventh most common and sixth in terms of mortality worldwide. Despite the advances in therapeutic modalities, the management of patients with EC remains challenging, with a 5-year survival rate of only 25% and a limited eligibility for curative surgery due to its late diagnosis. Conventional screening methods are impractical for the early detection of EC, given their either invasive or insensitive nature. The advent of liquid biopsy, with a focus on circulating tumor cells, circulating tumor DNA, and exosomes, heralds a non-invasive avenue for cancer detection. Exosomes, small vesicles involved in intercellular communication, are highlighted as potential biomarkers for EC diagnosis and prognosis. Along with a diverse cargo encompassing various types of RNA, DNA molecules, proteins, and metabolites, exosomes emerge as key players in tumorigenesis, tumor development, and metastasis. Their significance extends to carrying distinctive biomarkers, including microRNAs (miRNAs), long non-coding RNAs, and circular RNAs, underscoring their potential diagnostic and prognostic value. Furthermore, exosomes may be utilized for therapeutic purposes in the context of EC treatment, serving as efficient delivery vehicles for therapeutic agents such as chemotherapeutic medicines and miRNAs. In this editorial we delve into the applications of exosomes for the early detection and treatment of EC, as well as the future perspectives.

Key Words: Exosomes; Esophageal cancer; Diagnostic methods; Novel therapies

Core Tip: Esophageal cancer (EC) is a global health concern ranking seventh in incidence and sixth in mortality worldwide, with over 604000 new cases and 544000 deaths in 2020. Despite advancements in therapies, nearly half of EC patients experience distant metastasis or therapeutic resistance, resulting in a 5-year survival rate below 25%. Traditional screening methods are limited, necessitating the need for non-invasive alternatives. Liquid biopsy, particularly focusing on exosomes, emerges as a promising option for early EC detection. Exosomes, small vesicles for intercellular communication, carry diverse biomarkers and play a crucial role in tumorigenesis. Notably, exosomal microRNAs, long non-coding RNAs, and circular RNAs show potential as diagnostic and prognostic markers for esophageal squamous cell carcinoma. Beyond diagnosis, exosomes serve as effective delivery tools for therapeutic agents, exhibiting advantages in immunotherapy, gene therapy, and drug delivery, presenting a multifaceted approach in the battle against EC.

Citation: Christodoulidis G, Koumarelas KE, Kouliou MN. Pivotal role of exosomes in diagnosis and treatment of esophageal cancer in a new era of precision medicine. *World J Methodol* 2024; 14(1): 90624
URL: https://www.wjgnet.com/2222-0682/full/v14/i1/90624.htm
DOI: https://dx.doi.org/10.5662/wjm.v14.i1.90624

INTRODUCTION

Esophageal cancer represents a prevalent malignancy in the digestive system, ranking seventh globally in incidence and sixth in mortality, accounting for over 604000 new cases and more than 544000 deaths worldwide in 2020[1-5]. This malignancy predominantly manifests in two histological subtypes: Esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC). EAC typically arises in the lower part of the esophagus and is often associated with chronic gastroesophageal reflux disease (GERD). It is characterized by changes in the lining of the esophagus, known as Barrett's esophagus. On the other hand, ESCC typically originates in the upper or middle part of the esophagus and is closely linked to factors such as tobacco and alcohol consumption[1,4]. ESCC primarily affects people in East and Central Asia, while EAC is presented more in Western Europe and North America. Notably, China with its high prevalence, contributes to over 50% of newly diagnosed EC cases worldwide[1-3].

Despite advancements in surgical and systemic drug therapies, EC poses considerable challenges, with nearly half of patients experiencing distant metastasis or therapeutic resistance post-treatment. The 5-year survival rate for patients remains below 25%, with a median survival time ranging from 13.6 to 19.3 mo. Surgery stands as the cornerstone treatment, yet only around 30% of newly diagnosed patients are eligible for curative resection[1-3,5]. Presently, there is a scarcity of effective biomarkers for early-stage ESCC detection. Moreover, a significant percentage of patients experience loco-regional recurrence following surgical resection aimed at cure. While adjuvant radiotherapy and chemotherapy hold importance for ESCC, their clinical efficacy remains a subject of debate, emphasizing the critical need for earlier diagnosis [1,3,5].

Endoscopic examination with biopsy and imaging studies, while being two of the main screening methods for EC, are invasive and insensitive, respectively. Emerging minimally invasive technologies like cytosponge or transnasal endoscopy face barriers related to cost and discomfort, limiting their widespread acceptance as screening methods for ESCC[5].

Liquid biopsy, which focuses on circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and extracellular vesicles (EVs), particularly exosomes, is a potential early detection method[2,5]. Exosomes, microvesicles, and apoptotic bodies are three types of EVs, with exosomes having dimensions ranging from 30 to 150 nm and playing an important role in intercellular communication. Exosomes carry a diverse cargo of RNA types (miRNAs, regulatory mRNAs, piwi-interacting RNA, small nucleolar RNA, tRNA-derived small RNAs (tsRNAs), long noncoding RNAs (lncRNAs), and unidentified small RNAs), DNA sequences, proteins, lipids, glycoconjugates, and metabolites, and are present in multiple biological fluids, such as blood, saliva, urine, malignant ascites, and cerebrospinal fluid, offering a non-invasive avenue for cancer detection compared to methods requiring phlebotomy, like ctDNA and CTCs[1-6].

Recent studies underscore the significant role of exosomes in tumorigenesis, development, and metastasis, and they participate in processes such as angiogenesis, extracellular matrix remodeling, and miRNA transfer[1,3]. Furthermore, exosomes harbor lncRNAs, pivotal in intercellular material exchange, signal transduction, and regulation of crucial oncological behaviors. The dysregulation of exosomal lncRNAs shows promise as diagnostic and prognostic biomarkers across various cancers[3].

In-depth comprehension of esophageal cancer epidemiology, subtypes, and associated biomarkers, with a particular focus on the role of exosomes, presents invaluable insights for advancing early detection, prognostication, and therapeutic strategies, aiming for enhanced outcomes among EC patients.

EXOSOMES AND DIAGNOSIS

Exosomes play pivotal roles in various stages of the tumor development cascade, encompassing tumor proliferation, angiogenesis, epithelial-mesenchymal transition, migration, microenvironment remodeling, and therapeutic resistance[1,2,7,8]. The early diagnosis and accurate prediction of therapy outcomes using exosomes have the potential to significantly

enhance the prognosis of patients with ESCC.

Exosomes emerge as potent cancer biomarkers due to their ease of extraction from most biofluids, exceptional stability, and capacity to convey dynamic information regarding the tumor state[1,2]. Zhao *et al*[3] observed a notable upregulation in the levels of circulating exosomes (CEs) from ESCC patients, which demonstrated a sensitivity of 75% and specificity of 85% in distinguishing ESCC patients from healthy individuals. The elevated level of CEs independently serves as a prognostic marker for ESCC patients.

Exosomal miRNAs, including miR-652-5p, miRNA-21, miR-766-3p, and miR-182, exhibit potential as diagnostic and prognostic biomarkers for ESCC. Particularly, miR-182 depletion in the postoperative period renders it a promising biomarker post-surgery. The serum miR-25/miR-203 ratio is significantly higher in ESCC patients, presenting potential for monitoring the effect of tumor resection and recurrence (sensitivity = 71.9%, specificity = 96.6%). Following surgery, the expression level of miR-25/miR-203 significantly decreases, as indicated by the receiver operating characteristic curve (sensitivity = 97.4%, specificity = 65.8%)[2,5,9-11].

Exosomal Dicer assumes a significant role in miRNA synthesis; thus, monitoring exosomal Dicer may prove more efficient than miRNA for diagnosing esophageal cancer[2]. Small RNAs derived from tRNA (tsRNAs), such as tRNA-GlyGCC-5 and sRESE in exosomes, display potential as pre-operative biomarkers for ESCC, predicting prognosis and response to adjuvant therapy. The combined sensitivity of tRNA-GlyGCC-5 and sRESE reaches 90.5%, with a specificity of 94.2%[5].

LncRNAs are extensively investigated as crucial biomarkers in various cancer types. Exosomal related-lncRNAs (ER-lncRNAs), including AC082651.3, AP000487.1, PLA2G4E-AS1, C8orf49, and AL356056.2, are identified as potential markers for ESCC, diminishing after surgery. ER-lncRNA pairs exhibit superior predictive value compared to traditional clinical indicators for median survival time[3].

ER-lncRNAs, such as PCAT1, UCA1, POU3F3, ESCCAL-1, and PEG10, are promising biomarkers for ESCC diagnosis and prognosis. Specifically, the lncRNA UCA1 serves as a potent diagnostic marker with a sensitivity and specificity of 86.7% and 70.2%, respectively. These lncRNAs, as a diagnostic panel, provide an accurate diagnosis of early esophageal cancer[12]. Candidate lncRNAs, including AC098818.2, RASSF8-AS1, LINC00958, GMDS-DT, and AL591721.1, are significantly overexpressed in ESCC patients' blood serum samples and cancerous tissues compared to healthy donors or other cancer types, indicating increased specificity for screening[13]. The expression levels of lncRNAs NR_039819, NR_036133, NR_003353, ENST00000442416.1, and ENST00000416100.1 increase in patients with ESCC and decrease after surgery. Serum lncRNA RASSF8-AS1 has been identified as the most reliable diagnostic marker for ESCC[14].

CircRNAs also exhibit potential as diagnostic biomarkers. Has-circ-0001946 and has-circ-0043603, secreted by ESCC cells, may serve as diagnostic biomarkers for ESCC, with combined detection showing improved accuracy compared to single detection. Serum exosomal has-circ-0026611 emerges as a novel predictor of ESCC prognosis[15].

Exosomes, as carriers of various types of non-coding RNAs and a multitude of molecules, present a comprehensive landscape for potential biomarkers. Zhu *et al* investigated four exosomal metabolites in ESCC patients, revealing a marker panel, including 3'-UMP, palmitoleic acid, palmitaldehyde, and isobutyl decanoate, with excellent diagnostic performance (area under the curve = 0.98) for predicting ESCC recurrence. Among these, 3'-UMP is identified as the most crucial for diagnosis[4]. Rao *et al*[16] observed that intercellular adhesion molecule-1 (CD54) is upregulated in cancer tissues as well as the exosomal CD54, thus making it a great option for diagnostic biomarker. Utilizing exosomal CD54 as a biomarker, they observed a sensitivity and specificity of 66.13% and 71.31%, respectively. Exosomes exhibit advantages over other cancer biomarkers, such as circulating tumor cells and circulating tumor DNA, owing to their ample quantity, robust stability, and ease of accessibility.

UTILITY OF EXOSOMES IN TREATMENT

Exosomes serve as delivery tools, transporting miRNAs, mRNAs, lncRNAs, and proteins selectively to target cells. They possess unique natural advantages including immune-escape, easy penetration of cell membranes, and specific recognition by receptor cells. Their low immunogenicity, high biocompatibility, long circulating half-life, less toxicity, and the ability to cross biological barriers, render them potent vectors for therapeutic agents[1,6,12].

Exosomes encapsulate various therapeutic agents, such as chemotherapeutic medicines, proteins, siRNAs, lncRNAs, and miRNAs. Exosome-derived miR-154-5p attenuates the invasion of EC cells and inhibits their angiogenic capability *in vitro*, curbing the malignant progression of ESCC. The exosomal lncRNA UCA1 is observed to inhibit EC progression, and miR-339-5p transferred *via* exosomes induces radiosensitivity in ESCC cells. Certain exosomal miRNAs, like miR-339-5p, promote sensitivity to radiation therapy, downregulating Cdc25A. Thus, high levels of miR-339-5p in tumor tissues and serum indicate a good prognosis. Exosomal derived miR-375 promotes apoptosis and suppresses proliferation, invasion, and migration of tumor cells, explaining its potential in tumor treatment[1,17,18]. Exosomal miR-19b-3p transferred to cells reverses the inhibitory effect of PTEN overexpression on cell invasion. PTEN overexpression downregulates MMP-2 and vimentin, and upregulates E-cadherin. Thus, PTEN upregulation through exosomal miRNA seems promising[12,19]. Exosomes play a significant role in gene therapy, and exosomal ECRG4 mRNA is a potential target for EC gene therapy, suppressing cell proliferation *in vitro* and inhibiting tumor growth *in vivo*. Moreover, exosomes can exert antitumor effects from immune cells by displaying specific surface antigens, activating T cells vital for tumor immunity[2]. Specifically, engineered M1 macrophage-derived exosomes inhibit tumor growth and transform M2-type tumor-associated macrophages into M1-like macrophages[1]. Exosomes are also superior drug delivery systems compared to liposomes, polymers, *etc*., due to their biocompatibility, biodegradability, and better target specificity. Adriamycin- and paclitaxel-loaded exosomes exhibit low immunogenicity and toxicity, improving efficacy in treating

multidrug-resistant cancer cells[1,12]. Engineered exosomes, with specific molecules attached, enhance targeting, increase production, and offer various lipid and protein compositions. The utilization of exosomes in immunotherapy is an important aspect. Dendritic cell-derived exosomes loaded with tumor antigens induce anti-tumor immune responses, showing potential in cancer therapy. Exosomes may play a role in inducing senescence, representing a promising strategy for ESCC treatment.

CONCLUSION

EC is a major worldwide health issue, with high incidence and mortality. At present, the only definitive therapy is surgery, but by the time the tumor is diagnosed, it is at an advanced stage. Early identification and diagnosis may be the key to a better prognosis, and exosomal miRNAs, lncRNAs, and circular RNAs show promise as diagnostic and prognostic indicators for ESCC. Exosomes are an appealing option for enhancing the accuracy of early diagnosis and prognosis prediction due to their ease extraction, stability, and ability to communicate dynamic information about the tumor status. Furthermore, the utilization of exosomes in the therapy of EC as a therapeutic drug delivery method, is under investigation. Their unique natural advantages, including immune-escape and specific recognition by receptor cells, rendering them as potent vectors for therapeutic interventions. In summary, the potential of exosomes in early diagnosis and as carriers of therapeutic agents opens new avenues for precision medicine in the management of esophageal cancer.

FOOTNOTES

Author contributions: Christodoulidis G, Konstantinos-Eleftherios K, and Marina-Nektaria K contributed to the preparation of this paper; Christodoulidis G designed the overall concept and outline of the manuscript; Christodoulidis G, Konstantinos-Eleftherios K, and Marina-Nektaria K contributed to the discussion and design of the manuscript; Christodoulidis G, Konstantinos-Eleftherios K, and Marina-Nektaria K contributed to the writing and editing the manuscript, and review of the literature.

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

Country/Territory of origin: Greece

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S-Editor: Liu JH
L-Editor: Wang TQ
P-Editor: Yuan YY

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World Journal of Methodology

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World J Methodol 2024 March 20; 14(1): 90316

DOI: 10.5662/wjm.v14.i1.90316

ISSN 2222-0682 (online)

EVIDENCE REVIEW

Continuous glucose monitoring metrics in pregnancy with type 1 diabetes mellitus

Mohammad Sadiq Jeeyavudeen, Mairi Crosby, Joseph M Pappachan

Specialty type: Endocrinology and metabolism

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Belosludtseva NV, Russia; Yu H, China

Received: November 29, 2023
Peer-review started: November 29, 2023
First decision: December 12, 2023
Revised: December 17, 2023
Accepted: January 16, 2024
Article in press: January 16, 2024
Published online: March 20, 2024

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Abstract

Managing diabetes during pregnancy is challenging, given the significant risk it poses for both maternal and foetal health outcomes. While traditional methods involve capillary self-monitoring of blood glucose level monitoring and periodic HbA1c tests, the advent of continuous glucose monitoring (CGM) systems has revolutionized the approach. These devices offer a safe and reliable means of tracking glucose levels in real-time, benefiting both women with diabetes during pregnancy and the healthcare providers. Moreover, CGM systems have shown a low rate of side effects and high feasibility when used in pregnancies complicated by diabetes, especially when paired with continuous subcutaneous insulin infusion pump as hybrid closed loop device. Such a combined approach has been demonstrated to improve overall blood sugar control, lessen the occurrence of preeclampsia and neonatal hypoglycaemia, and minimize the duration of neonatal intensive care unit stays. This paper aims to offer a comprehensive evaluation of CGM metrics specifically tailored for pregnancies impacted by type 1 diabetes mellitus.

Key Words: Type 1 diabetes mellitus; Continuous glucose monitoring; Pregnancy;

Glycaemic control; Continuous glucose monitoring system

Core Tip: Intense glucose monitoring during pregnancy is crucial for the management of women with type 1 diabetes mellitus (T1DM) to ensure optimal maternal and foetal health outcomes. Continuous glucose monitoring (CGM) techniques are revolutionising diabetes care in patients with T1DM in recent years owing to its higher efficacy, relatively easier use for younger generation for testing compared to the cumbersome finger-prick capillary self-monitoring of blood glucose, and the options for integration CGM to continuous subcutaneous insulin infusion pump settings for simulating artificial pancreas. Understanding the CGM metrics is highly important for the correct management of these new technological advancements, which is the theme of this clinical update review.

Citation: Jeeyavudeen MS, Crosby M, Pappachan JM. Continuous glucose monitoring metrics in pregnancy with type 1 diabetes mellitus. *World J Methodol* 2024; 14(1): 90316
URL: https://www.wjgnet.com/2222-0682/full/v14/i1/90316.htm
DOI: https://dx.doi.org/10.5662/wjm.v14.i1.90316

INTRODUCTION

Sensors in healthcare, ranging from thermometers to wearable tech, have evolved significantly since the 19th century. Key developments include the introduction of electrocardiograms, implantable devices, and digital technology, leading to miniaturized, more accurate sensors[1]. The late 20th century saw advancements in glucose monitoring technology with wearable sensors revolutionizing the diabetes care[2]. The glucose sensor represents a pivotal advancement in biomedical engineering, integrating electrochemical principles to achieve real-time, non-invasive blood glucose monitoring. This innovation, crucial for diabetes management, emerged from extensive research into enzyme-based electrochemical sensors, harmonizing biocompatibility with analytical precision. Pioneered in the late 1990s of 20th century, these sensors utilized glucose oxidase to catalyse the oxidation of glucose, generating an electrical signal proportional to glucose concentration[3]. This breakthrough not only revolutionized diabetic care but also set the foundation for the development of wearable health monitoring technologies and personalized medicine[2].

A tiny sensor that is commonly placed on the arm or abdomen for continuous glucose monitoring (CGM) measures blood sugar levels every five minutes, day and night, and transmits the results to an external device[3,4]. People with diabetes can more easily keep track of their blood sugar levels over time[5,6]. CGM provides up to 288 blood glucose readings daily, providing detailed information about changes in blood glucose levels over 24 h[7,8]. Figure 1 shows the basic operational mechanisms of CGM sensor. Monitoring blood glucose levels provides information and understanding about high blood glucose levels, low blood glucose levels, for titration of medication and insulin. On long term it has been shown to reduce the incidence of occurrence of microvascular and macrovascular complications in type 1 diabetes and type 2 diabetes[9-11]. CGM use in diabetes during pregnancy is challenging with rapid changes in the blood volume and fluid shift across body compartments, growing foetus in the abdomen, and different pregnancy specific glucose targets [12]. Pregnancy also alters insulin sensitivity and glucose tolerance in a dynamic state of continual metabolic adjustment [8,13]. With CGM becoming increasingly used in pregnant women with type 1 diabetes, it becomes very important to understand the various parameters, and how it reflects in the pregnancy outcome, which we have detailed in this review.

CGM EFFECT ON PREGNANCY AND NEONATAL OUTCOMES

CGM plays a pivotal role in managing type 1 diabetes mellitus (T1DM) during pregnancy. The frequent and critical therapeutic decisions in T1DM pregnancies are primarily driven by glucose data, necessitating more rigorous monitoring than in non-pregnant individuals with T1DM[14,15]. Poor glycaemic control in pregnancy can have detrimental consequences not only for the mother but also for the developing foetus[16]. Therefore, CGM emerges as a protective tool for achieving favourable obstetric outcomes in pregnancies complicated by T1DM[17]. Studies have shown that pregnant women using CGM exhibit improved glycaemic control. For instance, Feig *et al*[18], reported a small yet significant difference in HbA1c levels (mean difference -0.19%; 95%CI -0.34 to -0.03) among pregnant women utilizing CGM compared to T1DM pregnancies managed by usual care. Additionally, these individuals spent a greater proportion of time in the target glucose range (68% with CGM *vs* 61% without) and less time in hyperglycaemia (27% with CGM *vs* 32% without)[18]. Scott *et al*[19], further emphasized that the primary efficacy of CGM was demonstrated by the increased duration pregnant users spent within the target glucose range.

Neonatal hypoglycaemia, a common complication in infants born to mothers with diabetes, can have long-lasting effects[20]. Stenninger *et al*[21], in their elegant study described that neonatal hypoglycaemia is usually a consequence of maternal hyperglycaemia especially during the labour. CONCEPTT trial, a landmark study, revealed that the use of CGM

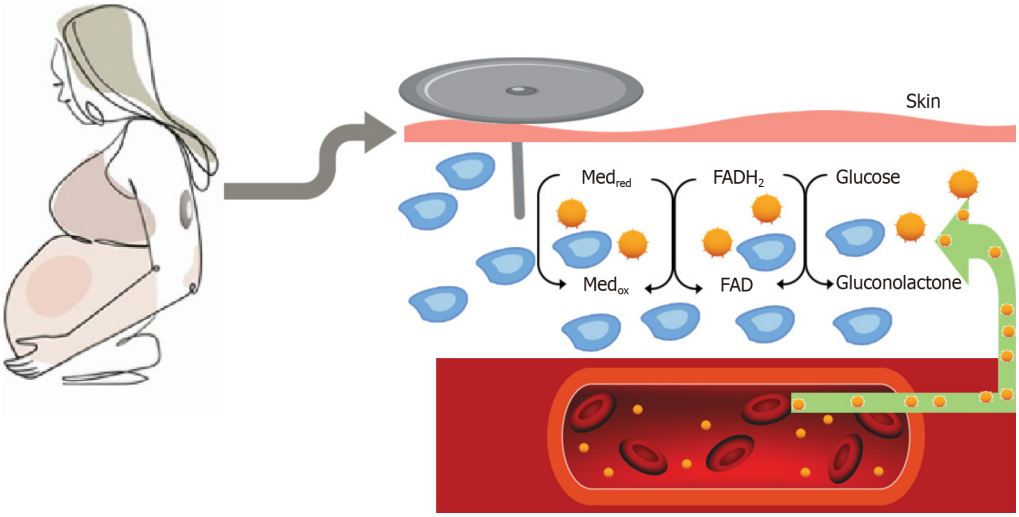

Figure 1 Basic operational mechanism of continuous glucose monitoring sensor measuring interstitial glucose. Med: Artificial redox mediators such as Ferrocene or Ferricyanide, red-reduced form, ox-oxidized form; FAD: Flavin Adenine Dinucleotide; FADH2: Flavin Adenine Dinucleotide, reduced form.

in pregnant women with T1DM was associated with better glycaemic control and reduced incidences of neonatal hypoglycaemia[18]. The study reported a lower rate of neonatal intensive care unit admissions and shorter hospital stays for newborns, highlighting the direct impact of maternal glycaemic control on neonatal health. Additionally, CGM use has been linked to a decrease in the incidence of large for gestational age (LGA) babies, a common complication associated with maternal hyperglycaemia[18]. Beyond the immediate neonatal outcomes, the implications of using CGM during pregnancy extend to long-term health benefits for the child. Maintaining optimal blood glucose levels through continuous monitoring can help to prevent complications that have lasting effects on the child's development and health [22]. Better maternal glucose control achieved through CGM has been correlated with lower risks of childhood obesity and metabolic disorders, which are often higher in children born to mothers with poorly controlled diabetes[23].

Moreover, the psychological benefits for the mother, such as reduced anxiety over managing diabetes during pregnancy, can contribute to a healthier prenatal environment[24,25]. This aspect, although indirect, plays a significant role in the overall well-being of both the mother and the foetus[26]. It is important to note that while CGM offers significant benefits, its effectiveness is maximized when combined with comprehensive diabetes education and support, ensuring that pregnant women with T1DM can effectively interpret and act upon the data provided by these devices[27].

CLINICAL TARGETS FOR CGM MONITORING DATA IN PREGNANCY

CGM has revolutionized the management of diabetes in pregnancy by providing direct observation of glycaemic excursions, diurnal profiles, and the ability to detect patterns of hypoglycaemia and hyperglycaemia[12]. This real-time monitoring enables the implementation of appropriate treatment decisions and lifestyle changes on a day-to-day basis, enhancing overall diabetes management[15,28]. Despite its availability since the late 1990, the usage of CGM was initially limited due to the absence of clear, established targets for its application in clinical care. To address this gap, the Advanced Technologies & Treatments for Diabetes (ATTD) Congress in February 2019 convened an international panel comprising physicians, researchers, and individuals with diabetes proficient in CGM technologies[27]. This global panel, which included diabetics, medical professionals, and research experts in CGM, aimed to formulate standards to aid clinicians, researchers, and individuals with diabetes in the utilization, understanding, and reporting of CGM data in both routine clinical care and research settings. The consensus reached by this panel, known as the 2019 International Consensus on CGM metrics, has since become the foundation for current clinical care standards[27]. The panel's recommendations were inclusive and generalizable, thanks to the involvement of individuals with diabetes. This consensus statement standardized CGM metrics in pregnancy, establishing targets such as time in range (TIR), time above range (TAR), and time below range (TBR). Additional metrics included the glucose management indicator (GMI), mean glucose, and glycaemic variability. These metrics provide a comprehensive framework for assessing and managing glycaemic control in pregnancy[29].

Moreover, the consensus also highlighted the importance of other CGM-derived data, such as the number of days the device is worn, device capture rate and ambulatory glucose profile (AGP)[27]. AGP provides an average time plot of glucose with percentile confidence intervals, offers an overarching view of glucose control over weeks, enabling a more nuanced understanding of the patient's glycaemic profile[30]. CGM metrics and targets by the ATTD consensus has significantly enhanced the utility of CGM in pregnancy[26]. These standardized metrics have become instrumental in guiding clinicians and researchers in optimizing diabetes care for pregnant women, ensuring that treatment and monitoring strategies are both effective and tailored to individual needs.

CORRELATION OF EACH PARAMETER TO PREGNANCY AND NEONATAL OUTCOMES

In the realm of managing type 1 diabetes during pregnancy, CGM provides critical data through various parameters. Understanding the correlation between these parameters and pregnancy and neonatal outcomes is essential. Each CGM metric offers unique insights into the glycaemic environment of the mother, which in turn can have significant implications for both maternal and foetal health. This section delves into how specific CGM metrics are correlated with pregnancy and neonatal outcomes, providing a comprehensive understanding of their impact and importance.

Number of days CGM worn

The duration of CGM wear in pregnant women with type 1 diabetes critically influences maternal and neonatal outcomes [29]. Consistent use of CGM in pregnancy leads to more effective glucose control, which is pivotal in reducing risks associated with T1DM. Studies have repeatedly highlighted that longer periods of CGM use correlate with better glycaemic control. Hughes *et al*[31], showed that, women who consistently used CGM for more than four days per week throughout their pregnancy demonstrated significant improvements in maintaining glucose levels within the target range. Consistent CGM monitoring, characterized by at least 96 h of data including nocturnal readings, is essential for effective diabetes management during pregnancy[32]. This continuous monitoring is crucial for detecting and addressing periods of hyperglycaemia and hypoglycaemia, especially common in the first trimester. The use of real-time CGM for 6 days at crucial stages of pregnancy (weeks 8, 12, 21, and 33) provided important insights into glucose trends, aiding in timely therapeutic interventions[33]. However, intermittent or short-term CGM use was found to be less effective in significantly reducing maternal hyperglycaemia by the third trimester, suggesting the need for prolonged and continuous CGM application[34].

The number of days CGM is worn also has direct implications not only for maternal health but also for neonatal outcomes. Prolonged CGM usage has been associated with lower risks of neonatal complications such as LGA infants and preterm births[18]. These findings underline the importance of longer and consistent use of CGM in managing glucose levels effectively throughout pregnancy, thereby supporting healthier pregnancy outcomes for both the mother and the child.

Percentage of time CGM is operational

While there is a lack of specific data regarding the operational time of CGM in pregnant women with type 1 diabetes, insights can be drawn from studies conducted on adult individuals with type 1 and type 2 diabetes[35]. These studies suggest that for comprehensive glucose monitoring and accurate derivation of CGM metrics, the device should be operational for at least 70% of the time[36]. Translated into practical terms, this would mean that the sensor should be active for a minimum of 16 h and 48 min in a day or 10 d within a 14-d period. The significance of maintaining this level of operational consistency becomes more pronounced in the context of pregnancy. Incomplete data capture can result in a loss of critical information regarding glucose levels. This gap in data is particularly concerning during pregnancy, as it could lead to poor judgment in treatment decisions. For expectant mothers with type 1 diabetes, this might mean missed opportunities for timely interventions or adjustments in their diabetes management plan, potentially impacting both maternal and foetal health. Furthermore, the importance of consistent CGM use in pregnancy is underscored by the dynamic nature of glucose levels during this period[37].

Mean glucose

The metric of mean glucose in CGM systems often receives less attention compared to other CGM metrics, yet it holds significant clinical relevance, especially in the management of type 1 diabetes during pregnancy. Mean glucose levels, as recorded by CGM, play a pivotal role in the calculation of the GMI, a parameter we will explore in detail later[38]. Notably, any substantial fluctuation in mean glucose levels is reflected in the GMI and HbA1c values, which are crucial for assessing glycaemic control[39]. In pregnancies complicated by type 1 diabetes, defining an exact target for mean glucose can be challenging. However, for practical purposes, it is generally advised that the mean glucose should align with the target blood glucose range[27]. A strong correlation exists between HbA1c, a marker of hyperglycaemia, and mean glucose levels, although this correlation is less pronounced with hypoglycaemia[38]. Clinical studies have shed light on the implications of mean glucose levels during pregnancy. For instance, a significant difference was observed in mean overnight glucose levels in pregnancies resulting in LGA status. Pregnancies with LGA babies exhibited considerably higher mean overnight glucose levels [6.0 ± 1.0 mmol/L (108.0 ± 18 mg/dL)] compared to those without LGA [5.5 ± 0.8 mmol/L (99.0 ± 14.4 mg/dL)][40]. In the same study, 162 women with Gestational Diabetes Mellitus (GDM) reported higher mean glucose levels in pregnancies with LGA status [6.2 *vs* 5.8 mmol/L (111.6 *vs* 104.4 mg/dL)][40]. The potential of CGM in managing mean glucose levels during pregnancy is further highlighted by the work of Petrovski *et al* [4], which revealed that CGM users in their first trimester had significantly lower mean blood glucose levels compared to those using self-monitoring blood glucose (SMBG) (6.92 ± 2.1 mmol/L *vs* 7.42 ± 3.4 mmol/L). This evidence underscores the effectiveness of CGM in providing tight glucose control, which is particularly crucial during pregnancy in managing type 1 diabetes.

GMI

The transition from the estimated A1C (eA1C) to the GMI marks a significant advancement in diabetes management, particularly in the context of pregnancy. While eA1C served as an earlier method to estimate average glucose levels, it was replaced by GMI due to its derivation from a larger, more representative dataset[38]. This new dataset provided a more accurate correlation with laboratory-measured HbA1c values, enhancing the precision of glucose monitoring. GMI

is calculated using mean glucose levels obtained from CGM systems, offering a more direct and immediate assessment of an individual's glucose control[41]. This method diverges from the HbA1c approach, which depends on the glycation of haemoglobin over longer periods. GMI's ability to provide real-time analysis makes it particularly valuable during pregnancy, where rapid fluctuations in glucose levels can occur due to physiological changes[38].

The significance of GMI in pregnancy is underscored by studies that demonstrate its correlation with TIR and its ability to reflect glycaemic control more accurately than HbA1c alone[39]. For example, Bergenstal *et al*[38], observed that GMI, calculated using CGM data, showed a strong correlation with TIR, especially in the second and third trimesters of pregnancy. Shah *et al*[39], further supported this by highlighting a notable negative association between TIR with GMI providing a clearer picture of glucose management. One significant benefit of GMI is its reduced susceptibility to the physiological changes that occur during pregnancy[41]. Unlike HbA1c levels, which can be influenced by the accelerated turnover of haemoglobin in pregnancy, GMI remains a more stable and reliable indicator of glucose control[38,41]. This stability is crucial in managing the dynamic glycaemic environment of pregnancy, where rapid changes in glucose levels can significantly impact maternal and foetal health. Moreover, GMI is derived from sensor-based average glucose readings, representing a cost-effective solution, particularly in resource-limited settings. This aspect of GMI is especially important considering the financial constraints and accessibility issues that can limit the use of extensive laboratory testing in some regions. The ability to estimate GMI directly from CGM data eliminates the need for frequent laboratory visits and blood draws, thereby reducing the overall cost and burden on healthcare systems and patients.

Another critical aspect of GMI in pregnancy management is the emphasis on trends rather than single-point measurements. The trend in GMI values provides a more comprehensive picture of glucose control over time, allowing for more nuanced and effective management strategies[41]. This is particularly relevant in pregnancy, where continuous monitoring and adjustments are vital to ensure both maternal and foetal well-being. The ability to track GMI trends enables healthcare providers to make more informed decisions, potentially leading to better outcomes by promptly addressing any adverse glycaemic patterns. In summary, GMI's resilience to physiological changes in pregnancy, its cost-effectiveness in glucose monitoring, and its focus on trends rather than isolated values, make it an indispensable tool in the management of type 1 diabetes during pregnancy.

Glycaemic variability

Glycaemic variability (GV) is an essential aspect of managing type 1 diabetes during pregnancy, characterized by the degree of fluctuation in blood glucose levels. CGM provides an invaluable tool for detailed analysis of these fluctuations, which are crucial for the health and well-being of both the mother and the developing foetus. GV is traditionally assessed using two primary metrics: Glucose standard deviation (SD) and coefficient of variation (CV)[42]. SD measures the extent of blood glucose fluctuations around the mean glucose level, with a high SD indicating larger swings[43]. These fluctuations are particularly significant in pregnancy due to potential impacts on foetal development. Kovatchev *et al*[43], have highlighted the importance of SD in CGM, emphasizing its strong correlation with mean glucose and HbA1c levels. On the other hand, CV offers a dimensionless measure of glucose variability relative to the mean glucose level. Its independence from mean glucose or HbA1c renders CV a unique and valuable tool in assessing glycaemic stability[44].

The clinical implications of GV during pregnancy are profound. GV has been identified as a potential risk factor for pregnancy complications, such as large for gestational age (LGA) infants[37]. Studies have shown that women with GDM exhibit higher GV, as indicated by increased SD and mean amplitude of glycaemic excursion (MAGE) values[28]. Quah *et al*[45] and Shindo *et al*[46], revealed that participants with GDM had significantly higher SD and MAGE values in both the first and second trimesters compared to those without GDM. Further research underscores the impact of GV on maternal and foetal health. Rodbard *et al*[47], found that women with GDM using CGM experienced less glucose variability and better glycaemic control compared to those not using CGM. This finding is supported by Dalfrà *et al*[42], who identified a relationship between macrosomia and maternal glycaemic variability in diabetic pregnancies. Additional studies by Feig *et al*[18] and Wei *et al*[48] have demonstrated that CGM users exhibit significantly lower glucose standard deviation and MAGE compared to SMBG users, indicating the efficacy of CGM in managing GV. The distinction in GV between type 1 and type 2 diabetes, as highlighted by El-Laboudi *et al*[49], points to the variability in glucose profiles and the need for tailored management strategies in pregnancy. Their study reported significantly higher CV in type 1 diabetic patients compared to those with type 2 diabetes[49]. This difference underscores the complexity and individualized nature of glucose management in type 1 diabetes pregnancy. Current research suggests that a CV value below 36% indicates a stable glucose profile, while values of 36% or higher suggest higher variability and an unstable profile[27].

Despite the evident association between GV and pregnancy outcomes, some studies have presented nuanced findings. For example, Dalfrà *et al*[42] in 2011 showed that women using CGM experienced reduced glycaemic variability, as indicated by lower SD and MAGE. However, a retrospective cohort study by Mulla *et al*[50] did not find trimester-specific relationships between GV and birth weight in women with type 1 diabetes, suggesting the multifaceted nature of GV's impact on pregnancy outcomes. In summary, GV, as assessed through CGM, plays a pivotal role in the management of type 1 diabetes during pregnancy. The metrics of SD and CV provide essential insights into glucose fluctuations, which are critical for both maternal and foetal health. The nuanced and variable impact of GV on pregnancy outcomes underscores the need for individualized monitoring and management strategies[42]. As research continues to evolve, the role of CGM in understanding and managing GV in pregnancy remains a vital component of diabetes care.

TAR (> 10.0 mmol/L)

In the management of type 1 diabetes during pregnancy, CGM offers critical insights into glucose control, particularly in assessing TAR. TAR, an indicator of hyperglycaemia, is categorized into two distinct levels: Level 1 (mild hyperglycaemia, > 180 mg/dL to 250 mg/dL or 10.1–13.9 mmol/L) and Level 2 (significant hyperglycaemia, > 250 mg/dL or > 13.9 mmol/L)[38]. However, for pregnant individuals with type 1 diabetes, the threshold for TAR is more stringent,

defined by sensor glucose values exceeding 140 mg/dL (> 7.8 mmol/L)[27]. This adjustment acknowledges the critical need for tighter glycaemic control to mitigate risks associated with maternal and foetal hyperglycaemia. Clinical guidelines recommend minimizing TAR, aiming for it to constitute no more than 25% of the time, equivalent to less than 6 h per day[27]. This target is imperative given the heightened risk of ketosis and diabetic ketoacidosis in pregnancy, conditions exacerbated by the physiological state of accelerated starvation inherent to this period[51].

Murphy *et al*[34], demonstrated the effectiveness of CGM in managing TAR, with CGM users showing a significantly lower percentage of time above range (27%) compared to SMBG users (32%). This reduction in TAR is of paramount importance in the context of pregnancy, where sustained hyperglycaemia can have detrimental effects on both maternal and foetal health. Further elucidating the impact of TAR on neonatal outcomes, research by Yamamoto *et al*[26], provided further insights into the neonatal impacts of TAR. Their study found that in cases of neonatal hypoglycaemia, maternal plasma glucose in the second trimester spent significantly less time within normal ranges (46% ± 14% *vs* 53% ± 15%) and more time above the optimal range (50% ± 16% *vs* 42% ± 17%) compared to infants without hypoglycaemia[26]. Similar trends were observed in the third trimester, with the percentage of time in range at 60% ± 16% *vs* 66 ± 14%, and time above range at 35% ± 16% *vs* 29% ± 14% for the respective groups[26]. Additionally, Scott *et al*[19] reported notable differences in glucose management with CGM use. Patients using CGM spent a greater proportion of time within the glucose goal range (67.6% ± 12.6% *vs* 61.3% ± 15.5%) and significantly less time above the target range (27.9% ± 13.4% *vs* 33.1% ± 15.0%) compared to SMBG users[19]. These findings underscore the superiority of CGM in achieving and maintaining optimal glucose levels during pregnancy.

Meticulous management of TAR is a crucial aspect of diabetes care in pregnancy as it is a critical component of optimal glycaemic control, with CGM emerging as an indispensable tool in this endeavour. The ability of CGM to accurately track and reduce TAR enhances the management strategies for diabetes in pregnancy, thereby playing a crucial role in promoting favourable maternal and neonatal outcomes. The continued investigation and application of CGM in this domain underscore its significance as a cornerstone in the management of diabetes during pregnancy.

TIR: (3.9–10.0 mmol/L)

TIR is increasingly recognized as a pivotal marker in managing type 1 diabetes during pregnancy. It offers comprehensive insights into the glucose profile by indicating the duration blood glucose levels stay within the target range of 63–140 mg/dL (3.5–7.8 mmol/L)[27]. This range is notably lower than in non-pregnant individuals, reflecting the physiological adaptations where glucose levels are generally lower in pregnancy[52]. Achieving a TIR of more than 70% of the time, equivalent to over 16 h and 48 minutes daily, is highly recommended[27]. This emphasis on maintaining a higher TIR underscores the importance of minimizing time spent in hyperglycaemic or hypoglycaemic states.

The relationship between TIR and pregnancy outcomes has been substantiated through various studies. For instance, Murphy *et al*[53], noted that every 5% increase in TIR is associated with improved neonatal outcomes. This finding highlights the direct impact of glycaemic control on foetal health, emphasizing the need for meticulous monitoring and management of blood glucose levels during pregnancy. The CONCEPTT study provides critical evidence on the effectiveness of CGM in improving TIR among pregnant women with type 1 diabetes[18]. This randomized trial included 215 pregnant women and 110 women planning pregnancy, comparing SMBG with CGM use. Remarkably, the TIR was significantly higher in the CGM group (68% with CGM *vs* 61% with SMBG), translating to an approximate difference of 1.5 h per day[18]. This study underscores the superiority of CGM over traditional SMBG in achieving optimal glucose control. The CONCEPTT study further revealed that women who had previously used CGM experienced a marked improvement in TIR during the first trimester, from 40% (10 h per day) in the early postpartum period to 55% (13.2 h per day) by the end of the first trimester[32]. Although the increase in TIR during the second trimester was minimal, a 5-percentage point gain in the third trimester elevated the TIR to 60% (14.4 h per day)[18,32]. These longitudinal improvements highlight the benefits of early and continued CGM use throughout pregnancy.

The focus on TIR in pregnancy management is not just about numerical targets; it embodies a broader strategy to ensure the health and well-being of both the mother and the foetus. Higher TIR correlates with reduced risks of pregnancy-related complications, such as preterm birth, preeclampsia, and neonatal hypoglycaemia[54]. Additionally, maintaining glucose levels within this targeted range can alleviate the psychological burden on expectant mothers, reducing anxiety and stress associated with diabetes management during this critical period[55,56]. The data from various studies, including the influential CONCEPTT trial, provide compelling evidence of the benefits of maintaining a high TIR. The focus on achieving and sustaining a TIR above 70% not only enhances maternal and foetal health outcomes but also sets a new standard in the approach to diabetes care during pregnancy.

TBR

TBR is a critical metric in CGM, particularly for pregnant women with type 1 diabetes, as it indicates periods of hypoglycaemia. TBR is categorized into two levels: Level 1 (mild hypoglycaemia, between 54 and 63 mg/dL or 3.0–3.5 mmol/L) and level 2 (significant hypoglycaemia, less than 54 mg/dL or < 3.0 mmol/L)[27]. These thresholds are lower than those for non-pregnant individuals, reflecting the physiological changes in pregnancy[52]. This adaptation was acknowledged in large clinical trials such as the Swedish and CONCEPTT trials[18,26]. Moreover, the occurrence of hypoglycaemia or decreasing insulin requirement, especially in the third trimester, has been strongly linked to uteroplacental insufficiency, making it crucial to monitor these levels for critical decision-making, such as considering early induction of labour[57].

Kristensen *et al*[58] observed a significant rise in the percentage of time spent below the threshold of 3.5 mmol/L, starting at 6 wk and peaking at 12-16 wk of gestation. This period coincides with an increased risk of severe hypoglycaemia in mothers. These findings suggest that minimizing the time that blood glucose levels fall below 3.5 mmol/L to less than 4% (less than 1 h per day) is particularly challenging in early pregnancy due to the limiting factor of

maternal hypoglycaemia in achieving stringent glycaemic goals. The CONCEPTT study further highlights the dynamics of TBR during pregnancy[18,32]. Although severe hypoglycaemia events were too infrequent for detailed correlation analysis with CGM time below range criteria, a notable trend was observed. Between 12 and 34 wk of pregnancy, the amount of time spent below 3.5 mmol/L decreased by half for both insulin pump and multiple daily injection users (from 6% to 3% and from 8% to 4%, respectively)[18]. This decrease indicates an evolving glycaemic profile as pregnancy progresses, emphasizing the need for continuous monitoring and adjustment of diabetes management strategies.

The imperative to maintain TBR values below critical thresholds (< 4%; <1 h below 63 mg/dL or value < 3.5 mmol/L, and < 1% or < 15 min below 54 mg/dL or < 3.0 mmol/L) is paramount in pregnancy[27]. Effective management of TBR is essential not only for maternal health but also for foetal well-being, as fluctuations in maternal glucose levels can have direct implications for foetal development[42]. In managing Type 1 diabetes during pregnancy, TBR as assessed through CGM plays a vital role in navigating the risks of hypoglycaemia. Continuous and vigilant monitoring of TBR, especially in the context of the changing glycaemic landscape of pregnancy, is crucial for achieving optimal maternal and neonatal outcomes. Figure 2 shows the graphical CGM metrics during pregnancy.

PITFALLS OF USING CGM IN PREGNANCY

CGM has become a vital tool in managing type 1 diabetes during pregnancy, yet it presents several pitfalls that necessitate careful consideration. One of the primary concerns lies in the realm of accuracy. CGM sensors, which measure glucose in the interstitial fluid, can sometimes lag behind actual blood glucose levels. This delay is particularly problematic given the rapid glucose fluctuations typical in pregnancy[8]. Klonoff *et al*[59], highlighted that the accuracy of CGM systems, especially in extreme glucose ranges, could vary, potentially leading to mismanagement of hyperglycaemia or hypoglycaemia. Furthermore, technical challenges such as sensor adhesion issues, exacerbated by physiological changes during pregnancy, can lead to gaps in monitoring[60]. The necessity for regular calibration in previous generation sensor posed additional hurdles, which has now been mostly resolved with factory calibrated sensors.

User-related issues and psychological impacts constitute another set of challenges. The phenomenon of alarm fatigue, where users become desensitized to frequent alerts, can lead to critical glucose changes being overlooked[61]. A survey by Polsky *et al*[12], highlighted that approximately 30% of CGM users experienced alarm fatigue, risking overlooked hypoglycaemic or hyperglycaemic events. Additionally, the constant stream of data and the need for continual decision-making can heighten anxiety and stress in pregnant women, potentially impacting their overall health. Economic and accessibility constraints also play a crucial role. The financial burden of CGM, not universally covered by insurance plans, can limit its accessibility. Cost and insurance limitations are significant barriers to wider CGM adoption, impacting its feasibility for many pregnant women with type 1 diabetes[62,63].

Clinical management challenges and the risk of over-treatment are further pitfalls in using CGM during pregnancy [64]. The interpretation of CGM data requires expertise and a nuanced understanding of diabetes management, a challenge for both patients and healthcare providers. The changing physiological landscape of pregnancy necessitates frequent adjustments in CGM settings, a complex task that can lead to either over-treatment or under-treatment. For instance, the CONCEPTT trial, highlighted the intricacies of managing insulin dosages based on CGM data, underscoring the need for specialized knowledge and continuous monitoring[18]. Additionally, indirect impacts on foetal health due to misinterpretation of CGM data or technical issues can have lasting consequences, emphasizing the need for accurate and reliable use of this technology. Hence, effective use of CGM requires a comprehensive understanding of these pitfalls, continuous education, and support for healthcare providers and patients alike[64]. Addressing these challenges is crucial to harness the full potential of CGM and ensure optimal maternal and foetal health outcomes in pregnancies complicated by type 1 diabetes. More data is necessary regarding how twin or multiple pregnancies affect utility of CGM metrics in pregnancy as there is inadequate evidence available currently.

PRACTICAL APPROACH TO OPTIMIZING CGM USE IN PREGNANCY

The first and foremost step in effectively using CGM during pregnancy is to actively engage the patient in the process. Open dialogues where the patient's opinions and observations are valued play a crucial role. This collaborative approach not only empowers the patient but also provides valuable insights into individualized management. Always check the sensor site and the injection or pump site. This step is crucial to ensure proper device functioning and to rule out any technical issues contributing to glycaemic variations. When opening the results data view, ensure that the cut-offs specific to pregnancy are set, as the default range can differ. Here are the steps that we commonly advocate for a complete assessment of the CGM data.

Data review and analysis

Data availability: Begin by confirming the adequacy of available data. For current CGM users, it's ideal to have at least 70% of data over a two-week period. In cases of significant hypoglycaemia or hyperglycaemia, a shorter period may suffice for analysis.

Pattern identification using AGP: Utilize the AGP to discern overarching patterns within the two-week data. Engage the patient in identifying factors contributing to these trends. This process is not just diagnostic but educational, helping

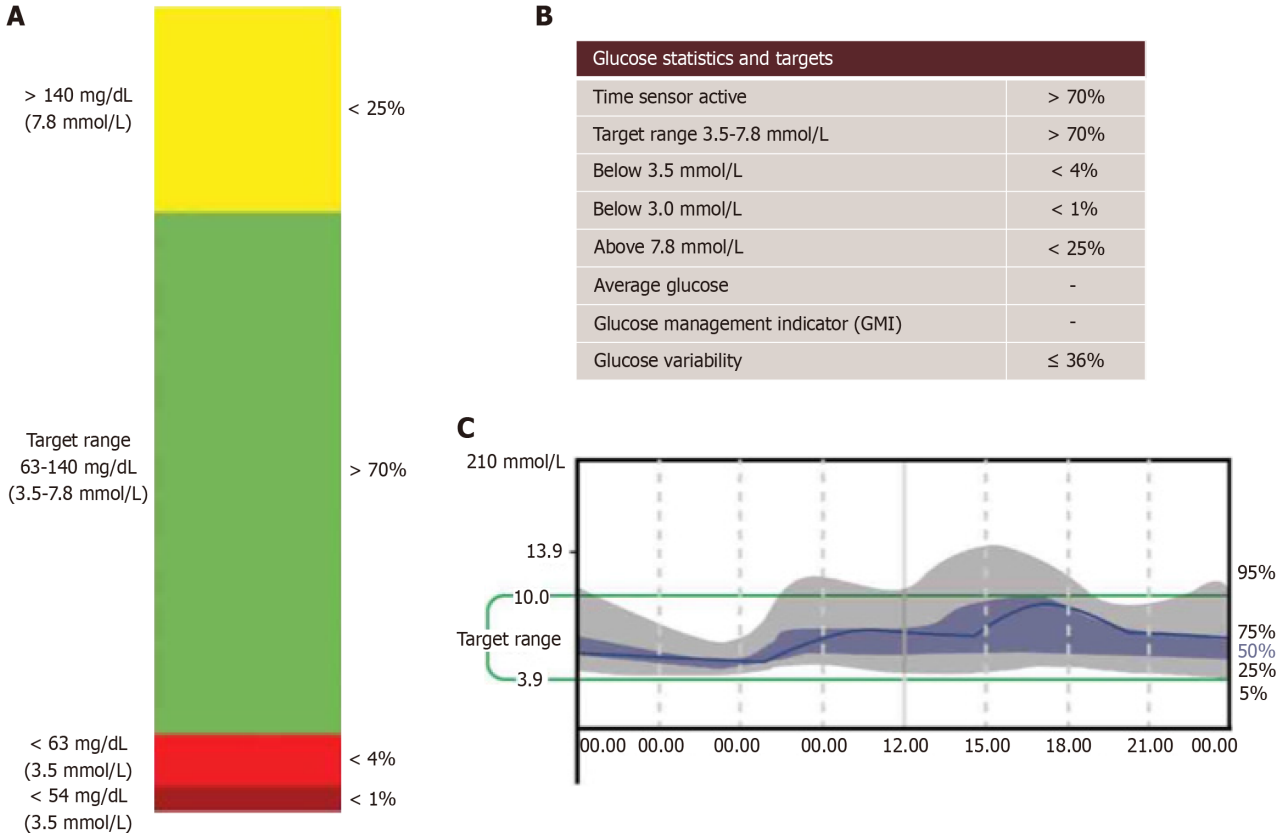

Figure 2 Shows the graphical continuous glucose monitoring metrics during pregnancy. A and B: Recommended metrics for continuous glucose monitoring during pregnancy; C: Illustrates a typical ambulatory glucose profile (AGP) model. When AGP is generated on certain devices, it defaults to standard time-in-range settings for non-pregnant individuals. Therefore, careful interpretation is necessary, or settings should be adjusted to reflect pregnancy-specific parameters prior to generating the AGP.

patients understand the interplay between insulin, diet, lifestyle, and glucose levels.

Prioritizing glycaemic patterns

Time in range assessment: Assess the TIR to quantify the average duration the patient spends within the target glucose levels each day. This metric is crucial in evaluating the effectiveness of current management strategies.

Identifying problematic patterns: Focus on identifying problematic glycaemic patterns in order of priority: Firstly, episodes of hypoglycaemia; secondly, periods of hyperglycaemia; and thirdly, instances of wide glycaemic variability. Review the overall glucose profile to pinpoint specific times of day when these patterns occur.

Daily graph review: Delve into daily graphs to verify if these patterns are isolated incidents or part of a recurring trend. This step is crucial in understanding the consistency and triggers of glycaemic fluctuations.

Collaborative solution development

Patient reflection and solution discussion: Encourage patients to reflect on potential causes for observed glycaemic issues and engage in a discussion to brainstorm potential solutions.

Action plan formulation: Develop a collaborative action plan with the patient. Ensure that they fully understand and are equipped with the necessary skills to implement the plan effectively.

Action plan documentation: Provide the patient with a copy of the action plan. Given the complexity and volume of information, this step is vital to ensure they have a reference to rely on.

The practical application of CGM in the management of type 1 diabetes during pregnancy requires a meticulous and patient-centred approach. By engaging patients in the process, thoroughly analysing CGM data, prioritizing glycaemic patterns, and collaboratively developing action plans, healthcare providers can enhance the efficacy of CGM. This approach not only improves glycaemic control but also empowers patients with the knowledge and skills necessary for successful diabetes management during this crucial phase of their lives.

CONCLUSION

CGM stands as a transformative tool in the management of type 1 diabetes during pregnancy. CGM's real-time glucose monitoring capability offers unparalleled benefits in optimizing glycaemic control, a crucial factor for ensuring the health and well-being of both the mother and the foetus. The implementation of CGM in pregnancy has demonstrated significant improvements in key metrics such as TIR, TAR, and TBR. These metrics provide a nuanced view of the patient's glycaemic profile, allowing for more precise adjustments in diabetes management strategies. The ability of CGM to identify patterns of glycaemic variability and to facilitate early interventions in cases of hypo- or hyperglycaemia is instrumental in mitigating risks associated with diabetes in pregnancy. However, the utilization of CGM is not without its challenges. Accuracy concerns, technical limitations, and the need for proper patient education and engagement are critical considerations. The importance of a patient-centred approach in CGM use cannot be overstated. By involving patients in the decision-making process, addressing their concerns, and ensuring they understand and can respond to their CGM data, healthcare providers can enhance the effectiveness of this technology. Furthermore, the economic and accessibility aspects of CGM use, along with the need for healthcare providers to stay updated with evolving technology, are areas that require ongoing attention and resources. Despite these challenges, the benefits of CGM in the context of pregnancy are clear and impactful. In conclusion, CGM represents a significant advancement in the management of Type 1 diabetes during pregnancy. Its comprehensive monitoring capability, coupled with a patient-centred approach, paves the way for more effective, personalized diabetes care, ultimately leading to improved maternal and neonatal outcomes.

FOOTNOTES

Co-first authors: Mohammad Sadiq Jeeyavudeen and Mairi Crosby.

Author contributions: Jeeyavudeen MS and Crossby M substantially contributed to the conception of the work and performed literature search, interpretation of relevant literature, article drafting, revision and Figure preparation and share the first authorship; Pappachan JM contributed to the literature search and revision of the article critically for important intellectual content; All authors have read and approved the final version of the manuscript. Jeeyavudeen MS and Pappachan JM are highly experienced clinicians specializing in the management of diabetes during pregnancy. Their extensive clinical work in Type 1 Diabetes Mellitus (T1DM) has significantly influenced the care of pregnant patients with diabetes. Both Jeeyavudeen MS and Pappachan JM have contributed to the field through numerous publications related to T1DM and are active members of the national societies and working group for diabetes management in pregnancy, reflecting their commitment to improving clinical practices and guidelines. In addition, Crossby M's role in the team is characterized by a strong focus on systematic reviews, demonstrating an enthusiasm for comprehensive, evidence-based approaches. Crossby M's work in synthesizing research findings complements the clinical insights provided by Jeeyavudeen MS and Pappachan JM, collectively forming a comprehensive approach to T1DM management in pregnancy. This integration of clinical expertise and research-based knowledge ensures that patient care is both effective and aligned with the latest scientific advancements.

Conflict-of-interest statement: The authors have nothing to declare.

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S-Editor: Liu JH
L-Editor: A
P-Editor: Zhao YQ

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World Journal of Methodology

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

World J Methodol 2024 March 20; 14(1): 89196

DOI: 10.5662/wjm.v14.i1.89196

ISSN 2222-0682 (online)

REVIEW

Update on the gut microbiome in health and diseases

Maurizio Salvadori, Giuseppina Rosso

Specialty type: Medicine, general and internal

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C, C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Fu M, China; Meng Y, China; Qureshi W, India

Received: October 23, 2023
Peer-review started: October 23, 2023
First decision: December 6, 2023
Revised: December 18, 2023
Accepted: January 27, 2024
Article in press: January 27, 2024
Published online: March 20, 2024

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Abstract

The Human Microbiome Project, Earth Microbiome Project, and next-generation sequencing have advanced novel genome association, host genetic linkages, and pathogen identification. The microbiome is the sum of the microbes, their genetic information, and their ecological niche. This study will describe how millions of bacteria in the gut affect the human body in health and disease. The gut microbiome changes in relation with age, with an increase in *Bacteroidetes* and *Firmicutes* . Host and environmental factors affecting the gut microbiome are diet, drugs, age, smoking, exercise, and host genetics. In addition, changes in the gut microbiome may affect the local gut immune system and systemic immune system. In this study, we discuss how the microbiome may affect the metabolism of healthy subjects or may affect the pathogenesis of metabolism-generating metabolic diseases. Due to the high number of publications on the argument, from a methodologically point of view, we decided to select the best papers published in referred journals in the last 3 years. Then we selected the previously published papers. The major goals of our study were to elucidate which microbiome and by which pathways are related to healthy and disease conditions.

Key Words: Gut microbiome; Dysbiosis; Pathobionts; Gut-brain axis; Heart-brain axis; Metabolic diseases; Omics techniques

Core Tip: Gut microbiome has relevant importance in healthy and diseased subjects. The production of several metabolites from the gut microbiome influences the immune system, brain, lung, heart, and metabolism. In the case of normal indigenous microbiota, metabolites produced have a benign action and contribute to the health. By contrast, the presence of pathobionts with their products may affect the different organs and produce diseases. The study of the gut microbiome is a difficult one and different omics technologies should be applied. The large quantity of studies highlights the relevance of the gut microbiome in health and disease.

Citation: Salvadori M, Rosso G. Update on the gut microbiome in health and diseases. *World J Methodol* 2024; 14(1): 89196
URL: https://www.wjgnet.com/2222-0682/full/v14/i1/89196.htm
DOI: https://dx.doi.org/10.5662/wjm.v14.i1.89196

INTRODUCTION

More than 150,000 papers with "Microbiome" in the title or abstract have been published since the term was introduced in 2001. Early-stage reports were cross-sectional studies of the microbiota at different body sites, associations with disease markers, and diseases themselves. More recently, three relevant projects, the Human Microbiome Project[1], the Earth Microbiome Project[2], and next-generating sequencing[3], have advanced novel genome associations, host genetic linkages, and pathogen identification. Current studies have principally focused on the functional or mechanistic aspects of differences in microbial composition.

In particular, a study from Gao *et al*[4] found that the more relevant studies cover six important aspects of microbiome research. They include best practices for analyzing microbiomes, the regulation of gut microbiomes in the human immune system, how microbiomes affect immune sensing, the role of microbiomes in the gut-brain interaction, the application of microbiomes in maternal and newborn health, and the study of nutri-microbiome epidemiology.

Due to the high number of publications on the argument, from a methodologically point of view, we decided to select the best papers published on referred journals in the last 3 years. Then, going in a backward way, we selected the previously published papers.

The aim of this study was to clarify the relationship between microbiome and healthy and disease conditions. In particular, we looked for which microbiome products and metabolites are involved in this relationship and which organs are strictly connected to microbiome normal or modified composition.

Before discussing the gut microbiome, and its function and its relationship with health and disease, exact definitions are needed for a correct understanding.

DEFINITIONS

Frequently, the terms microbiota and microbiome are mutually used, but they have a different significance. Table 1 shows the exact definitions needed to be understood.

Microbiota are defined as the microorganisms (bacteria, archaea, viruses, fungi) that live in the human body in healthy conditions. All of these microorganisms make up the microbiome. The microbiome is the sum of microbes, their genetic information, and their ecological niche. The metagenome is the totality of genes from the genomics of a mixed microbial population that provides information about genetic potential. Indigenous microbiota are the resident microbiota resident in the healthy subjects. Dysbiosis is the change in indigenous microbiota composition causing disease. Pathobionts are the microbiota causing disease.

GUT MICROBIOME

The microbiome is spread across different organs and tissues of the human body, but the most important and best studied is the gut microbiome. A total of 10^{14} bacteria already represent the gut microbiome, and 10^{11} bacteria flow each day from the pharynx to the stomach. Changes in the gut microbiome are associated with diseases, but frequently, it is not known if this is a cause or an effect.

Under normal conditions, formation of the adult microbiome occurs over the first 3 years of life and is affected by life events such as weaning, starting solid food, and primarily cessation of breastfeeding. At birth, the most common bacteria are aerobic bacteria such as *Enterococcus* and *Staphylococcus*. Later, anaerobes prevail, with a prevalence of *Firmicutes* and *Bacteroidetes*[5]. Several studies have documented the distribution of normal gut flora in the different parts of the intestine in adults as shown in Table 2[6-8].

Table 1 Definitions related to gut microbiome and its action

Terminology	Significance
Microbiota	All microorganisms living in the gut
Microbioma	Sum of microbes, their genetic information, and their ecological niche
Metagenome	Total genes providing information on genetic potential
Indigenous microbiota	Resident gut microbiota in the healthy subjects
Dysbiosis	Modification of the composition of gut microbiota causing diseases
Pathobionts	Gut microbiota causing diseases

Table 2 Distribution of normal gut flora in different parts of intestine

Intestine sections	Function	Normal flora
Stomach	Acid production, pepsin, amylase, CFU < 10^3/mL	*Lactobacillus; Streptococcus; Helicobacter pylori*
Small intestine: Duodenum, jejunum	Pancreatic enzymes, bicarbonate ions, bile salts, CFU: 10^3-10^4/mL	*Lactobacilli; Enterococci, Streptococci; Actinobacteria*
Small intestine: Ileum	CFU: 10^3-10^9/mL	*Enterococcus; Bacteroidetes; Lactobacillus; Clostridium; Corynebacteria*
Large intestine: Cecum, colon	Mucus and bicarbonate; CFU: 10^{10}-10^{12}/mL	*Bacteroidetes; Clostridium; Eubacterium; Ruminococcus; Streptococcus; Enterococcus; Lactobacillus; Fusobacteria*

CFU: Colony-forming unit.

FACTORS INFLUENCING THE GUT MICROBIOME

The Human Microbiome Project data suggest that the unperturbed microbiome is stable over short periods and that there is a degree of resilience of the microbiome due to several factors. In addition to age, several factors affect the composition of the gut microbiome, such as diet, host genetics, exercise, smoking, and drugs[9]. A vegetarian and rich fiber diet has a beneficial effect on the gut microbiome, favoring an increase in *Firmicutes* and *Bacteroidetes*[10,11]. Several genes associated with innate immunity influence the microbiome[12]. A recent study from Chen *et al*[13] documented the influence of host genetics on the gut microbiome. Exercise is associated with a beneficial effect on gut microbiome composition, as documented by Hughes *et al*[14] and Mailing *et al*[15]. It has also been documented that athletes have a reduced rate of inflammatory markers[16] and exercise has been proposed to reduce dysbiosis[17]. No smoker subjects showed an increase in the fecal microbiota of *Firmicutes* and *Actinobacteria*[18]. The same author in a different study found differences in the oral gut microbiota in smokers with respect to no smokers[19]. Several drugs have a powerful effect on microbiome composition. Antibiotics may damage the microbiome in two different ways. On the one hand, by destroying beneficial microbes, antibiotics may cause dysbiosis[20]. On the other hand, destroying beneficial microbes blocks the mechanism by which they inhibit pathogens[21]. These effects on the gut microbiome are related to the type of antibiotics and the treatment duration. Worse effects on the microbiome have been described with the use of clindamycin[22], clarithromycin, ciprofloxacin[23], and vancomycin[24]. All of these antibiotics cause a reduction in *Bacteroides* variety and *Ruminococcus*. Regarding non-antibiotic drugs, there is a complex bidirectional interaction between their use and the gut microbiome[25]. On the one hand, these commonly used drugs alter the gut microbiome composition and function; on the other hand, gut microbes can contribute to drug efficacy and safety by enzymatically transforming drug structure and altering drug bioavailability, bioactivity, or toxicity. Knowing these interactions enables interventions to modulate the gut microbiome and optimize treatment efficacy.

According to the Belgium Flemish cohort[26] and the Twins United Kingdom cohort[27], the most common no antibiotic drugs associated with microbiome modification and dysbiosis are proton pump inhibitors (PPIs), statins, laxatives, metformin, and angiotensin-converting enzyme inhibitors. PPIs, by changing microbiome composition may favor the colonization of pathogens such as *Clostridium difficile* and *Salmonella*[28,29]. Metformin, used to treat type 2 diabetes, increases *Escherichia coli* and reduces *Intestinibacter*, favoring dysbiosis and causing gastrointestinal side effects [30,31]. Studies conducted in the United Kingdom, the Netherlands, and Belgium have documented modifications in microbiome composition after the prolonged assumption of laxatives, statins, and antidepressants[26,27,32].

FUNCTIONS OF THE MICROBIOME

Metabolic function

The gut microbiota has the capacity to metabolize dietary fibers not metabolized by digestive enzymes[33]. In this way, the microbiome provides additional energy by metabolizing large polysaccharides and alcohols. The MEROPS database showed that the microbiome produces proteases that are able to metabolize different substances in the large intestine[34,35]. Additionally, beneficial effects produced by the microbiome are related to the production of several vitamins[36,37]. A study by Afzaal *et al*[38] showed the principal metabolites produced by gut microbiota in normal conditions and their functions (Table 3)[39-49].

Structural function

Under normal conditions, the microbiome contributes to maintaining the integrity of the gut epithelium. In this condition, cytokines present in the gut lumen do not pass through the gut epithelium. This function may be altered by pathogens such as *E. coli* and *C. difficile*. The dysbiosis produced by these bacteria facilitates the back diffusion of cytokines[50,51].

Protective function

The intestinal surface represents an important barrier, and the microbiome contributes to its stability[52]. The production of short-chain fatty acids (SCFAs) by the microbiome provides further energy for the epithelium and strengthens this barrier[53,54]. Table 4 shows examples of gut microbiome-derived metabolites and their beneficial effects on healthy conditions. In order of the metabolites, the pathway involved, the microbial agent responsible, and the health benefits produced, this information is shown in Table 4[55-70].

Relationship between the gut microbiome and immune system

The gut microbiome has important effects on the local and general immune systems. The local gut microbiome drives the maturation of gut-associated lymphoid tissue (GALT)[71] and maintains barrier function by mucus production and antimicrobial peptide production. In addition, GALT has specific influences on inflammatory *vs* no inflammatory cell phenotypes. One-sixth of the cells of the gut epithelium are represented by T lymphocytes. In addition, B lymphocytes, dendritic cells, and plasma cells are present in lymphoid tissue, principally in the colon mucosa or in Peyer's patches[72,73].

Indigenous microbiota or pathobionts may differentiate T helper (Th) cells into Th1, Th2, Th17, and regulatory T (Treg) cells *via* the production of microbiota metabolites. Filamentous bacteria induce the growth of Th17 and Th1; *Clostridia* stimulate Tregs and the anti-inflammatory cytokine interleukin 10 (IL-10). *B. fragilis* stimulates IL-10 and Tregs. By contrast, excessive stimulation of Th1 and Th2 cells due to the condition of dysbiosis may cause excessive production of proinflammatory cytokines[74]. Intestinal colonization has an important role in the development of tolerance[75]. This fact is relevant in the prevention of immune-mediated diseases. Indeed, the loss of immune tolerance may cause the development of allergic diseases or autoimmune diseases. Dysbiosis caused by *E. coli* or *C. difficile* is associated with eczema or atopic dermatitis. The production of SCFAs has a double effect. They constitute the main energy substrate for enterocytes and stimulate the maturation and correct function of Tregs[76]. Several axes have been established between the gut microbiome and different organs. In the case of dysbiosis they can generate diseases. The gut-brain axis may generate stress, anxiety, depression, schizophrenia, cognitive decline, and autism. The gut-brain endocrine axis generates regulatory, metabolic, behavioral, and hormonal disorders. The gut-heart axis generates cardiovascular diseases, atherosclerosis, thrombotic events, and hypertension. The gut-lung axis generates chronic obstructive pulmonary disease. The gut-liver axis generates liver inflammation, hepatocellular carcinoma, and non-alcoholic fatty liver. The gut-pancreas axis generates diabetes and pancreas cell inflammation. The gut-bone axis generates bone demineralization and osteoporosis. The gut-muscle axis generates muscle impairment, fragility, and sarcopenia. The gut-skin axis generates acne, psoriasis, atopic dermatitis, wrinkles, and aging. The gut-reproductive axis generates infertility, ovarian dysfunction, ovarian cancer, and postmenopausal osteoporosis. The gut-kidney axis generates chronic kidney disease, acute kidney injury, inflammation, nephrolithiasis, and nephropathy. The gut-bladder axis generates urinary tract infection and an overactive painful bladder. Some of these axes will be discussed below.

Gut microbiome and the brain

Recently, significant studies have documented the existence of the so-called "gut-brain axis." This is a bidirectional communication system between the gut and brain. On the one hand, the brain may control gastrointestinal functions such as peristalsis, mucus production, and the gut immune system[77]. On the other hand, the gut microbiome releases SCFAs and other metabolites influencing brain function, whereas several neurotransmitters are involved in the bidirectional communication between the host and the microbiota[78].

The gut microbiome may affect the brain directly by the gut nervous system, sending signals to the brain or indirectly by the production of intestinal hormones or transforming diet components into substances such as SCFAs, neurotransmitters such as serotonins, gamma amino butyric acid (GABA), tryptophan, and vitamins that influence the blood-brain barrier (BBB) and cerebral functions[79].

In healthy conditions, bacteria such as *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Streptococcus* are among the principal producers of neurotransmitters[80,81]. In addition, SCFAs affect the BBB, and several other immune pathways affect behavior, memory, and locomotion[82,83].

Table 3 Metabolites produced by gut microbiota and their functions

Metabolites	Functions	Ref.
Bile acid metabolites; including deoxycholic acid and lithocholic acid	Regulate bile acid, cholesterol, lipid, glucose, and activate host nuclear receptors and cell signaling pathways	Ramírez-Macías *et al*[39]
Short-chain fatty acids metabolites	Regulate food intake and insulin secretion, also aid in maintaining body weight	Psichas *et al* [40]
Branched-chain fatty acids including isobutyrate	Histone deacetylase inhibition, increased histone acetylation	Mischke *et al* [41]
Indole derivatives including indoxyl sulfate and IPA	IPA exhibits neuroprotective effects, acts as a powerful antioxidant and regulates intestinal barrier function	Hendrikx *et al* [42]
Lipopolysaccharide, peptidoglycan, lipoteicholic acid	Epigenetic regulation of genes in colorectal cancer, modulation of chromatine structure and transcriptional activity	Lightfoot *et al* [43]
Phenolic derivatives include 4-OH phenylacetic acid, urolithins, enterodiol and 9-prenylaringenin	Exhibit antimicrobial effect, maintain intestinal health and protect against oxidative stress	Larrosa *et al* [44]
Choline metabolites include choline, trimethylamine N-oxide, and betaine	Regulating lipid metabolism, and glucose synthesis contribute to the development of cardiovascular disease	Smallwood *et al*[45]
Polyamines include putrescine, spermidine and spermine	Sustaining the high proliferation rate of intestinal epithelial cells enhances intestinal barrier integrity and enhances the systematic adaptive immune system	Rooks *et al*[46]
Vitamins including thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6) panthotenic acid (B5), biotin (B7), folate (B11, B9), cobalamin (B12), and menaquinone (K2)	Help in red blood cell formation, DNA replication, and repair, work as an enzymatic co-factor, and enhance immune functioning	Nicholson *et al* [47]
Ethanol	Protein fermentation metabolism may be involved in NAFLD progression	Yao *et al*[48]
Hydrogen sulfide	Reduction/neutralization of reactive oxygen species	Afanas'ev *et al* [49]

IPA: Indole-3-propionic acid; NAFLD: Non-alcoholic fatty liver disease.

Table 4 Examples of gut microbiota-derived metabolites and their beneficial effects on human health

Metabolite	Pathway	Microbial agent	Health benefits
Butyrate	Carbohydrate metabolism	*Clostridia; Faecalibacterium prausnitzii; Coprococcus catus; Anaerostipes hadrus*	Increased intestinal barrier function; Modulate intestinal macrophage function; Suppression of colonic inflammation; Improvements in insulin sensitivity
Propionate	Carbohydrate metabolism	*Blautia obeum; Coprococcus catus; Roseburia inulinivorans; Prevotella copri*	Suppression of colonic inflammation; Decreased innate immune response to microbial stimulation; Protection from allergic airway inflammation; Improvements in insulin sensitivity and weight control in obese mice
Indole	Tryptophan metabolism	*Lactobacillus; Bifinobacterium longum; Bacteroides fragilis*	Maintenance of host-microbe homeostasis at mucosal surfaces *via* IL-22; Increased barrier function; Modulation of host metabolism
Indole-3-aldehyde	Tryptophan metabolism	*Lactobacillus*	Maintenance of mucosal homeostasis and intestinal barrier function *via* increased IL-22 production; Protection against intestinal inflammation in mouse models of colitis
Indole-3-propionate	Tryptophan metabolism	*Clostridium sporogenes*	Maintenance of intestinal barrier function and mucosal homeostasis; Increased production of antioxidant and neuroprotectant products
10-hydroxy-cis-12-octadecoate	Linoleic acid derivative (lipid metabolism)	*Lactobacillus*	Maintenance of intestinal barrier function; Decreased inflammation; Increased intestinal IgA production

IgA: Immunoglobulin A; IL-22: Interleukin 22.

It should also be highlighted that the Mediterranean diet rich in vegetables and fibers stimulates the activity and growth of beneficial bacteria for the brain[84]. More than 20% of patients with gut dysbiosis are affected by sleep disorders and depression[85,86].

Similarly, recent studies have documented that microbiota composition differs significantly between healthy controls and patients affected by neurovegetative disorders such as multiple sclerosis (MS), Alzheimer's disease (AD), and Parkinson's disease (PD)[87]. In MS, a higher abundance of *Firmicutes* and the absence of *Fusobacteria* are frequently found [88]. The stool microbial profile of AD patients has a decreased number of *Firmicutes* and *Actinobacteria*. *Firmicutes*, such as

Ruminococcoceae and *Turicibacteriaceae*, are less abundant in these patients[89]. PD patients have a lower production of SCFAs and fewer gut bacteria, such as *Lechnospiraceae* and *Faecalibacterium prausnitzii* producing these substances[90].

In conclusion, several recent studies have documented that a different composition of the gut microbiome may contribute to the development of neurodegenerative disorders causing chronic inflammation of neuronal cells and loss of the BBB.

MICROBIOME IN HEALTH AND DISEASE

Table 4 shows the gut microbiome in healthy conditions. A metabolically healthy microbiota (mainly achieved by a high fiber, low animal fat, and low protein diet and other aforementioned environmental factors) is shown in Figure 1. Microbial production of SCFAs provides an energy source for colonocytes and causes a decrease in luminal pH. The SCFAs acetate, butyrate, and propionate can bind to the G protein-coupled receptor 41 (GPR41) and GPR43, which are expressed on enteroendocrine L cells, and subsequently induce the secretion of glucagon-like peptide 1 and peptide YY, which contribute to increased energy expenditure, reduced food intake, and improved glucose metabolism and insulin secretion[91]. Butyrate is an activator of peroxisome proliferator-activated receptor gamma and a stimulator of β-oxidation and oxygen consumption in the gut, which maintains an anaerobic environment in the gut lumen[92].

Pathobionts often responsible for dysbiosis are shown in Table 5. Figure 2 shows the metabolic pathways induced by gut dysbiosis. A dysbiotic microbiota is often associated with a prolonged colonic transit time, resulting in a shift in colonic metabolism leading to increased microbial proteolysis. Even though the preferred substrate for bacterial fermentation is fermentable dietary fibers, bacteria will not switch to protein metabolism until fermentable polysaccharides are depleted. As a result of increased protein fermentation, branched-chain fatty acids (2-methylbutirate, isobutyrate, and isovalerate), trimethylamine, organic acids, gases, and trace amounts of phenols, amines, indoles, and ammonia are produced, causing an increase in luminal pH[93]. Together, such changes in the microbial environment and metabolites cause leakage of pathogen-associated molecular patterns, including lipopolysaccharides (LPS), which increase in the blood and trigger systemic low-grade inflammation and insulin resistance[94]. It should be noted, however, that some indole derivatives, such as 3-indolepropionic acid, produced by the fermentation of dietary fibers have been shown to improve glucose metabolism[95].

DISEASES ASSOCIATED OR RELATED TO DYSBIOSIS

Some diseases associated with gut microbiota abnormalities are shown in Table 6. Neurological diseases associated with gut dysbiosis have already been discussed. Other diseases associated with gut dysbiosis are allergic diseases, inflammatory bowel syndromes or diseases, and metabolic diseases.

Allergic diseases: Gut microbiota dysbiosis is reportedly associated with allergic diseases such as eczema and asthma [96]. Lower levels of gut *S. aureus* and *Clostridium* and higher levels of *Bifidobacterium* are present in children affected by allergic symptoms[97]. The cause has been ascribed to LPS that cause a reduced immune system response[98]. The relationship of gut dysbiosis and asthma or other lung syndromes justifies the term "gut-lung axis"[99].

Irritable and inflammatory bowel syndromes: Irritable bowel syndrome (IBS) is a condition affecting 10% to 20% of adults, and children may also be affected. Bennet *et al*[100] described alterations in the gut microbiome of patients affected by IBS. *Firmicutes* are increased with a reduction in *Ruminococcus* and *B. fragilis*. The result is an excessive increase in SCFAs with consequent increase in serotonin that alters intestinal motility. Inflammatory bowel disease (IBD) is a more severe condition. The most significant types of IBD are ulcerative colitis and Crohn's disease[101]. Several types of gut dysbiosis have been found in patients affected by IBD. *Bacteroidetes* and *Firmicutes* are decreased[102]. Recently Zhu *et al* [103] found an increase in Proteobacteria and *E. coli*. One of the consequences of such dysbiosis is a reduction in mucus production that allows gut flora to pass more easily across the intestinal barrier, thus enhancing the inflammatory process [104].

Gut dysbiosis and metabolic diseases: The gut microbiota participates in material metabolism to produce metabolites. The gut microbiota can affect the metabolism of glucose, lipids, and proteins by generating a series of metabolites and activating downstream signaling pathways as shown in Figure 3.

Obesity: In addition to genetic and behavioral factors, the gut microbiome has an important role in the genesis of obesity [105]. Obese patient gut flora have higher levels of *Firmicutes* and lower levels of *Bacteroidetes*[106]. Other bacteria found in the gut of obese patients are *Bacteroides*, *Ruminococcus*, and *Staphylococcus*[107]. These bacteria cause the increased degradation of β-glucuronide and aromatic amino acids, higher generation of organic acids and H_2, and higher biosynthesis of phenylalanine, tyrosine, and tryptophan[108]. In this condition, chronic inflammation is generated by the production of IL-1, tumor necrosis factor alpha, monocyte chemoattractant protein-1, and IL-6. In addition, LPS are produced that bind to the cluster of differentiation 14 receptor on the surface of immune cells to produce further inflammatory factors[109,110].

Type 2 diabetes mellitus: Several studies have documented the influence of the gut microbiome on the pathophysiology of type 2 diabetes mellitus. In this condition, pathogenic flora prevail over protective flora. Gurung *et al*[111] analyzed 42

Table 5 Some examples of potentially harmful gut microbiota bacterial species

Bacteria	Associated physiologic changes	Associated diseases states
Bacteroides	Activate CD4+ T cells	Increased with animal-based diet; Increased in obesity
Bilophila	Promote pro-inflammatory immunity	Increased in colitis; Decreased in autism
Clostridium	Promote generation Th17 cells	Increased after smoke exposure; Increased in autism and Rett syndrome; Positive correlation with plasma insulin and weight gain; Increased in type 2 diabetes; *Clostridium perfrigens* increased in old age
Escherichia coli	TLR activation	Increased in inflammatory bowel disease; Increased in type 2 diabetes
Neisseria	Sugar fermentation	Only two species are pathogenic: *Neisseria meningitides* and *Neisseria gonorrhoeae*

Th17: T helper 17: TLR: Toll-like receptor.

Table 6 Diseases associated with gut microbiota abnormalities

Disease	Features
Irritable bowel syndrome	An abundance of *Firmicutes* and a decrease of *Bacteroidetes*
Type I diabetes	In genetically predisposed individuals, autoimmune against pancreatic β-cells. Deficient development or alteration of the microbiota may contribute to dysfunctional immunity with the devastation of autoimmune β-cells and increased leakiness of the intestinal barrier. Variability of microbiomes reduced
Asthma	Outbreaks of *Chlamidophila pneumonia* during bronchitis and pneumonia development affect the airway microbiome. Gut microbiota is influenced by the introduction of microbiota to the environment, particularly in early life, which helps immune function growth and the development of defending against allergic sensitization
Food-borne pathogens and food poisoning	Opportunistic pathogens (*Campylobacter, Salmonella, Escherichia coli, Shigella*) disturb the microbiome's balance leading to dysbiosis
Malnutrition	Decrease or missing species that either process food categories efficiently or produce vitamins may reduce the absorption of nutrients. An overabundance of *Enetrobacteriaceae* can lead to epithelial damage, diarrhea, and limited absorption of nutrients
Depression	In physiologic system, *Bifidobacterium infantis*, generally found in infants' gastrointestinal tract and administered probiotic drugs, can have antidepressant effects
Anxiety	Oral administration of *Campylobacter jejuni* subclinical doses in murine models induced anxiety like behavior without stimulating immunity

studies and found that the protective bacteria were *Bifidobacterium* and *Bacteroides* as well as *Rosaburia* and *Faecalibacterium*. By contrast, *Ruminococcus*, *Fusobacterium*, and *Blautia* are pathobionts that, through the production of LPS, increase the permeability of the intestinal epithelium. In this condition, inflammatory molecules are produced, which increases insulin resistance (Figure 4)[112]. By contrast, protective flora produce IL-10 and other anti-inflammatory cytokines. In addition, protective flora exert their effect through the production of SCFAs and butyrate[113]. SCFAs also act as substrates for lipogenesis and gluconeogenesis in the liver.

Heart disease: Recent studies have also allowed identified a heart-gut axis. As in similar conditions, the effects of this axis are bivalent. Indeed, on the one hand, heart failure induces an increase in permeability of the gut barrier (the so-called leaky gut) with consequent passage of gut microbiota and its products in the circulation inducing inflammation. On the other hand, several products of the gut microbiota entering the blood may induce hypertension, atherosclerosis, and heart failure. The gut flora that is able to induce cardiovascular diseases is composed as follows[108]. There is an increase in Enterobacteriaceae as *E. coli* and *Klebsiella*, and a decrease in *Roseburia* and *F. prausnizii*[114]. Several metabolic pathways are involved in the gut-heart axis: trimethylamine (TMA) and trimethylamine n-oxide (TMAO), SCFAs, and bile acids [115]. Dietary sources including choline and l-carnitine provide substrates for microbiota-mediated production of trimethylamine. TMA after entering the portal circulation is converted by the hepatic host flavin-containing monooxygenase to TMAO. TMAO can promote atherogenesis and heart failure development. Adverse cardiac remodeling is also associated with elevated TMAO levels[116-118]. A beneficial effect is exerted by SCFAs. SCFAs improve intestinal barrier function by promoting mucous production. In addition, they improve vascular tone through G protein-coupled receptor signaling. Finally, SCFAs activate histone acetyltransferase and inhibit histone deacetylase, thereby inhibiting inflammation and modulating immune cell activation[119].

Bile acids: Bile acids may be modified by the microbiome. They are produced by the liver, and a small portion are metabolized by colon microbiota with the production of secondary bile acids such as deoxycholate, lithocholate, and

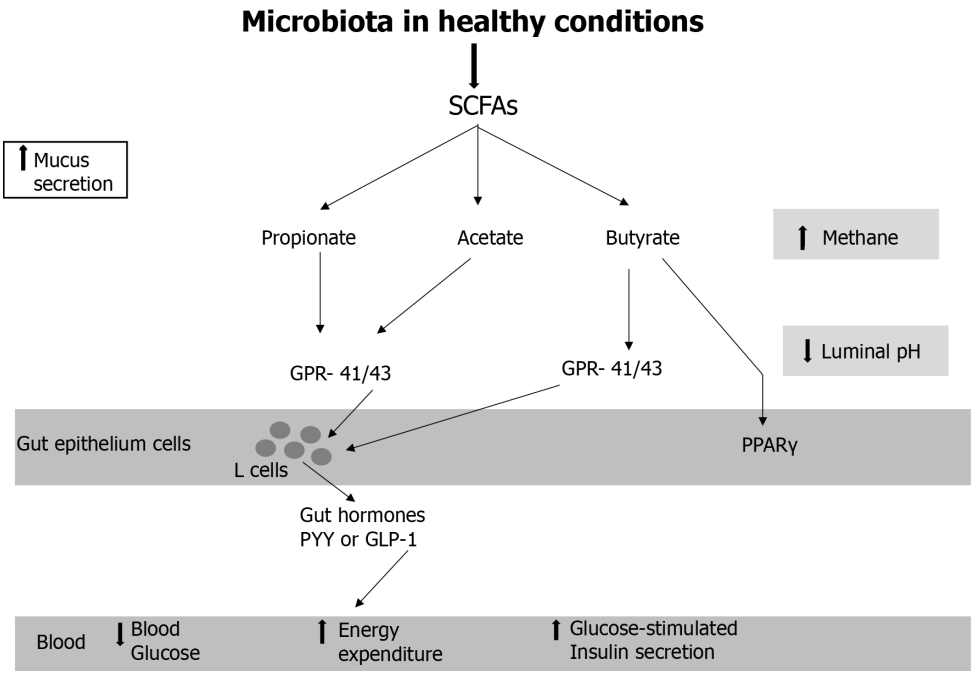

Figure 1 Microbiota in healthy conditions. GLP-1: Glucagon-like peptide 1; GPCR: G protein-coupled receptor; PPARγ: Peroxisome proliferator-activated receptor gamma; PYY: Peptide YY; SCFA: Short chain fatty acid.

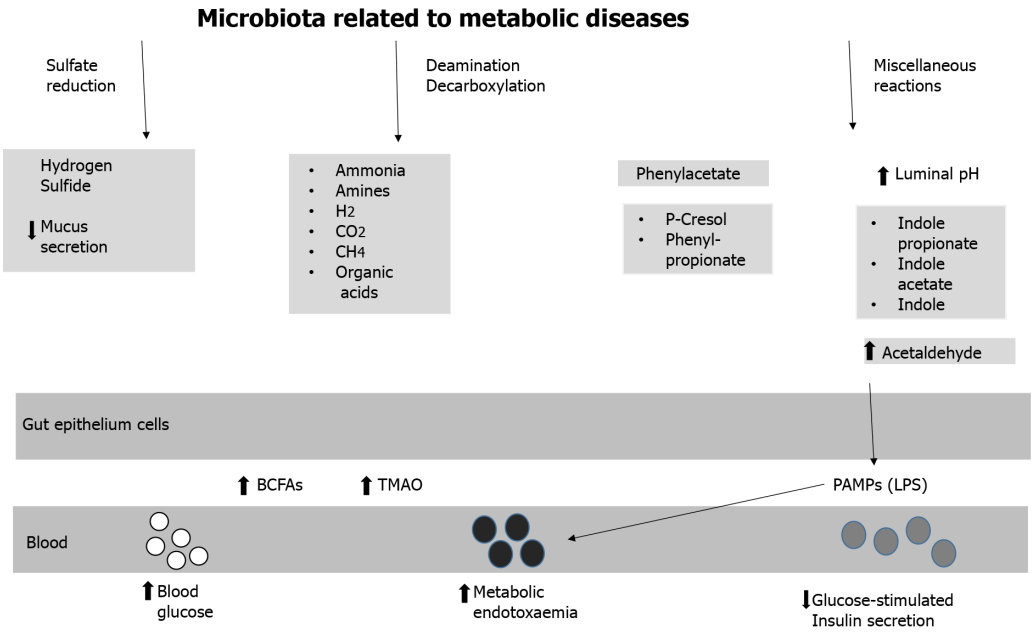

Figure 2 Microbiota related to metabolic diseases. BCFA: Branched-chain fatty acid; LPS: Lipopolysaccharides; PAMP: Pathogen-associated molecular pattern; TMAO: Trimethilamine oxide.

ursodeoxycholate. These secondary bile acids are powerful agonists of the farnesoid nuclear receptor (FXR), which modulates metabolism and inflammation. Therefore, bile acids inhibit the anti-inflammatory activity of FXR. At the cardiovascular level, bile acids favor atherosclerotic disease, cardiac hypertrophy and hypertension[120].

All of these studies are important, but often they are conflicting or have some drawbacks. Some examples are as follows.

The *Firmicutes*/*Bacteroidetes* ratio is cited in several studies as an important marker of health or disease; however, several of these studies have relevant bias due to methodological procedures or discrepancies in enrolling subjects for the study.

Modification in gut microbiota seems to cause irritable bowel disease, but the microbiota responsible and their metabolites are poorly understood. The same is true for the interrelation between not well defined microbiota and brain

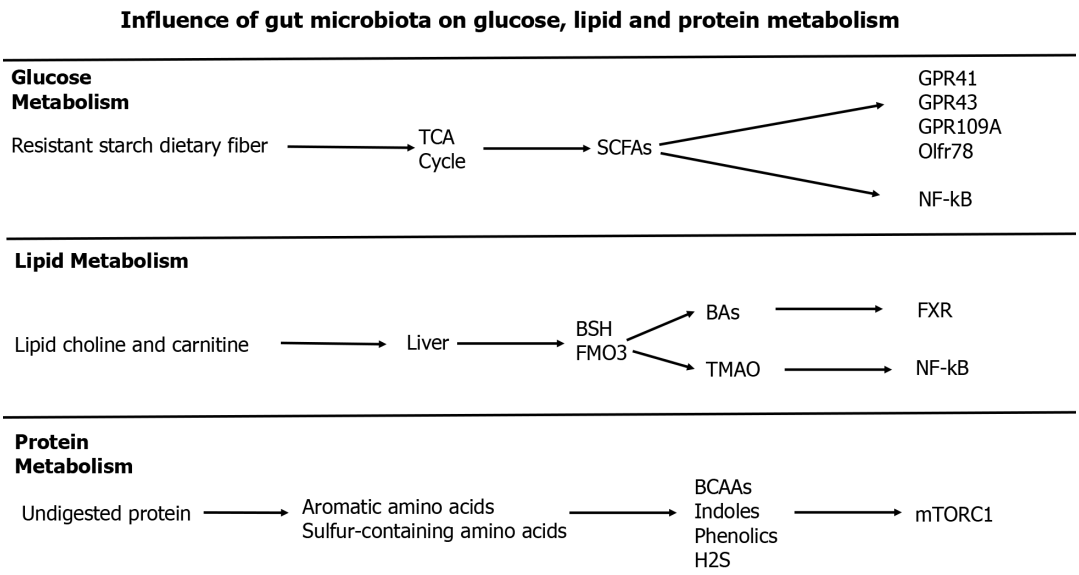

Figure 3 Influence of gut microbiota on glucose, lipid, and protein metabolism. BCAAs: Branched-chain amino acids; BSH: Bile salt hydrolase; FMO3: Flavin monooxigenase 3; FXR: Farnesoid X receptor; GPR: G protein-coupled receptor; Olftr: Olfactory receptor 78; mTORC1: Mammalian target of rapamycin complex 1; NF-kB: Nuclear factor kappa B; SCFAs: Short-chain fatty acids; TGR5: Takeda G protein-coupled receptor 5; TMAO: Trimethylamine N-oxide.

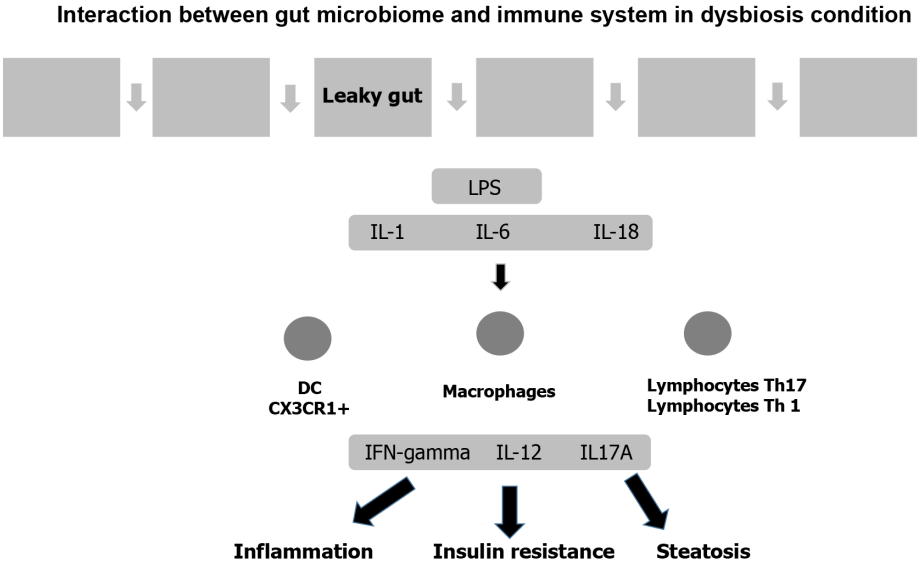

Figure 4 Interaction between gut microbiome and immune system in dysbiosis condition. DC: Dendritic cell; IFN-γ: Interferon gamma; IL-1: Interleukin 1; IL-6: Interleukin 6; IL-18: Interleukin 18; LPS: Lipopolysaccharides.

diseases such as PD and autism. Again, methodological problems and presence of confounders may be the cause.

More importantly, these problems concern the treatment. Indeed, meta-analyses on the use of prebiotics and probiotics have given contradictory results and to date, the majority of authors retain that large-scale randomized controlled studies are needed to evaluate the efficacy of these treatments.

TREATMENT AND PERSPECTIVES

Dysbiosis treatment is based on the use of prebiotics or probiotics. In addition, fecal microbiota transplantation has been proposed. Prebiotics are dietary products that may change the composition and functions of microbiota by enhancing the presence of indigenous microbiota or by favoring the growth of specific bacteria. Probiotics are live beneficial bacteria. Among these favorable effects to re-equilibrate, gut microbiota dysbiosis has been documented by *Akkermansia muciniphila*, *F. prausnizii*, *B. uniformis*, predator bacteria, and phage therapy[8]. Fecal microbiota transplantation is the process of transplantation of fecal microorganisms from healthy people to re-equilibrate gut microbiota dysbiosis. In the future we can imagine the use of sequestrate or binding resins to eliminate harmful products or to sequestrate microbial

metabolites. Other approach is the use of 3, 3-dimethyl-1-butanol to reduce TMAO or TMA-lyase inhibitors. Overall, several problems remain to be resolved.

There is substantial heterogeneity in the microbiota in both normal conditions and dysbiosis. In general, there is not a single bacterium but a collection of bacteria. This collection will likely not be best defined by the individual bacteria but rather by their metabolic capacities. This refers to the enzymes that are expressed, functioning and determining downstream metabolism.

CONCLUSION

The human gut possesses millions of microorganisms that is called microbiota. Microbiota in normal conditions exerts beneficial effects over the whole body and is connected with many organs forming different axis. Several factors may modify the microbiota composition and favor the presence of dangerous microorganisms, better known as pathobionts. This condition is called dysbiosis and is linked with different diseases, such as neurological diseases, metabolic diseases, and circulatory diseases. An understanding of microbiota in both the healthy subject and sick patient and understanding of the biological pathway connecting the gut microbiota with different organs are essential to finding therapeutic measures. The use of probiotics, prebiotics, phages, and feces transplantation represent to date the therapeutic measures more frequently adopted. However, several trials are ongoing looking for new, more efficient therapeutic strategies.

Future directions, among others could be as follows: The development of multiomics techniques such as metagenomics, metabolomics, and metatranscriptomes should be further developed to answer the critical questions of which microbiota are involved in health and disease. Indeed, in addition to metagenomics, the microbiome should also be analyzed by metabolomics studies in order to find its metabolic organization.

In addition, studies on genome-wide association will be used to correlate different genotypes with diseases phenotypes, and studies of metabolome wide association will correlate metabolic phenotypes with disease phenotypes.

The key findings of our study were as follows: (1) In normal conditions, the gut microbiome exerts a beneficial effect on the organism. (2) This is related to the production of several metabolites. (3) Several axis connect the microbiome with several organs. (4) Gut microbiome modifications with the appearance of pathobionts are at the basis of several diseases in different organs.

FOOTNOTES

Author contributions: Salvadori M and Rosso G equally contributed to the development of the manuscript; Salvadori M and Rosso G wrote the manuscript; Rosso G looked for new references; Both authors reviewed the final manuscript.

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

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S-Editor: Liu JH
L-Editor: Filipodia
P-Editor: Guo X

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World Journal of Methodology

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

World J Methodol 2024 March 20; 14(1): 88519

DOI: 10.5662/wjm.v14.i1.88519

ISSN 2222-0682 (online)

MINIREVIEWS

Gut microbiome in alcohol use disorder: Implications for health outcomes and therapeutic strategies-a literature review

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Specialty type: Medical laboratory technology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Liu C, China; Stachowska E, Poland; Wang YD, China

Received: September 27, 2023
Peer-review started: September 27, 2023
First decision: December 7, 2023
Revised: December 22, 2023
Accepted: January 24, 2024
Article in press: January 24, 2024
Published online: March 20, 2024

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Abstract

Alcohol use disorder (AUD) represents a major public health issue which affects millions of people globally and consist a chronic relapsing condition associated with substantial morbidity and mortality. The gut microbiome plays a crucial role in maintaining overall health and has emerged as a significant contributor to the pathophysiology of various psychiatric disorders. Recent evidence suggests that the gut microbiome is intimately linked to the development and progression of AUD, with alcohol consumption directly impacting its composition and function. This review article aims to explore the intricate relationship between the gut microbiome and AUD, focusing on the implications for mental health outcomes and potential therapeutic strategies. We discuss the bidirectional communication between the gut microbiome and the brain, highlighting the role of microbiota-derived metabolites in neuroinflammation, neurotransmission, and mood regulation. Furthermore, we examine the influence of AUD-related factors, such as alcohol-induced gut dysbiosis and increased intestinal permeability, on mental health outcomes. Finally, we explore emerging therapeutic avenues targeting the gut microbiome in the management of AUD, including prebiotics, probiotics, and fecal microbiota transplantation. Understanding the complex interplay between the gut microbiome and AUD holds promise for developing novel interventions that could improve mental health outcomes in individuals with AUD.

Key Words: Alcohol use disorder; Gut microbiome; Dysbiosis

Core Tip: The emerging field of research on the gut microbiome's role in alcohol use disorder (AUD) has revealed significant implications for health outcomes and potential therapeutic strategies. Alcohol consumption has profound effects on the gut microbiome, leading to dysbiosis and increased systemic inflammation but their association has been found bidirectional. The gut microbiome represents a promising therapeutic target for the treatment of AUD, with dietary interventions such as probiotics and prebiotics, as well as fecal transplantation showing potential in improving gut dysbiosis and reducing inflammation.

Citation: Koutromanos I, Legaki E, Gazouli M, Vasilopoulos E, Kouzoupis A, Tzavellas E. Gut microbiome in alcohol use disorder: Implications for health outcomes and therapeutic strategies-a literature review. *World J Methodol* 2024; 14(1): 88519
URL: https://www.wjgnet.com/2222-0682/full/v14/i1/88519.htm
DOI: https://dx.doi.org/10.5662/wjm.v14.i1.88519

INTRODUCTION

Alcohol use disorder (AUD) represents a major public health issue which affects millions of people globally. AUD is characterized by excessive drinking and persistent alcohol-seeking behavior. It has been described as a single spectrum of problematic use and clinically significant impairment based on endorsement of at least two of a total of 12 criteria that assess behavioral and physical manifestations of heavy alcohol consumption according to the Diagnostic and Statistical Manual of Mental Disorders. The terms alcohol abuse and alcohol dependence fall under the umbrella of the general term AUD, and can be can be classified as mild (if patient meet 2 or 3 criteria), moderate (if patient meet 4 or 5 criteria), or severe AUD (if patient meet more than 6 criteria)[1].

The prevalence of AUD is increased in high/upper middle-income countries in both males and females. Some estimates show almost 6% of individuals meet the AUD criteria, leading to significant socioeconomic problems and public health losses[1,2].

The worldwide prevalence of heavy episodic drinking surpassed 18% of total population in 2016[3]. Nevertheless, alcohol use and its effects present substantial variations across different countries. The European Union is the region with the highest alcohol consumption at a global scale, with 87% of its adolescents having consumed alcohol at least once during their lifetime, even higher compared to the United States' 70% of adolescents[4].

Alcohol abuse is responsible for approximately 3 million deaths per year (5.3% of all deaths), along with more than 5% of the disease burden globally according to the WHO[5]. AUD is attributed as a causal factor for a plethora of diseases, which include communicable diseases, such as maternal, perinatal and nutritional conditions, and non-communicable diseases, such as epilepsy, cancer, cardiovascular, digestive diseases and injuries[3]. While the physical health consequences of AUD have been well-described, the impact of alcohol on mental health is a matter of ongoing deliberation. The interplay between alcohol consumption, mental health, and physical health outcomes is complex and multifaceted. AUD syndrome is the result of cumulative effects caused by excessive alcohol consumption, a person's genetic susceptibility, and several environmental factors, as such, deep understanding of its pathophysiology could be vital for the development of an effective treatment[6].

Abundant evidence have highlighted that alcohol dependence (alcoholism) is a complex genetic disease, with a heritability estimate as high as 50%, and a large number of variants across the genome influencing the onset and development of a person's addiction to alcohol, with some of these genes being involved in alcohol metabolism[1]. Acquiring a better understanding of the way that the environment influences genetic risk which contributes to the onset of alcoholism is of major importance in deciphering the underlying mechanisms of AUDs[7].

The gut microbiome, the complex ecosystem of microorganisms residing in the gastrointestinal tract, has risen as a fascinating field of study, as it affects several physiological processes, including digestion, metabolism, and immune function. Recently, the gut microbiome is referred to hold a substantial role in the pathophysiology of mental illnesses such as schizophrenia, bipolar disorder, anxiety disorders, depression and AUD. Preclinical studies have indicated the influence of gut microbiota in the gut-brain axis (GBA) and the bidirectional interactions between the central nervous system, the enteric nervous system, and the gastrointestinal tract, potentially affecting mental health outcomes[8].

In this review, we will discuss the latest findings regarding the changes in gut microbiome associated with AUD, and how they contribute to the development and progression of the disorder. We will further discuss the potential mechanisms through which gut dysbiosis leads to implications on health outcomes. Additionally, we will delve into the potential therapeutic approaches targeting the gut microbiome for the treatment of AUD, such as probiotics, prebiotics, dietary interventions and fecal transplantation. By considering the gut microbiome when evaluating and treating individuals with AUD, clinicians may be able to improve the health outcomes of these patients and reduce the burden of disease associated with AUD.

METHODLOGY

A comprehensive literature search was conducted using the PubMed database to identify relevant articles for this review. The following search terms were used: "gut microbiome," "alcohol use disorder," "alcohol abuse," "alcohol consumption," "microbiota," and "microbiome." This review focused on articles published in the English language between 2010 and 2022, to ensure the inclusion of recent research while capturing significant developments in the field.

The initial search yielded a broad range of articles related to the gut microbiome and AUD. After careful evaluation, articles were selected based on their relevance to the topic. Studies that investigated the changes in gut microbiome composition and function in individuals with AUD, as well as those which explored the impact of alcohol on gut dysbiosis and associated health outcomes were included. Additionally, articles focusing on therapeutic strategies targeting the gut microbiome for the treatment of AUD were considered.

The selected articles were thoroughly reviewed, and the key findings, methodologies, and conclusions were extracted. Data related to the mechanisms linking alcohol consumption to gut dysbiosis, the implications of gut dysbiosis on health outcomes, and the potential therapeutic approaches targeting the gut microbiome were synthesized and organized in a coherent manner.

By analyzing the available literature and synthesizing the findings, this review intends to contribute to the growing body of knowledge on the gut microbiome's role in AUD and provide valuable insights for future research and clinical practice.

THE COMPOSITION OF GUT MICROBIOME IN HEALTHY VS AUD INDIVIDUALS

The human body is inhabited by a vast number of microorganisms that live in concordance with their host and are commonly referred to as human microbiota or microflora. The largest proportion of microbiota, approximately 70%, can be found in the gut. Intestinal microflora is involved in host's physiology, regulating digestion, vitamin production, metabolism of xenobiotics, immunological responses and at the same time conferring protection against pathogen perturbation[9,10].

Although it was originally believed that gut contains about 1000 bacterial species, a large scale study has estimated that the collective human gut microbiota is composed of over 35000 bacterial species. Overall, the healthy gut microbiota is predominantly constituted by strict anaerobes that dominate over anaerobes from the phyla Firmicutes and Bacteroidetes. This is followed by the phyla Actinobacteria, and Proteobacteria, with minor proportions of species belonging to the phyla of Fusobacteria, Tenericutes, and Verrucomicrobia. Despite the constant appearance of this general profile remains constant, gut microbiota can exhibit temporal and spatial differences regarding distribution at the genus level and higher classification[10,11].

A microbiome that has been linked with a healthy host, requires an extent of resistance towards external (*e.g.* dietary, pharmaceutical) and internal (*e.g.* age) changes and the ability of resilience afterwards. Thus, microbial health does not reflect a static but rather a dynamic state. Any such disturbance could jeopardize the balance of the composition and/or regulation of microbial communities, a condition that is commonly described as dysbiosis. This condition is far more likely to occur due to inadequate presence of commensal microorganisms, possible alteration of regular microbial diversity, and competition between commensal and pathogenic species for a particular body region or nutrients. Additional external factors that favor the progression of a dysbiotic state involve malnutrition, as in the case of low dietary fibers or vitamins, food additives (*e.g.* preservatives, emulsifers), chronic alcohol consumption, drug abuse or certain medication (most often antibiotics, over-the-counter anti-inflammatories and chemotherapeutics), exposure to hazardous environmental agents (toxins, heavy metals or radiation), and increased stress levels (anxiety, depression). Current literature suggests that mental health disorders (as in the case of drug and alcohol abuse) are often correlated with dysbiosis[12].

Alcohol abuse is known to cause deleterious effects all over human body. Despite that, it remains to be proved whether alcohol drinking is the cause or the consequence of changes in the gut microbiota. As stated above, alcohol consumption can cause significant imbalances to the gut microbiome such as the promotion of potentially pathogenic bacteria in alcoholics with and without liver disease, resulting in dysbiosis. de Timary *et al*[13], observed no differences in alcohol intake between the dysbiotic and non-dysbiotic group, while the total concentration in bacteria and in most bacterial families, genera or species failed to recover after detoxification, suggesting that the difference in composition of the gut microbiota might not be the consequence of drinking, which raises the issue of whether the alteration in the gut microbiota could be a precursor to the development of alcohol-dependence in some subjects. The majority of data deriving from human-based studies show a link between alcohol intake and bacterial abundance that does not imply causality. Current knowledge suggests that innate gut bacteria may bey key influencers of voluntary alcohol intake in rats that have been selectively bred for their increased ethanol intake. Administration of antibiotics that are non-absorbable prior to ethanol access has been reported to inhibit approximately 70% of volunteray ethanol intake. This effect has been fully observed during the first day of access to alcohol. The available data suggest that the firewall mechanisms that typically prevent increased alcohol intake tend to be suppressed by endogenous microbiota of a rat strain that is selected for their preference towards alcohol[14]. Carbia *et al*[15] demonstrated that the most typical patten of alcohol misuse in adolescence is linked with alterations of the gut microbiome, even prior to the development of addiction. An animal-based study of Segovia-Rodríguez *et al*[16] shed light to whether intestinal microbiota could be the cause of increased alcohol intake in animals that received fecal transplantation from alcohol-dependent laboratory animals, leading to a higher alcohol consumption and to a reduced spontaneous locomotor activity compared to animals which received

transplant from control animals or those treated with the buffer without feces[17]. This finding indicated that alterations that were induced by microbiota would affect the host in a more universal fashion. Authors proposed a synergistic mechanism of interaction between the new microbiota received and alcohol consumption. A plausible explanation of the bidirectional effect is that in the presence of alcohol, microbiota can promote a positive feedback mechanism that favors the abundance of bacteria that benefit from alcohol intake[17]. These alterations reflect a dysregulation in the microbiome-gut brain axis that could further trigger dysregulation and lead to an even more increased risk of psychopathology and especially when appearing during key windows across a person's lifetime[15].

Most data about the influence of alcohol on the relative abundance of gut microbiome comes from animal-based studies; expose to alcohol does not alter the abundance of gut microbiota, but remarkably alternates its composition. In animal models, alcohol intake decreases the relative abundance of the genera Lactobacillus (or Sporolactobacillus) and promotes the relative abundance of the genera Blautia, Allobaculum[18], Ruminococcus, Coprococcus[19], Adlercreutzia and Turicibacter[20], Alistipes and Odoribacter[21], resulting in memory loss, and neuropsychiatric behaviors, like anxiety and depression-like disorders[18,20,21].

Different patterns of drinking, such as chronic drinking (chronic) or recent heavy drinking (acute) could lead to a different composition of gut microbiota[22]. Specifically, in a mouse model with acute alcohol consumption, an upregulation of the *phyla Actinobacteria* and *Verrucomicrobia* and the genera *Bacteroidales* and *Lachnospiraceae* was recorded[23], while in chronic alcohol consumption *Bacteroidetes*, *Bacteroides genus*, and *Akkermansia* genus were present at a higher proportion[22]. Furthermore, acute alcohol consumption decreased the levels of *Lactobacillus*, *Escherichia-Shigella*, and *Turicibacter*[23], while in chronic alcohol intake the relative abundance of *Firmicutes phylum*, *Lactococcus*, *Pediococcus*, *Lactobacillus*, and *Leuconostoc genus* was downregulated[22].

Even though alcohol consumption could be a critical factor that influences gut microbiome in terms of function and composition, alcohol metabolism itself, along with its effects on the patient/consumer can be influenced by the microbiome, establishing a bidirectional relationship. However, existing data on alcohol's effect on gut microbiome in humans are scarce. The first studies dealing with changes in the gut microbiome of individuals with AUD concluded a reduction in the abundance of beneficial bacteria such as *Bacteroidetes*, *Lactobacillus* and *Bifidobacterium*, and an increase in potentially harmful bacteria, such as *Enterobacteriaceae*, *Streptococcus* and *Proteobacteria*[24,25].

On the other side, alcohol dependents with higher intestinal permeability seems to present a more unique profile with a sharp reduction in the abundance of Ruminoccaceae family (and in particular in Rumminococcus, Faecalibacterium, Subdoligranulum. Oscillibacter and Anaerofilum), and an increase in Lachnospiraceae and the genera Blautia and Megasphaera[25].

Dubinkina *et al*[26] were the first to describe the gut metagenome of patients with alcoholic disorder using shotgun (whole-genome) metagenomic sequencing. They found significant differences on the gut microbial community in gut dysbiosis, when comparing alcoholics with and without liver disease, as opposed to Mutlu *et al*[24], who reported similarities between the two groups. Dubinkina *et al*[26] reported only a slight overlap in the metagenomic signature of alcoholics with and without liver disease. Increased populations of Klebsiella and decreased Coprococcus, *Faecalibacterium prausnitzii*, and unclassified Clostridiales were associated with alcoholics without liver cirrhosis, whereas both groups were characterized by reduced *Acidaminococcus sp*, as well as increased Lactobacilli and Bifidobacterium members, however with different species in each group. Most recently, Bjørkhaug *et al*[27] confirmed *via* sequencing the higher relative abundance of *Proteobacteria* in alcohol overconsumers in a dose independent manner. Their results revealed reduced relative abundance of Faecalibacterium, a plethora of taxonomic groups inside the Firmicutes phylum, and specifically in the Clostridia and Actinobacteria class in the group of alcohol overconsumers, and a higher relative abundance of Sutterella, Clostridium, and Holdemania[27].

A more recent meta-analysis highlighted the causal role of alcohol in gut dysbiosis confirming that alcohol consumption could favor the proliferation of some bacterial species in the gut, such as the already mentioned Bacteroidetes and Proteobacteria and suppress other species like the probiotic Lactobacillus and Bifidobacterium and the physcobiotics *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*[28].

However, the gut microbiome does not consist of bacteria only, but also includes viruses and fungi. Major differences have been observed regarding the fecal viromes of AUD patients, specifically in the composition of bacteriophage species. At least 18 bacteriophages were found to be more abundant in the control subjects, including eight bacteriophages that target Propionibacterium, five that target Enterobacteria, while the remaining seem to target Salmonella, Lactobacillus, Cronobacter, Escherichia, and Leuconostoc. Concerning the bacteriophage species which were more abundant in the AUD patient population, the findings include two bacteriophages that target Streptococcus and two that target Lactococcus[29]. Concerning mycobiome, there are scarce data investigating fungal abundance differentiation in AUD patients compared to non-alcoholic individuals. An increase in the abundance of Candida[30-33], Pichia[30,33] Kluyveromyces, Issatchenkia and Scopulariopsis[33], genus level has been reported, while Saccharomyces, Penicillium [31], and Epicoccum[30], presented lower levels. Literature data are conflicting regarding Debaryomyces[30,31,33] (Figure 1).

Implications of gut dysbiosis in AUD

The dysbiosis observed in the gut microbiome of individuals with AUD can have significant implications for health outcomes. One of the most notable consequences is the increased risk of developing liver disease. Chronic alcohol consumption leads to imbalanced gut microbiome, which in turn causes hepatocytes damage, leading to the development of alcoholic liver disease (ALD), ranging from steatosis to cirrhosis. Gut dysbiosis has also been linked to the development of other chronic diseases, including cardiovascular disease and type 2 diabetes. Gut microbiota may influence brain function through neural, endocrine, and immune pathways[34] related to vagus nerve signaling[35]. In order to ameliorate the health outcomes of gut dysbiosis, it is essential to understand the role of the gut microbiome in

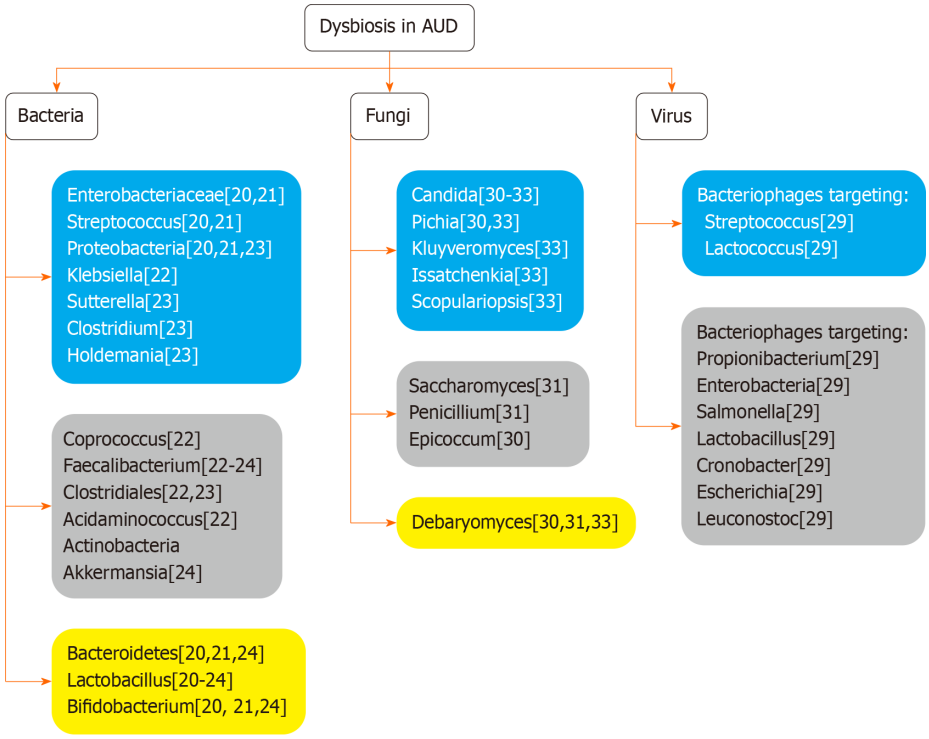

Figure 1 Variation in gut microbiome in alcohol use disorder individuals. Blue boxes present gut microbiome with higher abundance in alcohol use disorder patients. Grey boxes show gut microbiome with decreased number in alcohol use disorder patients. Yellow boxes present gut microbiome for which data are controversial. AUD: Alcohol use disorder.

health maintenance, and how an imbalance could affect the human body.

Inflammation, a hallmark of gut dysbiosis, plays a pivotal role in the development of these diseases. Research has shown that gut dysbiosis can lead to the production of pro-inflammatory cytokines, lipopolysaccharides (LPS), and bacterial endotoxins, resulting in increased inflammation, oxidative stress, insulin resistance and endothelial dysfunction, and other widespread effects on the body, further exacerbating the detrimental health outcomes associated with AUD [36]. The intestinal mucosa can be detrimentally affected by inflammation caused by microbiome metabolites or ethanol, leading to damage and increased permeability. Although the exact mechanism has not yet been characterized, alcohol may lead to gut inflammation *via* alterations in the gut microbiota by promoting an increase in pro-inflammatory bacteria and/or decrease in anti-inflammatory bacteria, and affecting cytokine expression. Accordingly, in an animal model treated with human gut microbiota, the relative population of a pro-inflammatory bacterium (Clostridium cluster XIVa) increased, whereas there was a decrease in anti-inflammatory bacteria (*Akkermansia muciniphila*, Atopobium, *Faecalibacterium prausnitzii*), compared to untreated[37]. Interestingly, this kind of alcohol-induced dysbiosis was found to mediate intestinal barrier dysfunction, as well as ALD, *via* activation of tumor necrosis factor receptor I, in intestinal epithelial cells. Inflammation is primarily facilitated *via* introduction of leukocytes, as well as the presence of inflammation mediators, such as histamine, reactive oxygen species (ROS) and leukotriene. Mucin can be altered by reduced Mucin 2 (MUC-2) expression, which in turn is caused by increased matrix metalloproteinase-9 (MMP-9) expression by epithelial cells. MMP-9 can also be induced by Claudin-1 up-regulation, inhibiting goblet cell differentiation *via* Notch signalling, also leading to inflammation, due to the resulting reduction in MUC-2 expression[37].

The amount of episodes that involve binge drinking are associated with increased responsiveness of the stimulated cytokines [interleukin (IL)-6 and IL-8]. Moreover, blood cytokine response is stimulated (mostly increases *via* the Toll-like receptor 4 (TLR4) triggered cytokines IL-6, IL-8, Il-1b), as the frequency of the binge drinking events increases. Additionally, craving behavior is linked to increased levels of circulating markers of inflammation in AUD patients[15].

Release of endotoxins by dysbiotic microbiota in the gut was also found to induce production of pro-inflammatory cytokines and ROS by the stimulated hepatic Kupffer cells[38,39]. Even a sole binge drinking event can elevate serum endotoxin, most possibly due to translocation of products derived from gut bacteria and disturbance of the innate immune response, which contribute to the deleterious effects of binge drinking[15]. For instance, gut microbiota possibly affect the intestinal barrier's integrity. The following release of cytokines could signal to the brain *via* activation of the vagus nerve or *via* signaling across the blood–brain barrier. In parallel, substances that are produced by gut microbiota are possibly absorbed reaching the brain through the bloodstream. Subsequently, the brain, can affect the gut microbiota through neuronal and endocrine mechanisms and by the adoption of health behaviors. Thus, it is obvious that a possible imbalance of gut microbiota may affect the brain and lead to dysfunctions in the form of psychiatric disorders including as emotional and cognitive alterations.

AUD patients have enhanced levels of LPS, as a result of the increased abundance of the gram-negative bacteria which produce it, and have been shown to trigger significant inflammation; high levels of LPS can cause sepsis, even septic shock, and health implications including neurodegenerative disorders such as Alzheimer's disease, *via* chronic neuroin-

flammation[40]. Previous studies have shown that alcohol and gut dysbiosis elevate the LPS serum levels that derive from bacteria[22,41]. LPS can release inflammatory factors such as tumour necrosis factor alpha, IL-1, IL-6, IL-8, IL-10, interferon gamma (IFN-γ), and MMP-9 and induce inflammation by activating TLR4 complexes[42] and subsequently switches on the hypothalamic-pituitary-adrenal axis (HPA) axis to influence the brain. The brain can affect the intestine *via* the HPA axis which releases adrenocorticotropic hormones, leading to elevated intestinal permeability. In alcoholics, the higher relative abundance of gram-negative bacteria also includes Enterobacteriaceae[43] and Proteobacteria[26]; their increased population highly correlates with elevated LPS levels, and results in a more potent immunological response, compared to other phyla. These, combined with increased gut permeability, are significant contributors of AUD-related inflammation[44].

Metabolites that derive from the gut microbiome can exert diverse functions in the body with the majority of them involved in pathways of the digestive system formation and function. These molecules range from short chain fatty acids (SCFAs) and neurotransmitters to precursors of neurotransmitters, bile acids, hormones and vitamins. Each one of these metabolites has important impact on the brain function of the host. Notably, a decrease in the metabolic potential of alcohol dependent patients microbiome has been marked, characterized with an overall functional decrease in pathways related to methane metabolism, bacterial chemotaxis, and pyrimidine metabolism, and increase in metabolic pathways related to the phosphotransferase system[26].

The intestinal microbiome produces a range of small molecules representing most major metabolite classes, allowing them to ferment complex dietary polysaccharides and carbohydrates, as well as dietary fibers, producing SCFAs[45]. SCFAs (acetate, propionate, and butyrate) constitute a significant energy source for gut epithelial cells[46], and have been associated with improvement in the function and maintenance of the intestinal barrier's integrity, for an overall protective effect against pathogens[47]. Their anti-inflammatory nature supports epithelia cell proliferation, as well as T cell differentiation in the colon, contributing to gut homeostasis[48]. The bacterial SCFA butyrate can enhance tight junction integrity by inducing the production of relevant proteins, including claudin, occludin and zonula occludes, which as a result inhibits bacterial translocation[49]. Since both the intestinal bacteria and the levels of produced metabolites have such interlinked functions with the human body, affecting barrier integrity, there should be substantial caution of the altered populations to avoid collateral harm[50]. SCFAs are part of a satiety-inducing mechanism, with propionate enhancing gluconeogenesis, and the aforementioned butyrate playing a role in increasing glucagon-like peptide-a *via* gut cell stimulation[51]. Importantly, through the GBA, each metabolite present a neuromodulatory function, regulating the levels of critical neurotransmitters, including enteroendocrine serotonin, noradrenaline, and dopamine,) which affect numerous functions of the nervous system, as well as range of emotions, behaviors and other central nervous system (CNS) functions. Even gamma aminobutyric acid, a predominant inhibitory neurotransmitter, has been found to be secreted by bacterial strains, such a Bifidobacterium and Lactobacillus[24]. Lower levels of isovalerate in fecal SCFAs correlated with higher alcohol consumption in AUD patients, compared to controls, and were successfully reversed post-faecal microbiota transplantation (FMT) treatment[15,52,53]. The therapeutic efficacy may be attributed to recovery in the levels of bacteria such as Alistipes, Faecalibacterium, Ruminococcus, and Ocillibacter, which have been previously associated with increased isovalerate[54].

A metagenomic analysis in AUD patients by Litwinowicz *et al*[55] (concluded to a significant increase in facultative anaerobes (such as the Enterobacteriaceae family), that may be a result of a simultaneous decrease in levels of butyrate-producing bacteria (Butyricicoccaceae, Lachnospiraceae, Ruminococcaceae, Oscillospiraceae), thus reducing beta-oxidation, which in turn ends up increasing oxygen levels. Most of the produced butyrate gets used up for energy, with only approximately 5% remaining in circulation, however, this small fraction appears to be capable of inducing a potent anti-inflammatory response, through various pathways, including through the G-protein-coupled receptors 41 and 43 [56], inhibition of IFN-γ signaling, and induction of nuclear factor kappaB[57]. Butyrate's functions extends to strengthening the intestinal barrier *via* permeability reduction, therefore lower levels of bacteria producing it (Ruminococcaceae, Lachnospiraceae), could negatively affect AUD progression[55,58].

The connection among gut dysbiosis and brain function is firm given the ability of the gut microbiome to affect the functions of the central nervous system. SCFAs and the condition of the gut have been shown to influence a plethora of psycho-neurological conditions that include depression, anxiety, stress, Autism Spectrum Disorder (ASD), schizophrenia, and Parkinson disease[59,60]. Apart from negatively affecting the progress of AUD itself, intestinal microbiota can have a detrimental effect in various factors associated with relapse, such as alcohol craving and negative emotional states, such as depression and anxiety[13]. Of note, the level of intestinal permeability was associated with behaviors, depression recovery, anxiety, and craving levels observed in individuals with reduced intestinal permeability, but not in individuals with increased intestinal permeability. This finding seems to suggest a role of the microbiome GBA in AUD. Both addiction and withdrawal are known to arise from modifications in the neuronal function. The GBA is regarded as the bidirectional communication pathway that links the brain with the gastrointestinal tract. Different pathways for communication between the gut and the brain involve neuronal signaling *via* the vagus nerve, endocrine actions *via* the HPA, and stimulation of neural inflammation, or a wide range of metabolic changes. Several studies have proved that bacteria affect the GBA and interfere in conditions and phenotypes such as ASDs, social behavior, anxiety, depression, eating habits as well as the amount of food consumed[61].

Therapeutic strategies targeting the gut microbiome in AUD

Treatment outcomes in AUD can vary across patients and different medications. Most pharmacotherapies focus on limiting alcohol craving through neuromodulation, including opioid, glutamate, gamma-aminobutyric acid, and serotonin systems. While achieving abstinence is the desirable result, its rarely achieved, by merely 16% of AUD patients. The three approved AUD medications (disulfiram, acamprosate, and naltrexone) have shown moderate therapeutic efficacy, and with AUD being a heterogenous disorder, a single medication is unlikely to be effective for every patient

[62]. As it is increasingly recognized that the gut microbiome is correlated with AUDs, it has emerged as a potential therapeutic target for the treatment of AUD, aiming to reverse the dysbiotic state and reduce inflammation. Dysbiosis of microbiota can be restored through different approaches.

PRO-/PRE-BIOTICS

Dietary interventions have been investigated as a means to improve gut dysbiosis in individuals with various pathologies including AUD. A Mediterranean-style diet, rich in fruits, vegetables, whole grains, and healthy fats, is widely known for its beneficial effects on overall health, promotion of maintenance of a diverse and beneficial gut microbiota, increase in the concentration of SCFAs, and protection of the intestinal mucus layer[63]. Conversely, another study found that a diet with unsaturated fats affected the intestinal barrier, inducing inflammation and liver injury in mice exposed to chronic alcohol consumption[64].

Another approach to modulate gut microbiota are the dietary supplements in the form of either probiotics or prebiotics since it has been proposed as an emerging therapeutic target for the management of cognitive and/or behavior pathology. The definition of probiotics states that they are "living microorganisms which, when administered in adequate quantities, present an overall benefit to the host's health"[65]. Due to probiotics' potential benefits to the CNS and mental disorders, it has been proposed they be characterized as "psychobiotic," expecting low side effects, and anti-inflammatory, antidepressant, and anti-anxiety effects, ameliorating mental functions in Alzheimer's disease and ASD[66].

The administration of probiotics in health subjects has been linked with alterations in brain activity related to emotional memory and decision-making procedures as well as changes in functional connectivity.

The beneficial use of probiotics has been indicated in impaired social cognition and emotional functioning disorders, which have been linked to AUDs[67,68]. The administration of probiotics in healthy individual has been linked with alterations in brain activity related to emotional memory and decision-making procedures[69], as well as changes in functional connectivity during the performance of different emotional tasks[70,71], while stress conditions leading to rapid reaction times in an emotional recognition task and reduction of proinflammatory cytokines[72]. In autism-animal model, a probiotic administration could reverse social behavior deficits[73]. A very early study (1995) showed that consuming 100 mL of a Bacillus natto-fermented product, could reduce breath alcohol (44% reduction) and aldehyde (45% reduction) concentrations in a group of participants 1 h after drinking whisky, compared to a control group of rats [74].

Most data about the benefits of probiotics use in alcohol disorders arise from studies of alcohol liver disease with a focus on the improvement of the liver tissue. Probiotic supplementation restores the number of fecal Bifidobacteria, Lactobacilli, and Enterococci in alcoholic patients. The proposed mechanism of action is *via* modulation of dysbiosis and balance restoration, which in turn promotes an anti-inflammatory microenvironment allowing the reduction of the intestinal permeability and the translocation of bacterial components (LPS) to the systemic circulation. Furthermore, endotoxemia is found to be reduced while at the same time, probiotics can prevent bacterial metabolites from reaching the liver and triggering inflammatory responses[75]. By reducing systemic proinflammatory status and neuroinflammation, probiotics also offer an excellent alternative to relieve CNS damage reinforcing beneficial effects on addiction and, consequently, alcohol consumption[76].

Lim *et al*[77] studied the effects of 19 probiotic species on alcohol and acetaldehyde metabolism identifying four probiotic species, which present a relatively higher tolerance to alcohol, and a more effective alcohol and acetaldehyde metabolism: *Lactobacillus gasseri* CBT LGA1, *Lactobacillus casei* CBT LC5, *Bifidobacterium lactis* CBT BL3, and *Bifidobacterium breve* CBT BR3. These species also showed high mRNA expression levels of alcohol and acetaldehyde dehydrogenase (ALDH). A mixture of these four probiotics species and excipients, the ProAP4, was then administered to rats for 2 wk in advance of acute alcohol administration. The serum alcohol and acetaldehyde concentrations were significantly decreased in the treated group than in the control. Thus, the administration of these four probiotic species, rapidly reduced blood alcohol and acetaldehyde levels in an alcohol and ALDH-dependent fashion. Subsequently, another randomized placebo-cotroled crossover study examined the effect of Duolac ProAP4 supplementation on alcohol detoxification in humans, which lead to a reduction in both blood alcohol and acetaldehyde levels in *ALDH2* 2 hetorozygotes[78].

A number of prebiotics and probiotics alone or their combination could hold the potential of regulating brain's neurotransmition and in turn could attenuate alcohol-related addictive processes, and the related affective and cognitive-behavioral modifications. Accordingly, synbiotic supplementation was reported by Pizarro *et al*[79] to induce alterations in the relative population of gut's bacteria, tryptophan derivatives, g-aminobutyric acid and norepinephrine levels in the hippocampus and prefrontal cortex of mice, post alcohol withdrawal. Interestingly, although alcohol appeared to induce a detrimental effect in long-term memory and mobility in female mice (which was reduced by synbiotics), male mice showed no significant changes, indicating a higher alcohol tolerance in males. A double-blind, placebo-controlled trial randomized participants in four groups supplemented with placebo, prebiotics, probiotics and synbiotics, respectively. This study highlighted the increase of the number of supplement-specific bacteria after probiotic administration in healthy individuals, but no improvement in the metabolism of an acute dose of alcohol was reported[80]. Furthermore, the combination of drugs that inhibit the hyper-glutamatergic state [N-acetylcysteine (NAC) and acetylsalicylic acid (ASA)] with probiotics (*Lactobacillus rhamnosus*) markedly inhibit relapse ethanol intake. Two mechanisms were induced by these treatments; NAC + ASA reduced the glutamatergic tone and antibiotic + LGG reduced the dopaminergic tone, that reduced the alcohol binge–drinking relapse effect independently and complementary[81].

Most recently, a recombinant probiotic *Lactococcus lactis* expressing human ADH1B (hADH1B) was constructed, to enhance alcohol degradation in the intestinal tract after oral administration. Alcohol's metabolism includes its

decomposition from the enzyme alcohol dehydrogenase the action of which is followed by the liver enzyme ALDH; thus, as expected, the administration of hADH1B-expressing probiotic reduced alcohol absorption, and extended alcohol tolerance time and a reduction of recovery time and protected the intestine and liver from damage after acute alcohol consumption in mice[82].

FECAL TRANSPLANTATION

A number of recent studies (both preclinical and clinical) have shown that transplantation of fecal microbiota from AUD patient is capable to alter the intestinal barrier and modify brain function. In total, important alterations in the gut microbiome in time occur as a response to chronic alcohol exposure and correspond to severe intestinal barrier dysfunction and ALD development. Moreover, the altered bacterial communities of the gut may serve as a significant therapeutic target for the prevention/treatment of chronic alcohol intake induced intestinal barrier dysfunction and liver disease[83]. An animal model study showed that when sensitive to alcohol mice were fed with intestinal microbiota from resistant mice, the development of alcohol-induced liver lesions was prevented and a better gut homeostasis was observed. In total FMT treated mice indicated a similar intestinal microbiota profile to alcohol-resistant mice[84].

A phase 1 randomised clinical trial of FMT for AUD demonstrated short-term and long-term changes in AUD patients with cirrhosis using microbial manipulation. The FMT group presented a higher number of Alistipes and Roseburia, and increased production of SCFA. In contrast, there was a reduction in stool isovalerate and 2- methylbutyrate in placebo. Alcohol craving was negatively associated with Ruminococcaceae genera after FMT, and the reverse pattern was seen with Proteobacteria genera, such as Pseudomonas and with other potential pathobionts, such as Enterococcus. Ethanoligenens, which is associated with endogenous alcohol production, was also negatively linked with FMT. Potentially beneficial genera and those higher in post-FMT, such as *Bilophila* and *Ruminococcus*, were associated with lower alcohol-craving score after FMT. FMT subjects also showed reduced intestinal permeability, short-term decreases in inflammation, and decreased lipopolysaccharide binding protein, all of which supports FMT as a beneficial treatment for AUD patients, that often suffer from impaired intestinal barrier function[53].

A later study in germ-free mice, colonized with microbiota from post-FMT humans, showed a striking decrease in both initial ethanol acceptance as well as ethanol preference, compared to control group. The beneficial impact was attributed to changes in the microbial taxa (increased Lachnospiraceae and Ruminococcaceae, decreased Enterobacteriaceae), which appeared comparable to those in post-FMT humans, and was complemented with improvements in intestinal barrier function, increase in SCFAs, and lower butyrate. Notably, changes in gene expression were limited in the intestine, but not the liver or prefrontal cortex, and were associated primarily with inflammation and immune response, proliferation of epithelial cells, as well as response to oxidative stress. These results promote gut microbiota and the intestinal interface as therapeutic targets to lower alcohol intake in AUD patients[85].

CONCLUSION

The emerging field of research on the gut microbiome's role in AUD has revealed significant implications for health outcomes and potential therapeutic strategies. Alcohol consumption has profound effects on the gut microbiome, leading to dysbiosis and increased systemic inflammation. These alterations have significant implications for health outcomes, including the development of liver disease, cardiovascular disease, and type 2 diabetes. The gut microbiome represents a promising therapeutic target for the treatment of AUD, with interventions such as probiotics, prebiotics, and dietary modifications showing potential in improving gut dysbiosis and reducing inflammation. However, further research is needed to fully understand the intricate interactions between alcohol consumption and the gut microbiome, and to develop effective interventions that can mitigate the detrimental effects of AUD on gut health.

FOOTNOTES

Author contributions: Koutromanos I, Gazouli M, Tzavellas E study conception and design; Koutromanos I, Legaki E, Vasilopoulos E, Kouzoupis A data collection; Gazouli M, Tzavellas E analysis and interpretation of results; Koutromanos I, Legaki E, Vasilopoulos E, Kouzoupis A draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other coauthors contributed their efforts in this manuscript.

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S-Editor: Qu XL
L-Editor: A
P-Editor: Zhao YQ

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World Journal of Methodology

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

World J Methodol 2024 March 20; 14(1): 90127

DOI: 10.5662/wjm.v14.i1.90127

ISSN 2222-0682 (online)

MINIREVIEWS

Therapeutic role of yoga in hypertension

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Specialty type: Medicine, general and internal

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Wang KJ, China

Received: November 23, 2023
Peer-review started: November 23, 2023
First decision: November 30, 2023
Revised: December 19, 2023
Accepted: January 16, 2024
Article in press: January 16, 2024
Published online: March 20, 2024

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Abstract

Systemic hypertension is an established risk factor for coronary artery disease and cerebrovascular accident and control of blood pressure reduces the risk of a major cardiovascular event. Both non-pharmacological and pharmacological treatment options are available to treat hypertension. Yoga, recently received more attention as a treatment modality for various lifestyle disorders, even though practiced in India since ancient times. In this review, we are analyzing the role of yoga in the treatment of systemic hypertension.

Key Words: Yoga; Hypertension; Blood pressure; Asana; Pranayama; Meditation

Core Tip: Lifestyle modification is a vital component in the management of hypertension in addition to pharmacotherapy. However, it is infrequently practiced in day-to-day life. Yoga practice helps in clinically relevant reduction in blood pressure in people with hypertension. However, there is a scarcity of the necessary information and awareness not only among the general population but also among healthcare professionals regarding the benefits of yoga in hypertension. Awareness of various yoga practices suitable for hypertension, their respective effects, and the necessary precautions when performing them helps in utilizing this in day-to-day life.

Citation: Joshi AM, Raveendran AV, Arumugam M. Therapeutic role of yoga in hypertension. *World J Methodol* 2024; 14(1): 90127
URL: https://www.wjgnet.com/2222-0682/full/v14/i1/90127.htm
DOI: https://dx.doi.org/10.5662/wjm.v14.i1.90127

INTRODUCTION

Systemic hypertension is a major public health issue and of the total hypertensives, only about 1 in 4 adults (24%) have well-controlled hypertension[1]. It is an established risk factor for coronary artery disease, cerebrovascular accident, cardiac failure, atrial fibrillation, chronic kidney disease, heart valve diseases, aortic syndromes, and dementia[2].

Hypertension accounts for 14% of deaths and control of hypertension is associated with a 35% to 40% reduction in the risk of cerebrovascular accident, a 50% reduction in the risk of heart failure, and a 20%-25% reduction in the risk of myocardial infarction[3,4]. Uncontrolled hypertension contributes to 49% of coronary artery disease and 62% of cerebrovascular disease[2]. On average, a 5 mm of Hg reduction in systolic blood pressure (BP) reduces the risk of a major cardiovascular event by about 10%[5].

Lifestyle modifications like reduced dietary sodium intake, increased intake of fruits and vegetables, and regular physical exercise help in the prevention and treatment of hypertension in addition to pharmacological agents. Yoga is becoming increasingly popular not only among the general population but also among healthcare professionals as a modality to tackle various lifestyle diseases like hypertension, diabetes, and dyslipidemia.

WHAT IS YOGA?

Yoga is an art, a science, and a philosophy of living a healthy lifestyle. Originating in India over 5000 years ago, yoga was primarily about spiritual progress. "Yoga" means union, and signifies the merging of Atman, the individual consciousness, with Paramatman, the universal consciousness. On a practical level, yoga practice aims to enhance holistic health, happiness, and harmony while overcoming the sufferings of human life. Yoga is a complex intervention with multiple components such as shatkriya (cleansing processes), asana (postures), pranayama (breathing exercises), bandha (locks), mudra (subtle gestures), dhyana (meditation), relaxation, chanting mantras, dietary guidelines, a code of conduct, philosophy, and spirituality.

YOGIC WAY OF LIFE

Yoga practices have the potential to influence somatic and psychological functions on different levels. Therefore, yoga interventions should be planned and performed accordingly. Apart from 'doing' yoga postures or breathing practices, the cultivation of the right values and attitudes towards day-to-day stressors and adopting a yogic lifestyle is vital in reducing stress and managing hypertension[6].

The classical yoga texts describe the concept of chitta prasadanam (happiness of mind) and ways to achieve it. The mental qualities of maitri, karuna, mudita, and upeksha are suggested to achieve a blissful state of mind. They provide a yogic way of approaching a wide variety of situations in everyday life.

Maitri is an attitude of friendliness toward the happiness of others. It is the ability to share another person's happiness instead of being jealous. Having such a perspective makes it possible for us to celebrate the beauty of human experiences. We can learn, understand, and grow through the friendship of happy and positive people. Karuna means compassion for the sorrows of those suffering. This is essential for spiritual and personal growth. It means helping if someone is upset, and comforting them. Compassion teaches us to be less judgmental and accept others as they are. It also develops an emotional understanding and bond. Mudita is a joyfulness towards the good deeds of others. It is about appreciating the virtuous qualities of others and finding inspiration to cultivate them. Upeksha refers to indifference towards the negative behavior of others. It is the quality of developing equanimity or neutrality towards those who were unkind, or those who have hurt us. Taking the opposite view towards negative thoughts and actions (pratipaksha bhavanam) also gives important insights into the management of emotions.

Mindfulness qualities such as living in the present moment, acceptance, letting go, gratitude, and loving-kindness are also effective antidotes for stress and anxiety.

YOGA PRACTICES FOR HYPERTENSION

Practical considerations

Sherman[7] proposed consideration of the domains such as style of yoga, the dose and delivery of yoga, components of the yoga intervention, specific class sequences, dealing with modifications, selection of instructors, facilitation of home practice and measurement of intervention fidelity over time while developing an appropriate yoga protocol. The yoga therapy prescription is based on the individual requirements of the patients. The following are some yoga practices

recommended for hypertension (Table 1 and Figure 1). An additional file provides details of recommended yoga practices in hypertension with images (see Supplementary material).

Shatkriya (Six cleansing practices): These are yogic cleansing techniques described in the Hatha Yoga texts. It is believed that regular internal cleansing enhances the functional capacity of the organs. There is limited scientific evidence on the efficacy of these practices in hypertension. Trataka (concentrated gazing) practice has been recommended for hypertension[8]. Jala neti (nasal cleansing with warm saline water) can be practiced once a week, however, other cleansing techniques are contraindicated[9].

Sharir Sanchalana (Warm-up practices): These are loosening exercises that relieve stiffness and prepare the body for the practice of asana. They ease tension accumulated in different body areas and improve blood circulation. For optimal pose performance and injury prevention, it is vital to perform sharir sanchalana before yoga practice.

Surya Namaskara (Sun salutation): It involves a series of dynamic yoga postures performed in a specific sequence. Slow-paced practice, according to the individual capacity, is advised for pre- and stage I hypertension[10]. Performing surya namaskara at a fast pace is comparable to aerobic exercise, resulting in increased strength and endurance. In contrast, slow-paced practice results in a decline in cardiovascular parameters to normal levels, similar to yoga training.

Asana (Yoga postures): Asana practiced with awareness is capable of bringing about the stability of body and mind. Asana practice improves the flow of vital energy through the body, resulting in a positive sense of well-being. It is a preparatory practice for meditation that fosters a quieting of the mind. The yoga postures should be modified to suit individual needs, considering other associated comorbidities.

Pandey *et al*[11] suggested the potential of gentle restorative yoga as a therapeutic option for hypertensive patients. In this practice, props are used to facilitate stretching, provide support, and induce relaxation in a yoga pose. Shavasana (corpse pose) is the most important posture described widely in the management of hypertension[12]. While relaxation poses and gentle restorative yoga postures are mostly effective in hypertension, other asanas may be performed as additional practices. An additional file provides details of additional yoga practices in hypertension with images (see Supplementary material).

Pranayama (Regulated breathing practices): Pranayama is a vital component of yoga and is associated with the regulation of breathing. Pranayama can be added as a supportive therapy with drugs in mild or moderate cases of hypertension[13]. Slow breathing practices are effective in reducing BP and are recommended as the first treatment for low-risk hypertensive and prehypertensive patients who are reluctant to start medication[14]. Ujjayi pranayama (ocean breath) without breath retention significantly decreases stress-induced changes in cardiorespiratory parameters and decreases BP[15]. Studies have shown immediate benefits of sukha pranayama (easy, comfortable breathing)[16], nadi shodhana (alternate nostril breathing)[17], slow-paced bhastrika (bellows breath)[18], chandra nadi (left unilateral forced nostril breathing)[19], bhramari (humming breath)[20], sheetali (cooling breath)[21], and pranava (Aum chanting)[13] pranayama practices in reduction of BP.

Pranayama and meditation can be practiced while sitting in a chair, sukhasana (comfort pose), ardha padmasana (half lotus pose), padmasana (lotus pose), or vajrasana (thunderbolt pose). In these meditative postures, the heart rate and the breath slow down, which lowers the BP.

Mudra (Subtle gestures): Mudra is a term meaning a 'bodily position' or 'subtle gesture'. Mudra can be practiced independently or incorporated into yoga postures, pranayama, and meditation. The effects of yoga practices are enhanced with mudra, as they deepen awareness and concentration.

Dhyana (Meditation): There are two major types of meditation, concentration and open awareness. Concentration meditation involves a focus on a word, sound, prayer, or phrase. Expansive, open awareness or mindfulness meditations emphasize developing a passive attitude towards intruding thoughts, emotions, or body sensations, and a return to focus. Both types have a significant effect in reducing BP[22,23]. Mindfulness meditations can be practiced as formal, dedicated practice, or informal, integrated practice during the activities of daily life. While the formal practice deepens the meditation experience, the informal practice emphasizes meditation as a way of living.

Yoga Nidra (Yogic sleep or effortless relaxation): Yoga nidra is a comprehensive, profound relaxation technique for removing physical, mental, and emotional tensions. This practice includes awareness of different body parts, relaxation, breath awareness, auto-suggestions, and imagery. It reduces anxiety, and stress and improves autonomic functions in hypertensive patients[24].

MECHANISM OF YOGA IN HYPERTENSION

Various mechanisms (Figure 2) by which yoga practices reduce BP include:

Effect on stress

Yoga, by reducing mental stress and autonomic imbalance, helps to prevent and control hypertension[24,25]. Mental stress and sympathetic overactivity contribute to the development of systemic hypertension and cardiovascular morbidity[26]. Yoga modulates the physiological response to stress *via* neurohumoral activation[27]. It optimally balances

Table 1 Recommended yoga practice in hypertension

Yoga practice	Details of the yoga practice	Duration[1]
Shatkriya (Yogic cleansing practices)		
Shatkriya	Trataka (concentrated gazing)	10 to 15 min
	Jala neti (nasal cleansing with warm saline water)	5 min (Once a week)
Sharir sanchalana (Warm-up practices)		
Sharir sanchalana (Warm-up practices)	Toe bending, ankle bending, knee bending, half butterfly, finger bending, wrist bending, elbow bending, shoulder rotation, and neck movements in a seated position with synchronization of breathing	5 to 10 min
Surya namaskara (Sun salutations)		
Surya namaskara (Sun salutations)	Series of dynamic yoga postures in a specific sequence with meditative awareness, practiced at slow-speed with breath synchronization	3–7 rounds as per capacity; The postures can be held for a short period, without straining[6]
Asana (Yoga postures) for relaxation		
Shavasana (Corpse pose)	Shavasana with slow, rhythmic diaphragmatic breathing, awareness of the sensation at the nostrils, the temperature of the inhaled and exhaled air, relaxing the muscles, and feeling the heaviness of different parts of the body	5 to 30 min
Restorative Yoga (Conventional poses with props)	Supported and modified stretching to suit individual needs[11]; Shavasana (Corpse pose); Supta baddha konasana (Reclined bound angle pose); Upvistha konasana (Wide-angled seated forward bend); Balasana (Child's pose); Pashchimottanasana (Seated forward bend); Salamba kapotasana (Supported pigeon pose)	15 min[11]
Other Asana (Additional practices)		
Standing postures	Tadasana (Palm tree pose); Tiryak tadasana (Swaying palm tree pose); Katichakrasana (Standing spinal twist); Ardhachakrasana (Standing backward bend)	Synchronizing the movements with the breathing cycle; The postures can be held for a short period, without straining
Seated postures	Vakrasana (Seated spinal twist); Gomukhasana (Cow face Pose); Ardha Ushtrasana (Half camel pose); Pashchimottanasana (Seated forward bend); Marjarasana (Cat stretch)	
Prone postures	Bhujangasana (Cobra pose); Tiryak bhujangasana (Swaying cobra pose); Ardha Shalabhasana (Half locust pose)	
Supine postures	Pavanmuktasana (Wind releasing pose); Eka and Dwipada uttaanpadasana (straight single and both legs raising)	
Meditative Postures for Pranayama and Meditation		
Meditative postures (Spine erect, shoulders relaxed, face softened)	Seated in a chair; Sukhasana (Comfort pose); Vajrasana (Thunderbolt pose); Ardha padmasana (Half lotus pose); Padmasana (Lotus pose)	During pranayama and meditation
Pranayama (Regulated breathing practices)		
Deep abdominal breathing	Gentle deep inhalation and slow longer exhalation	5 min
Sukha pranayama (Easy comfortable breathing)	Conscious, slow and deep breathing with equal duration for inhalation and exhalation) at the rate of 6 breaths/min	5 min[16]
Anulom Vilom/Nadi shodhan pranayama (Alternate nostril breathing)	Gentle inhalation through the left nostril followed by exhalation through the right nostril. Next inhalation through the right nostril and exhalation through the left nostril	Upto 20 min[17]
Chandra nadi pranayama/Chandra bhedan pranayama (Left nostril breathing)	Unilateral left nostril breathing with inhalation and exhalation for an equal count of five; Variation: Inhalation through the left nostril followed by exhalation through the right nostril	27 rounds of 6 breaths/min[19]
Slow bhastrika pranayama (Bellows breath)	Deep inhalation and slow exhalation (Respiratory rate 6/min), imagine the open blue sky while breathing[18]	5 min[18]
Ujjayi pranayama (Ocean breath)	Slow inhalation and exhalation with a slight contraction around the glottis; Can be practiced in seated, supine, or standing position	10 min[15]
Bhramari pranayama (Humming bee breath)	Slow deep inhalation followed by exhalation with a humming sound	5 min[20]
Sheetali pranayama (Cooling breath)	Inhalation through curled tongue in the form of a tube, slow exhalation through the nose	9 rounds each[9]
Pranava pranayama/ AUM chanting	Slow and deep inhalation with complete yogic breathing followed by the audible vibratory resonance of a prolonged AUM chant; Can be practiced in a	5 min[13]

	supine position	
Mudra (Subtle gestures)		
Shanmukhi mudra (Closing the seven gates)	Closing the ears with the thumbs, placing the index fingers on the eyes, the middle fingers near the nostrils, the ring fingers, and the little fingers above and below the lips	Practiced during bhramari pranayama
Brahma mudra (Divine spiritual gesture)	Synchronizing neck movements with deep breathing and vibration sounds	3 to 9 rounds of this practice at each sitting
Apan Vayu Mudra (Mudra of the heart)	Placing the tip of the index finger at the base of the thumb and joining the tips of the middle finger, ring finger, and thumb, the little finger extended	15 min
Gyan mudra (Mudra of wisdom)	Joining the tips of the index finger and the thumb, other fingers extended	Practiced during pranayama and meditation; Up to 30 min a day
Meditation (Formal practice)		
Concentration Meditation (Focus on a word, sound, prayer, mantra, or phrase)	Examples: AUM japa; Ajapa japa; Anahata (heart) chakra meditation with the mantra 'YAM'	10-15 min
Mindfulness meditation (Open awareness of the present moment with kindness, acceptance, and curiosity)	Examples: Body scan meditation (Awareness of body parts); Mindful breathing meditation (Breath awareness); Loving-kindness meditation (having unconditional compassion towards ourselves and all other beings)	
Meditation (Informal/Integrated practice)		
Mindfulness (Non-judgmental present-moment awareness during daily activities)	Examples: Mindful yoga (Yoga postures with awareness); Mindful Eating	Can be practiced anytime, anywhere in a day-to-day life
Yoga nidra (yogic sleep or effortless relaxation)		
Yoga nidra	Relaxation practice that includes awareness of different body parts and breath, relaxation, auto-suggestions, and imagery	Up to 45 min[29]

[1]The durations mentioned are not necessarily to be practiced during a single yoga session. A customized yoga sequence as per individual requirements is recommended.

the sympatho-vagal stress response[27]. Yoga cultivates psychosomatic harmony, induces relaxation, and reduces stress. As a result, emotional stability occurs, the somatization symptoms are relieved, and the systolic and diastolic BP is reduced[23]. Chronic stress-induced sustained muscular contraction reduces the lumen/diameter of blood vessels in the muscles. This can lead to increased BP. Yoga induces relaxation and decreases arterial tone and peripheral resistance. Yoga is also helpful in various stress-induced psychological disorders like anxiety, depression, and insomnia[24].

Autonomic regulation and parasympathetic dominance

Yoga practices specifically affect cardiovascular autonomic regulation and tend to normalize it[28]. They control the autonomic nervous system and influence the brain's electrical rhythms, heart rate, and systolic and diastolic BP[29]. Yoga relaxation[12], and slow-paced pranayama techniques[17,20,21] decrease sympathetic activity and enhance parasympathetic activity. These practices optimally balance sympatho-vagal stress response and enhance healthy cardiovascular functioning[16,27,30].

Improved baroreceptor sensitivity

Various yogic practices also reduce chemoreceptor response and improve baroreceptor sensitivity, which restores BP to normal in patients with essential hypertension[25]. Baroreceptor sensitivity can be enhanced significantly by slow breathing practices of yoga[18]. Ujjayi pranayama (ocean breath) exerts gentle pressure on the carotid sinuses. Many baroreceptors in the carotid sinus serve as 'sampling areas' for homeostatic mechanisms that maintain BP. Improvement in baroreflex sensitivity results in the normalizing of autonomic cardiovascular rhythms[24].

Effect on hormone secretions

Some yoga postures exert controlled pressure on the kidneys and the adrenals, regulating blood supply to them, which mainly regulates BP through secretions of hormones like renin, angiotensin, adrenalin, *etc*[31].

Beneficial effect on comorbid conditions

Yoga-based lifestyle intervention is an important treatment modality in the reduction of BP, as is found to be beneficial in various other cardiovascular risk factors like obesity, dyslipidemia, and diabetes mellitus[32,33].

Improvement in sleep quality

Studies have shown an association between insomnia and elevated BP of stage 1 and 2 hypertension[34]. Yoga practices

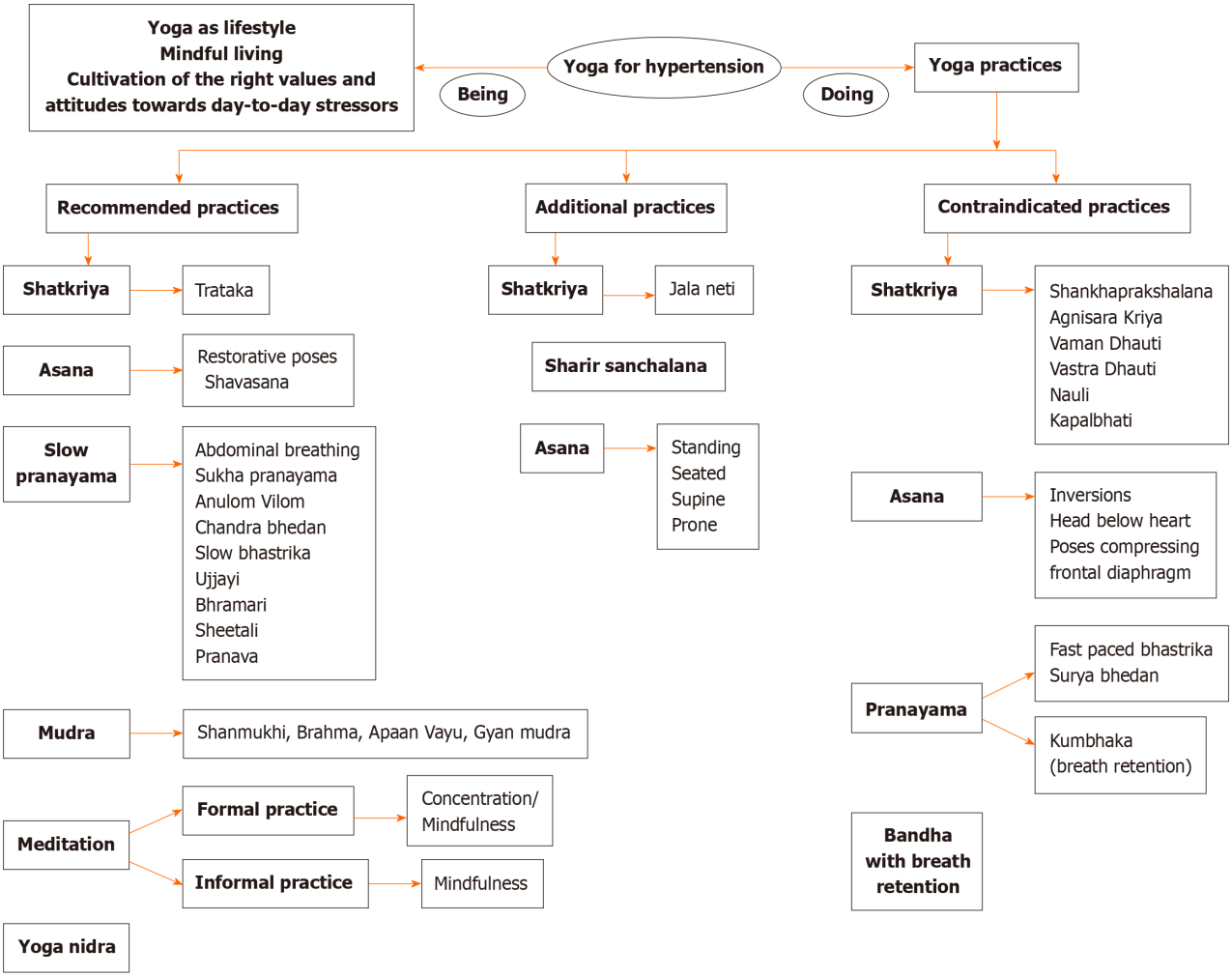

Figure 1 Various yoga practices recommended for hypertension.

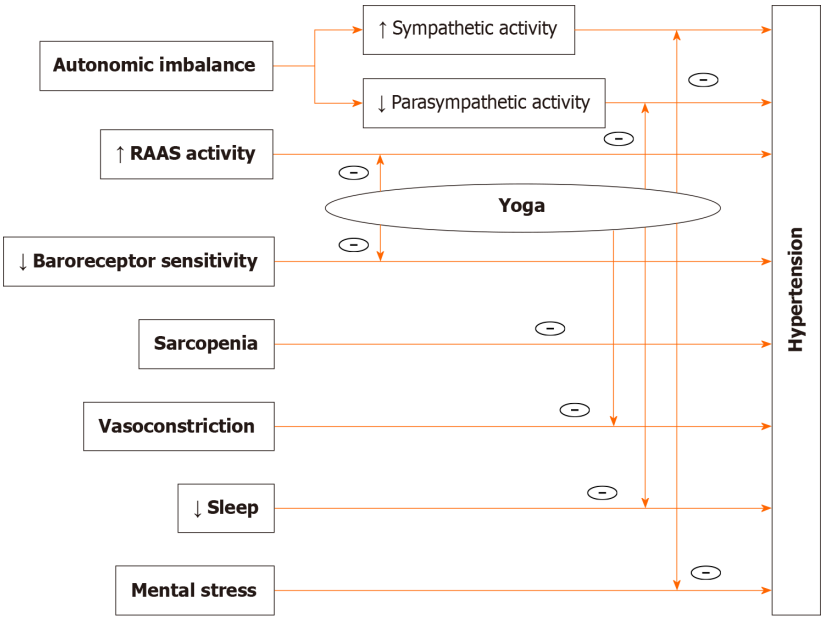

Figure 2 Mechanism of yoga in hypertension. RAAS: Renin-Angiotensin-Aldosterone System.

such as Om chanting and yoga nidra are beneficial in improving the sleep quality of chronic insomnia patients[24]. Yoga has been suggested as an adjuvant nonpharmacological option for making effective and sustainable changes in patients suffering from sleep disorders.

Beneficial effect on sarcopenia

Sarcopenia, the age-related loss of muscle mass and muscle function, is associated with hypertension in older adults[35]. Yoga practice is beneficial to prevent and attenuate the age-related deterioration of physical health, muscular strength, and flexibility[36]. Holding yoga postures for a few seconds builds strength by using body weight for resistance, which in turn slows the onset of sarcopenia. They also strengthen the skeletal muscles and improve bone density. The effects of various yogic practices on managing hypertension and their mechanisms are mentioned in Table 2.

SAFETY OF YOGA FOR HYPERTENSION

Population-based surveys and clinical trials suggest that yoga is a relatively safe intervention as compared to other forms of physical activity. However, the most commonly reported adverse effects of yoga are pain and soreness, muscle injuries, and fatigue[37]. To avoid the adverse effects, yoga should be learned under the guidance of a qualified yoga professional. Since the benefits of yoga are influenced by frequency, duration, and method of practice, the recommended yoga therapy protocol should be followed to gain optimum benefits[37].

Although yoga is an effective and promising approach to the management of hypertension, it should be used as a complementary therapy and not as a substitute for professional medical advice, diagnosis, and treatment of hypertension. It must be noted that yoga is not for medical emergencies.

Those who are having any health problems must get a medical opinion before proceeding with various yoga practices. Even if the BP is maintained at acceptable normal levels with medication, safety precautions should be followed.

The room where yoga is practiced should not be too hot as in 'hot yoga,' since it increases cardiovascular strain, raising BP.

PRECAUTIONS AND CONTRAINDICATIONS

The following is a brief description of precautions and contraindications of yoga practices in hypertension (Table 3).

Asana (Yoga postures)

Hypertensive practitioners have even higher BP during an exercise and in holding a static yoga posture, proportionate to the effort required to practice. Overstraining and excess muscular efforts stimulate the sympathetic nervous system, resulting in increased BP. Therefore, it is advised to refrain from the stronger forms of practices and holding static postures for long periods[38]. Relaxation in shavasana at the end of asana practice is beneficial.

Aggressive and sudden changes in yoga postures such as standing up quickly from a lying down position should be avoided as they may be stressful. Care should be taken while practicing backbends, especially in a standing position. Breath should not be held during a yoga posture. Strenuous practice should also be avoided in the elderly or people with high ocular pressure disorders. During yoga practice, if the breath is rapid, and the person feels agitated, flushed, dizzy, or uncomfortable, it is advised to come out of the pose and rest in shavasana. Supported and modified stretching using blankets, bolsters, or a chair for support is also recommended.

Although inversions and head-below-heart postures improve health and reduce anxiety and stress, they are not recommended for people suffering from high BP[9,38]. They can cause a significant rise in both systolic and diastolic arterial pressures. In inverted yoga poses, the intra-thoracic pressure is increased. Pumping against gravity increases the cardiovascular strain, resulting in increased BP. Modifications in some of these yoga poses are recommended. For example, in balasana (child's pose), or adho mukha shvanasana (downward facing dog pose), the head can be supported with folded blankets or pillows, so that it is at the level of the heart. In viparita karni (yoga inversion pose), supported legs up the wall position can give inversion benefits of lymphatic drainage and improved venous return from the lower extremities without adding the risk of increased BP. Other asanas such as mayurasana (peacock pose), and dhanurasana (bow pose) compress the front of the diaphragm, which can raise BP.

Pranayama (Regulated breathing practices)

Pranayama is a safe practice suitable for all age groups. However, if practiced in the wrong way, it may cause harm or complications. Judicious practice under expert guidance is a must. Caution is required, especially for vulnerable patients and those having health concerns. Gentle, soothing, and relaxing pranayama practices are effective in the management of hypertension. No violent or fast breathing should be practiced. Overstraining is not recommended. The ratio of the inhalation and exhalation should not be forced.

Hyperventilation practices such as fast-paced bhastrika pranayama (bellows breath) may be unsafe in patients with hypertension and cardiovascular disease, as they cause vasoconstriction and increase BP[39]. Suryabhedan pranayama (right nostril breathing) has a sympathetic stimulating effect and should be avoided[10]. Practicing kumbhaka (breath retention) during pranayama can result in a significant increase in systolic, diastolic, and mean arterial pressure[40]. It may be due to the combined effect of an increased level of heart rate and total peripheral resistance during kumbhaka. It

Table 2 Effect of yoga practices on hypertension and their mechanism of action

Yoga Practice	Effect on hypertension	Mechanism of action
Shatkriya (Cleansing practices)		
Trataka kriya	Significant reduction in BP and HR in patients with primary hypertension[8]	Induces calmness similar to a mental state during meditation[8]
Neti kriya	Clear up the head and neck region producing a sense of lightness[6]; Beneficial in reducing anxiety and depression	Reduce toxic accumulation in head and neck region
Sharir Sanchalana (Warm-up practices)		
Sharir Sanchalana	Warm-up exercises prepare the body for the practice of asana	Removes stiffness in the muscles and joints; Improve blood circulation
Surya Namaskara (Sun salutations)		
Slow practice of Surya Namaskara	Recommended for pre and stage I hypertension[10]	Produce psychosomatic harmony[6]
Asana (Yoga postures)		
Asana practiced with awareness and breath synchronization	Prevent and attenuate the age-related deterioration of physical health, muscular strength, and flexibility[36]; Capable of bringing about the stability of body and mind; Improves the flow of vital energy through the body, resulting in a positive sense of well-being; Preparatory practice for meditation that fosters a quieting of the mind	Holding yoga postures for a few seconds build strength by using body weight for resistance, which in turn slows the onset of sarcopenia[36]; Mental relaxation and a state of "restful awareness"; Changes the long-held patterns of feelings and emotions; Exert controlled pressure on the kidneys and the adrenals, which regulates BP through secretions of hormones like renin, angiotensin, adrenaline, *etc*.[31]
Restorative Yoga	More effective with significantly greater reductions in BP and HR as compared to stretching[11]	Facilitates stretching, provides support, and induces relaxation. Releases habitual stress patterns that are stored in the body as areas of tension
Shavasana (corpse pose) with breath awareness	Effective in BP reduction[12]	Reduction in the load on the heart by blunting the sympathetic response along with an enhanced parasympathetic activity[12]; Allows the body and mind to integrate through mindful stillness
Forward bending poses	"Head-down" postures confer stress-reducing, calming, and mind-quietening benefits	Forward bends with head supported brings a sense of calm
Backward bending poses	Elevate low mood and have energizing effect	Chest opening poses that allow expansive inhalation; Stimulate the heart chakra associated with emotions and balance
Twisting postures	Release tension, and energize; Helps in de-stressing, relaxing, and unwinding	Relieve spinal stiffness and clear emotional blockages
Meditative postures	Maintain a steady comfortable position for a long time without conscious effort; Improve concentration and peace of mind	The heart rate and the breath slow down, which lowers the BP. Enhance feelings of being grounded, centred, and balanced
Pranayama (Regulated breathing practices)		
Slow-paced pranayama	A modest reduction in BP[14]	Optimally balances sympathovagal stress response [27]; Baroreceptor sensitivity can be enhanced significantly by slow breathing[18]
Deep slow abdominal breathing	Effective in reduction of systolic BP	Improve vagal tone, increase parasympathetic dominance, and decrease sympathetic discharges[30]
Sukha pranayama (Easy, comfortable breathing)	A significant reduction in Heart rate, systolic pressure, pulse pressure, and mean arterial pressure[16]	Normalization of autonomic cardiovascular rhythms as a result of increased vagal modulation and/or decreased sympathetic activity and improved baroreflex sensitivity[16]
Immediate Effect of Nadi shodhana pranayama (Alternate nostril breathing)	Reduce the BP and HR[17]	Activating the parasympathetic nervous system and enhancing healthy cardiovascular functioning[17]
Slow pace (respiratory rate 6/min) bhastrika pranayama (Bellows breath)	Reduction in systolic and diastolic BP[18]	Enhanced parasympathetic activity[18]
Chandra nadi pranayama (Left unilateral forced nostril breathing)	An immediate decrease in cardiovascular parameters with the decrease in HR, systolic pressure, and pulse pressure[19]	Normalization of autonomic cardiovascular rhythms with increased vagal modulation and/or decreased sympathetic activity along with improvement in baroreflex sensitivity[19]
Bhramari pranayama (Humming breath)	Immediate positive effect on reducing the systolic BP [20]	Parasympathetic dominance[20]

Ujjayi pranayama (Ocean breath) without breath retention or bandhas	Significantly decreases stress-induced changes in cardiorespiratory parameters and decreases BP[15]	Shift autonomic nervous control toward the parasympathetic side[15]; Exerts gentle pressure on the carotid sinuses that regulates BP through homeostatic mechanisms
Sheetali pranayama (Cooling breath)	The immediate effect: reduce the systolic and diastolic BP in hypertensive patients[21]	Decreasing the sympathetic activity[21]
Pranava pranayama/AUM chanting	Five min of AUM chanting reduces systolic and diastolic BP[13]; Reduces depression, anxiety, and stress[24]; Improves quality of sleep[24]; Promotes relaxation, and provides calmness	Improvement in baroreflex sensitivity resulting in the normalizing of autonomic cardiovascular rhythms[24]
Mudra (Subtle gestures)		
Brahma mudra (Divine spiritual gesture)	Induces a sense of relaxation and reinvigoration in the head and neck region that reduces stress[6]	Cultivates psychosomatic harmony, induces relaxation, reduces stress
Apaan vayu mudra (Mudra of the heart)	Reduction in systolic and diastolic BP	The finger position sensitizes nerves in the palm and wrist area resulting in a systemic effect on the cardiovascular system
Shanmukhi mudra (Closing the 7 gates)	Produces a sense of inner calm[6]	This mudra encourages 'pratyahara', which means withdrawing the mind inwards by blocking some of the sensory distractions of the surroundings
Gyan mudra (Mudra of wisdom)	Reduces mental stress, indicated in hypertension	Deepens awareness; Enhances meditation experience; Regulates the fire and air elements in the body
Meditation		
Formal meditation		
Concentration meditation	Significant decrease in the HR and systolic and diastolic BP[22]; Reduces stress and anxiety; Induces relaxation	Modulates the physiological response to stress *via* neurohumoral activation[22]; Decreases the arterial tone and the peripheral resistance
Mindfulness Meditation (Non-judgmental awareness of the present moment with kindness, acceptance, and curiosity)	Significant effect on reducing systolic and diastolic BP in patients with hypertension[23]	Focus on the moment with "acceptance" and "non-criticism" helps in regulating the emotional state. Emotional stability relieves the somatization symptoms and the BP is reduced[23]
Informal meditation		
Mindfulness	Helps to cope face day-to-day challenges and stressful situations	Serves as a 'micro-meditation' technique for quick stress relief
Yoga nidra (Yogic sleep or effortless relaxation)		
Yoga nidra	Reduces systolic and diastolic BP[29]; Reduces depression, anxiety, and stress[24]; Improves quality of sleep[24]	Improve autonomic functions[24,29]; Influence the brain's electrical rhythms[29]

BP: Blood pressure; HR: Heart rate.

is contraindicated in hypertension, heart disease, and individuals recovering from an illness, surgery, or injury.

Bandha (Lock)

Bandha, which means to lock, close off, or stop, are yoga practices that redirect the flow of blood and lymph to other parts of the body. They consist of neuro-muscular locks and involve changes in internal pressure to a very high degree. The practice of bandha with long retention of breath strains the heart and is not recommended for hypertension[9]. Bandha practices during pranayama should also be avoided.

Shatkriya (Six cleansing practices)

Kapalbhati (Skull shining breath), a popular breathing technique that consists of forceful exhalations followed by passive inhalations, increases diastolic BP, suggesting sympathetic stimulation[41]. Shankha prakshalana (alimentary tract cleansing) involves repeating rounds of drinking salt water, performing a set of asanas, and evacuating the bowels. This kriya (set of practices) can be risky for individuals with hypertension because water intake may lead to a rise in blood volume and, thus, cardiac output. The physical exertion involved in the procedure may further increase the heart rate. A short and simplified version of this kriya, known as laghu shankha prakshālana, requires a lesser physical strain. Preliminary evidence suggests its safety in patients with mild to moderate essential hypertension[42]. An additional file provides a brief description of contraindicated yoga practices in hypertension with images (see Supplementary material).

Table 3 Yoga for hypertension: Precautions and contraindications

Yoga practice	Details of yoga practice	Precautions and contraindications
Shatkriya (Yoga cleansing practices)		
Shatkriya (Yoga cleansing practices)	Shankhaprakshalana (Alimentary tract cleansing); Agnisara Kriya (Activating the digestive fire); Vaman dhauti (Regurgitative cleansing); Vastra dhauti (Cloth cleansing); Nauli kriya (Abdominal massaging); Basti kriya (Yogic enema); Kapalbhati (Skull shining breath)	Contraindicated in hypertension[9]; Kapalbhati increases diastolic BP suggesting sympathetic stimulation[41]; Laghu shankhaprakshalana, the simplified and shorter version of shankhaprakshalana is suggested in mild to moderate hypertension[42]
Asana (Yoga postures)		
Inversions and head below the heart postures	Shirshasana (Headstand pose); Sarvangasana (Shoulderstand pose); Chakrasana (Wheel pose); Halasana (Plough pose); Adhomukh shvanasana (Downward facing dog pose); Prasarit Padottanasana (Wide-Legged standing forward bend)	Cause a significant rise in both the systolic and diastolic arterial pressures; Contraindicated in hypertension[9,38]; Increase intrathoracic pressure and strain the cardiovascular system; Pooling of the blood in the head and neck region resulting in the rise of BP
Other asanas	Mayurasana (peacock pose); Dhanurasana (bow pose)	Compress the front of the diaphragm, which can raise the BP
Pranayama (Regulated breathing practices)		
Pranayama pace	Fast breathing	Rapid breath practices may be unsafe in patients with hypertension and cardiovascular disease
Pranayama type	Bhastrika pranayama (Bellows breath) with rapid forceful inhalation and exhalation	Increases HR and BP, producing vasoconstriction[39]
Pranayama type	Surya bhedan/surya nadi pranayama (Right nostril breathing)	Sympathetic stimulating effect[39]
Pranayama technique	Kumbhaka (breath retention)	Significant increase in systolic, diastolic BP, and mean arterial pressure[40] due to combined effect of increased level of HR and total peripheral resistance during kumbhaka
Bandha (lock)		
Bandha (lock); Practiced with breath retention	Mula Bandha (Root lock, pulling the perineum inward); Uddiyan Bandha (Abdominal lock, lifting of the diaphragm); Jalandhar Bandha (Chin Lock, pressing the chin on the chest and contracting the throat)	Long retention of breath strains the heart and is not recommended for hypertension[9]; If the jalandhar bandha is not performed properly, the BP is raised

BP: Blood pressure; HR: Heart rate.

REAL-WORLD DATA

A meta-analysis showed that yoga had a modest and significant effect on BP control (systolic 4.17 and diastolic 3.26 mm of Hg)[43]. It was also observed that yoga had a significant effect on BP when compared to those not taking any treatment but had no effect in comparison with the exercise group. Subgroup analysis showed that maximum benefit was seen in those who were practicing postures, meditation, and breathing. Studies incorporating yoga postures, meditation, and breathing resulted in higher BP reduction (systolic 8.17 and diastolic 6.14 mm of Hg)[43]. However, according to some studies, the suggested key components of yoga intervention for hypertensive patients are breathing and meditation rather than physical activity[44]. In a meta-analysis, different yoga interventions were compared, and it was observed that only the studies that included breathing and/or meditation techniques without postures had significant effects on hypertension[45]. BP reduction with yoga is compatible with other non-pharmacological interventions like exercise, and salt restriction[46]. Even this small reduction in BP in people with hypertension reduces the risk of cardiovascular events [47].

Yoga reduces BP significantly (systolic 7.96 and diastolic 5.52 mm of Hg) as compared to the no-treatment group and is comparable to exercise and other non-pharmacological treatment groups. Exercise and other non-pharmacological treatments reduce BP in the range of 3-9 mm of Hg as compared to the no-treatment group[43].

The average BP reduction with a single antihypertensive agent is about 10/5 mm of Hg and the addition of the second drug results in lower BP reduction[48]. Considering these facts, the amount of BP reduction achieved by yoga is significant and clinically relevant. Yoga practices combined with antihypertensive drugs have been shown to reduce BP and pulse rate during resting conditions, during stimulus-induced conditions, and in mild-to-moderate hypertension. In most hypertensive patients, it reduces the dose of antihypertensive drugs required[28].

LIMITATIONS

There are studies showing no beneficial effect of yoga on BP. There are a lot of limitations in published studies, including small sample size, the absence of uniform yoga practices, the absence of adequate controls, and the absence of blinding and non-uniform research methodologies, making it difficult to compare various research protocols. Yoga practices, the

duration of recommended practices, the techniques, and in some cases, even the names of yoga practices differ with different schools of yoga.

Standardized yoga therapy protocols for specific conditions are a debatable topic. A uniform set of practices cannot be recommended to every patient, as it requires a customized, tailor-made approach.

Yoga is sometimes termed as 'a laborious way to well-being' because of the time, effort, and motivation required for the practice. Yoga requires the person's willingness and cooperation for the practice. Patients' participation determines the treatment outcome in yoga therapy. Finally, it must be emphasized, that yoga is not a substitute for standard medical care and it is not for medical emergencies.

CONCLUSION

Yoga is an effective, time-honored, and promising approach to the management of hypertension. It is a safe intervention if practiced according to prescribed safety guidelines. Yoga practices influence various somatic and psychological functions and help to bring a state of physiological and psychological balance. This helps in clinically relevant reduction in BP in hypertension with a reduction in the dose of antihypertensive drugs required. Yoga postures, breathing practices, and meditation are the three most important components in the effective management of hypertension. Yoga therapy is not just about performing yoga, but also about the cultivation of the right values and attitudes towards day-to-day stressors. Yoga needs to be incorporated as a way of living a yogic lifestyle.

FOOTNOTES

Author contributions: Joshi AM and Raveendran AV designed the manuscript, collected data, and wrote the manuscript; Arumugam M contributed to data collection and manuscript writing; Joshi AM designed and prepared the yoga practice manuals (additional files); Joshi AM, Raveendran AV, Arumugam M contributed to the manuscript revision.

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

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Corresponding Author's Membership in Professional Societies: Association of Physicians of India, L-16571.

S-Editor: Liu JH
L-Editor: A
P-Editor: Zhao YQ

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World Journal of Methodology

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World J Methodol 2024 March 20; 14(1): 90590

DOI: 10.5662/wjm.v14.i1.90590

ISSN 2222-0682 (online)

MINIREVIEWS

Can propensity score matching replace randomized controlled trials?

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Specialty type: Methodology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Nooripour R, Iran

Received: December 7, 2023
Peer-review started: December 7, 2023
First decision: December 23, 2023
Revised: January 5, 2024
Accepted: February 23, 2024
Article in press: February 23, 2024
Published online: March 20, 2024

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Abstract

Randomized controlled trials (RCTs) have long been recognized as the gold standard for establishing causal relationships in clinical research. Despite that, various limitations of RCTs prevent its widespread implementation, ranging from the ethicality of withholding potentially-lifesaving treatment from a group to relatively poor external validity due to stringent inclusion criteria, amongst others. However, with the introduction of propensity score matching (PSM) as a retrospective statistical tool, new frontiers in establishing causation in clinical research were opened up. PSM predicts treatment effects using observational data from existing sources such as registries or electronic health records, to create a matched sample of participants who received or did not receive the intervention based on their propensity scores, which takes into account characteristics such as age, gender and comorbidities. Given its retrospective nature and its use of observational data from existing sources, PSM circumvents the aforementioned ethical issues faced by RCTs. Majority of RCTs exclude elderly, pregnant women and young children; thus, evidence of therapy efficacy is rarely proven by robust clinical research for this population. On the other hand, by matching study patient characteristics to that of the population of interest, including the elderly, pregnant women and young children, PSM allows for generalization of results to the wider population and hence greatly increases the external validity. Instead of replacing RCTs with PSM, the synergistic integration of PSM into RCTs stands to provide better research outcomes with both methods complementing each other. For example, in an RCT investigating the impact of mannitol on outcomes among participants of the Intensive Blood Pressure Reduction in Acute Cerebral

Hemorrhage Trial, the baseline characteristics of comorbidities and current medications between treatment and control arms were significantly different despite the randomization protocol. Therefore, PSM was incorporated in its analysis to create samples from the treatment and control arms that were matched in terms of these baseline characteristics, thus providing a fairer comparison for the impact of mannitol. This literature review reports the applications, advantages, and considerations of using PSM with RCTs, illustrating its utility in refining randomization, improving external validity, and accounting for non-compliance to protocol. Future research should consider integrating the use of PSM in RCTs to better generalize outcomes to target populations for clinical practice and thereby benefit a wider range of patients, while maintaining the robustness of randomization offered by RCTs.

Key Words: Propensity score matching; Randomized controlled trials; Randomization; Clinical practice; Validity; Ethics

Core Tip: Several studies in the literature compare treatment effect estimates in propensity score matching studies and randomized controlled trials (RCTs), but few employ both methods synergistically in determining treatment outcomes. This is a first review to report and provide examples on how propensity score matching can be integrated into RCTs to refine randomization, account for non-compliance to protocol and improve external validity to produce more comprehensive and generalizable evidence for informed clinical decision making.

Citation: Liau MYQ, Toh EQ, Muhamed S, Selvakumar SV, Shelat VG. Can propensity score matching replace randomized controlled trials? *World J Methodol* 2024; 14(1): 90590
URL: https://www.wjgnet.com/2222-0682/full/v14/i1/90590.htm
DOI: https://dx.doi.org/10.5662/wjm.v14.i1.90590

INTRODUCTION

In the paradigm of clinical research, establishing causality is vital in helping clinicians better grasp the efficacy, or harm, of potentially groundbreaking interventions. The foundation for evidence-based medicine depends on the influence of interventions on patients' health. To attain the best outcomes for patients, causal relationships must be studied objectively. In this regard, randomized controlled trials (RCTs) have been well-established as the gold standard for establishing causal relationships in clinical research, largely due to the randomization of participants which eliminates confounders[1]. Despite this, there still exist concerns about the use of RCTs in clinical research. This includes the ethicality of withholding potentially-lifesaving treatment from a group, relatively poor external validity of RCTs due to stringent inclusion criteria, need for resources to conduct trials, and lack of feasibility to continue trials for a prolonged duration due to manpower and resource constraints[2]. In particular, a retrospective cohort study of 1017 RCTs found that poor recruitment is the most frequently reported reason for RCT termination, accounting for up to 40% of the total discontinuations[3]. This in turn leads to a considerable waste of scarce research resources. However, with the inception of propensity score matching (PSM) as a retrospective statistical methodology by Rosenbaum and Rubin[4] in 1983, new frontiers have opened to establish causality in clinical research. PSM predicts treatment effects using observational data from registries to create a matched sample of participants who received or did not receive the intervention based on their propensity scores, which is associated with baseline characteristics such as age. Since then, newer models of computing propensity scores have emerged and the uptake of PSM in research has increased exponentially, owing to its ability to estimate causal effects when random assignment of treatments is unethical or not feasible[5-9]. Recently, an increasing number of studies have begun to adopt an integrated approach, increasing the generalizability of their results with PSM while maintaining the robustness of randomization offered by RCTs, demonstrating the potential benefits of using both methods synergistically[10-12]. Therefore, this literature review aims to explore the integration of PSM studies as a potent adjunct to RCTs in establishing causality in healthcare, potentially circumventing the concerns and quandaries surrounding RCTs as a research modality. We report the advantages and limitations of RCTs and PSM studies, as well as their synergistic implementation and its advantages as compared to either method alone. It is hoped that the findings of our review would help guide researchers and clinicians alike to consider adopting the use of PSM in RCTs in future clinical research.

METHODOLOGY

A comprehensive literature search was conducted on PubMed, Web of Science, CENTRAL, Scopus and Embase from inception to 25 November 2023 with the keywords: "randomized controlled trial", "propensity score matching", "observational studies", and "advantages". The inclusion criteria included studies with PSM or RCTs as their methodology, or if both methods were used concurrently. Non-English articles, animal studies, conference abstracts, oral

and poster presentations were excluded. If an institution published more than one study, the most recent article was selected for analysis. Relevant information regarding the advantages and limitations of RCTs, PSM and their synergistic implementation were extracted. The included articles also served as key examples to support the use of each method or the integration of PSM into RCTs respectively.

RANDOMIZED CONTROLLED TRIALS

An RCT is an experimental study randomly assigning human participants to a control and treatment group, and is typically used to establish cause and effect in medical treatments such as novel drugs, devices and surgical techniques. Crucially, the randomization process ensures that participants are allocated to groups comparable in terms of baseline characteristics and potential confounders, and that the observed differences in outcomes are due to the treatment effect [13,14]. different types and methods of randomization are performed in RCTs. Some common methods are shown in Table 1[15,16]. The choice of randomization method depends on the characteristics and objectives of the study, such as the sample size, the number of treatment groups, the presence of covariates, and the primary outcome. The randomization method should be specified in the study protocol and implemented with adequate concealment to ensure the validity and reliability of the results[17].

In double blinded studies, blinding is also incorporated to ensure that neither patients nor doctors administering the treatment are aware of the treatment allocation[18,19]. In triple blinded studies, data analysts assessing the outcomes are also blinded to reduce bias further. This is crucial as knowledge of which treatment the patient receives could lead to the behavioral changes of patients and doctors who might be biased towards the provision of the newer treatment instead of the placebo, decreasing the objectivity and credibility of the study[20]. Blinding can be achieved through the certain means including double-dummy designs, central randomization and independent outcome assessors. Furthermore, it is recommended to use pre-specified and transparent protocols, registration, and reporting guidelines to ensure the integrity of the blinding process. Despite that, some studies cannot be blinded[21]. For example, blinding cannot be achieved in a trial comparing different types of psychotherapy for depression as the patients and therapists would know which type of therapy they are receiving or providing[22]. Overall, randomization and blinding seek to reduce allocation and selection biases within RCTs. Figure 1A summarizes the major steps taken in an RCT study.

PROPENSITY SCORE MATCHING

An alternative to estimating causal relationships when RCTs cannot be performed is PSM. PSM is a statistical technique that predicts treatment or interventional effects using observational data from existing sources such as registries or electronic health records, to create a matched sample of participants who received or did not receive the intervention based on their propensity scores[4]. Propensity scores are the probabilities of receiving the intervention given the observed characteristics of the participants such as age, gender and comorbidities, and attempts to reduce the bias and confounders inherently present in such studies[23]. This is due to participants being assigned treatments and interventions based on clinical needs, mostly influenced by patient-centered factors including but not limited to patient comorbidities, and not through random allocation[4]. For example, in a study comparing the effectiveness of a new drug *vs* a placebo, the participants who choose to take the new drug may differ from those who do not in terms of their age, health status, or other characteristics that may affect the outcome of interest. These differences may confound the causal relationship between the treatment and the outcome, and make the comparison between the groups invalid.

Various models are used to compute such probabilities, which are outlined in Table 2. The logistic regression model is most commonly used due to its simplicity. However, it assumes a linear relationship between the covariates and the log-odds of the treatment, an assumption that does not always hold true for all variables in medicine[24]. Body mass index (BMI), for instance, has a nonlinear relationship with mortality, with both low and high BMI being associated with a high mortality rate[25]. In such cases, other models can be used assuming the variables fulfill the model's assumptions[26]. It is important to note that different models used will generate different results in a finite sample. Although many models exist, there are no guidelines for their choice. However, one could consider the guidelines proposed by Baser[27] based on five criteria which aims to select the best model based on their ability to reduce selection bias in that given data sample.

PSM then involves matching the participants who received the intervention with those who did not based on their propensity scores, so that they are theorized to have similar characteristics and would thus be comparable. PSM can use different matching methods depending on the availability and balance of the data[28]. Table 3 shows the possible matching methods available while Figure 1B summarizes the major steps taken in a study employing PSM.

CAN PROPENSITY SCORE MATCHING REPLACE RANDOMIZED CONTROLLED TRIALS?

Although PSM and RCTs are used to establish a causal link between interventions and eventual health outcomes, it is important to note the differences between these methodologies. Fundamentally, RCTs are conducted to determine the efficacy of new treatments compared to an existing control treatment[29]. In contrast, PSM studies serve as a retrospective method of evaluating the efficacy of a treatment. This is done by using the baseline characteristics of patients to match individualized treatment groups against newly formed control groups *via* assigning a propensity score to each group[30].

Table 1 Comparison of randomization methods for clinical trials

Method	Description	Ref.
Simple randomization	Each participant has an equal chance of being assigned to any of the treatment groups. This method is easy to implement and unpredictable, but it may result in unequal group sizes or imbalances in important covariates, especially in small studies	Grimm and Müller[75], 1999
Block randomization	Participants are allocated to treatment groups in blocks of fixed size, such as 4 or 6. This method ensures that the group sizes are balanced at any point of the study, but it may introduce some predictability if the block size is known or guessed by the investigators	Sreedevi *et al* [76], 2017
Stratified randomization	Participants are first stratified by one or more relevant factors, such as age, gender, or disease severity, and then randomized within each stratum. This method ensures that the treatment groups are balanced with respect to the stratification factors, but it may increase the complexity and cost of the randomization process	Kahan and Morris[21], 2012
Minimization	Participants are allocated to the treatment group that minimizes the imbalance in a set of predefined factors, such as prognostic variables or previous treatments. This method is adaptive and can achieve better balance than stratified randomization, but it may also introduce some predictability and bias if the allocation is not concealed	Treasure and MacRae[77], 1998

Table 2 Comparison of methods of computing propensity scores

Method	Advantages	Disadvantages	Ref.
Logistic regression	Simple and widely used	May not capture complex or nonlinear relationships	Otok *et al*[6], 2017
	Can handle binary and continuous covariates	May be sensitive to model misspecification	
	Can estimate the propensity score and the treatment effect in one model	May not balance all covariates well	
Discriminant analysis	Can handle multiclass treatment	May not capture nonlinear relationships	Rudner and Johnette [7], 2006
	Can capture linear combinations of covariates	May be sensitive to outliers and distributional assumptions	
	Can handle multicollinearity among covariates	May not balance all covariates well	
Random forests	Can handle complex and nonlinear relationships	May be computationally intensive	Zhao *et al*[8], 2016
	Can handle binary, categorical, and continuous covariates	May overfit the data	
	Can balance all covariates well	May not estimate the propensity score and the treatment effect in one model	

Table 3 Possible matching methods utilized in propensity score matching studies

Matching method	Indication
One-to-one	This method matches each treated unit with one control unit that has the closest propensity score. This method is simple and intuitive, but it may discard some units that are not matched
One-to-many	This method matches each treated unit with more than one control unit that has similar propensity scores. This method can increase the sample size and precision, but it may also introduce more bias due to imperfect matches
Nearest neighbor	This method matches each treated unit with the control unit that has the nearest propensity score, within a specified caliper or threshold. This method can reduce bias by excluding poor matches, but it may also reduce efficiency by excluding good matches
Caliper	This method matches each treated unit with the control unit that has the propensity score within a specified range or distance. This method can ensure a high degree of similarity between the matched pairs, but it may also result in a loss of observations if the caliper is too narrow
Stratification	This method divides the propensity score distribution into a number of strata or intervals, and then compares the outcomes of the treated and control units within each stratum. This method can balance the covariates across the strata, but it may also produce heterogeneous treatment effects across the strata

As will be explored further in the paper, specific advantages and disadvantages to both study methodologies would favour clinicians and researchers to choose one method over the other (Table 4). However, it is also important to contextualize the considerations behind the methodology choice[31].

Context and objectives

By the nature of RCTs (Figure 1A), the division of participants into two distinct groups, encompassing one control group and one treatment group, necessitates certain ethical considerations, particularly concerning the control group and their

Table 4 Summary of the advantages of propensity score matching and randomized controlled trials

Propensity score matching	RCTs
Allows for utilization of retrospective data where randomization was not done	Gold standard for causal inference by eliminating bias
Improves efficiency of subject enrolment in prospective studies	Required as part of regulatory requirements
Allows analysis of causal inference in investigations where ethical considerations forbid RCTs	Allows researchers to conduct targeted studies to answer specific questions
Better external validity and generalizability	Better internal validity
Avoidance of type II errors	
Shorter timeline to study completion	

RCTs: Randomized controlled trials.

access to a potential novel treatment or intervention. Firstly, due to the presence of strict inclusion and exclusion criteria that governs those who are eligible for participation within an RCT, there is a chance that certain populations or groups may be consistently excluded from taking part in these studies that would determine the efficacy of potentially life-saving treatments. Consequently, the results obtained from the study cannot be universalizable, as the effects of the treatment and intervention cannot be accurately assessed in these ignored groups[32].

This can be observed in studies such as the one done by Leinonen *et al*[33], which demonstrates a discrepancy in the biodata of RCT subjects as compared to the general population, particularly concerning the use of acetylcholinesterase inhibitors as a treatment for Alzheimer's disease. The study found that RCT participants were significantly younger, due to the stringency of exclusion criteria that prevented the recruitment of older individuals into the study, who usually comprise the bulk of the demographic who are usually afflicted with Alzheimer's disease. As such, the use of acetylcholinesterase inhibitors as a treatment option in older adults cannot be fully validated. Were PSM to be used instead, it would follow that the generalizability of the results would allow for findings to be applied across various age groups, including older adults, who would likely benefit the most from treatment. Further, strict selection criteria of RCT might lead to more favorable treatment outcomes for one group compared to another group. For example, Gui *et al*[34] reported that surgical resection had superior 3-year and 5-year disease free survival compared to transarterial chemoembolization plus radiofrequency ablation for hepatocellular carcinoma. When analyzing only the PSM data, the difference was not significant.

Moreover, there is the need to consider the ethicality of using placebos, which is often done in the control group in RCTs. Especially in the presence of established first-line treatment or intervention options for whichever disease or condition is being studied, the use of placebos in place of this established treatment option would be ethically frowned upon, more so if the condition being studied is potentially deadly or debilitating and swift, definitive intervention would be necessary[35,36]. Due to the retrospective nature of PSM studies, which use already-existing data to determine the efficacy of treatments, it is possible to eliminate these ethical concerns as the study design circumvents the need to recruit participants to assess interventional outcomes[37]. A key example is examining the cardiovascular safety of common oral hypoglycemic agents for type 2 diabetes mellitus. As the study aimed to investigate the adverse effects of drug therapies, it would be unethical to expose patients to the risk of harm in RCTs. Instead, an algorithm is developed based on generalized PSM to estimate the effects of various diabetic medications individually or with metformin on cardiovascular events[38]. A case could be made for PSM studies replacing RCTs in such instances.

Despite the advantages that PSM has over the ethical considerations of RCTs, it is also important not to discount the ethical considerations of PSM itself, particularly regarding data privacy and the use of patient data from electronic health records and registries. As it is often not practical to seek the consent of individuals to utilize observational data for retrospective studies, institutional review boards usually waive the need to obtain consent for using personal data in most circumstances. This is provided that the results are published in the form that does not identify the individual. Despite that, the issue of data privacy in the face of the retrospective nature of PSM studies must not be ignored. Nevertheless, the advent of methods such as propensity score-based pooling and combining distributed linear regression with propensity score modelling can avoid the need for individual-level data while maintaining analytic integrity, thereby offering protection of patient privacy[39,40]. Hence, PSM could still prove to be the better method when ethical issues arise.

Feasibility

Although RCTs are known for their robustness in determining the efficacy of new treatments, they are also known to take a longer time to complete, especially due to the 'real-time' nature of tracking participant progress and long-term outcomes. The entire RCT progress, as well as its duration, is dependent on the recruitment of appropriate participants for the study. This process requires the development of strict criteria that would enable only the most suitable subjects to participate in the study. The development of such criteria, together with the time it takes to recruit sufficient suitable participants to participate in the study, would understandably take a long time[41]. In addition to this, one must consider that participants must be followed up for an extended period because treatments themselves have several potential outcomes and side effects that may take months or even years to manifest. Additional time would also need to be taken to

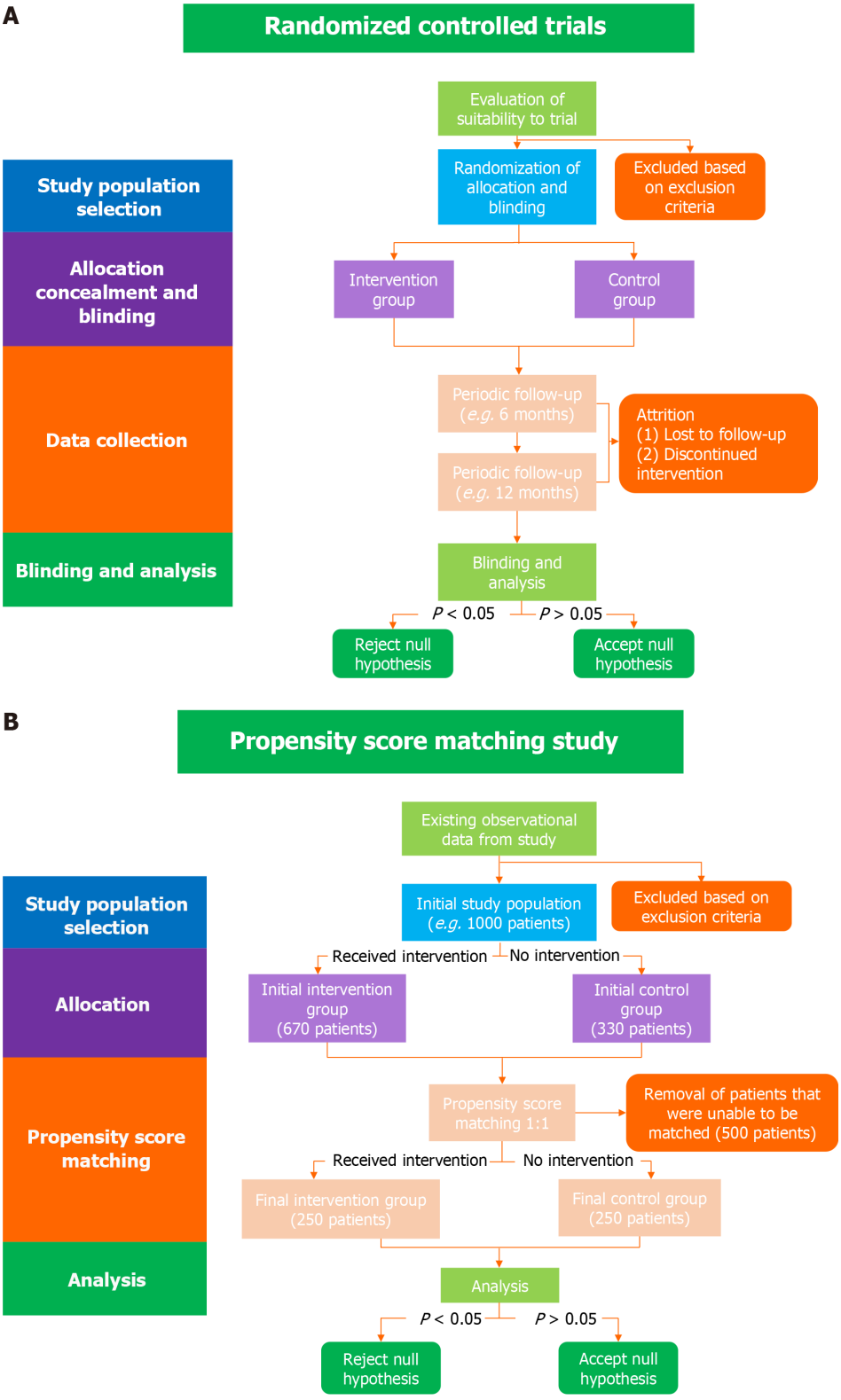

Figure 1 Flowchart overviewing the steps. A: Flowchart overviewing the steps taken in a randomized controlled trial, randomization of allocation and blinding is key to reduce bias in randomized controlled trials; B: Flowchart overviewing the steps taken in a propensity score matching study, propensity score matching creates a matched sample of patients who received or did not receive the intervention based on their propensity scores, which takes into account the patients' baseline characteristics.

obtain data of interest and perform statistical analysis to form conclusions regarding treatment efficacy[42]. Majority of research grants have an expiry date of couple of years by which a RCT has to be concluded. Thus, it is a common observation that recurrence free survival (RFS) is commonly used as a surrogate of overall survival (OS) in oncology trials as RFS can be determined much earlier as compared to OS.

On the other hand, PSM studies can be conducted on pre-existing data and would take much shorter time to complete. In instances where a treatment for a condition would be required with some urgency, such as in the case of vaccines or antidotes for epidemics and pandemics, it is possible that PSM studies could replace RCTs. Consider this study by Hsu *et al*[43], which uses a PSM methodology with data from a previous cohort study to suggest new influenza vaccination guidelines for the elderly. Especially given the morbidity of the infection in older populations, as well as the dynamism of the infection itself due to high mutation rates and the existence of multiple strains of the same virus, vaccination recommendations would have to be generated rather quickly to adapt to an ever-changing seasonal infection. This was possible due to PSM, whose methodology allowed for retrospective comparison between vaccinated and non-vaccinated individuals, and the effects of vaccination specifically on those vaccinated. Therefore, PSM studies are usually more feasible and quicker to implement due to their retrospective nature which precludes the need for recruitment and monitoring.

Better external validity

As stringent prerequisites are used in RCTs regarding participant selection, a strict set of inclusion and exclusion criteria are often needed. This, verily, would have implications for the validity of RCTs. Such exacting criteria would mean that RCTs generally have poorer external validity, which means that study findings may not be generalizable to the rest of the population, or different contexts[44]. Majority of RCTs exclude elderly, pregnant women and young children; thus, evidence of therapy efficacy is rarely proven by robust clinical research for this population. On the other hand, the validity of PSM studies depends on how closely the study sample represents the population of concern. This is ultimately influenced by multiple factors, including the study context, confounders, and the statistical model used to yield the actual propensity score. It is generally accepted that the external validity of PSM studies is quite robust, as the results from PSM studies can be generalized to other populations given that the model assumptions are accounted for. The sample size is sufficient enough for a large statistical power[45]. Studies exist that show that, at the very least, PSM provides an external validity that is comparable, if not better, than that of RCTs. For example, a study by Kuss *et al*[46] compared PSM and RCT as methodologies for assessing outcomes following coronary artery bypass grafting. The study validated that any differences observed in the findings between the RCT and PSM methodologies in this specific context were statistically insignificant. Thus, owing to better external validity, PSM can be employed to better generalize outcomes of studies to patient populations for translation to clinical practice.

Addressing lack of randomization in retrospective studies

Retrospective studies are an important area where the advantages of PSM are demonstrated. They use existing data recorded for purposes other than research and patients usually do not undergo interventions *via* randomization[47]. These data are thus best analyzed *via* PSM. For example, in a retrospective cohort comparison study of cervical total disc replacement performed as an outpatient *vs* inpatient procedure, patients were not randomized to either outpatient or inpatient groups unlike in RCTs. Pre-existing variables thus likely influenced the type of intervention they received. To eliminate their influence, PSM was used to control for variances in patient characteristics. Every patient was assigned a propensity score based on variables such as age and gender, among others. Each outpatient case was then systematically matched with three inpatient cases with similar propensity scores to compare intervention outcomes[48]. After adjusting for the inherent confounding factors, any differences in observed outcomes can be attributed to the intervention itself although randomization is not performed[28]. Therefore, PSM can make use of retrospective data in a way to analyze the causal effects of the intervention itself.

The advent of PSM also allows better application of findings generated from retrospective analyses. Although robust, RCTs are challenging to conduct and often generate results that may not apply to a real-world setting. This may be due to either the complexity of the intervention or the selection process for participants yielding a population different from that seen in general clinical practice[49]. Unlike RCTs, PSM makes use of data that have been collected from actual patients undergoing interventions in real-world practices. This gives the analysis a more realistic touch and makes it more applicable to clinical practice.

Resource efficiency

PSM has its role in prospective cohort studies as well. PSM can be used for patient enrolment in prospective studies to improve statistical and logistical efficiency. In a novel approach to PSM, a propensity score model is developed based on pre-existing patient data. The study tapped on data from two groups of patients-those who were referred for acupuncture and those receiving the usual care, to compare the effectiveness of the two interventions to manage chronic musculoskeletal pain. These patients are not randomized to either group. Patients are then matched by their propensity scores for recruitment into the prospective cohort study, where patient-reported outcomes are collected through an interview. Without PSM, patients that would have otherwise been recruited would ultimately be excluded from analysis due to a lack of propensity score overlap[50]. This thus improves study precision and maximizes resources.

Another example where PSM can be used to increase efficiency in terms of patient enrolment is the Diabetes Prevention Program (DPP)[51]. DPP is a multicenter RCT designed to compare diet and exercise against medications on preventing or delaying the onset of type 2 diabetes. However, the process of subject selection was highly inefficient. The 158177 subjects had to be screened before 3819 subjects were finally randomized to one of the four original arms. If PSM had been employed to recruit the subjects in a more targeted fashion, less resources could have been expended.

Error avoidance

With the use of PSM, it may be possible to avoid the type II error that often affects the statistical power of RCTs. RCTs

have a high risk of type II error, failing to reject the null hypothesis when it is false[52]. In other words, RCTs may falsely report no significant difference between the intervention and the control groups. This may be due to the rigorous nature of RCTs, which require careful planning, ethical approval, recruitment, randomization, intervention delivery, follow-up, data collection, analysis, and reporting. These processes can introduce limitations that can reduce the power and precision of RCTs, such as low sample size, high attrition, poor adherence, crossover, contamination, protocol deviations, and measurement error. Despite using various protocols such as intention-to-treat, as-treated, or per-protocol analysis, RCTs may still fail to detect or report clinically significant changes in the outcome of interest[44]. Conversely, PSM utilizes existing data sets, thus circumventing the issues related to low statistical power that plague RCTs, especially in small sample sizes and high attrition rates.

WHEN ARE RANDOMIZED CONTROLLED TRIALS BETTER?

RCTs have been the acknowledged standard in evidence-based medicine for decades, only second to systematic reviews and meta-analyses[53]. The performance of a RCT is robust and requires strict specification of study conditions in all aspects of its conduct, including participant selection, treatment and control assignment arms, inclusion and exclusion criteria, randomization method, outcome measurement, among others[49]. It first emerged in 1948 to investigate streptomycin treatment of pulmonary tuberculosis[54] and is recognized as the standard method for "rational therapeutics" in medicine by the 20th century[55]. Although novel methods of proving causal effects have emerged, RCTs are still highly regarded due to their various advantages which will be discussed below and summarized in Table 4.

Elimination of bias: Gold standard for causal inference

RCTs have the unique advantage of randomization which eliminates accidental bias, including selection bias. This adjusts for inherent features that may have increased the likelihood of subjects being allocated to treatment or control groups. Randomization thus eliminates any systematic differences between the two groups. This promotes comparability of the study groups, creating a basis for more accurate comparison[56], which has not been possible in other study designs[1]. As a result, any outcome differences can be attributed to the intervention rather than confounding factors. This contributes to the high internal validity of RCTs as a study design. To top it all off, RCTs can provide high statistical power, detecting and quantifying meaningful effect size differences between the intervention and the control groups, proving the causal relationship between intervention and outcomes more robustly[57].

While observational studies may use statistical methods to try to account for possible bias, some biases are very hard to correct[58]. A 2020 systematic review by Lantz[59] of 46 evaluations of interventions targeted at healthcare super-utilizers warned of this caveat. Methodological and study design weaknesses, especially regression to the mean, called into question supposed positive findings. Interestingly, observational studies of super-utilizer programs tended to report positive outcomes post-intervention. Yet on the other hand, RCTs reported no significant difference between intervention and control groups. The "positive" outcomes of these observational studies were likely biased by regression to the mean. This refers to the statistical tendency for patients incurring unusually high costs at a particular point in time to move closer to the average over time[60]. Despite statistical methods in place to correct for bias, this may not always be successful, depending on the inherent features of the data set. Therefore, this further strengthens the gold standard status of RCTs for causal inference.

While PSM can correct for confounders, it still has its shortcomings especially when compared to RCTs. Most importantly, it assumes that all relevant confounders are measured and included in the propensity score model. This is known as the ignorability or unconfoundedness assumption, and it is often untestable and may be violated in practice [61]. If unobserved or unmeasured confounders are present but not accounted for by the propensity scores, the matching may not eliminate them. Consequently, causal estimates may be biased or inconsistent[62]. Therefore, without a careful selection and measurement of the covariates based on substantive knowledge and theory, PSM may remain inferior to RCTs.

Although PSM is a thorough process, the possibility of bias due to matching errors or model misspecification cannot be overlooked. PSM estimates propensity scores with a statistical model, such as logistic regression, discriminant analysis, or random forests. These models may be misspecified or inaccurate, thus not capturing the true relationship between the covariates and the treatment assignment[63]. The process may produce mismatched pairs with poorly estimated propensity scores, increasing the comparison's imbalance or bias[64]. Therefore, RCTs may retain their role as the gold standard for causal inference, until such systematic shortcomings in the alternatives are accounted for.

Regulatory requirements

Given its status as the gold standard for causal inference, RCTs have a long-standing role in regulatory requirements. Since 1962, in the wake of the thalidomide crisis in which an anti-nausea and sedative drug widely used was found to cause severe congenital disabilities, evidence of efficacy is required before a drug can be approved[65]. The Food and Drug Administration (FDA) in the United States is a key player in the approval of drugs and medical devices[66]. Under FDA regulations, a series of clinical trials are conducted with the medication, to determine if the findings support the manufacturer's efficacy claims and demonstrate that the drug is safe. The early drug approval statute in 1962 generally required at least two adequate and well-controlled randomized investigations[67]. Although regulatory guidelines have evolved over the past decades to allow non-RCTs as well as to include a range of concessions, RCTs continue to have a long-lasting importance in this field, given their rigor and advantage of randomization.

Targeted studies

A clear difference between RCTs and observational studies is that when ethical and feasible, RCTs allow researchers to design a study to investigate questions they want answered, rather than the questions they can answer with naturally occurring data. For instance, the impact of physician-patient race concordance on patient's health behavior is notoriously challenging to determine using observational data[68]. As most individuals choose their primary care physician, selection is already present in concordant *vs* discordant dyads. Further, long-standing structural inequalities have it such that many disadvantaged individuals, who tend to be of a minority race, do not have a primary care physician[58]. If researchers were to rely on existing data through observational studies alone, this question would never be answered sufficiently. In contrast, RCTs have the capacity to provide a satisfactory response to this. Researchers created a pop-up clinic where Black male patients were randomly assigned to see either a Black or non-Black physician[69]. It was found that those randomly assigned to Black physicians were 18% more likely to use preventive health services after the interaction than those assigned to a racially discordant doctor. This example thus illustrates an added advantage of RCTs in addressing questions that cannot otherwise be answered with regular observational studies.

USE OF PROPENSITY SCORE MATCHING IN RANDOMIZED CONTROLLED TRIALS

While RCTs are the gold standard for causal inference, challenges such as ethical considerations and real-world applicability can limit the scope and generalizability of RCT findings. This is when PSM can complement RCTs, offering a solution to address these issues and enhance the validity of the results. The synergy between RCTs and PSM can be powerful, and several examples illustrate how these two methodologies can work together effectively.

RCTs with imperfect randomization

In some RCTs, the process of randomization may not achieve perfect balance in baseline covariates, especially in small samples. This can lead to residual confounding and affect the internal validity of the trial. PSM can be employed further to improve the balance between the treatment and control groups, enhancing the reliability of the RCT results. For example, the Bracing in Adolescent Idiopathic Scoliosis Trial was initially designed solely as a randomized trial[70]. However, there was a slower than anticipated enrollment of participants due to most participants preferring one treatment over the other and thus declining randomization. Therefore, a preference cohort was included thereafter and PSM was used to control for potential selection bias due to the nonrandom treatment assignment in the preference cohort. This helped to refine the treatment and control arms in both the randomized and preference cohorts in terms of baseline characteristics, and facilitates integration of the data for fair comparison. In another study by Wang *et al*[71] investigating the impact of mannitol on outcome among participants of the Intensive Blood Pressure Reduction in Acute Cerebral Hemorrhage Trial (INTERACT2), it was found that there was significant variability in baseline covariates between patients treated with and without mannitol. With the use of propensity score methods, the baseline characteristics of both cohorts can be adjusted for, and a fairer comparison can be made to determine the effect of mannitol. Van Groenestijn *et al* [72] also used PSM to correct for baseline inequalities in a RCT studying the effectiveness of aerobic exercise therapy on disease-specific and generic health-related quality of life in ambulatory patients with amyotrophic lateral sclerosis. Hence, the use of propensity score analyses and multivariate models can be synergistic with RCTs to establish causal relationships while enhancing validity.

Non-compliance to protocol

In RCTs, there are often participants who do not adhere to the assigned treatment. In such scenarios, PSM can be applied to account for non-compliance or deviations in treatment received, and thus provide a more comprehensive understanding of the treatment's impact. The Odyssey Outcomes trial compared the cardiovascular outcomes of treatment using alirocumab with placebo in patients with recent acute coronary syndrome receiving intensive statin treatment[73]. Despite being prescribed specific doses of alirocumab, some patients did not adhere to the prescribed dose or frequency of medication, which would affect the perceived effectiveness of the drug. To account for this, PSM was used to adjust for patients' compliance to the prescribed drug regime so that a better comparison between alirocumab and placebo could be performed. Therefore, propensity score methods may be useful in accounting for nonadherence or deviations in protocol which may be inevitable in large clinical studies.

Translation of RCT data to clinical practice

RCTs are designed to establish causal relationships under controlled conditions, but the extrapolation of their results to broader patient populations and diverse clinical settings poses several challenges, such as the use of stringent inclusion and exclusion criteria to enhance internal validity while compromising external validity, limiting the generalizability of findings to real-world patient populations. In addition, clinical practice involves diverse patient populations with varying comorbidities, demographics, and treatment responses. RCTs may not capture this heterogeneity adequately[74]. Paradoxically, many clinical practices are rapidly adopted by medical practitioners despite no evidence from RCTs. For example, laparoscopic cholecystectomy, a current gold-standard procedure for removing gallbladders, is not supported by RCT evidence.

PSM addresses the challenges of translating RCT data to clinical practice by facilitating a more nuanced comparison of treatment and control groups. By accounting for observed confounding variables, PSM helps create matched cohorts that closely resemble the characteristics of the broader patient population encountered in clinical settings. For example, RCTs

have demonstrated that the Songling Xuemaikang capsule (SXC) is effective in reducing blood pressure in essential hypertension. However, the efficacy of SXC in actual clinical settings is still unknown. Using a PSM approach, Lai *et al*[10] compared the results of patients treated with SXC monotherapy from both real world and RCT cohorts and found that SXC monotherapy is at least as effective in real-world settings as within the RCT. Similarly, Chung *et al*[11] used propensity score-based poststratification to generalize the results of the Flexibility in Duty Hour Requirements for Surgical Trainees Trial to the nonrepresentative samples. In addition, Godley *et al*[12] used propensity score based methods to assess the impact of dosage levels of Volunteer Recovery Support for Adolescents across measures such as frequency of substance used and emotional problems. Therefore, PSM employed using data from RCTs to create a matched cohort reflective of the broader patient population can allow for a more realistic assessment of the intervention's effectiveness in routine clinical settings.

LIMITATIONS

There are some limitations to our review. The studies reviewed in this article were not able to do a direct comparison between PSM and RCTs due to the nature of their investigation. Nevertheless, these studies were able to describe the advantages and disadvantages of each method collectively. In addition, there is a lack in reporting of the disadvantages of integrating PSM into RCTs. Further studies are therefore required to examine the limitations of the synergistic implementation of PSM and RCTs concurrently.

CONCLUSION

More studies adopting the synergistic implementation of PSM and RCTs concurrently are emerging, demonstrating the feasibility and advantages the integration of both methods have to offer. PSM offers an ethical and practical alternative in situations where RCTs are not feasible or ethical. RCTs, on the other hand, continue to be the gold standard for establishing causal relationships, offering the highest level of internal validity and have a role in regulatory requirement for novel medical treatment. Ultimately, the choice between PSM and RCTs should be made carefully, considering the specific goals and constraints of the research context applied. Rather than a binary choice, the integration of PSM into RCTs should also be considered if possible. The combined implementation of both approaches can help improve the generalizability of results to a wider range of patients and specific patient populations of interest for translation to clinical practice, while maintaining the robustness of randomization and high internal validity. Therefore, the synergistic integration of PSM into RCTs should be considered for future research when possible.

FOOTNOTES

Author contributions: Liau MYQ, Toh EQ, Muhamed S, Selvakumar SV and Shelat VG designed the research study; Liau MYQ, Toh EQ, Muhamed S, Selvakumar SV and Shelat VG performed the research; Liau MYQ, Toh EQ, Muhamed S, Selvakumar SV and Shelat VG analyzed the data and wrote the manuscript; All authors have read and approve the final manuscript.

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

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S-Editor: Li L
L-Editor: A
P-Editor: Zhao S

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World Journal of Methodology

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

World J Methodol 2024 March 20; 14(1): 89709

DOI: 10.5662/wjm.v14.i1.89709

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Retrospective Study

Billroth II anastomosis combined with brown anastomosis reduce reflux gastritis in gastric cancer patients

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Specialty type: Surgery

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Yu WB, China

Received: November 9, 2023
Peer-review started: November 9, 2023
First decision: December 12, 2023
Revised: December 21, 2023
Accepted: January 24, 2024
Article in press: January 24, 2024
Published online: March 20, 2024

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Abstract

BACKGROUND

The surgeon performing a distal gastrectomy, has an arsenal of reconstruction techniques at his disposal, Billroth II among them. Braun anastomosis performed during a Billroth II procedure has shown evidence of superiority over typical Billroth II, in terms of survival, with no impact on postoperative morbidity and mortality.

AIM

To compare Billroth II *vs* Billroth II and Braun following distal gastrectomy, regarding their postoperative course.

METHODS

Patients who underwent distal gastrectomy during 2002-2021, were separated into two groups, depending on the surgical technique used (Billroth II: 74 patients and Billroth II and Braun: 28 patients). The daily output of the nasogastric tube (NGT), the postoperative day that NGT was removed and the day the patient started per os feeding were recorded. Postoperative complications were at the same time noted. Data were then statistically analyzed.

RESULTS

There was difference in the mean NGT removal day and the mean start feeding day. Mean total postoperative NGT output was lower in Braun group (399.17 mL *vs* 1102.78 mL) and it was statistically significant ($P < 0.0001$). Mean daily postoperative NGT output was also statistically significantly lower in Braun group. According to the postoperative follow up 40 patient experienced bile reflux and alkaline gastritis from the Billroth II group, while 9 patients who underwent

Billroth II and Braun anastomosis were presented with the same conditions ($P < 0.05$).

CONCLUSION
There was evidence of superiority of Billroth II and Braun *vs* typical Billroth II in terms of bile reflux, alkaline gastritis and NGT output.

Key Words: Billroth II; Billroth II and Braun; Reconstruction techniques; Gastrectomy; Distal gastrectomy

Core Tip: This is a retrospective study to evaluate the efficacy of the addition of Braun enteroenteroanastomosis to Bilroth II reconstruction compared to Billroth II alone in terms of the postoperative outcomes of these surgical techniques, following distal gastrectomy. The addition of Braun anastomosis demonstrated superiority in terms of survival without impacting complications or mortality. The study highlights the significance of considering bile reflux and alkaline gastritis in postoperative quality of life after gastrectomy, emphasizing the role of Braun's anastomosis in reducing bile reflux and associated complications.

Citation: Christodoulidis G, Kouliou MN, Koumarelas KE, Argyriou K, Karali GA, Tepetes K. Billroth II anastomosis combined with brown anastomosis reduce reflux gastritis in gastric cancer patients. *World J Methodol* 2024; 14(1): 89709
URL: https://www.wjgnet.com/2222-0682/full/v14/i1/89709.htm
DOI: https://dx.doi.org/10.5662/wjm.v14.i1.89709

INTRODUCTION

Theodor Billroth (1829-1894) was a prominent figure of surgery during the 19th century, being the first to perform a subtotal gastrectomy[1,2]. Among his heritage, Billroth II[1], an operation, in which, after a partial gastrectomy and closure of duodenum stump, a side-to-side anastomosis is performed between jejunum and the greater curvature of the stomach. Although Heinrich Braun (1862-1934), another surgical pioneer, was the first to describe the formation of an ulcer in the jejunum after a gastroenterostomy, he is widely known for the homonymous enteroenterostomy[3]. Braun enteroenterostomy[4], is defined as the anastomosis between the afferent and efferent loops of jejunum, distal to a gastroenterostomy. The purpose of a Braun anastomosis, originally introduced in 1892[5], is to reduce the reflux of bile and pancreatic secretions into the stomach[6], as well as the possibility of ileus[7] and divert oral intake from the afferent limb[8], which is crucial, given the fact that bile reflux is one of the most important factors that determine the postoperative quality of life after gastrectomy[9]. Furthermore, alkaline gastritis is correlated with esophagitis, Barrett's esophagus and with the emergence of metachronous cancer, therefore the management of reflux gastritis is fundamental [10,11]. Due to its alkaline protecting effect, Braun's anastomosis is today widely applied to distal gastrectomy and pancreaticoduodenectomy[5].

Distal gastrectomy[12-14] remains the operation of choice for distal-third gastric cancer, as followed by lower mortality and morbidity rates, higher quality of life and no significant difference as far as long-term survival rates are concerned, compared to total gastrectomy, with the efforts now leaning on maintaining the continuity of the Gastrointestinal tract[15, 16]. Moreover, the surgeon performing a distal gastrectomy, has a variety of reconstruction techniques, Billroth-I, Billroth-II, and Roux-en-Y, each with its respective advantages and disadvantages, at his disposal[5,10-12,16-18]. Among those, Billroth II with or without Braun anastomosis is often preferred worldwide[19]. Braun anastomosis performed during a Billroth II procedure has shown evidence of superiority over typical Billroth II, in terms of survival, with no impact on postoperative complications and mortality[20].

Therefore, this study compared the two above mentioned surgical techniques regarding their postoperative course.

MATERIALS AND METHODS

For the purpose of this retrospective study, data were collected from patients undergoing distal gastrectomy at the Department of Surgery, University Hospital of Larissa, during 2002-2021. No patients were excluded based on their underlying disease. As far as primary diagnosis is concerned, from the entire sample, 5 patients were diagnosed with gastrointestinal stromal tumor, 6 patients with gastric ulcer, and all the remaining patients suffered from gastric adenocarcinoma. They were then separated into two groups, depending on the surgical technique used (Billroth II: 74 patients, mean age: 70.75 years, 44 male, 30 female; and Billroth II and Braun: 28 patients, mean age: 70.41 years, 21 male, 7 female). As minimum and maximum age in the sample was 42 and 92 years respectively, patients were also divided into two subgroups (≤ 67 years and > 67 years). There was no categorization, on the basis of the way the anastomoses were performed, for example hand sewn or with the use of a mechanical stapler. Demographic data, including age and

gender, the output of the nasogastric tube (NGT), the postoperative day (POD) that NGT was removed, the day the patient started feeding and the total postoperative hospitalization days (PHD) were recorded. Patients on their 5th POD underwent gastroscopy in order to investigate any possible development of alkaline gastritis and bile reflux. NGT output was measured on a daily basis at a fixed hour and data were collected until the 10th POD. Patients with NGT ≥ 10th POD or need for NGT reinsertion after 10th POD were excluded from the study. Before comparisons between the two surgical techniques were made, the data from each subgroup underwent Shapiro-Wilk test for normality ($P < 0.05$)[21-26]. Due to the fact that normality could not be proven, Mann-Whitney *U* test ($P < 0.05$) was used[27]. Aligned Rank Transform three way ANOVA was then performed (ARTool) for the effects of age, gender and surgical method on hospitalization days and NGT total output, to be examined[28]. All statistical analysis was conducted using IBM SPSS statistics v22 software.

RESULTS

The study outcomes are displayed in Table 1. The mean PHD for Billroth II was 13.09, while for Billroth II and Braun was 10.17 ($P < 0.0001$). Moreover, there was statistically significant difference between the two methods as far as feeding start day and NGT removal day are concerned ($P < 0.0001$). Data from BII and Braun for NGT removal day follow the normal distribution, but since BII data do not, Mann Whitney was applied. NGT output mean is systematically lower for BII + B group during all POD (Figures 1 and 2). Moreover, patients from BII + B group had their NGT removed by the 6th POD due to lack of drainage, while patients from BII group had still an increased drainage volume due to reflux gastritis.

According to the postoperative complications, out of the 74 patients who underwent Billroth II, forty presented with bile reflux and alkaline gastritis (54%), and from the group of patients who underwent BII and Braun these complications were observed in only 9 patients (32%) ($P < 0.05$) (Table 2). These findings were mainly confirmed by the patients gastroscope report during their postoperative follow-up and the NGT output.

In our study BII outweighed BII and Braun in terms of operation time, with a mean operating time of 226.4 min ± 41.6 min. for the BII group *vs* a duration of 255.8 min ± 66.2 min. for the BII and Braun group ($P < 0.05$). However, there were no statistically significant difference in blood loss during surgery (Table 3).

An Aligned Rank Transform three-way ANOVA was conducted and examined the effect of age, gender and surgical method on hospitalization days and NGT total output (Table 4). There was a statistically significant interaction between age and gender in total NGT output ($P < 0.05$) or PHD ($P < 0.05$). Similarly, there was an interaction effect on NGT output or PHD between age and method, gender and method or age, gender, and method ($P < 0.05$). This means that younger and male patients had smaller values of NGT output as well as less hospitalization days. Moreover, whichever of the independent factors were combined with BII and Braun anastomosis had also a better outcome.

Patients on their follow-up were given questionnaires for the postoperative quality-of-life assessing data about patients recovery, in terms of physical, emotional and cognitive behavior. A short analysis of the data acquired indicated that the patients of the two groups had a similar postoperative status.

DISCUSSION

Gastric malignancies account for 930000 over 1000000 new cases and 700000 deaths annually[15,29]. Gastric cancer is considered the third deadliest, while being the fifth most commonly diagnosed[15]. Considering the progress in earlier diagnosis a more preservative attitude towards distal gastric cancer resection has been recently adopted, since there is no difference regarding long term survival rates between distal and total gastrectomy[12,15]. The extent of the portion of the stomach removed, given adequate oncologic margins (≥ 3 cm for T2 tumors or types 1 and 2 and ≥ 5 cm for types 3 and 4) does not constitute a prognostic factor, unlike perigastric lymph node clearance. Regarding lymph node clearance during distal gastrectomy, JGCA recommends D1 or D1+ for cT1N0 and D2 for cT2-T4 tumors[13]. Due to the fact that the most important factor affecting the decision between distal or total gastrectomy is the proximal resection margin, patients suffering from malignancy in the middle part of the stomach can also be submitted to distal gastrectomy, thus counting almost for 23%-70% of all cancer gastrectomies in Europe and Asia[12,30,31]. Although five-year survival rates of gastrectomy range between 33%-50%, patients can suffer from ongoing gastrointestinal symptoms for up to 6 months postoperatively[29,32].

The selection of reconstruction methods following distal gastrectomy presents a significant dilemma. Options such as Billroth I, Billroth II, and Roux-en-Y are available, with the latter gaining prominence in the 1970s and 1980s as a response to the elevated incidence of post-gastrectomy alkaline reflux gastritis[33]. Roux-en-Y exhibits superiority over Billroth II in terms of functional and endoscopic outcomes, attributed to the mitigated risks of gastroduodenal and duodenogastroesophageal reflux (DGER), identified as precipitating factors for malignancy development based on reflux gastritis and esophagitis[7,17,18]. However, Roux-en-Y anastomosis entails certain drawbacks, such as a potential occurrence of Roux stasis syndrome (observed in approximately 0-13% of patients), leading to vomiting, stomach dilation, and prolonged hospitalization[18]. Additionally, the procedure necessitates an extended operation time and is associated with increased intraoperative blood loss and greater postoperative weight loss compared to Billroth II and Braun[10,15]. Billroth II with Braun is often proposed as the primary surgical approach, with Roux-en-Y considered a secondary option in case of Braun's failure[34].

A retrospective analysis involving 720 patients with gastric malignancy from 1997-2011 suggested that Billroth II and Braun may enhance lifespan without escalating postoperative complications and mortality rates[20]. The literature, including a Randomised Clinical Trial and a prospective randomized trial, underscores the comparable acceptability of

Table 1 Study outcomes

	Operation	*n*	Mean	SD	*P* value
Postoperative hospitalization days	Billroth II	74	13.09	1.41	< 0.0001
	Billroth II + Braun	28	10.17	2.01	
Feeding start day	Billroth II	74	6.33	0.66	< 0.0001
	Billroth II + Braun	28	5.17	0.41	
NGT removal day	Billroth II	74	4.31	0.31	< 0.0001
	Billroth II + Braun	28	4.00	0.67	
NGT output (day 0)	Billroth II	74	183.56	56.29	< 0.0001
	Billroth II + Braun	26	73.64	25.70	
NGT output (day 1)	Billroth II	72	265.80	50.08	< 0.0001
	Billroth II + Braun	26	168.18	76.95	
NGT output (day 2)	Billroth II	61	234.59	44.82	< 0.0001
	Billroth II + Braun	21	85.56	43.08	
NGT output (day 3)	Billroth II	49	280.00	57.76	< 0.0001
	Billroth II + Braun	16	47.14	17.82	
NGT output (day 4)	Billroth II	30	251.67	70.48	< 0.0001
	Billroth II + Braun	12	136.00	93.25	
NGT output (day 5)	Billroth II	21	234.62	65.08	< 0.0001
	Billroth II + Braun	7	116.67	92.80	
NGT output (day 6)	Billroth II	13	322.50	76.11	< 0.0001
	Billroth II + Braun	1	0	NA	
NGT output (sum)	Billroth II	74	1102.78	203.94	< 0.0001
	Billroth II + Braun	28	399.17	140.18	

NGT: Nasogastric tube; NA: Not available.

Table 2 Postoperative complications

Postoperative complications	Billroth II	Billroth II with Braun	*P* value
Bile reflux	40	9	0.048
Alkaline gastritis	40	9	0.048
Anastomotic bleeding	2	1	0.820
Anastomotic fistula	1	1	0.470

Billroth II and Braun to Roux-en-Y gastrojejunostomy, emphasizing the significance of considering operation time and blood loss in critically ill patients during the selection of the appropriate procedure[10,15,17]. However, a prospective randomized trial exhibited statistically significant differences in favor of Roux-en-Y regarding the degree and extent of gastritis and bile reflux, though no distinctions were observed in the overall Gastrointestinal Quality of Life Index score [17,35,36].

The application of Braun anastomosis in pancreaticoduodenectomy (PD) has garnered substantial attention, with studies indicating its potential benefits. Comparative analyses between Child non-Braun and Child Braun cohorts revealed statistically significant reductions in DGER rates in the Braun group, positioning Braun enteroenterostomy as a significant independent factor in mitigating DGER[37]. These findings align with similar results in pylorus-preserving pancreatoduodenectomy, affirming the advantageous role of Braun anastomosis in minimizing postoperative DGER incidence[38]. A recent meta-analysis of ten studies comprising 1614 patients reported no significant differences in mortality, intraoperative blood loss, postoperative pancreatic fistula, bile leakage, gastrointestinal hemorrhage, intra-abdominal abscesses, wound complications, and overall hospital stay between Braun PD and typical PD. Nevertheless, the Braun group exhibited lower rates of reoperation, morbidity, clinically relevant DGER, postoperative NGT

Table 3 Patients' characteristics

Characteristics	Billroth II	Billroth II and Braun
Age	70.75	70.41
Gender		
Male	44	21
Female	30	7
Operation time (min)	226.4 ± 41.6	255.8 ± 66.2
Blood loss (mL)	175.4 ± 121.3	148.7 ± 96.8

Table 4 Aligned Rank Transform three-way ANOVA results ($P < 0.05$)

	NGT output (sum)	Postoperative hospitalization days
Age	0.929	0.339
Gender	0.325	0.093
Method	0.170	0.469
Interaction age and gender	0.861	0.288
Interaction age and method	0.946	0.635
Interaction gender and method	0.579	0.177
Interaction age, gender, and method	0.983	0.998

NGT: Nasogastric tube.

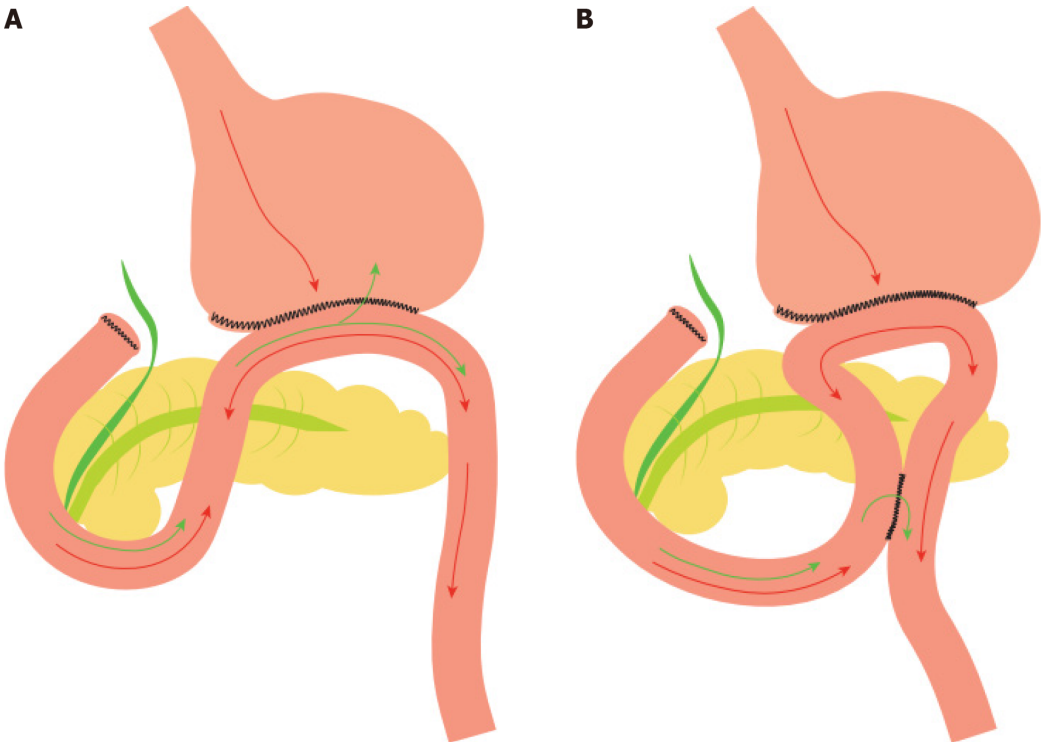

Figure 1 Billroth II and Braun figure. A: Billroth II; B: Billroth II and Braun.

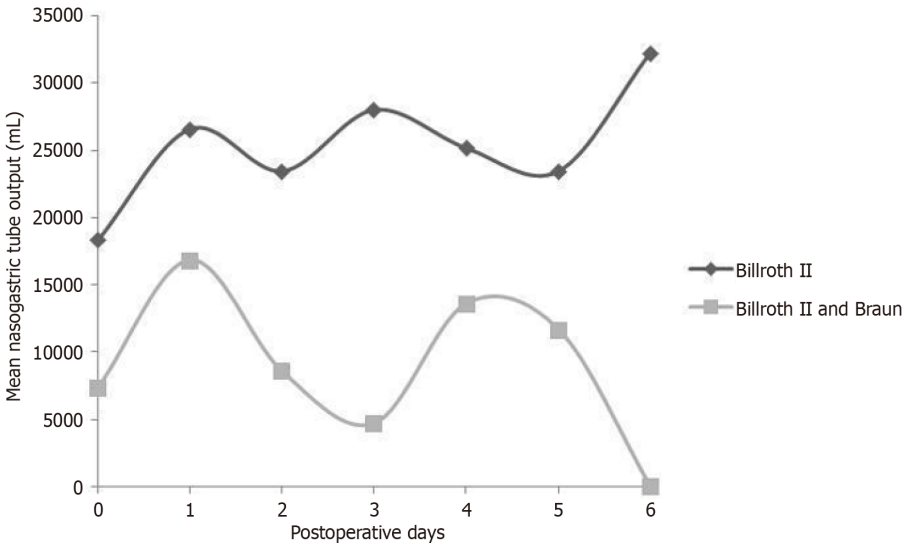

Figure 2 Mean daily nasogastric tube output.

reinsertion, and vomiting[6].

Our study, albeit contributing valuable insights, is not without limitations. It bears the inherent biases of a retrospective, non-randomized trial conducted over a nineteen-year span, during which accumulated experience may have exerted an influential role. Notably, the study group lacked stratification based on anastomosis techniques (manual suturing or the use of a stapling device), surgery type (open *vs* laparoscopic) and the specific disease leading to gastrectomy, the latter due to a constrained sample size. The uneven distribution of participants between the two groups may introduce non-homogeneity biases. It is pertinent to acknowledge that gastric reflux was not quantitatively assessed using imaging methods, such as radionuclide biliary scanning. Despite these limitations, the existing literature supports the utility of Braun anastomosis, emphasizing the exigency for well-designed randomized controlled trials to further delineate its merits.

CONCLUSION

In conclusion, there were evidence of superiority of Billroth II and Braun against typical Billroth II, in terms of bile reflux, alkaline gastritis and NGT output. These results were statistical significant, eventhough the several study limitations. The need for randomized controlled trials is highlighted.

ARTICLE HIGHLIGHTS

Research background

The study focuses on comparing two reconstruction techniques, Billroth II and Billroth II with Braun anastomosis, commonly used after distal gastrectomy, examining their impact on postoperative outcomes. The retrospective study collected data from patients undergoing distal gastrectomy, dividing them into two groups based on the reconstruction technique used. The significance of our research lies to the close follow-up in accordance with the gastroenterologists to confirm the diagnosis of alkaline reflux gastritis.

Research motivation

The research is motivated by the debate on the optimal reconstruction technique following distal gastrectomy for gastric cancer. The study aims to contribute valuable insights by comparing the postoperative outcomes of the two reconstruction methods, Billroth II and Billroth II with Braun anastomosis, in order to inform clinical decision-making and potentially improve patient outcomes in the treatment of distal gastric cancer.

Research objectives

To evaluate and compare the postoperative course of patients undergoing distal gastrectomy with either Billroth II or Billroth II with Braun anastomosis. Specific outcomes under scrutiny involve factors such as postoperative hospitalization days (PHD), feeding initiation, nasogastric tube (NGT) removal, and the occurrence of complications like bile reflux and alkaline gastritis, aiming to discern potential advantages between the two reconstruction techniques.

Research methods

The study employed a retrospective design, collecting data from patients who underwent distal gastrectomy at the Department of Surgery, University Hospital of Larissa, spanning from 2002 to 2021. Patients were categorized based on the reconstruction technique used (Billroth II or Billroth II with Braun), and statistical analyses, including Mann-Whitney *U* test and Aligned Rank Transform three-way ANOVA, were performed to assess variables such as PHD, feeding start day, NGT removal, and complications.

Research results

The research revealed that distal gastrectomy with Billroth II and Braun anastomosis demonstrated superiority over typical Billroth II in terms of postoperative outcomes. Statistically significant differences were observed, including shorter PHD, earlier feeding initiation, quicker NGT removal, and a lower incidence of complications such as bile reflux and alkaline gastritis, highlighting potential benefits of the Billroth II and Braun anastomosis technique in the surgical management of distal gastric cancer.

Research conclusions

In conclusion, the study suggests evidence of the superiority of Billroth II with Braun anastomosis over typical Billroth II in the context of distal gastrectomy for gastric cancer. Despite inherent limitations in the retrospective design, the findings emphasize the potential benefits of the specific reconstruction technique, such as reduced postoperative complications and improved outcomes.

Research perspectives

Future research should focus on addressing limitations such as sample size constraints, variations in surgical techniques, and the absence of quantitative assessments for gastric reflux, aiming to provide more conclusive evidence on the optimal reconstruction method for enhanced postoperative outcomes in patients with distal gastric cancer.

FOOTNOTES

Author contributions: Tepetes K and Christodoulidis G designed the study; Christodoulidis G, Kouliou MN, Koumarelas KE, and Karali O collected the data; Kouliou MN, Koumarelas KE, and Karali O wrote the manuscript; Argyriou K performed the statistical analysis and contributed to the analysis; Tepetes K, Christodoulidis G, and Argyriou K supervised the report.

Institutional review board statement: Due to the retrospective nature of our study, we didn't require approval by the institution.

Informed consent statement: The informed consent statement is not required due to the retrospective nature of our study.

Conflict-of-interest statement: The authors declare no conflict of interest.

Data sharing statement: Data is available from the corresponding author at gregsurg@yahoo.gr.

Country/Territory of origin: Greece

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S-Editor: Chen YL
L-Editor: A
P-Editor: Zhao YQ

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World Journal of Methodology

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World J Methodol 2024 March 20; 14(1): 88850

DOI: 10.5662/wjm.v14.i1.88850

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Observational Study

Convenient model of hard tissue simulation for dental radiographic research and instruction

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Specialty type: Dentistry, oral surgery and medicine

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Mostafavinia A, Iran

Received: October 13, 2023
Peer-review started: October 13, 2023
First decision: December 7, 2023
Revised: December 20, 2023
Accepted: January 19, 2024
Article in press: January 19, 2024
Published online: March 20, 2024

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Abstract

BACKGROUND

The authors describe a technique for building an alternative jawbone phantom using dental gypsum and rice for research and dental radiology instruction.

AIM

To investigate the potential of an alternative phantom to simulate the trabecular bone aspect of the human maxilla in periapical radiographs.

METHODS

Half-maxillary phantoms built from gypsum-ground rice were exposed to X-rays, and the resulting images (experimental group) were compared to standardized radiographic images produced from dry human maxillary bone (control group) (n = 7). The images were blindly assessed according to strict criteria by three examiners for the usual trabecular aspects of the surrounding bone, and significant differences between groups and in assessment reliability were compared using Fisher's exact and kappa tests ($\alpha = 0.05$).

RESULTS

The differences in the trabecular aspects between groups were not statistically significant. In addition, interobserver agreement among observers was 0.43 and 0.51 for the control and experimental groups, respectively, whereas intraobserver agreement was 0.71 and 0.73, respectively.

CONCLUSION

The tested phantom seemed to demonstrate potential for trabecular bone image

simulation on maxillary periapical radiographs.

Key Words: Phantom; Radiology; Education; Endodontic treatment; Bone trabecular

Core Tip: The successful interpretation of radiographs is a complex process that relies on the clinician's understanding of the radiographic image and ability to recognize the range of appearances of hard and soft tissues. To improve radiographic technique and image interpretation, the assimilation of normal appearances of hard tissues is fundamental in the research and teaching of dentomaxillofacial radiology. The authors describe a technique to build an alternative jawbone phantom using dental gypsum and rice for research and dental radiology instruction. The tested phantom seemed to have potential for trabecular bone image simulation on maxillary periapical radiographs.

Citation: Munhoz EA, Xavier CRG, Salles RP, Capelozza ALA, Bodanezi AV. Convenient model of hard tissue simulation for dental radiographic research and instruction. *World J Methodol* 2024; 14(1): 88850
URL: https://www.wjgnet.com/2222-0682/full/v14/i1/88850.htm
DOI: https://dx.doi.org/10.5662/wjm.v14.i1.88850

INTRODUCTION

Dental radiographs provide crucial and objective information that is unseen during clinical examination but that aids dentists in performing diagnosis, therapeutic planning, and treatment. Thus, an accurate analysis of a radiographic image depends on the quality of acquisition, a visual inspection, and an interpretation of the findings[1]. However, the successful interpretation of radiographs is a complex process relying on a combination of technical factors, anatomical and pathological knowledge, and clinician experience and expertise to understand the images and correlate them with perceived signs and symptoms[2]. By recognizing the difference between normal and varied radiographic tissue conditions, clinicians can identify changes caused by diseases that affect the teeth and jaws.

The process of learning, building, and improving radiographic diagnostic skills is a structured and rigorous undertaking that requires extensive human training. This should involve practicing radiologic technical skills, expanding scientific knowledge about diseases, and exposure to clinical environments[3].

Anthropomorphic devices, such as manufactured head phantoms, are proper alternatives commonly used to simulate the radiographic characteristics of maxillofacial tissues and to enable extensivelaboratory experimentation and practice. These simulators help to prevent unnecessary repetitions and patient X-ray overexposure in clinical settings[4], but they are also expensive, and the resulting bone trabeculae aspect is often insufficiently realistic. For this reason, cadaveric dried human skulls[5-11] or animal hard and soft tissue[12,13] are generally preferred for research and teaching, but they are extremely difficult to obtain. This is because ethical concerns involved in the fabrication and adjustment often needed for human or animal phantoms, such as cutting and grinding, demand considerable expertise and commonly produce irreversible changes that restrict their use in other applications[9-12].

Trabecular bone is highly visible, dominating images of the alveolar structure and adding complexity to human radiographic analysis[3,14]. Nevertheless, most *in vitro* studies on the reliability of the radiographic method are carried out only on uncovered extracted teeth[15-19], which may increase the differences related to clinical study results[3].

To date, no hard tissue simulation model can be manufactured in a simple and individualized way to mimic human bone for radiological training nor studies. As such, this study aimed to investigate the potential of an alternative phantom to simulate the trabecular appearance of the human maxilla in conventional periapical radiographs.

MATERIALS AND METHODS

This study was approved by the local ethics committee, which is in compliance with the Declaration of Helsinki.

Custom phantom preparation

In total, 20 human teeth (13 to 18 and 23 to 28), extracted for therapeutic reasons and kept in 0.5% thymol solution, were scaled, polished with water/pumice, and dried with absorbent paper. After, the roots were covered with two layers of paraffin wax (Surgipath Medical Ind., Richmond, IL, United States) and heated to 56 °C in a water bath to simulate the periodontal ligament space on radiographs.

Right and left polyvinyl siloxane silicone negative molds (Elite Double 8, Zhermack, Badia Polesine, Italy) were obtained from the maxilla of a dental training manikin (Buyamag Inc., Carlsbad, CA, United States), which was previously split in half. The teeth were positioned upside down inside the right and left molds (n = 7), and the coronal part of each extracted tooth was inserted into its respective negative locus.

To make each gypsum-rice phantom piece, regular white rice was ground in a blender operating at medium speed (15000 revolutions per min) for 15 s, and the crushed grains were separated into four different fractions using 0.50-, 1.00-, and 2.00-mm mesh sieves. Smaller fragments were discarded, and the remaining larger fractions were mixed in equal-weight proportions. Thereafter, 40 g of a type V high-strength dental stone (Jade Stone, Whip Mix Co., Fort Collins, CO, United States) was mixed with water (0.19 L/P ratio) and homogenized in a bowl, together with 60 g of the processed hydrated rice. The resulting mixture was then poured into the silicone negative mold, and 24 h later, the set model was removed and kept in a controlled environment (25 °C and 75% relative humidity) until use. The filling process was repeated until seven phantoms were obtained.

The control group was composed of seven dry human skulls that were routinely used for educational training and radiographic research purposes, with at least four teeth in one of the maxillary sides (n = 7). No history or background on the skulls was available.

Radiographic procedure

The experimental phantoms were fixed behind a 15-mm-thick vertical acrylic barrier intended to simulate the effect of soft tissues during X-ray exposure[11]. A periapical E film (Insight, Kodak Co., Rochester, NY, United States) was positioned behind each phantom using a specifically designed attaching device (Figure 1).

For the radiographic procedure involving the human skulls, a Rinn-XCP posterior horizontal holder (Dentsply Rinn, Elgin, IL, United States) was applied, and the film packet was placed in the palate next to the premolar and molar teeth. A cotton roll was positioned between the bite block and the occlusal surface of the teeth to keep the acrylic bite block away from the film exposure area. To simulate soft tissue, a 15-mm-thick vertical acrylic barrier was positioned at the front of the skulls[13].

Periapical radiographs of both the experimental and control groups were obtained in the buccolingual direction using the paralleling technique and a long spacer cone. The exposure time was 0.5 s, the focus-object distance was 50 cm, and the object-receptor distance was 2 cm. All radiographic images were obtained with a dental X-ray unit (X-707, Yoshida Dental MFC Co. Ltd, Tokyo, Japan) at 70 kVp and 8 mA.

The exposed films were immersed in developer solution for 3 min at 21 °C and rinsed with water for 10 s before being submerged in the fixer solution for 8 min. The developed films were subsequently washed with running water for 10 min and allowed to dry in a dust-free atmosphere.

Image evaluation

Three radiologists with at least five years of experience were asked to judge whether the images of bone trabeculae patterns in the 14 periapical radiographs, mounted in random order, resemble that experienced in daily clinical practice (Figure 2). Such features as intertrabecular distance, trabecular bone coarseness and striae, and mineral bone density were made available to the examiners as evaluation criteria. Then, before each evaluation, volunteers were instructed to disregard the presence of anatomical details, such as the maxillary sinus floor, the maxillary zygomatic process, and the lamina dura. Signs of previous dental interventions, such as root canal fillings, dental restorations, or absent teeth, were also ignored by the examiners.

All radiographic exams were performed with the aid of an ethyl-vinyl-acetate mask positioned over a fluorescent cold light box (Medalight LP-400, Hong Kong, China) in the same light-controlled room. Use of magnifying glasses was not allowed. The assessments were recorded in a proper form, and the viewing time was unlimited. A second evaluation was executed 4 wk after the first assessment. Then, the final assessment of each radiographic image was obtained from two or more similar assessments, as assigned by the examiners.

Statistical analysis

Differences between groups were compared using Fisher's exact test, adjusted to the 5% significance level, whereas inter- and intraobserver agreements were determined by Cohen's kappa statistic. Further, a statistical evaluation of all tests was performed using the SPSS database software (SPSS v. 18.0 for Windows, Chicago, IL, United States).

RESULTS

The number of images in the experimental group (3 of 7) from which examiners concluded that the maxillary trabecular pattern in the periapical radiograph resembled that experienced in daily clinical practice was not significantly different from that from the control group (6 of 7) (P = 0.559). In addition, the agreement between observers (interobserver reliability) was 0.43 (moderate) when experimental group images were analyzed and 0.51 (moderate) when control group radiographs were examined. Further, the stability of the responses from each examiner at different time points (intraobserver reliability) was 0.73 (substantial) for the experimental group images and 0.71 (substantial) for the control group radiographs, and the generated data are summarized in Table 1.

DISCUSSION

Detailed knowledge of the appearances of normal radiographic bone and anatomical structures is mandatory for the identification of abnormal appearances, such as those due to infections or systemic diseases[6,14,20-23]. Thus, perceiving

Table 1 Generated data

	Dry human maxillary bone	Rice maxillary phantom
Trabecular pattern		
Usual	6	3
Not usual	1	4
Total	7	7
Agreement test		
Interobserver	0.41	0.34
Intraobserver	0.73	0.71

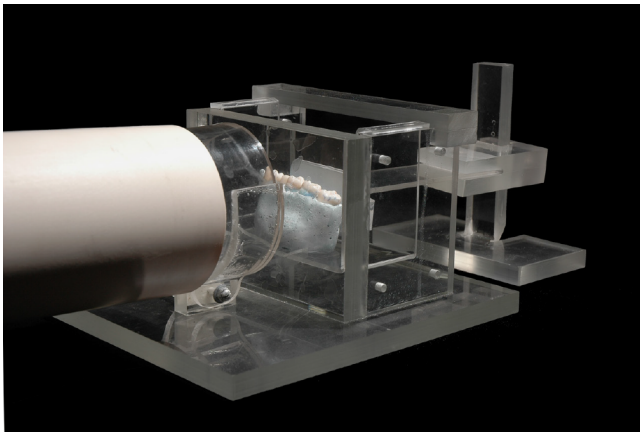

Figure 1 Acrylic device used to standardize the radiographic exposure of the gypsum-rice phantom and to simulate the effect of soft tissues on radiography. Oblique aspect and lateral aspect.

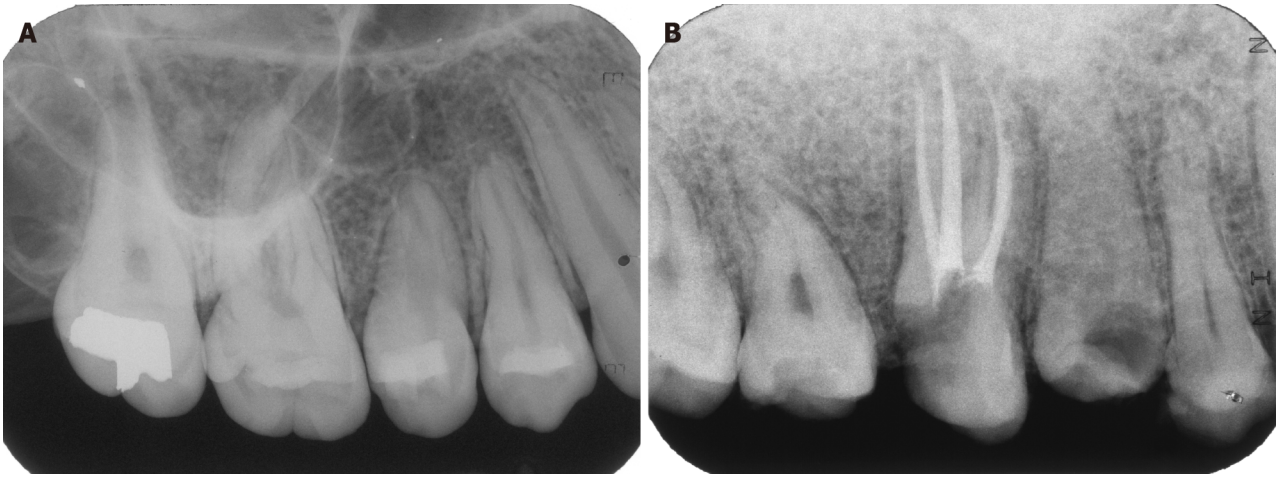

Figure 2 Radiographic image. A: Dry human maxillary bone periapical radiographic image; B: Gypsum-rice maxillary phantom periapical radiographic image.

bone aspects in radiographs is complex because it requires the simultaneous assessment of diverse features, including the bone trabecular pattern, density, thickness, horizontal alignment, and space between trabeculae[2,10,11].

As well, the absence of significant differences between groups may have occurred because of the subjectivity involved in assessing each of these characteristics. Further, a wide variation in observer performance in the periapical radiological diagnoses has been widely recognized[24,25], and the previous experience of each observer may have influenced the interpretation of early periapical intraoral radiographs, as explained by Patel *et al*[22].

In this study, the interexaminer agreement for the bone aspect (0.43) was fair, even though the radiographs were viewed in the same light box and with the same radiographic mask, which are considered to improve interobserver agreement[22]. In addition, substantial variations in individuals' interpretations of radiographic images were common-

place, confirming the results of previous studies[5]. However, this fair reliability may suggest that the radiographic aspects of bone were unclear in the examiners' minds, possibly because a well-established bone pattern was unavailable to form a mental image. Further, variations in the thicknesses of the maxillary and mandibular trabecular bone[10,11,26] detected in the tested skulls may have accounted for this obscurity.

Moreover, the intentional lack of examiner calibration before the evaluations possibly contributed to this fair reliability. As such, prior clarification of the criteria by presenting human maxillary radiographic images to the examiners, including those from the control group, was expected to reduce the subjectivity of the analysis, as the control group's radiograph evaluations would seem clearer to the examiners[27]. Another possible reason for the lack of significant differences between groups was likely due to the low number of intact human skulls available for comparison. Thus, most authors have adopted mandibular phantoms for their studies[10,11,13,14,28-31], as its flat anatomical nature facilitates the use of periapical films and positioning during radiographic imaging[2].

We presented an inanimate anthropomorphic phantom that was intended to simulate bone trabeculae in periapical radiographs of the maxilla. The superimposition of anatomical landmarks, such as the maxillary sinus floor and the maxillary zygomatic process, however, could not be reproduced in the radiographic images that were provided by this custom method. Thus, different results could be expected when applying this method to reproduce mandibular phantoms. In addition, in the maxilla, the trabeculae tend to be finer, more widely spaced, and homogenous, as observed in the experimental phantom, whereas in the mandible, the trabeculae tend to be relatively thick, closely packed, and often aligned horizontally[2].

In addition to simulating human anatomical structures, the tested phantoms are lightweight, easy to build, and low cost, and they have a reasonable size, features considered essential for a radiographic phantom[27]. In addition, the gypsum/rice mixture is poured into the mold, adapting to all root surfaces, including those of multi-rooted furcation teeth, thus eliminating alveoli cuts or grindings, as required for the use of dry human or animal mandible or maxilla[7,8,10-12]. In addition, the rice phantoms possess sufficient mechanical strength to withstand research or didactical handling, but due to the porous structure of the rice grains, the phantom can become fragile following careless repeated use.

According to the herein study results, it can be inferred that the narrow beam attenuation and scattering properties facilitated by the thickness of the tested gypsum-rice combination resemble those of human bone. These findings are thus non-definitive, as this is a preliminary report, and the composition of a larger sample whose radiographic appearance is analyzed by a system that produces quantifiable data may generate stronger evidence of the validity of gypsum-rice phantoms to mimic the radiographic appearance of human bone.

CONCLUSION

According to the preliminary results presented, the phantom constructed from dental gypsum and rice has the potential to simulate maxillary trabecular bone on laboratory periapical radiographs.

ARTICLE HIGHLIGHTS

Research background

The process of learning, building, and improving radiographic diagnostic skills is a structured and rigorous undertaking that requires extensive human training. This should involve practicing radiologic technical skills, expanding scientific knowledge about diseases, and exposure to clinical environments.

Research motivation

Trabecular bone is highly visible, dominating images of the alveolar structure and adding complexity to human radiographic analysis. Nevertheless, most *in vitro* studies on the reliability of the radiographic method are carried out only on uncovered extracted teeth, which may increase the differences related to clinical study results. To date, no hard tissue simulation model can be manufactured in a simple and individualized way to mimic human bone for radiological training nor studies.

Research objectives

To investigate the potential of an alternative phantom to simulate the trabecular appearance of the human maxilla in conventional periapical radiographs.

Research methods

Half-maxillary phantoms built from gypsum-ground rice were exposed to X-rays, and the resulting images (experimental group) were compared to standardized radiographic images produced from dry human maxillary bone (control group) (n = 7). The images were blindly assessed according to strict criteria by three examiners for the usual trabecular aspects of the surrounding bone, and significant differences between groups and in assessment reliability were compared using Fisher's exact and kappa tests ($\alpha = 0.05$).

Research results

The differences in the trabecular aspects between groups were not statistically significant. In addition, interobserver agreement among observers was 0.43 and 0.51 for the control and experimental groups, respectively, whereas intraobserver agreement was 0.71 and 0.73, respectively.

Research conclusions

According to the preliminary results presented, the phantom constructed from dental gypsum and rice has the potential to simulate maxillary trabecular bone on laboratory periapical radiographs.

Research perspectives

The perspectives are to improve the technique using rice as well as the creation of techniques using other accessible materials.

FOOTNOTES

Author contributions: Bodanezi AV, Munhoz EA, and Capelozza ALA contributed on conception and design of the work, the acquisition, analysis and interpretation of data for the work; Bodanezi AV and Munhoz EA also contributed on drafting the work and revising it critically for important intellectual content; Xavier CRG and Salles RP contributed on collecting and analyzing data for the work.

Institutional review board statement: This study was reviewed and approved by the Local Ethics Committee in Brazil (Approval No. 153).

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: There is no conflict of interest at this manuscript.

Data sharing statement: No additional data are available.

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.

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S-Editor: Chen YL
L-Editor: A
P-Editor: Yuan YY

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World Journal of Methodology

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World J Methodol 2024 March 20; 14(1): 89853

DOI: 10.5662/wjm.v14.i1.89853

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Observational Study

Artificial night light and thyroid cancer

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Specialty type: Medical laboratory technology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Qin Y, China; Tzeng IS, Taiwan

Received: November 14, 2023
Peer-review started: November 14, 2023
First decision: November 30, 2023
Revised: December 6, 2023
Accepted: February 2, 2024
Article in press: February 2, 2024
Published online: March 20, 2024

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Abstract

BACKGROUND

The occurrence of thyroid cancer (TC) has increased in recent decades. Exposure to outdoor artificial light at night (ALN) is associated with an increased risk of cancer.

AIM

To investigated the impact of ALN, as a significant environmental pollutant, on TC incidence worldwide.

METHODS

The assessment involved analyzing satellite ALN data in conjunction with TC incidence data [adjusted standardized rate (ASR)], while considering the quality of cancer registries (QCR), gross domestic product (GDP) per person, and health expenditure per person (HEP) for each country.

RESULTS

Results indicated a correlation between higher ASR and ALN exposure percentages, particularly in countries with higher GDP or HEP quartiles (all $P<$ 0.05). Significant differences in ASR were observed across QCR levels, both high and low quality (all $P < 0.05$), but not in countries without registry activity. However, when evaluating ASR against ALN exposure percentages while considering GDP/HEP quartiles or QCR levels, no significant associations were found (all $P > 0.10$).

CONCLUSION

The findings suggest a potential link between higher GDP and adverse health conditions, serving as possible risk factors for TC, rather than a direct association with ALN. Limitations include the use of cross-sectional data, temporal misalign-

ment, and reliance on ALN as a socioeconomic proxy. It is proposed that light pollution might be connected to a lifestyle conducive to carcinogenesis. Additionally, the presence of higher GDP/HEP could enhance access to diagnostic resources, potentially facilitating TC diagnosis and inclusion in cancer registries.

Key Words: Lighting; Human; Epidemiology; Thyroid; Cancer

Core Tip: We explored the impact of outdoor artificial light at night (ALN) on thyroid cancer (TC) worldwide. While a correlation was found between higher TC rates and ALN exposure in countries with greater economic indicators [gross domestic product (GDP) and health expenditure per person (HEP)], the association disappeared when accounting for registry quality. The findings suggest that high GDP may be more closely linked to health conditions and TC risk factors than ALN, possibly indicating a lifestyle connection to carcinogenesis. While correlations between ALN and economic factors are observed, a direct link of ALN to TC remains unconfirmed. Additionally, higher GDP/HEP could contribute to better diagnostic access, aiding TC diagnosis and registry inclusion.

Citation: Tselebis A, Koukkou E, Milionis C, Zabuliene L, Pachi A, Ilias I. Artificial night light and thyroid cancer. *World J Methodol* 2024; 14(1): 89853
URL: https://www.wjgnet.com/2222-0682/full/v14/i1/89853.htm
DOI: https://dx.doi.org/10.5662/wjm.v14.i1.89853

INTRODUCTION

The occurrence of thyroid cancer (TC) has increased in recent decades, in contrast to the incidence of most solid tumors in developed countries, which either remains stable or decreases. The reasons for this increase are still unclear; this epidemiological discrepancy compared to other neoplasias merits further research. Outdoor artificial light at night (ALN) is ubiquitous in the modern world and exposure to it is one of the major environmental pollutants[1]. Light pollution has increased to such an extent that it no longer affects not only residents of large cities, but also those living in more remote areas. Exposure to ALN is associated with an increased risk of cancer[2]. The association of ALN with carcinogenesis is relatively novel. It has been suggested that exposure to ALN reduces nocturnal production of melatonin, which acts as a tumor suppressor[3]. However, little data focused on TC in relation to light pollution have been presented in the literature so far[3]. In this study we aimed to assess, worldwide, the potential impact of ALN on TC, using available satellite ALN data and reported TC epidemiological data.

MATERIALS AND METHODS

To study the potential impact of ALN on TC, published satellite data on light pollution worldwide in 173 countries were used[1] (for a global image of ALN circa 2016, Earth at Night (Black Marble) 2016 Color Maps). In particular, exposure levels-per (%) population and per (%) surface area of each country- to ALN > 87 μcd/m^2 and ALN > 688 μcd/m^2 (levels at which the ability to view the natural night sky is lost and where the Milky Way is no longer visible, respectively) - were used. These thresholds were chosen because the 87 μcd/m^2 level corresponds to 50% more night luminance compared to normal, whereas the 688 μcd/m^2 level denotes the total loss of the natural appearance of the night sky[1]. Light pollution data were estimated with reference to the corresponding per country TC incidence data as provided by the World Health Organization and the Global Initiative for Cancer Registry Development (https://gco.iarc.fr/). In particular, we used the standardized per age and per 100.000 population of each country TC incidence adjusted standardized rate (ASR). The normality of the data distribution was assessed by the Kolmogorov-Smirnoff test. To assess financial influences (as an indirect measure of living conditions and lifestyle) data were collected for gross domestic product (GDP) per person for each country (https://data.worldbank.org/indicator/NY.GDP.PCAP.CD) and health expenditure per person (HEP) for each country (https://data.worldbank.org/indicator/SH.XPD.CHEX.PC.CD) from the World Bank. The quality of cancer registries (QCR), classified in three groups as either high quality registries, registries of lower quality or no registry activity, per the Global Initiative for Cancer Registry Development; https://gco.iarc.fr/) was also noted. Comparisons of ASR and ALN exposure percentages were done according to GDP/HEP quartiles or QCR levels with the Kruskall Wallis test (KW, with statistical significance set at $P < 0.05$). The ASR was evaluated against ALN exposure percentages, conditioned for GDP/HEP quartiles and QCR levels, with Kendall's Tau test (KT, due to non-normal data distribution, with statistical significance set at $P < 0.05$). Statistical analyses were done with Minitab v.17.1 (Minitab Inc, State College, PA, United States, 2010) and JASP v0.15 (JASP Team, University of Amsterdam, NL, 2021).

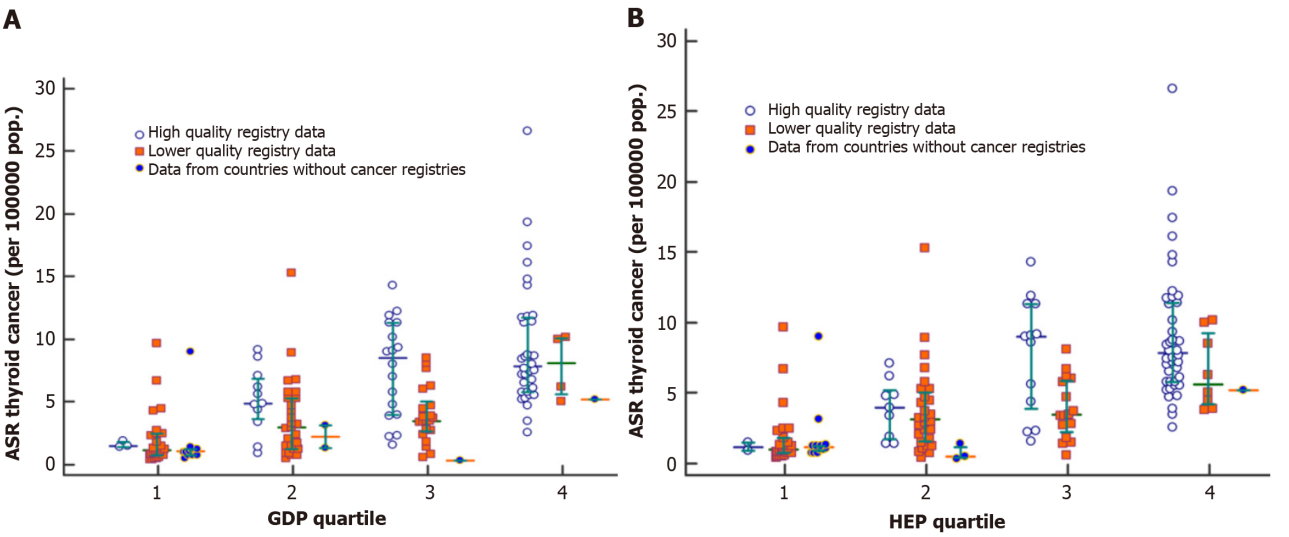

Figure 1 Plots of adjusted standardized rate for thyroid cancer, taking into account quality of cancer registries level. A: By gross domestic product per person quartile; B: By health expenditure per person quartile; horizontal lines indicate the 25th and 75th percentiles, respectively, with the median in between. GDP: Gross domestic product; HEP: Health expenditure per person.

RESULTS

The median value worldwide, per (%) population or per (%) area, of ALN > 87 μcd/m², was 67.3% and 7.5%, while ALN > 688 μcd/m² was 34.6% and 0.7%, respectively. The median ASR was 4.2/100000 population. There were significant differences of ASR and ALN exposure percentages by GDP quartiles or HEP quartiles (Figures 1 and 2).

Higher ASR and ALN exposure percentages were noted with higher GDP quartiles or HEP quartiles (all $P < 0.05$, KW). Differences in ASR were significant for QCR of high quality and lower quality (all $P < 0.05$, KW), but not for data with countries with no registry activity. Evaluation of ASR against ALN exposure percentages, taking into account GDP/ HEP quartiles or QCR levels did not yield significant results (KT ranged from -0.145 to + 0.272, all $P > 0.10$) (Figure 3, Supplementary Figure 1).

DISCUSSION

The global scale of the ALN problem is illustrated by the fact that, according to available data, 83% of the world's population lives under conditions of severe light pollution[1]. In our study we aimed to clarify the nature of the relationship between ALN and TC, taking into account GDP or HEP and level of QCR. We noted differences in ASR by GDP quantiles and HEP quantiles worldwide; however ASR was not associated with exposure to ALN. Moreover, the higher the QCR was the higher ASR was noted. Thus, financial indicators were associated with the incidence of TC, whereas ALN was not associated with its incidence.

Currently, the annual rate of TC's new cases worldwide is increasing, estimated at about 20% from 1990 to 2013. The rise recorded is similar in Europe, the United States of America, Canada and Australia, although changes in increased incidence are greater in low income countries compared to high income countries[4,5]. The causes of this "epidemic" remain largely unclear[6]. It may represent a true increase or simply an increase in diagnoses of subclinical tumors that would otherwise have caused no symptoms, had they gone undetected[7]. Possibly, the ease of diagnosing very small tumors, due to advances in medical technology and screening programs, may play a role[8-10]. Perhaps, however, some factors related to lifestyle are also to blame.

Research has already identified some factors that affect the likelihood of developing TC. Genetics, exposure to ionizing radiation and iodine intake have been found to increase the risk[4]. There are also studies that have associated air pollution, obesity, smoking and alcohol intake to TC[11,12]. Recently, a study published also inculpated light pollution [3]. The researchers reported that city lights at night suppress melatonin (which may regulate estrogen activity, has antineoplastic properties and assists in the adaptability of humans to their environment) and disrupts circadian rhythms, which is also a risk factor for carcinogenesis[13].

Regarding the data that we used, the satellite night illumination data used in this work were from 2016; these were obtained with Visible Infrared Imaging Radiometer Suite (VIIRS), and are considered to be very accurate[1]. The VIIRS ALN data are also considered to be a proxy of socioeconomic conditions. Data for the latter, which we used, were from 2019 and 2020, mostly before or at the beginning of the coronavirus disease 2019 pandemic. The TC incidence data which were used were also from 2019-2020. Experts argue that the latency period from the beginning of the neoplastic process to diagnosis of TC is approximately 2.5 years[14]. Thus, for ALN *vs* TC incidence, this study takes into consideration this lag time. However, the temporal misalignment of cross-sectional data from different years (2016 for ALN, 2019-2020 for TC incidence, and 2019-2020 for economic data) may have affected the accuracy of the associations studied, especially

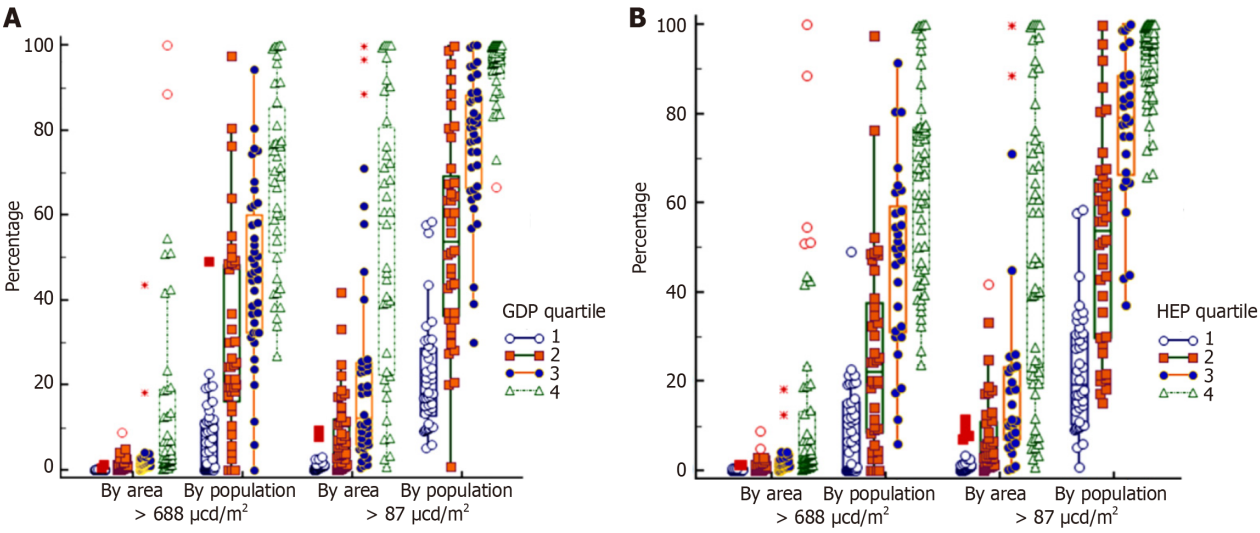

Figure 2 Plots of artificial light at night exposure percentages (vertical scale), by area or population. A: By gross domestic product per person quartile; B: By health expenditure per person quartile; boxplots are framed by the 25th and 75th percentile. GDP: Gross domestic product; HEP: Health expenditure per person.

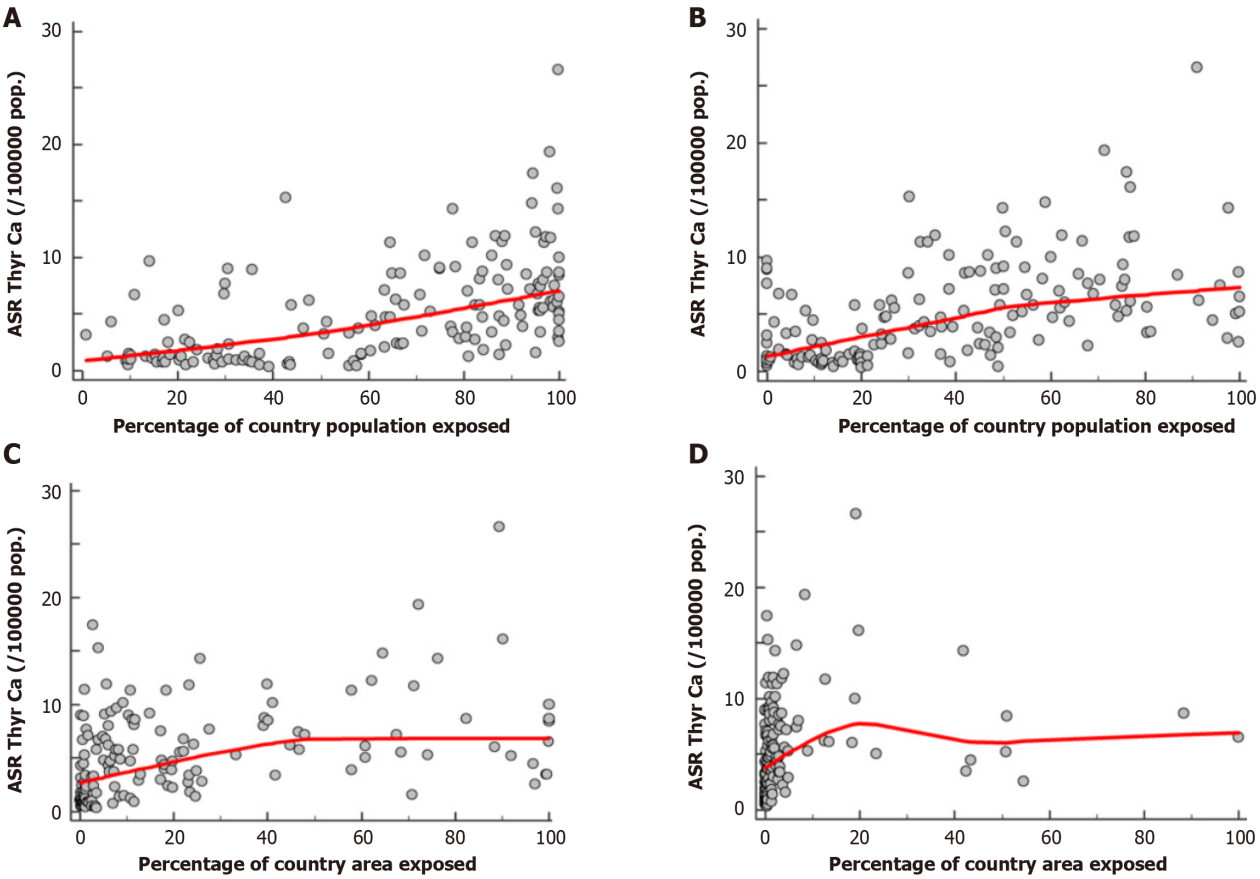

Figure 3 Scatterplots of thyroid cancer incidence by level of exposure to artificial light at night, with locally weighted scatterplot smoothing curves. A: Thyroid cancer (TC) incidence versus percentage of country population exposed to artificial light at night > 87 µcd/m²; B: TC incidence versus percentage of country population exposed to artificial light at night > 688 µcd/m²; C: TC incidence versus percentage of country area exposed to artificial light at night > 87 µcd/m²; D: TC incidence versus percentage of country area exposed to artificial light at night > 688 µcd/m²; ASR Thyr Ca: Adjusted standardized rate of TC incidence per 100000 population.

considering the latency period for TC. Another limitation of the study is that the accuracy of epidemiological data for cancer incidence may not be satisfactory in countries with low quality cancer registries or without cancer registries. This introduces potential bias, as the accuracy of TC data may vary widely between countries. The VIIRS ALN data may be a proxy of socioeconomic conditions[15,16], but to a different degree depending on the country[17,18]; the relationship may be more accurate in richer vis-à-vis poorer ones[19]. Thus, the relationship between ALN data and socioeconomic conditions may not be uniform across all countries; while we recognize this, we have to accept a consequent degree of uncertainty in the interpretation of results. According to our results, light pollution might be associated with a lifestyle leading to carcinogenesis, but we were not able to delve deeply into specific lifestyle factors. Another caveat, regarding the analysis performed for this study is that all the data which were used were cross-sectional; while the use of such data is commonly implemented, particularly in the social sciences, experts argue that there are limits to the representability of such an approach.

CONCLUSION

Our findings imply that intense ALN is indeed associated to financial measures such as GDP but it is the latter, and not ALN, which may create conditions that are detrimental to health and a potential risk factor for TC. It is possible that light pollution is associated with a lifestyle that leads to carcinogenesis. Furthermore, higher GDP and HEP implies better access to diagnostic means, possible facilitation of TC diagnosis and better inclusion in cancer registries. Exploring the underlying mechanisms linking light pollution, socioeconomic status, and lifestyle factors to TC risk is crucial for a more comprehensive understanding of these associations. Physicians should be aware of the potential impact of lifestyle, including exposure to ALN, on cancer risk. Further research seems imperative to elucidate the intricate relationship between ALN, lifestyle factors, and TC. Future investigations should delve into specific aspects of lifestyle, such as sleep hygiene and circadian rhythm disruptions, to identify modifiable risk factors. The broader implications for public health should not be overlooked: public health initiatives aimed at reducing light pollution, promoting healthy sleep habits, and raising awareness about the potential impacts of ALN on health may contribute to overall cancer prevention strategies.

ARTICLE HIGHLIGHTS

Research background

The increasing incidence of thyroid cancer (TC) globally has sparked interest in identifying potential environmental factors contributing to this rise. While prior research has explored various risk factors, the association between artificial light at night (ALN) and TC remains an underexplored area. Present status: Current data indicate a notable increase in TC cases, along with a rise in exposure to ALN. Significance of the Study: Our findings imply that intense ALN is indeed associated to financial measures such as gross domestic product (GDP), but it is the latter, and not ALN, which may create conditions that are detrimental to health and a potential risk factor for TC.

Research motivation

The study is motivated by the need to comprehensively investigate the impact of ALN on TC at a global scale, recognizing the ubiquitous nature of light pollution in the modern world. Main topics and key problems: The primary focus is on evaluating the relationship between ALN exposure and TC incidence worldwide. The study delves into the prevalence of ALN using satellite data and examines whether there is a significant association with rates of TC. The research investigates the role of socioeconomic conditions, as indicated by GDP and health expenditure per person (HEP), in contributing to TC incidence. The study incorporates the quality of cancer registries (QCR) as a variable, recognizing the potential impact of data accuracy on the observed relationships. Significance for future research: By exploring the interplay between ALN exposure, socioeconomic factors, and TC, this study lays the groundwork for a more holistic understanding of the risk factors associated with TC. The study contributes to the broader field of environmental determinants of cancer, emphasizing the need for researchers to consider light pollution as a potential lifestyle-related factor impacting cancer risk. Acknowledging the limitations in exploring specific lifestyle factors in this study, future research can delve deeper into understanding the precise elements of lifestyle, such as sleep hygiene and circadian rhythm disruptions, which may contribute to TC risk.

Research objectives

Main objectives: To quantify and assess the prevalence of ALN exposure globally vis-à-vis TC epidemiology, using satellite data and cancer registries, respectively and controlling for financial conditions by country. Realized: Achieved through the analysis of ALN levels exceeding specific thresholds vis-à-vis TC epidemiology. Correlations among different financial indicators with both ALN exposure and TC incidence were noted, providing insights into potential socioeconomic influences. We integrated the QCR as a variable to account for potential variations in data accuracy. Future research: Significance: The study sets the stage for future research by highlighting the intricate relationship between ALN, socioeconomic factors, lifestyle, and TC risk. Future investigations can build upon these insights, delving deeper into specific lifestyle factors and refining preventive interventions.

Research methods

Research Methods Used-Novelty: The study leveraged ALN data to explore its potential association with TC, integrating ALN exposure data with socioeconomic indicators such as GDP and HEP. Moreover, it considered the QCR as a variable that potentially leads to variations in data accuracy. Statistical Analyses: The data were analyzed with the Kolmogorov-Smirnoff, Kruskall Wallis and Kendall's Tau tests.

Research results

The global prevalence of ALN exposure was assessed, revealing that 67.3% of the world's population experiences ALN levels surpassing 87 μcd/m², and 34.6% surpassing 688 μcd/m². Globally, TC incidence, measured by the Adjusted Standardized Rate (ASR), was found to be 4.2 per 100000 population. Significant variations in ASR and ALN exposure percentages were noted across GDP and HEP quartiles, with higher values correlating to higher economic indicators. Differences in ASR were observed concerning the QCR, showing higher ASR in high-quality registries compared to lower quality ones. ASR by GDP and HEP quartiles demonstrated higher rates with increased economic indicators, and ALN exposure percentages also rose with higher economic quartiles. Direct associations between ASR and ALN exposure percentages were not significant. The study underscores the intricate relationship between ALN, economic indicators, and TC, emphasizing the role of socioeconomic conditions in cancer epidemiology. Problems that Remain to be Solved: The study found no direct link between ALN and TC, emphasizing the need for further research to understand their complex relationship. Consideration of QCR is crucial, urging refined assessments of data accuracy.

Research conclusions

New theories proposed: While this study primarily focused on empirical investigations rather than proposing new theoretical frameworks, it introduced a nuanced perspective on the relationship between ALN, socioeconomic factors, and TC. The absence of a direct correlation between ALN and TC challenges existing theories that oversimplify the link between light pollution and cancer risk. The findings encourage a more complex understanding of environmental and socioeconomic influences on cancer incidence, prompting future theoretical developments in this field. New Methods Proposed: The study did not explicitly propose new research methods but demonstrated an innovative approach through the integration of diverse methodologies. Notably, the interdisciplinary analysis, global scale examination and incorporation of socioeconomic indicators represent methodological advancements. The study's emphasis on the QCR as a variable and its statistical analyses contribute to methodological robustness.

Research perspectives

Future research should delve deeper into understanding the complex dynamics between ALN exposure and cancer risk. Investigating specific patterns of light exposure, considering variations in intensity, duration, and timing, may provide a more nuanced understanding of how ALN influences cancer incidence. Future research could also investigate the role of economic factors in shaping lifestyle choices, healthcare access, and environmental exposures, refining our understanding of the socioeconomic mechanisms influencing cancer incidence. Research focusing on the improvement of cancer registry quality assessment methods is crucial. Developing strategies to enhance data accuracy, especially in regions with lower-quality registries, will contribute to more reliable and comparable cancer incidence data. This could involve collaborations to standardize data collection practices globally.

FOOTNOTES

Author contributions: Tselebis A and Ilias I designed this research work; Tselebis A, Koukkou E, Milionis C, Zabuliene L, Pachi A and Ilias I performed the research; Tselebis A and Ilias I analyzed the data; Tselebis A, Koukkou E, Milionis C, Zabuliene L, Pachi A and Ilias I wrote the paper; All authors have read and agreed to the published version of the manuscript.

Institutional review board statement: Since this work is based on available, anonymized epidemiological data no IRB approval was necessary.

Informed consent statement: Since this work was based on available anonymized epidemiological data no informed consent was required.

Conflict-of-interest statement: The authors report that they have no conflict of interest.

Data sharing statement: No additional data are available.

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/Territory of origin: Greece

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S-Editor: Qu XL
L-Editor: A
P-Editor: Guo X

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World Journal of Methodology

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World J Methodol 2024 March 20; 14(1): 90930

DOI: 10.5662/wjm.v14.i1.90930

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Observational Study

Technical note for intraoperative determination of proper acetabular cup size in primary total hip arthroplasty

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Specialty type: Orthopedics

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Yang FC, China

Received: December 17, 2023
Peer-review started: December 17, 2023
First decision: January 10, 2024
Revised: January 12, 2024
Accepted: February 20, 2024
Article in press: February 20, 2024
Published online: March 20, 2024

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Abstract

BACKGROUND

Selecting the optimal size of components is crucial when performing a primary total hip arthroplasty. Implanting the accurate size of the acetabular component can occasionally be exacting, chiefly for surgeons with little experience, whilst the complications of imprecise acetabular sizing or over-reaming can be potentially devastating.

AIM

To assist clinicians intraoperatively with a simple and repeatable tip in elucidating the ambivalence when determining the proper acetabular component size is not straightforwardly achieved, specifically when surgeons are inexperienced or preoperative templating is unavailable.

METHODS

This method was employed in 263 operations in our department from June 2021 to December 2022. All operations were performed by the same team of joint reconstruction surgeons, employing a typical posterior hip approach technique. The types of acetabular shells implanted were: The Dynasty® acetabular cup system (MicroPort Orthopedics, Shanghai, China) and the R3® acetabular system (Smith & Nephew, Watford, United Kingdom), which both feature cementless press-fit design.

RESULTS

The mean value of all cases was calculated and collated with each other. We distinguished as oversized an implanted acetabular shell when its size was > 2 mm larger than the size of the acetabular size indicator reamer (ASIR) or when the

implanted shell was larger than 4 mm compared to the preoperative planned cup. The median size of the implanted acetabular shell was 52 (48–54) mm, while the median size of the preoperatively planned cup was 50 (48–56) mm, and the median size of the ASIR was 52 (50–54) mm. The correlation coefficient between ASIR size and implanted acetabular component size exhibited a high positive correlation with $r = 0.719$ ($P < 0.001$). Contrariwise, intraoperative ASIR measurements precisely predicted the implanted cups' size or differed by only one size (2 mm) in 245 cases.

CONCLUSION

In our study, we demonstrated that the size of the first acetabular reamer not entering freely in the acetabular rim corroborates the final acetabular component size to implant. This was also corresponding in the majority of the cases with conventional preoperative templating. It can be featured as a valid tool for avoiding the potentially pernicious complications of acetabular cup over-reaming and over-sizing in primary total hip arthroplasty. It is a simple and reproducible technical note useful for confirming the predicted acetabular cup size preoperatively; thus, its application could be considered routinely, even in cases where preoperative templating is unavailable.

Key Words: Acetabular shell; Total hip arthroplasty; Hip; Acetabulum; Acetabular component; Primary hip arthroplasty

Core Tip: The technique mentioned can be featured as a valid tool for avoiding the potentially pernicious complications of acetabular cup over-reaming and over-sizing in primary total hip arthroplasty. It is a simple and reproducible technical note useful for confirming the predicted acetabular cup size preoperatively; thus, its application could be considered routinely, even in cases where preoperative templating is unavailable.

Citation: Karampinas P, Vlamis J, Galanis A, Vavourakis M, Krexi A, Sakellariou E, Patilas C, Pneumaticos S. Technical note for intraoperative determination of proper acetabular cup size in primary total hip arthroplasty. *World J Methodol* 2024; 14(1): 90930
URL: https://www.wjgnet.com/2222-0682/full/v14/i1/90930.htm
DOI: https://dx.doi.org/10.5662/wjm.v14.i1.90930

INTRODUCTION

Total hip arthroplasty (THA) is indubitably a successful and cost-effective surgical procedure. For obtaining reproducibly consummate results, apposite preoperative planning is a mandatory routine. This planning involves diligent physical examination and X-ray templating, aiding in appropriate component size selection. Intraoperatively, acetabular shell over-sizing, acetabular bony deficits arising from acetabular reaming and acetabular cup over-medialization are all conditions to avoid during a THA[1-3]. Consequently, precise reaming and acetabular cup sizing must be estimated and selected during the operation. Ben Lulu *et al*[1] propounded the intra-operative measurement of the femoral head as a tool for optimal acetabular size selection. Additionally, a single-center study by Muñoz-Mahamud *et al*[2] indicated that this simple tool might demonstrate analogous validity and accuracy as preoperative digital templating regarding determining the definitive implanted acetabular cup size in primary THA.

Perusing the existing literature, limited papers examine preoperative or intraoperative methods for measuring acetabulum size and the correlations with implanted acetabular cup size in primary THA. The current study's objective is to scrutinize the association between the intraoperative features of the last acetabular reamer utilized for the acetabulum preparation and the final acetabular cup implanted contrasted to the preoperative acetabular cup templating in primary THA and to bolster orthopaedic surgeons' intraoperative decision-making in terms of the selection of the final acetabular component by providing a simple and repeatable technical note.

MATERIALS AND METHODS

After acquiring approval from our Institution's review board, a prospective observational single-center study was conducted in our department. From June 2021 to December 2022, all patients admitted to our hospital for elective primary THA were prospectively registered in a database and retrospectively reviewed. All operations were performed by the same team of joint reconstruction surgeons, employing a typical posterior hip approach technique. The types of acetabular shells implanted were: The Dynasty® acetabular cup system (MicroPort Orthopedics, Shanghai, China) and the R3® acetabular system (Smith & Nephew, Watford, UK), which both feature cementless press-fit design. Data were recorded regarding demographics, body mass index (BMI), comorbidities, indication for THA, hip approach, templated socket size, implanted cup outer diameter and acetabular cup type. Patients with a history of congenital/developmental hip deformity (Perthes's disease, dysplasia), post-traumatic osteoarthritis, severe osteoarthritis with large acetabular

osteophytes, and acetabular protrusion cases, were excluded from the study.

Our Institution's picture archiving and communication system analyzed preoperative and postoperative radiographs. Regarding preoperative planning, acetabular size measurement was executed in traditional digital X-ray films (100% magnification) of anteroposterior pelvic and hip lateral views. To calibrate the image, we utilized known implanted femoral head component size. We then calculated the diameter of the contralateral native acetabulum size, assuming that patients' femoral heads were generally symmetrical, apart from those with congenital/developmental hip deformities, which were excluded. To bolster the reliability of the measures, all X-ray films were examined by two of the authors. Each author performed three measurements. The three calculations were averaged to create the final value for that author. The average of the final values for each of the two authors was utilized as the final measurement for the analysis.

The last acetabular bone reamer used was defined as the acetabular size indicator reamer (ASIR), featuring a larger diameter than the acetabular rim, hence, not entering freely into it without cutting bone first (Figure 1). The Pearson correlation coefficient (r) was utilized to discover whether the reamer's size correlated with the acetabular component size. The correlation size (Pearson correlation coefficient) was interpreted using the method delineated by Hinkle *et al*[4]. The level of statistical significance was set to $P < 0.05$, and all analyses were executed with the assistance of commercially available statistical software.

A conventional posterior approach was employed to expose the hip joint and acetabulum in all cases. After following the standardized steps of the operation, the acetabulum with bone reamers was prepared as described below: The first reamer utilized was the smaller one (43 mm), and then we continued by raising two numbers until the size of 47-48 mm. Until this point, all bone reamers enter the acetabulum cavity freely without resistance from the acetabular rim's periphery. Reaming preparation starts from the acetabular fossa, while the reamers' size is standardly rising symmetrically to the acetabulum until the subchondral bone is exposed. From the following sizes of acetabular reamers, 50 mm to 56 mm, the acetabulum periphery reaches a point where it is unattainable for the reamer to be inserted into the acetabulum cavity unobstructed because the maximum diameter of the reamer is bigger than that of the acetabular peripheral rim (Figure 2A). This exact reamer is the last used for the acetabular preparation after removing the acetabular bone periphery and small osteophytes. The size of the first reamer that is not feasible to be placed entirely into the acetabular cavity, gives us the size of the acetabular shell to implant (Figure 2B and C). This reamer has been defined as the ASIR. It is vitally important to underline that if the acetabular reaming process is carried on after that point, necessary bone from the acetabular periphery, acetabular rim, and the anterior and posterior walls is gradually removed, which could affect the implanted acetabular shell's primary support.

RESULTS

Out of 345 primary THAs performed, 263 cases were included in our study that met the inclusion criteria. The mean age of the patients was 68.1 years old (range 48-93). The majority (59%) of the patients were female, whilst mean BMI was 28.3 Kg/m^2. Indications for surgery were osteoarthritis (241 cases), ischemic necrosis of the femoral head (9 cases) and femoral neck fracture (13 cases). We collected the data from the templating measurements for every single case. We juxtaposed them with the size of the final acetabular bone reamer (the ASIR) and the acetabular shell implanted. The mean value of all cases was calculated and collated with each other. We distinguished as oversized an implanted acetabular shell when its size was > 2 mm larger than the size of the ASIR or when the implanted shell was larger than 4 mm compared to the preoperative planned cup. The median size of the implanted acetabular shell was 52 (48–54) mm, while the median size of the preoperatively planned cup was 50 (48–56) mm, and the median size of the ASIR was 52 (50–54) mm (Table 1). The correlation coefficient between ASIR size and implanted acetabular component size exhibited a high positive correlation with $r = 0.719$ ($P < 0.001$) (Figure 3).

Figure 4 depicts the correlation between the implanted acetabular shell and the preoperatively planned cup and intraoperative reaming measurement. The size of preoperatively planned cups precisely estimated the implanted shells' size or differed by one size (2 mm) in 198 cases. Contrariwise, intraoperative ASIR measurements precisely estimated the implanted cups' size or differed by one size (2 mm) in 245 cases (Table 2). The most frequently planned cup featured a 52 mm diameter in females, while 54 mm was the size most regularly implanted in males. Finally, it is paramount to accentuate that no alterations were discerned regarding the two types of acetabular implants employed in the study.

A few limitations apply to our technical note. First of all, our study group was limited to Caucasian patients living in Southern Europe. Furthermore, this technique may not be so accurate in patients with extremely severe osteoarthritis or in atypical cases such as congenital hip dysplasia. Finally, the technique was used by surgeons of the same institution/department and the postoperative follow-up was limited to 12 months.

DISCUSSION

Total hip arthroplasty aims for pertinent restoration of joint biomechanics. In terms of preoperative planning, precise and reliable evaluation of the appropriate acetabular component size is crucial. In general terms, the determination of the planned implant's size can be carried out by specific overlays on standard plain X-rays, digital templating with or without the utilization of calibration to advanced imaging with EOS, computed tomography (CT), magnetic resonance imaging, and and customized guides for each patient[1-3]. Conventional planning is considered at least as robust as digital planning; however, contemporary literature is still contentious[5]. Anteroposterior pelvic radiographs are customarily used in preoperative THA templating. Information concerning the pelvis and contralateral hip anatomy

Table 1 Mean values of acetabular templating, acetabular size indicator reamer and implanted cup size

Mean value templating	Mean value 'fine reamer'	Mean value cup implanted
50 (48-56) mm	52 (50-54) mm	52 (48-56) mm

Table 2 Percentage accuracy of preoperative and intraoperative measurements in comparison with final acetabular component's size

	Pre-operative templated cup size, %	Intra-operative reamer measurement, %
Percentage of accurately prediction	73.8	92.8
Percentage of discrepancy > 1 size	21.1	7.1
Percentage of discrepancy > 2 size	5.1	0.1

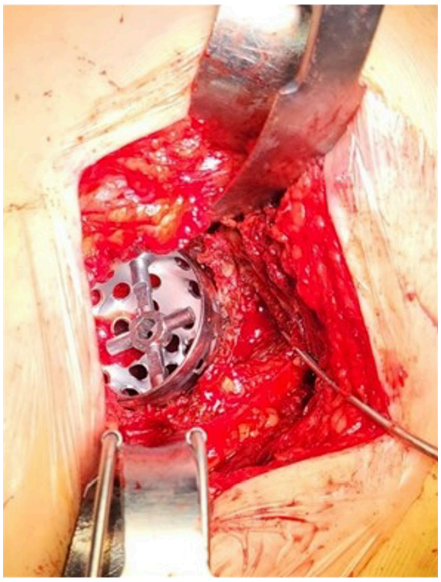

Figure 1 The defined acetabular size indicator reamer, not entering into the acetabulum cavity without cutting bone first.

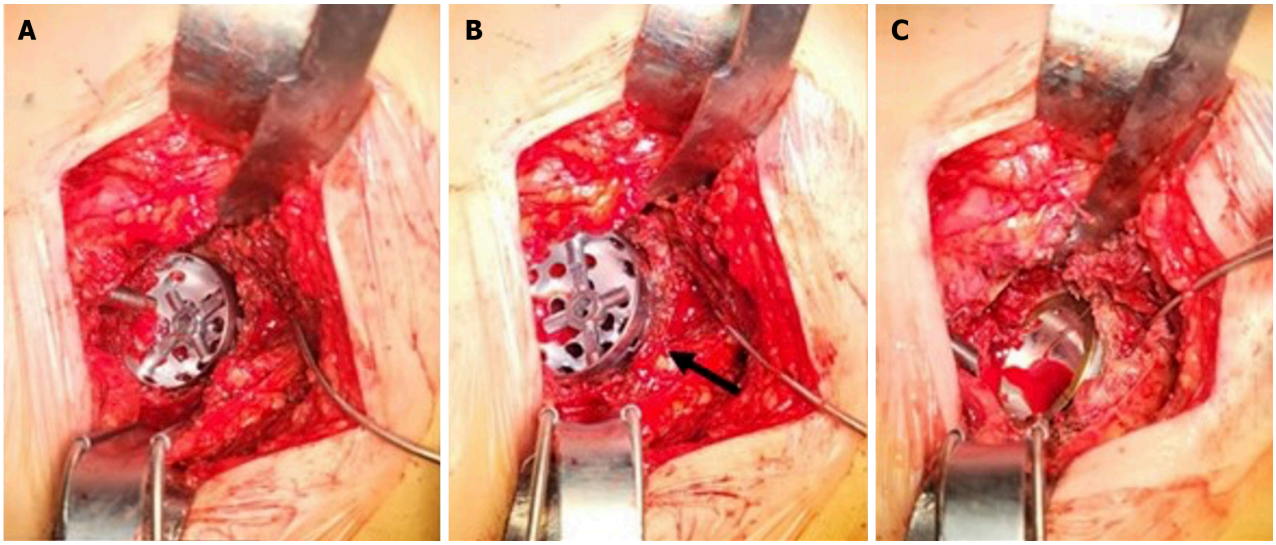

Figure 2 The acetabulum with bone reamers. A: Typical cotyloid cavity preparation with successive reaming; B: The acetabular size indicator reamer (ASIR); C: Providing the size of the acetabular component to implant. Black arrow indicates ASIR's position in the whole reaming procedure.

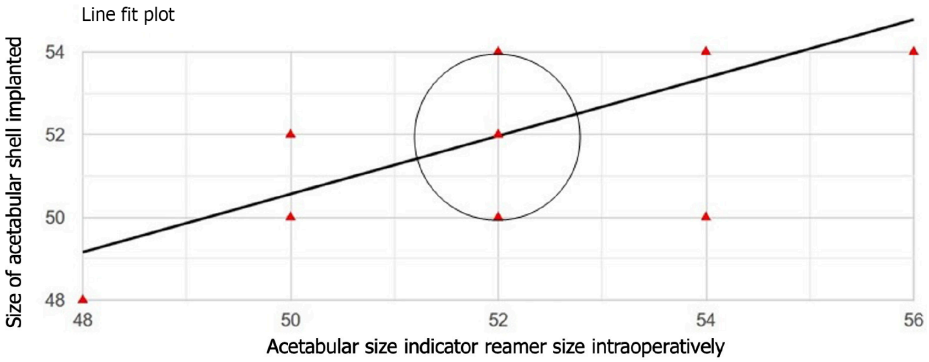

Figure 3 Coefficient correlation illustration depicting positive covariance.

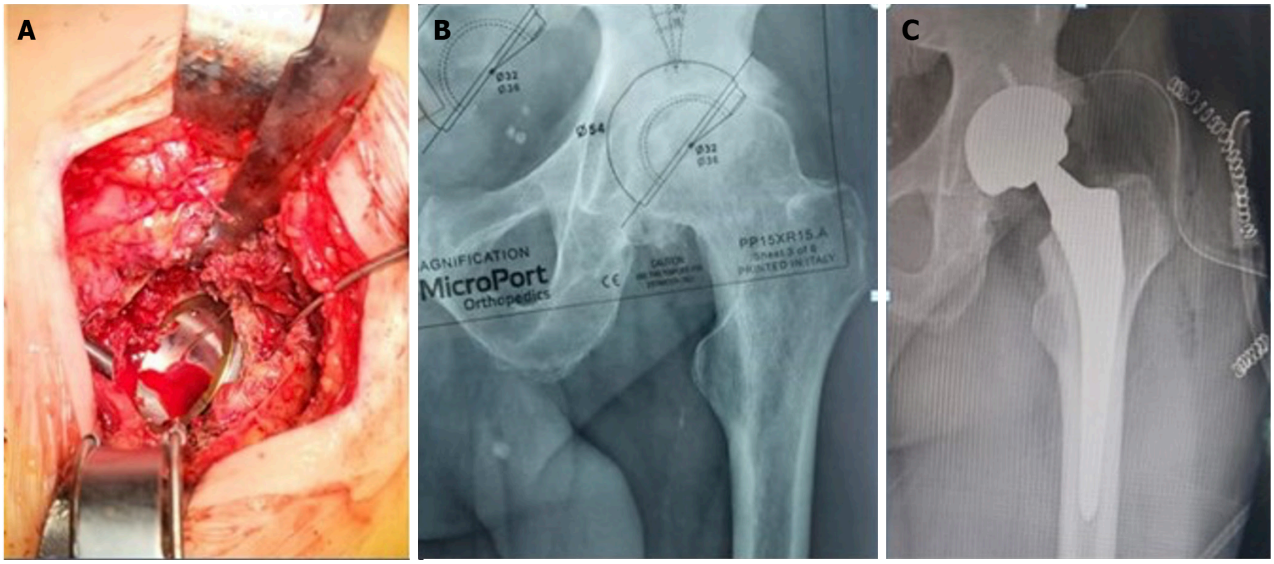

Figure 4 Correlation between. A: Acetabular size indicator reamer; B: Acetabular templating; C: Acetabular shell implanted.

enables the assessment of leg-length discrepancies[6]. The evaluation of bony morphology, arthritic wear pattern, and a proper implant design and size, is needed to restore joint's biomechanics in present-day THA[3,7]. Sex, height and weight have all been indicated to be considerably associated with implant size[8].

Regarding existing literature on acetabular offset, the well-established biomedical theory of medialization appears as most prevalent for a better patient's outcome. In this case, the acetabular preparation consists of reaming down to the true floor, thus medializing the hip's rotation center and decreasing acetabular offset. This medialization dwindles the body weight's lever arm during monopodial stance, diminishing the resultant force on the femoral head if every anatomic formation is intact[9]. Current literature focuses on reducing the acetabular offset in relation to the femoral offset, extensive criticism of this guideline has been observed[10-12]. Thorough preoperative planning may assist in avoiding excessive medialization of the acetabular cup, which is correlated to inferior outcomes following THA[10]. Orthopaedic surgeons should ream the medial cotyloid cavity to the floor until a suitable component size for implantation. This triggers medial and superior displacement of the hip's rotational center to achieve a comparatively normal acetabular offset and rotation center[9]. Additionally, the exact preoperative planning reduces surgical time and number of complications[3,12]. Sharkey *et al*[10] reported that acetabular shell oversizing might be associated with enhanced rates of periprosthetic acetabular fractures, whilst undersizing might be connected to early implant loosening arising from insufficient press-fit. An oversized cup has been implicated in postoperative pain deriving from psoas impingement and anterior overhang[13-15].

Regarding preoperative templating, predicting the definite implant size ± one size is regarded as acceptable[12]. Templating accuracy has been proven to upgrade with the training level of the surgeon[16]. Previous experience augments preoperative templating performance, as clinicians accomplish proficiency in planning the acetabular cup size after 50-100 attempts when a succinct algorithm and immediate feedback are provided[3]. A direct correlation between radiographically measured native femoral head size and implanted acetabular shell size, has been conjugated in primary THA[5]. In accordance with the literature, preoperative planning of acetabular component size features a high level of accuracy, predicting the definite implant within ± one size in 80% to 100% of cases[11-12,17-19]. A retrospective study of 277 patients undergoing primary THA by Pfeufer *et al*[5] revealed a relation between the acetabular component's size and radiographically measured contralateral native femoral head's size, with a discrepancy of 7 mm. Moreover, digital

radiographs' templating accuracy in predicting the implant size has been gauged and found to be sufficiently good[8,9]. Nonetheless, digital templating demands special software provided by the companies. As these digital templates exhibit confined availability, many centers worldwide have halted templating prior to surgery. Therefore, surgeons incapable of procuring special digital templating software can resort to the typical acetate templates on digital radiographs[6]. Ben Lulu *et al*[1] computed the femoral head's size intraoperatively, indicating a noteworthy association between the implanted shell's diameter and the removed femoral head's diameter calculated with calipers. Moreover, they recommended that measuring the femoral head's diameter during surgery could be applied as a considerable intraoperative monitoring tool and, along with preoperative templating figures, may contribute to increased precision rates. Additionally, Muñoz-Mahamud *et al*[2] drew the inference that measuring the femoral head's size intraoperatively is an uncomplicated and well-grounded method, availing surgeons selecting the optimal acetabular component size, being as reliable as preoperative templating regarding avoiding cup-oversizing in THA. Extreme caution is justified when the cup reamer is > 4 mm than the native head's anteroposterior diameter.

Even though these methods are considered established and adequate, it is exceedingly salient to give prominence to the remaining space for refinement of the general guidelines concerning assisting surgeons in opting for the right decision when determining acetabular component size in primary THA. Moreover, inexperienced surgeons may pronouncedly find it helpful defining the acetabular shell's size. Consequently, anybody involved in THA planning may reap the benefits from a simple tool aiding in predicting implant sizing in THA. The robust correlation we detected between the implanted acetabular shell's size and the acetabular component's size selected from the preparation of the acetabulum with our method, with a median difference of 2 mm, is of substantial clinical significance, as modern hip arthroplasty can benefit from the use of advanced technology by improving its accuracy and ensuring consistent and repeatable outcomes.

CONCLUSION

In our study, we demonstrated that the size of the first acetabular reamer not entering freely in the acetabular rim corroborates the final acetabular component size to implant. This was also corresponding in the majority of the cases with conventional preoperative templating. It can be featured as a valid tool for avoiding the potentially pernicious complications of acetabular cup over-reaming and over-sizing in primary THA. It is a simple and reproducible technical note useful for confirming the predicted acetabular cup size preoperatively; thus, its application could be considered routinely, even in cases where preoperative templating is unavailable.

ARTICLE HIGHLIGHTS

Research background

Selecting the optimal size of components is crucial when performing a primary total hip arthroplasty. Implanting the accurate size of the acetabular component can occasionally be exacting, chiefly for surgeons with little experience, whilst the complications of imprecise acetabular sizing or over-reaming can be potentially devastating.

Research motivation

This paper aims to assist clinicians intraoperatively with a simple and repeatable tip in elucidating the ambivalence when determining the proper acetabular component size is not straightforwardly achieved, specifically when surgeons are inexperienced or preoperative templating is unavailable.

Research objectives

This paper aims to assist clinicians intraoperatively with a simple and repeatable tip in elucidating the ambivalence when determining the proper acetabular component size is not straightforwardly achieved, specifically when surgeons are inexperienced or preoperative templating is unavailable.

Research methods

This method was employed in 263 operations in our department from June 2021 to December 2022. All operations were performed by the same team of joint reconstruction surgeons, employing a typical posterior hip approach technique. The types of acetabular shells implanted were: The Dynasty® acetabular cup system (MicroPort Orthopedics, Shanghai, China) and the R3® acetabular system (Smith & Nephew, Watford, United Kingdom), which both feature cementless press-fit design.

Research results

The mean value of all cases was calculated and collated with each other. We distinguished as oversized an implanted acetabular shell when its size was > 2 mm larger than the size of the acetabular size indicator reamer (ASIR) or when the implanted shell was larger than 4 mm compared to the preoperative planned cup. The median size of the implanted acetabular shell was 52 (48–54) mm, whereas the median size of the preoperatively planned cup was 50 (48–56) mm, and the median size of the ASIR was 52 (50–54) mm. The correlation coefficient between ASIR size and implanted acetabular component size exhibited a high positive correlation with $r = 0.719$ ($P < 0.001$). Contrariwise, intraoperative ASIR

measurements precisely predicted the implanted cups' size or differed by only one size (2 mm) in 245 cases.

Research conclusions

In our study, we demonstrated that the size of the first acetabular reamer not entering freely in the acetabular rim corroborates the final acetabular component size to implant. This was also corresponding in the majority of the cases with conventional preoperative templating. It can be featured as a valid tool for avoiding the potentially pernicious complications of acetabular cup over-reaming and over-sizing in primary THA. It is a simple and reproducible technical note useful for confirming the predicted acetabular cup size preoperatively; thus, its application could be considered routinely, even in cases where preoperative templating is unavailable.

Research perspectives

The robust correlation we detected between the implanted acetabular shell's size and the acetabular component's size selected from the preparation of the acetabulum with our method, with a median difference of 2 mm, is of substantial clinical significance, as it provides both the potential to enhance accuracy further and the ability to accomplish predictable and reproducible results in modern hip arthroplasty.

FOOTNOTES

Author contributions: Karampinas P, Vlamis J, Vavourakis M, and Patilas C contributed to study design; Karampinas P, Vlamis J, Vavourakis M, and Krexi A contributed to data collection and data interpretation; Karampinas P, Vlamis J, and Galanis A contributed to manuscript preparation – original draft presentation; Karampinas P, Vlamis J, Krexi A, Sakellariou E contributed to literature search; Patilas C and Pneumaticos S contributed to manuscript preparation – review and editing; Pneumaticos S contributed to supervision.

Institutional review board statement: Our institution's board committee with the No 25/28-09-2021 decision, approved this research.

Informed consent statement: Informed consent was obtained from all individual participants included in the study.

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

Data sharing statement: All raw data are available to access should they be requested.

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.

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S-Editor: Liu JH
L-Editor: A
P-Editor: Zhang XD

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World Journal of Methodology

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World J Methodol 2024 March 20; 14(1): 88518

DOI: 10.5662/wjm.v14.i1.88518

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Basic Study

Rikkunshito increases peripheral incretin-hormone levels in humans and rats

Hiroshi Kono, Shinji Furuya, Hidenori Akaike, Katsutoshi Shoda, Yoshihiko Kawaguchi, Hidetake Amemiya, Hiromichi Kawaida, Daisuke Ichikawa

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Lin SR, Taiwan

Received: September 29, 2023
Peer-review started: September 29, 2023
First decision: December 6, 2023
Revised: December 27, 2023
Accepted: February 3, 2024
Article in press: February 3, 2024
Published online: March 20, 2024

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Abstract

BACKGROUND

It was reported that rikkunshito (TJ-43) improved the cisplatin-induced decreases in the active form of ghrelin in plasma; however, other effects on gastrointestinal hormones have not been investigated.

AIM

To investigate the effects of TJ-43 on peripheral levels of incretin hormones, including gastric inhibitory polypeptide (GIP) and glucagon-like polypeptide-1 (GLP-1), in humans and rats.

METHODS

Patients were divided into two groups, namely patients who received TJ-43 immediately following surgery [TJ-43(+) group] and those who received TJ-43 on postoperative day 21 [TJ-43(-) group], and the plasma levels of active GIP and active GLP-1 were assessed. In animal experiments, rats were treated with TJ-43 [rat (r)TJ-43(+) group] or without [rTJ-43(−) group] by gavage for 4 wk, and the plasma active GIP and active GLP-1 levels were measured. The expression of incretin hormones in the gastrointestinal tract and insulin in the pancreas were investigated by immunohistochemistry. Furthermore, the cyclic adenosine monophosphate activities were assessed in pancreatic tissues from rats treated with or without TJ-43 *in vivo*, and the blood glucose levels and plasma insulin levels were measured in rats treated with or without TJ-43 in oral glucose tolerance tests.

RESULTS

In humans, the active incretin hormone levels increased, and values were significantly greater in the TJ-43(+) group compared those in the TJ-43(-) group. In

rats, the plasma active incretin levels significantly increased in the rTJ-43(+) group compared with those in the rTJ-43(-) group. GIP and GLP-1 expressions were enhanced by TJ-43 treatment. Moreover, plasma insulin levels increased and blood glucose levels were blunted in the rTJ-43(+) group.

CONCLUSION
The results show that TJ-43 may be beneficial for patients who undergo pancreatic surgery.

Key Words: Incretin hormone; Japanese traditional herbal medicine; Gastric inhibitory polypeptide; Glucagon-like polypeptide-1; Islet cells; Insulin

Core Tip: This study aimed to investigate the effects of rikkunshito (TJ-43) on gastrointestinal hormones, including gastric inhibitory polypeptide and glucagon-like peptide-1, in humans and rats. As a result, TJ-43 increased incretin hormones, and insulin expression, and suppressed increases in blood glucose levels in human and animal models. Thus, TJ-43 may provide benefits after pancreatic surgery.

Citation: Kono H, Furuya S, Akaike H, Shoda K, Kawaguchi Y, Amemiya H, Kawaida H, Ichikawa D. Rikkunshito increases peripheral incretin-hormone levels in humans and rats. *World J Methodol* 2024; 14(1): 88518
URL: https://www.wjgnet.com/2222-0682/full/v14/i1/88518.htm
DOI: https://dx.doi.org/10.5662/wjm.v14.i1.88518

INTRODUCTION

The Japanese traditional herbal medicine named "rikkunshito" (TJ-43) is generally obtained in the form of a powdered extract consisting of eight crude medicines[1] and is used to treat various gastrointestinal (GI) disturbances[2-4]. TJ-43 was also shown to promote gastric emptying in rats[5]. Furthermore, using TJ-43 in combination with an anti-emetic drug prevented anorexia and vomiting in advanced breast cancer patients undergoing chemotherapy[6]. The preventive effects of TJ-43 on chemotherapy-induced nausea, vomiting, and anorexia were also reported in patients treated with cisplatin-based chemotherapy[7]. Moreover, TJ-43 is administered to patients undergoing GI surgery, including pancreatico-duodenectomy[1], and significant effects of TJ-43 have been reported. TJ-43 increased peripheral acylated-ghrelin levels, improved delayed gastric emptying (DGE), and ameliorated postoperative GI symptoms in patients undergoing gastrectomy[8]. In patients undergoing pylorus-preserving pancreatico-duodenectomy (PpPD), postoperative oral food intake does not increase easily because of DGE. Considering that TJ-43 increases active ghrelin levels, which increases food intake, TJ-43 is often administered in patients undergoing PpPD. In addition, it is necessary to control blood glucose levels in patients undergoing PpPD.

The GI hormones constitute a group of hormones that control functions of the digestive organs and are secreted by enteroendocrine cells in the GI tract and pancreas[9]. They exert autocrine and paracrine actions that help integrate the GI functions. Most gut peptides, including secretin, cholecystokinin, or substance P, play the roles of neurotransmitters and neuromodulators in the central and peripheral nervous systems[10]. Enteroendocrine cells do not form glands but are spread throughout the GI tract. It is well known that TJ-43 increases the secretion of the GI hormone "ghrelin," an endogenous ligand of the growth hormone secretagogue receptor. It consists of 28 amino acids and is secreted mainly from the stomach[11].

Incretin hormones are also GI hormones and have received significant attention because of their important roles in glucose homeostasis, type 2 diabetes, and potentially other metabolic disorders[12]. Glucose intake leads to a stimulation of insulin secretion than an intravenous administration of glucose[13]. This is known as the incretin effect, which is attributed to the fact that oral glucose intake leads to the release of incretin hormones, including gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), from entero-endocrine cells in the gastrointestinal tract. In contrast, intravenous glucose administration does not induce the release of incretin hormones[13]. The GI hormones released in response to nutrient absorption act as endocrine signals to the pancreatic islet cells, augmenting insulin secretion. In this study, TJ-43 increased peripheral incretin-hormone levels in patients undergoing PpPD; however, the effects and significance of TJ-43 on GI hormones, including incretin hormones, which are related to insulin secretion, have not been elucidated in previous reports. Therefore, the effects of TJ-43 on incretin hormones were also investigated in rats herein.

MATERIALS AND METHODS

Patients and sample collection

This was a retrospective observational study. Blood samples were obtained from 41 patients who underwent PpPD at the University Hospital (Table 1). The study was conducted following the ethical standards outlined in the Declaration of Helsinki and approved by the University Ethics Committee (Chief, Prof. Zentaro Yamagata; No. 820). Upon admission, informed consent was obtained from all patients. The tumor stage was evaluated according to the Union for International Cancer Control classification[14]. Moreover, the histological and pathological diagnoses of the specimens were confirmed using the World Health Organization classification criteria.

The patients were preoperatively enrolled into the two groups based on treatments of TJ-43; the TJ-43(-) group (n = 20) was treated from the postoperative day (POD) 21 with TJ-43 (7.5 g/d) using an enteral feeding catheter or by oral administration, representing the conventional treatment, and the TJ-43(+) group (n = 21) was treated daily with TJ-43 (7.5 g/d) from POD 1, representing the modified treatment (Figure 1). Enteral feeding of 900 kcal/day was started from POD 1 and continued throughout the study period. The postoperative meals were started at POD 7 in all cases. Three weeks after surgery, the total oral calorie intake was evaluated in both groups, which represented the primary endpoint of this study.

For the definition of complications, DGE was defined based on international criteria[15]. Postoperative pancreatic fistula (POPF) was classified into grades based on the guidelines established by the International Study Group on Pancreatic Fistula[16,17]. Grade A POPF, known as a biochemical fistula, has no clinical impact on the normal postoperative pathway. Clinically significant POPFs are classified as grades B and C. Grade B POPF requires one of the following conditions: an endoscopic or radiological intervention, a drain *in situ* for longer than 3 wk, clinical symptoms without organ failure, or a clinically relevant change in POPF management. Whenever a major change in clinical management or deviation from the normal clinical pathway is required, or organ failure occurs, the classification shifts to grade C POPF[17].

Blood samples

Blood samples were collected before and after the operation designated time points. Plasma samples were stored at −80°C until further analysis.

Measurements of gastrointestinal hormones

The plasma levels of active GIP (RayBiotech Life Inc., Peachtree Corners, GA, United States), and GLP-1 (Invitrogen, Waltham, MA, United States) levels were evaluated[18].

Immunoreactive insulin levels and blood glucose levels

Immunoreactive insulin (IRI) was assessed by clinical laboratory analysis. The amount of glucose in the serum was measured using the Glucose B Test (Wako Pure Chemicals Co., Ltd., Tokyo, Japan).

Animals

The experiments were performed according to protocols approved by the university review board (#3-38). The protocols followed our institutional criteria and the National Research Council criteria for the care and use of laboratory animals in research. Male Sprague–Dawley rats (180–200 g body weight, Japan SLC Inc., Shizuoka, Japan) were used. Animals were housed in sterilized cages in a facility with a 12-h night/d cycle. The staff and veterinarians in the animal laboratory maintained the animal facilities and were always available to ensure animal health. All animals were provided humane care in compliance with governmental regulations and institutional guidelines.

Experimental protocols

Rats were treated with TJ-43 once a day (1 g/kg body weight) [rat (r)TJ-43(+) group] by gavage for 4 wk (Figure 1B). On the sacrifice date, the rats fasted for 8 h before TJ-43 treatment. The rats were fed regular chow diets 1 h after TJ-43 treatment. Blood samples were collected from the aorta 3 h after TJ-43 treatment. Immediately after blood collection, the blood samples were centrifuged. A DPP-4 inhibitor (Sigma-Aldrich, Milwaukee, WI) was added to the plasma at a concentration of 50 nM to 1 mL for measurement of incretin hormones. These samples were stored at −80°C.

Some tissue sections were stained with hematoxylin and eosin to assess inflammation and necrosis. Pathology was evaluated in a blinded fashion by an expert in rodent pathology.

Immunohistochemistry for GIP, GLP-1, and insulin

Tissue specimens were cut into 4-mm serial sections. Each section was treated in antigen retrieval solution for 15 min at 120°C using Dako REAL Target Retrieval Solution (Dako, Carpinteria, CA, United States). Then, sections were blocked using 5% normal blocking serum for 20 min and incubated with rabbit polyclonal antibody. The following antibodies were used: anti-GIP diluted 1:300 (bs-0098R, Bioss, Woburn, MA, United States), anti-insulin diluted 1:2000 (ab181547, Abcam, Cambridge, United Kingdom), and anti-GLP diluted 1:200 (ab218532, Abcam). Sections in which the primary antibody had been substituted by non-immune serum served as negative controls. Following incubation, immune-peroxidase staining was completed using a Vectastain ABC Elite kit (Vector Laboratories, Burlingame, CA, United States) and 3,3'-diaminobenzidine-tetrachloride as a chromogen[19].

Table 1 Clinicopathological characteristics, *n* (%)

Variable		TJ-43− (*n* = 20)	TJ-43+ (*n* = 21)	*P* value
Age	Year	67 7.0	66 7.7	0.962
Sex	Male	10 (50)	14 (67)	0.199
	Female	10 (50)	7 (33)	
Disease	Pancreas Ca. (Ph)	7 (35)	8 (38)	
	IPMC	2 (10)	2 (10)	
	IPMA	2 (10)	1 (5)	
	CBD Ca.	5 (25)	5 (24)	
	Vater Ca.	3 (15)	4 (19)	
	Panaceas-NET	1 (5)	0 (0)	
	GB Ca.	0 (0)	1 (5)	
UICC tumor stage; pancreas Ca. (0/I/IIA/IIB); other Ca. (0/I/II/III)	Pancreas Ca. (Ph)	(0/0/1/6)	(0/0/3/5)	
	IPMC	(1/0/1/0)	(0/0/1/1)	
	IPMA	N/A	N/A	
	CBD Ca.	(0/1/4/0)	(0/3/2/0)	
	Vater Ca.	(0/1/2/0)	(0/2/2/0)	
	P-NET	(0/1/0/0)	N/A	
	GB Ca.	N/A	(0/0/1/0)	
Tumor differentiation (well/mod./poor)	Pancreas Ca. (Ph)	(3/2/2)	(1/6/1)	
	IPMC	(0/2/0)	(1/0/1)	
	IPMA	N/A	N/A	
	CBD Ca.	(0/5/0)	(2/2/1)	
	Vater Ca.	(2/1/0)	(1/3/0)	
	P-NET	N/A	N/A	
	GB Ca.	N/A	(0/1/0)	
Time of operation	Min	500 ± 56	509 ± 0.72	0.299
Blood loss	mL	692 ± 0.54	959 ± 0.66	0.182
HbA1c	%	5.6 ± 2.2	5.7 ± 2.3	0.892
Tumor markers	CEA (ng/mL)	3.1 ± 1.3	3.7 ± 1.1	0.872
	CA19-9 (U/mL)	455 ± 23	451 ± 29	
DGE	%	25	19	0.773
POPF				0.886
Grade A		15 (75)	16 (76)	
Grades B and C		5 (25)	5 (24)	
Postoperative pneumonia		1 (5)	1 (4.8)	0.889

UICC: Union for International Cancer Control; Ph: Pancreas head; IPMC: Intraductal papillary mucinous carcinoma; IPMA: Intraductal papillary mucinous adenoma; CBD: Common bile duct; Ca.: Carcinoma; NET: Neuroendocrine tumor; GB: Gall bladder; Hb: Hemoglobin; DGE: Delayed gastric emptying; POPF: Postoperative pancreatic fistula; N/A: Not applicable; TJ-43: Rikkunshito.

The density of stained cells in three different areas per section was quantified and standardized (per 250000 mm^2) using the BZ-H3C measurement module (Hybrid Cell Count Ver.1.1, Keyence)[20,21].

Cyclic adenosine monophosphate activity in the pancreas

Cyclic adenosine monophosphate (cAMP) determination was performed using a cAMP ELISA kit (#581001, Cayman

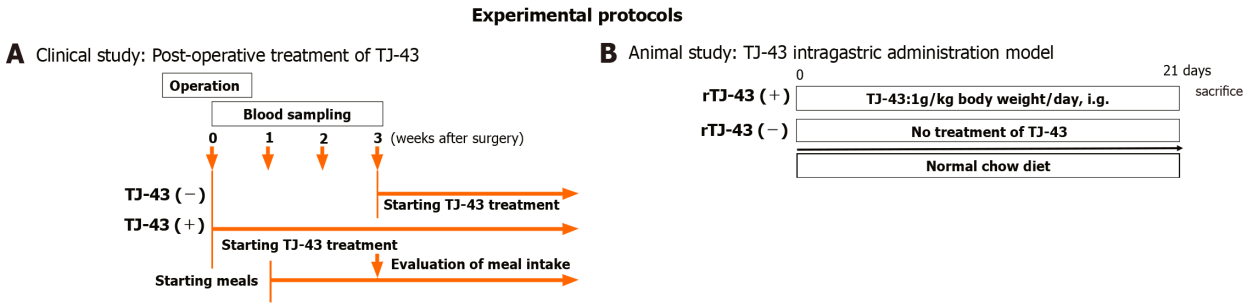

Figure 1 Experimental protocols. A: Clinical study; B: Animal study. Rikkunshito (TJ-43); TJ-43(-), patients without TJ-43 treatment; and TJ43(+), patients with TJ-43 treatment; rTJ-43(-), and rats without TJ-43 treatment; rTJ43(+), rats with TJ-43 treatment; and i.g.; intragastric administration. TJ-43: Rikkunshito.

Chemical Company, Ann Arber, MI, United States) following the manufacturer's instructions. In another set of experiments, pancreatic tissues were harvested from rats treated with TJ-43. The frozen tissues were weighed and dropped into volumes (5–10 mL of solution/gram of tissue) of 5% trichloroacetic acid (TCA) in water. The samples were homogenized on ice using a Polytron-type homogenizer. The precipitate was removed by centrifugation at 1500 g for 10 min, and the supernatant was carefully transferred to a clean test tube. The TCA was extracted from the samples using water-saturated ether. Five volumes of ether were added to one volume of supernatant and mixed for 10 s, and then the organic and aqueous phases were allowed to separate. The top of the ether layer was carefully removed and discarded. The extraction was repeated two more times. The residual ether was removed from the aqueous layer by heating the sample to 70°C for 5 min. All ether must be removed because even trace amounts can interfere with the assay. All the samples had a protein concentration greater than 1 mg/mL. Finally, 100 mL of each sample was assayed for cAMP levels according to the measurement method described in the handling instructions.

Oral glucose tolerance test and plasma insulin levels

In the rTJ-43(+) group, animals received intragastric TJ-43 treatment for 3 wk. All rats fasted for 12 h before experiments, in both groups. Then, glucose dissolved in distilled water (1 g/mL) was administered (2 g/kg body weight) by gavage 3 h after TJ-43 administration in the rTJ-43(+) and rTJ-43(-) groups.

Blood samples were taken from the tail vein before and 30, 60, and 120 min after the administration of glucose. The amount of glucose in the serum was measured using the Glucose B Test (Wako Pure Chemicals Co., Ltd., Tokyo, Japan).

Statistical analysis

Statistical analyses were performed using EZR software (Saitama Medical Center, Saitama, Japan)[22]. Data were presented as the mean ± standard error of the mean. $P < 0.05$ was considered a significant difference.

RESULTS

Treatment of TJ-43 after PpPD

Adverse events were not observed by TJ-43 treatments (Table 1).

Effects of TJ-43 on incretin hormone levels

Plasma incretin hormone levels after TJ-43 treatment were significantly greater compared with those before TJ-43 treatment (Figure 2).

Effects of TJ-43 on immunoreactive insulin and blood glucose levels

The IRI levels in the TJ-43(+) group were greater, but not significant, compared with those in the TJ-43(−) group (Figure 3A). There were no significant differences in blood glucose levels between the two group (Figure 3B).

Effects of TJ-43 on plasma levels of incretin hormones

Plasma incretin-hormone levels increased significantly after TJ-43 treatment (Figure 4).

Effects of TJ-43 on the expression of incretin hormones in the gastrointestinal tracts

Immunohistochemical analysis revealed that GIP expression was mainly detected in the glandular stomach in rats without TJ-43 treatment (Figure 5). After 4 wk of TJ-43 treatment, the expression markedly increased. Moreover, GLP-1 expression was mainly detected in the ileum and enhanced after TJ-43 treatment.

Effects of TJ-43 on the cAMP activity in the pancreatic tissues

The cAMP activity was measured in the pancreatic tissues of normal rats (Figure 6). However, the activity in the tissues of rats treated with TJ-43 significantly increased compared with those in the tissues of rats without TJ-43 treatment *in vivo*.

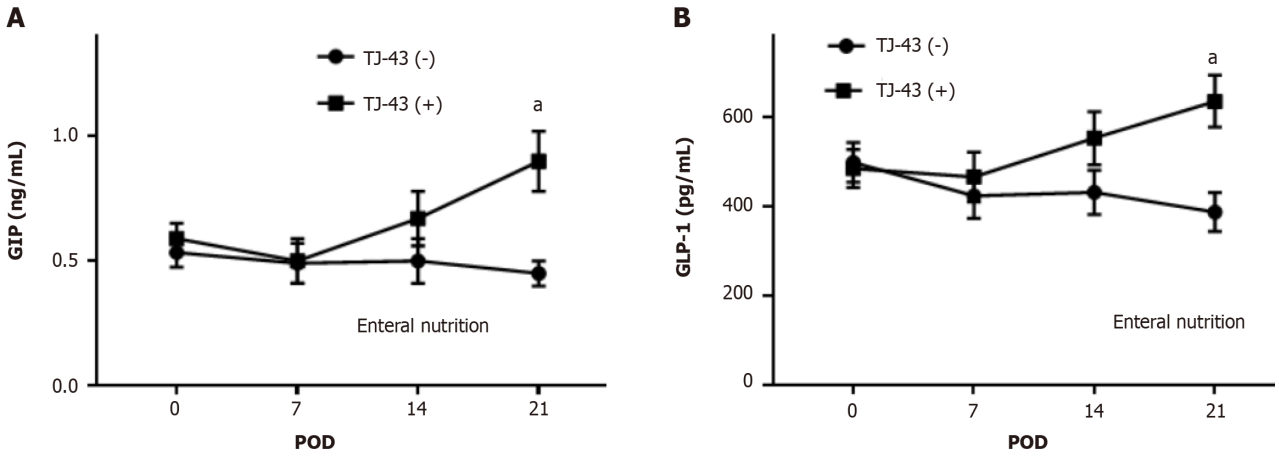

Figure 2 Effects of rikkunshito on plasma incretin levels. A and B: Plasma levels of (A): Gastric inhibitory peptide; and (B): Glucagon-like polypeptide-1 were measured by ELISA. Rikkunshito (TJ-43); TJ-43(−), patients without TJ-43 treatment; and TJ43(+), patients with TJ-43 treatment. $^{a}P < 0.05$ compared with the TJ-43(−) group by ANOVA with Bonferroni's *post-hoc* test. TJ-43: Rikkunshito; GIP: Gastric inhibitory peptide; GLP-1: Glucagon-like polypeptide-1; POD: Postoperative day.

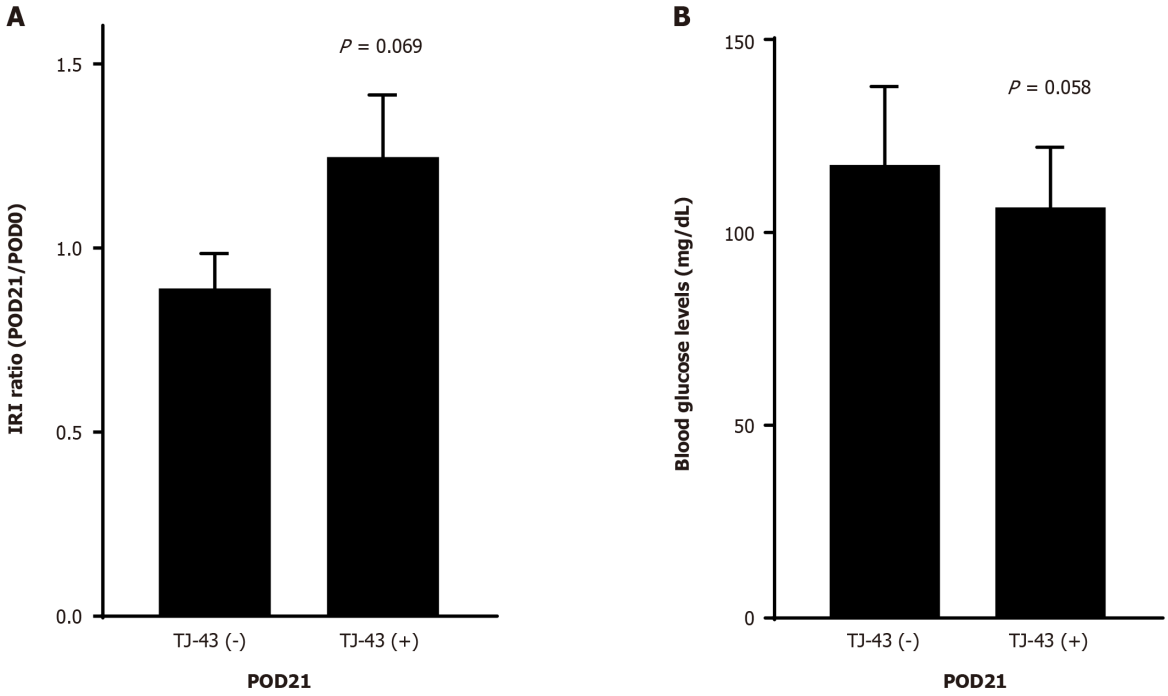

Figure 3 Effect of rikkunshito on insulin secretion and blood glucose levels. A and B: Immunoreactive insulin levels (A) and blood glucose levels (B) are shown. Rikkunshito (TJ-43); TJ-43(−), patients without TJ-43 treatment; TJ43(+), patients with TJ-43 treatment. TJ-43: Rikkunshito; POD: Postoperative day; IRI: Immunoreactive insulin.

Effects of TJ-43 on the expression of insulin in the pancreatic islet cells

As a result of the cAMP activity in the pancreatic tissues, the expression of insulin in the pancreatic islet cells was markedly enhanced in the cells isolated from animals treated with TJ-43 compared with those isolated from animals without TJ-43 treatment (Figure 5).

Effects of TJ-43 on blood glucose levels

The results of the simultaneous administration of glucose and TJ-43 to rats are shown in Figure 6. In the rTJ-43(−) group, the blood glucose levels rose immediately after administration, reaching a maximum value (232.5 ± 22.9 mg/dL) at 30 min and 211.2 ± 3.20 mg/dL at 60 min, followed by a gradual decrease, almost reaching the pre-administration level at 120 min.

In contrast, the blood glucose levels in the rTJ-43(+) group at 30 and 60 min after administration were 194.5 ± 26.1 and 194.0 ± 16.1 mg/dL, respectively. These values were significantly blunted compared with those in the rTJ-43(-) group ($P < 0.05$).

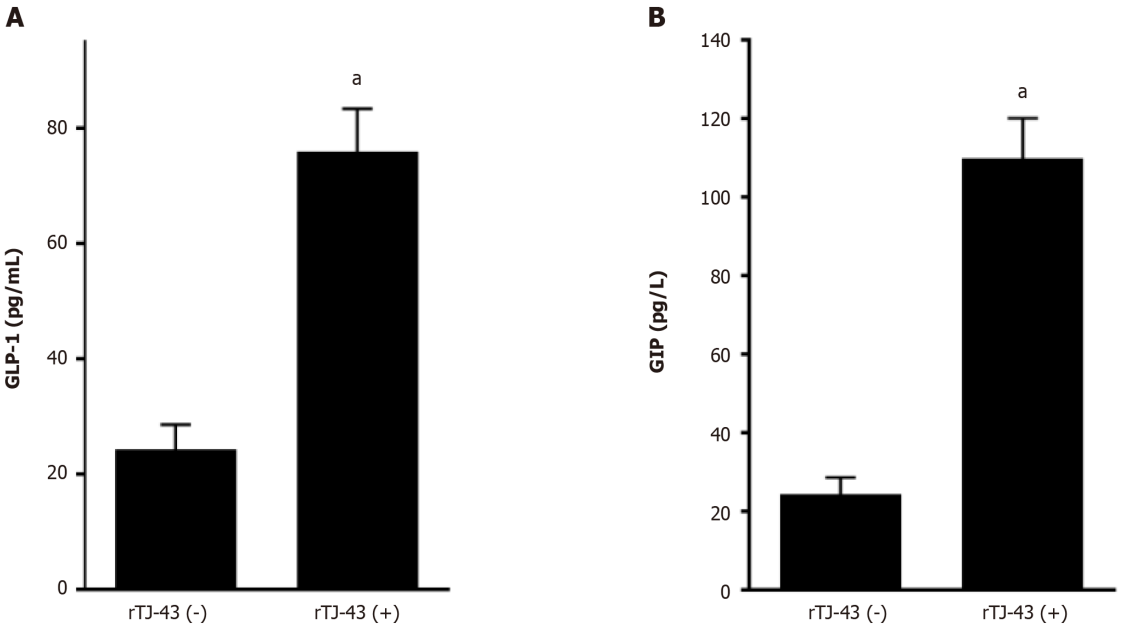

Figure 4 Effects of rikkunshito on plasma incretin hormone levels. A and B: Plasma levels of gastric inhibitory peptide; and glucagon-like polypeptide-1 were measured by ELISA. Rikkunshito (TJ-43); rTJ-43(−), rats without TJ-43 treatment; and TJ43(+), rats with TJ-43 treatment. $^{a}P < 0.05$ compared with the TJ-43(−) group by ANOVA with Bonferroni's *post-hoc* test. TJ-43: Rikkunshito; GLP: Glucagon-like polypeptide-1; GIP: Gastric inhibitory peptide.

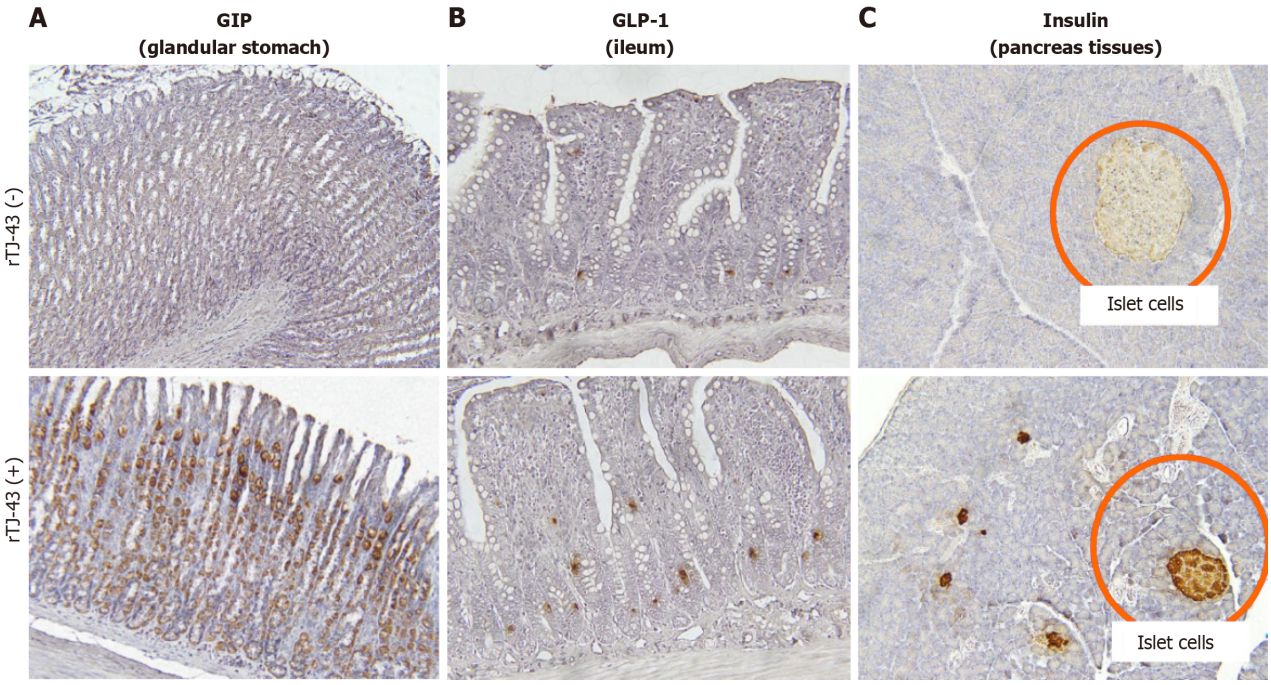

Figure 5 Effects of rikkunshito on expressions of incretin hormones in gastrointestinal and insulin in pancreatic tissues. A: Gastric inhibitory peptide in the glandular stomach; B: Glucagon-like polypeptide-1 in the ileum; C: Insulin in the pancreas. The expression of incretin hormones in the gastrointestinal tracts and insulin in the pancreas was investigated by immunohistochemistry. TJ-43: Rikkunshito; rTJ-43(−): Rats treated without TJ-43; rTJ43(+): Rats treated with TJ-43; GIP: Gastric inhibitory peptide; GLP-1: Glucagon-like polypeptide-1.

Effects of TJ-43 on blood insulin levels

The results of blood insulin levels in rats treated with the simultaneous administration of glucose are shown in Figure 6. In the rTJ-43(-) group, blood insulin levels rose immediately after glucose administration, reaching a maximum value (3110 ± 456 mg/dL) at 30 min and 2286 ± 298 mg/dL at 60 min. Then the levels gradually decreased to the pre-administration level at 120 min (data not shown).

Moreover, the blood insulin levels at 30 and 60 min after glucose loading in the rTJ-43(+) group were significantly greater than those in the rTJ-43(-) group ($P < 0.05$).

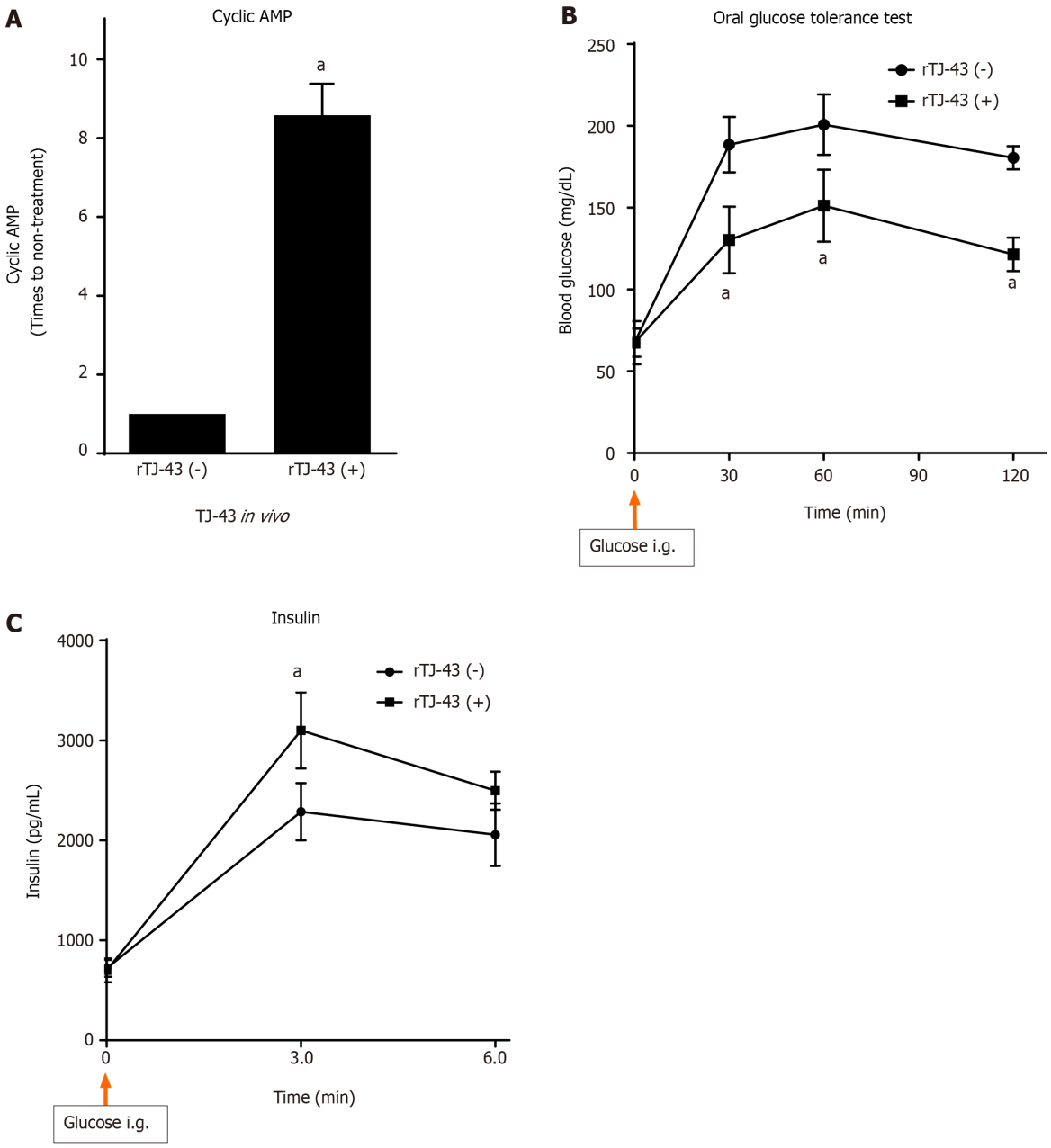

Figure 6 Effects of rikkunshito on cyclic adenosine monophosphate activities in pancreatic tissues, oral glucose tolerance test, and plasma insulin levels. A: Cyclic adenosine monophosphate activities in the pancreatic tissues; B: Oral glucose tolerance test; C: Plasma insulin levels. The cyclic adenosine monophosphate activities were investigated in the pancreatic tissues of rats treated with rikkunshito (TJ-43) for 3 wk or without treatment. Blood glucose levels are measured after intragastric glucose administration (2.0 g/kg) in rats treated with TJ-43 (1 g/kg) for 3 wk or without treatment at designated time points (n = 6 in each group). In the oral glucose tolerance test, plasma insulin levels are measured in rats treated with TJ-43(+) or without TJ-43(−) (n = 6 in each group) after glucose administration at designated time points. TJ-43, rikkunshito; rTJ-43(−), rats treated without TJ-43; and rTJ43(+), rats treated with TJ-43. $^{a}P < 0.05$ compared with the TJ-43(−) group by ANOVA with Bonferroni's post-hoc test. TJ-43: Rikkunshito; AMP: Adenosine monophosphate.

DISCUSSION

Incretins are GI polypeptide hormones secreted after nutrient intake[13,23]. One of the incretins, GIP, is secreted from K cells in the upper intestine. Another incretin, GLP-1, is secreted from L cells in the lower intestine. Insulin is secreted from the pancreatic islet b-cells in a blood glucose-dependent manner, suggesting that hypoglycemia rarely occurs without meal consumption. In addition, incretins inhibit gastric acid secretion but do not affect gastric emptying[24]. Thus, incretin hormones are attracting attention for their clinical application in diabetes treatment.

In this study, TJ-43 increased plasma incretin-hormone levels in the patients undergoing PpPD (Figure 2). Incretin is quickly destroyed by the DPP-4 enzyme. Therefore, incretin is less likely to be destroyed, and postprandial glucose levels after are reduced. The clinically available drug that suppresses this enzyme is referred to as a DPP-4 inhibitor. Although it is less effective than the direct injection of GLP-1, it can easily be started as an oral medication. The development of GLP-1 agonists gained more attention after it was demonstrated that exogenous GLP-1 can lower both fasting-induced and

postprandial glycemia[25]. During fasting, the glucose-lowering effect of GLP-1 is predominantly mediated by effects on islet cell function, increasing insulin[26]. Accordingly, incretin hormones and their agonists are unlikely to cause hypoglycemia. Collectively, these new drugs are called "incretin-related drugs." In this study, TJ-43 treatment increased the plasma incretin levels (Figures 2 and 4), and thus, TJ-43 treatment may be useful in combination with other incretin-related drugs.

Herbal medicines stimulate incretin secretion in mice[27,28]. In that study, TJ-43 did not affect postprandial glucose, active GLP-1, triglyceride, or remnant-like particle cholesterol responses[29]. In contrast, the plasma insulin levels 60 min after TJ-43 administration were significantly higher than those measured immediately after TJ-43 administration. Additionally, the free fatty acid levels were reduced 60 to 180 min after the intake of TJ-43. It was concluded that active GLP-1 does not contribute to enhanced insulin secretion 60 min after ingestion of solid test meals. A prokinetic effect of TJ-43 may alter insulin secretion after ingestion of solid test meals. In this study, TJ-43 increased the incretin levels after the pancreatic operation (Figure 2), but the increase in GIP was greater than that in active GLP-1 (Figure 2), suggesting that the effect of TJ-43 on the increase in GIP is stronger compared that in GLP-1.

Effects of TJ-43 on plasma levels of incretin hormones and insulin expression

Both GIP and GLP-1 hormones improve glucose tolerance. GIP appears to be the most effective, particularly regarding insulin secretion, whereas the action of GLP-1 is mainly associated with the inhibition of glucagon secretion[30]. In the present study, plasma active GIP and active GLP-1 Levels were increased by TJ-43 treatment in patients undergoing PpPD (Figure 2). Furthermore, insulin secretion increased, and blood glucose levels decreased in patients with TJ-43 treatment after PpPD (Figure 3), although there were no significant differences among the clinical cases. Notably, the effects of TJ-43 treatment still need to be investigated in normal physiological conditions. Moreover, an animal study is required to clarify whether the increases in incretin-hormone and IRI levels are caused by TJ-43 treatment. Therefore, a study using animal models is currently underway.

The plasma levels and intestinal expression of incretin hormones significantly increased in rats. Furthermore, the degree of increase in GIP was significantly higher than that in GLP-1. Moreover, the activation of cAMP, insulin expression in the pancreas tissues, and blood insulin levels were markedly enhanced by continuous TJ-43 administration by gavage (Figures 4-6). Conversely, the blood glucose levels were significantly lower in the rTJ-43(+) group compared with those in the rTJ-43(-) group after performing oral glucose tolerance tests (Figure 6). Thus, TJ-43 can increase plasma incretin hormone levels, the expression of insulin, and inhibit elevation of blood glucose levels. Hence, TJ-43 may be beneficial for patients who undergo pancreatic resection.

Although TJ-43 may have useful effects on insulin secretion after pancreatic surgery, only a small number of clinical cases were considered in this retrospective study. Therefore, it is difficult to establish a definite conclusion, and a randomized control study with a large number of clinical cases is needed, in addition to the animal study, to verify the effects of TJ-43 and clarify the mechanisms. Overall, oral dietary intake of TJ-43 may be advantageous for increasing blood levels of incretin hormones and acylated ghrelin after pancreatic surgery, which reduces the total volume of pancreas tissue.

CONCLUSION

The results suggest that TJ-43 is beneficial for patients who undergo pancreatic surgery.

ARTICLE HIGHLIGHTS

Research background

It was reported that rikkunshito (TJ-43) improved the cisplatin-induced decreases in the active form of ghrelin in plasma; however, other effects on gastrointestinal hormones have not been investigated. In patients undergoing pylorus-preserving pancreatico-duodenectomy (PpPD), postoperative oral food intake can be hindered by delayed gastric emptying (DGE). In addition to ghrelin, the effects of TJ-43 on gastrointestinal hormone levels are investigated herein.

Research motivation

It is necessary to resolve the issue of DGE after PpPD.

Research objectives

This basic study aimed to investigate the effects of TJ-43 on peripheral levels of incretin hormones, including gastric inhibitory polypeptide (GIP) and glucagon-like polypeptide-1 (GLP-1), in humans and rats.

Research methods

Patients were divided into two groups, namely patients who received TJ-43 immediately following surgery [TJ-43(+) group] and those who received TJ-43 on postoperative day (POD) 21 [TJ-43(-) group], and the plasma levels of active GIP and active GLP-1 were assessed. In animal experiments, rats were treated with TJ-43 [rat (r)TJ-43(+) group] or without [rTJ-43(−) group] by gavage for 4 wk, and the plasma active GIP and active GLP-1 Levels were measured. The expression

of incretin hormones in the gastrointestinal tract and insulin in the pancreas were investigated by immunohistochemistry. Furthermore, the cyclic adenosine monophosphate activities were assessed in pancreatic tissues from rats treated with or without TJ-43 *in vivo*, and the blood glucose levels and plasma insulin levels were measured in rats treated with or without TJ-43 in oral glucose tolerance tests.

Research results

The active GIP and active GLP-1 Levels increased, and the values at POD 21 were significantly greater in the TJ-43(+) group than those in the TJ-43(-) group. In the rat model, the plasma active incretin levels significantly increased in the rTJ-43(+) group compared with those in the rTJ-43(-) group, although GIP and GLP-1 expressions were enhanced by TJ-43 treatment in both groups. Moreover, plasma insulin levels increased and blood glucose levels were blunted in the rTJ-43(+) group.

Research conclusions

The results suggest that TJ-43 is beneficial for patients who undergo pancreatic surgery.

Research perspectives

To verify the effects and clarify the mechanisms of TJ-43 after pancreatic surgery, a prospective study is required.

FOOTNOTES

Author contributions: Kono H conducting and organizing this experiment; Furuya S, assessment of samples; Akaike H, assessment of samples; Shoda K, analyzing the data; Kawaguchi Y, analyzing the data; Amemiya H, assessment of samples; Kawaida H, collecting samples; Ichikawa D advising for these experiments.

Institutional review board statement: This study was reviewed and approved by the University of Yamanashi Hospital, Institutional Review Board: ID No. 820.

Institutional animal care and use committee statement: This study is approved by the Institutional Animal Care and Committee.

Conflict-of-interest statement: All authors have no conflicts of interest to disclose.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at (hkouno@yamanashi.ac.jp). Participants gave informed consent for data sharing.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Country/Territory of origin: Japan

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S-Editor: Liu JH
L-Editor: A
P-Editor: Guo X

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World Journal of Methodology

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

World J Methodol 2024 March 20; 14(1): 89723

DOI: 10.5662/wjm.v14.i1.89723

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Basic Study

Time-dependent impact of a high-fat diet on the intestinal barrier of male mice

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Specialty type: Medical laboratory technology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Duan SL, China

Received: November 10, 2023
Peer-review started: November 10, 2023
First decision: December 17, 2023
Revised: December 26, 2023
Accepted: February 18, 2024
Article in press: February 18, 2024
Published online: March 20, 2024

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Abstract

BACKGROUND

Excessive saturated fat intake compromises the integrity of the intestinal mucosa, leading to low-grade inflammation, impaired mucosal integrity, and increased intestinal permeability, resulting in the migration of lipopolysaccharide (LPS) to other tissues.

AIM

To evaluate the chronic effects (at 10 and 16 wk) of a high-fat diet (HFD) (with 50% energy as fat) on the phylogenetic gut microbiota distribution and intestinal barrier structure and protection in C57BL/6 mice.

METHODS

Forty adult male mice were divided into four nutritional groups, where the letters refer to the type of diet (control and HFD or HF) and the numbers refer to the period (in weeks) of diet administration: Control diet for 10 wk, HFD for 10 wk, control diet for 16 wk, and HFD for 16 wk. After sacrifice, biochemical, molecular, and stereological analyses were performed.

RESULTS

The HF groups were overweight, had gut dysbiosis, had a progressive decrease in occludin immunostaining, and had increased LPS concentrations. Dietary progression reduced the number of goblet cells per large intestine area and *Mucin2* expression in the HF16 group, consistent with a completely disarranged intestinal ultrastructure after 16 wk of HFD intake.

CONCLUSION

Chronic HFD intake causes overweight, gut dysbiosis, and morphological and

functional alterations of the intestinal barrier after 10 or 16 wk. Time-dependent reductions in goblet cell numerical density and mucus production have emerged as targets for countering obesity-driven intestinal damage.

Key Words: High-fat diet; Intestine; Ultrastructure; Goblet cells; Gut microbiota

Core Tip: There is great interest in the scientific community in the impact of unhealthy eating habits, such as excess saturated fatty acid intake, on the gut microbiota composition and metabolic disease onset. Here, we evaluated the progressive changes in the intestinal structural barrier and gut microbiota composition in mice fed a high-fat diet (HFD) for 10 or 16 wk. HFD administration resulted in gut dysbiosis, compensatory enhancement of goblet cell numerical density, and increased *Mucin2* expression after 10 wk. Continuous feeding reduced the goblet cell number and the expression of *Mucin2* and occludin, consistent with the impaired tight junction ultrastructure in the chronically obese HFD-fed mice after 16 wk.

Citation: Miranda CS, Santana-Oliveira DA, Vasques-Monteiro IL, Dantas-Miranda NS, Glauser JSO, Silva-Veiga FM, Souza-Mello V. Time-dependent impact of a high-fat diet on the intestinal barrier of male mice. *World J Methodol* 2024; 14(1): 89723
URL: https://www.wjgnet.com/2222-0682/full/v14/i1/89723.htm
DOI: https://dx.doi.org/10.5662/wjm.v14.i1.89723

INTRODUCTION

Obesity is an undeniable health challenge worldwide due to its increasing prevalence in recent decades. Although it is a multifactorial disease, a positive energy balance is a frequent trigger. Excessive accumulation or abnormal body fat distribution results in a state of low-grade chronic inflammation, which is a potential risk factor for many metabolic disorders, including oral glucose intolerance, insulin resistance, dyslipidemia, metabolic-associated fatty liver disease, and type 2 diabetes[1,2].

Recent evidence suggests that the gut barrier should be a new study target for understanding the obesity epidemic[3,4]. A high-fat diet (HFD) increases plasma lipopolysaccharide (LPS) concentrations by 2 to 3 times compared to a control diet[5]. Intestinal LPS can impair gut barrier integrity, leading to the leakage of LPS into the systemic circulation, inducing chronic inflammation, and contributing to metabolic endotoxemia and obesity[6].

The intestinal epithelial barrier regulates absorption, secretion, and protection to prevent systemic entry of antigens from the intestinal lumen[7,8]. Tight junctions (TJs), located in the apical portion of the lateral membrane of intestinal epithelial cells (IECs), are composed of multiple proteins, including the transmembrane protein occludin, that regulate intestinal permeability. Nutritional factors, such as irregular consumption of protein and casein peptides[3], may alter the dynamic regulation of intracellular occludin in TJs, specifically the permeability barrier to macromolecules[9]. A leaky gut facilitates the translocation of potentially harmful antigens into epithelial cells[10].

A hydrated gel composed of mucins secreted by goblet cells covers the luminal surface of the intestinal mucosa[11]. In an intact epithelium, this surface restricts the passage of most hydrophilic solutes[12]. However, studies on the effects of an HFD on gut barrier structure are contradictory. A previous study reported that HFD intake resulted in an increased number of goblet cells[13]. However, other studies have shown a significant reduction in goblet cell numerical density in HFD-fed mice[1,14]. These inconsistent results may be due to differences in the duration of HFD consumption and the age of the mice.

The present study hypothesized that HFD feeding elicits time-dependent changes in the gut microbiota composition, intestinal barrier structure, and protection. The majority of the mouse models were subjected to diet-induced obesity protocols (10 wk) or posttreatment (16 wk).

MATERIALS AND METHODS

Animals and ethics approval

All procedures performed in the present study complied with the Arrive Guidelines. The procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and Brazilian Federal Law No. 11.794/2008, in addition to adhering to European Union standards on animals used for scientific purposes protection. The local Ethics Committee (Institute of Biology, CEUA N° 017/2021) approved the experimental design.

Twenty adult male C57BL/6 mice were housed in cages (5 rats per cage). The cages were maintained at a controlled temperature (21 ± 2 °C) and humidity (60% ± 10%), and the mice had free access to water and food. The cages allowed for sufficient ventilation and were arranged on a rack (NexGen Mouse 500, Allentown, PA, United States). The environment included light-dark cycles of 12/12 h and air renewal (15 min/h).

The sample size was considered the minimum needed to reach statistical significance based on the Ethics Committee recommendation. There was no animal exclusion during the experiment. The first author was aware of each group allocation, and procedures were first applied to the control group.

Experimental design

Twenty adult mice (3 months old) were included in this study. Initially, the animals were randomly assigned to one of the two nutritional groups: (1) The control group (C) (n = 10) was fed a control diet (14% of the total energy was protein, 10% was fat, and 76% was carbohydrate; the total energy was 15 KJ/g); and (2) The HF (n = 10) group was fed an HFD (14% of the total energy was protein, 50% was fat, and 36% was carbohydrate; the total energy was 21 KJ/g).

After 10 wk, five animals from each group were sacrificed. The other five animals in each group were fed the same diet for 16 wk, resulting in four study groups (n = 5 per group): Animals fed the C diet for 10 wk (C10), animals fed the HFD for 10 wk (HF10), animals fed the C diet for 16 wk (C16), and animals fed the HFD for 16 wk (HF16). PragSoluções (www.pragsolucoes.com.br, Jaú, São Paulo, Brazil) produced the experimental diets according to the recommendations of the American Institute of Nutrition (AIN 93M)[15].

Sample preparation

Mice were fasted for 6 h and sacrificed under anesthesia with ketamine (240 mg/kg) and xylazine (30 mg/kg). Blood samples were harvested by cardiac puncture, and plasma was obtained through centrifugation (712 × g) for 15 min and frozen (-80 °C) for further analysis. The small and large intestines were carefully dissected, weighed, and fixed in Millonig formalin for microscopic analysis or frozen at -80 °C for molecular analysis.

Plasma analysis

Plasma concentrations of LPS (multispecies LPS ELISA Kit Cat #SEB526Ge-96T; Cloud-Clone Corp., Katy, United States) were analyzed in duplicate with commercially available ELISA kits using Fluostar Omega equipment (BMG LABTECH GmbH, Germany).

Histology and stereology

After dissection, the large intestines (cecum) of the animals were fixed in Millonig for 48 h, followed by incubation in Paraplast Plus (Sigma-Aldrich Co., St. Louis, MO, United States). The tissues were cut into 5-µm-thick sections and subjected to alcian blue staining. Photomicrographs were obtained with a Lumenera camera and Olympus BX51 microscope (Tokyo, Japan), followed by stereological estimation of the numerical density of goblet cells per area [Q_A (goblet cells)], which comprised the division of the total number of goblet cells counted in the test area, except those touching the forbidden line, by the test area (in mm^2)[1], obtained with the STEPanizer stereology tool (www.stepanizer.com).

Immunohistochemistry

For immunohistochemistry, slides from the small intestine (ileum) were deparaffinized, and after antigen retrieval (citrate buffer, pH = 6.0, at 60 °C for 20 min), peroxidase and nonspecific binding blocks were added, followed by incubation with a primary antibody against occludin (Invitrogen, 40-4700; Waltham, MA, United States; dilution 1:20) overnight at 4 °C. The antibody was diluted in 1.5% horse serum (Vector Laboratories, CA, United States). The slides were then incubated with a panspecific biotinylated secondary antibody for 10 min followed by incubation with streptavidin and peroxidase for 5 min. The sections were stained with DAB for 5 min (Vectastain Universal Quick HRP Kit, peroxidase, PK-7800, Vector Laboratories). The slides were counterstained with hematoxylin and mounted with Entellan (Merck, Darmstadt, Germany).

Transmission electron microscopy

Cecum fragments (1 mm^3, n = 3 per group), fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.2) and postfixed with 1% osmium tetroxide, were dehydrated in acetone and added to epoxy resin (48 h at 60/70 °C). Ultrathin sections (60-80 nm) contrasted with 5% uranyl acetate and 2% lead citrate were observed under a transmission electron microscope (FEI, Oregon, United States) at the National Institute of Biochemistry and Bioimaging Science and Technology (CENABIO - UFRJ).

Real-time reverse transcriptase-polymerase chain reaction

Total RNA was extracted from 70 mg of ileum tissue using 700 µL of TRIzol (Invitrogen, CA, United States). Spectrometry and Nanovue equipment (GE Life Sciences) were used to determine the RNA concentration, and cDNA was synthesized using oligo(dT) and Superscript III reverse transcriptase (Invitrogen, CA, United States). *Gapdh* was used as the endogenous control[16].

The efficiencies of the target gene and endogenous control were approximately equal and were calculated from a cDNA dilution series. Polymerase chain reaction (PCR) was performed as follows: Predenaturation and polymerase activation (4 min at 95 °C) with 40 cycles of 95 °C for 10 s and 60 °C for 15 s, followed by melting (60-95 °C with a heating rate of 0.1 °C/s). The cDNA was replaced with deionized water for the negative control. The relative mRNA expression ratio was calculated by the Equation $2^{-\Delta\Delta Ct}$, where -ΔCT represents the difference between the number of cycles of the target genes and the endogenous control. The nomenclature of the genes followed international standards, with the first letter in capital letters and italics. The primers used were generated using Primer3 online software (*Mucin2* - forward: GTAGTTTCCGTTGGAACAGTGAA, reverse: ATGCCCACCTCCTCAAAGAC; and *Gapdh* - forward: CATCACTGC-

CACCCAGAAGACTG, reverse: ATGCCAGTGAGCTTCCCGTTCAG).

16S rRNA gene amplification by qPCR

The feces found in the cecum were used to extract microbial DNA using a commercial kit (QIAamp Fast DNA Stool Mini Kit, Qiagen). Qubit (Life Technologies) and horizontal electrophoresis (1% agarose gel) were used for DNA quantification, and purity and concentration determination. The relative abundance of the main phyla found in the fecal microbiota (*Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria*) was assessed *via* real-time quantitative PCR assays by detecting 16S rRNA genes (indicators are listed in Table 1). The abundance of different phyla was normalized to the ΔΔ Ct value of the total amount of bacteria in the sample[17].

Data analysis

The data are presented as the mean and SD. Differences between the groups were tested by the Brown-Forsythe and Welch one-way ANOVA and Dunnett T3 *post hoc* test (GraphPad Prism version 10.1.2 for Windows, GraphPad Software, Boston, MA, United States). $P < 0.05$ was considered to indicate statistical significance.

RESULTS

HFD causes overweight and gut dysbiosis without altering food intake

The four groups started the experiment on mice with similar body masses. At the end of the study, the HF groups were heavier than their counterparts (HF10: +15% *vs* C10; HF16: +22% *vs* C16; Figure 1A). Moreover, compared to HF10 individuals, HF16 individuals were overweight (+6%, Figure 1A). Food intake (in grams) was similar throughout the experiment, irrespective of the diet (C: 2.96 ± 0.08, HF10: 3.00 ± 0.04, C16: 3.01 ± 0.03, and HF16: 3.08 ± 0.04). However, the HF groups had greater energy intake than the C groups (HF10: +33% *vs* C10; HF16: +34% *vs* C16; Figure 1B), which was expected due to the high energy density of the HFD.

Gut dysbiosis occurred in the HFD-fed groups (Figure 1C). Both the HF10 and HF16 groups presented an increased *Firmicutes* proportion concomitant with decreased *Bacteroidetes*, confirming that overweight augments the *Firmicutes*/*Bacteroidetes* ratio. Notably, the HFD promoted a progressive decrease in the abundance of *Proteobacteria* and *Actinobacteria*. Compared with the HF10 diet, the HF16 diet was associated with a lower proportion of *Proteobacteria* (-83%) and *Actinobacteria* (-76%).

HFD results in time-dependent structural modifications and protection of the intestinal barrier, and induces endotoxemia

High saturated fat intake provoked a time-dependent alteration in the distribution of cecal mucosal cells, as revealed by the reaction of the cells to alcian blue and PAS. Figure 2A shows more goblet cells (stained in blue) in the cecum of the HF10 group than in the cecum of the C10 group. Conversely, the progressive intake of the HFD decreased the Q_A (goblet cells) in the apical region of the crypts in the HF16 group, implying a reduction in mucus production compared to that in the age-matched group. Gut stereology confirmed these observations with the Q_A (goblet cells) results, which were greater in the HF10 group than in the C10 group (+23%), while the HF16 group presented a decrease in Q_A (goblet cells) compared to that in the C16 group (-16%, Figure 2B). The decrease in Q_A (goblet cells) was time-dependent for both diets: C16 and HF16 produced lower Q_A (goblet cells) of the intestine than C10 (-33%) and HF10 (-54%). Consistent with the stereology, the HF10 group presented 55% greater intestinal *Mucin 2* gene expression than the C10 group. On the other hand, the HF16 group did not differ from the C16 group (Figure 2C).

The modifications in the structure and protection of the intestinal barrier in the HF groups caused a marked increase in the plasma concentrations of LPS (HF10: +25% *vs* C10; HF16: +40% *vs* C16; Figure 2D), characterizing endotoxemia. In agreement with the previous results, immunohistochemistry staining for occludin revealed positive reactions in the C10, HF10, and C16 groups, suggesting progressive damage to TJs due to HFD feeding, as HF16 cells showed negative immunoreactions for occludin (Figure 3A).

HFD causes time-dependent damage to the intestinal ultrastructure

Analysis of the intestinal ultrastructure (Figure 3B) revealed that the C10 group had microvilli on the apical surface of the absorptive IEC, in addition to an active mucus layer, numerous mitochondria, and integrity of the cellular membrane. The C16 group also exhibited preservation of the intestinal ultrastructure, as indicated by the presence of intact TJs harboring two consecutive IECs. In contrast, the HF10 group exhibited lipid inclusions inside the IECs parallel to thickened and irregular microvilli, implying damage to cell function. In agreement with previous findings, the HF16 group exhibited a completely disarranged ultrastructure, almost no mitochondria, no microvilli, and permeable TJs.

DISCUSSION

In recent decades, the prevalence of overweight and obesity in developed and developing countries has been increasing due to changes in dietary patterns. Contemporary dietary habits include high energy intake, in forms such as saturated fats and added sugars, and reduced fiber, fruit, and vegetable intake. Therefore, nutritional habits associated with a

Table 1 Primers used for determination of the phylum or class of microorganisms of the gut microbiota

Phylum or class	Forward primer	Reverse primer
Actinobacteria	5′-TACGGCCGCAAGGCTA-3′	5′-TCRTCCCCACCTTCCTCCG-3′
Bacteroidetes	5′-CRAACAGGATTAGATACCCT-3′	5′-GGTAAGGTTCCTCGCGTAT-3′
Class-γ-proteobacteria	5′-TCGTCAGCTCGTGTYGTGA-3′	5′-CGTAAGGGCCATGATG-3′
Eubacteria (*all bacteria*)	5′-ACTCCTACGGGAGGCAGAGT-3′	5′-ATTACCGCGGCTGCTGGC-3′
Firmicutes	5′-TGAAACTYAAAGGAATTGACG-3′	5′-ACCATGCACCACCTGTC-3′

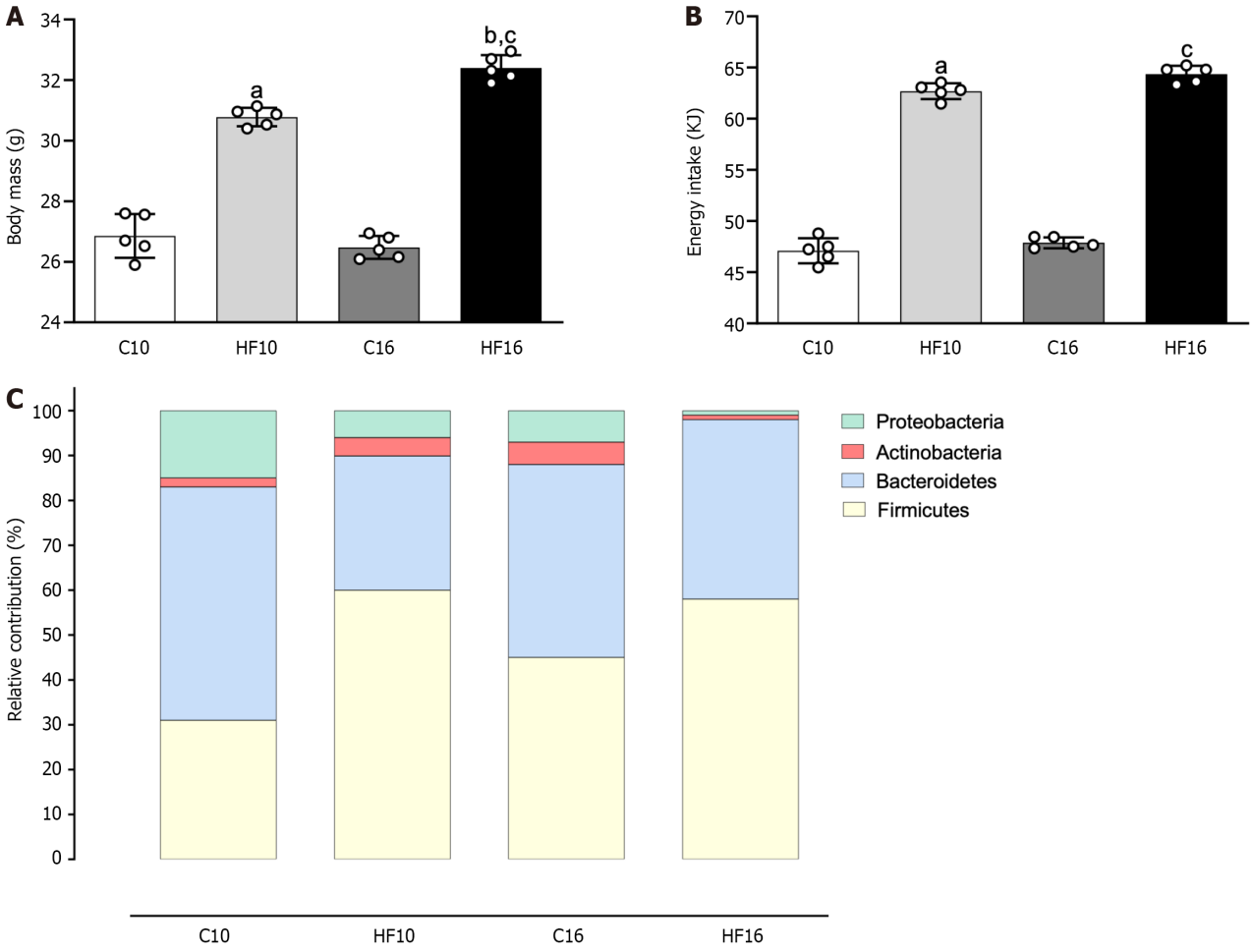

Figure 1 Body mass, energy intake, and phylogenetic microbiota distribution. A: Body mass; B: Energy intake; C: Phylogenetic microbiota distribution. Brown-Forsythe and Welch one-way ANOVA and Dunnett T3 *post hoc* test were used (mean ± SD, $n = 5$). [a]$P < 0.01$, compared with control; [b]$P < 0.01$, compared with high-fat diet for 10 wk; [c]$P < 0.01$ compared with control diet for 16 wk. C10: Control diet for 10 wk; C16: Control diet for 16 wk; HF10: High-fat diet for 10 wk; HF16: High-fat diet for 16 wk.

sedentary lifestyle are strongly related to the genesis and progression of metabolic diseases[18].

The data from this study showed that excessive intake of saturated fatty acids caused increased body mass in C57BL/6 mice fed an HFD for 10 or 16 wk. Chronic high saturated fat intake yields low-grade inflammation and impairs cellular carbohydrate and lipid metabolism, causing weight gain owing to the high energy density of saturated fat[19]. In contrast, there was no difference in food intake between the experimental groups, implying that the metabolic alterations could stem from excessive energy intake from saturated fatty acids.

Lipotoxicity provokes alterations in the intestinal barrier and gut microbiota, favoring obesity[6]. The human intestine is colonized by up to 100 trillion microorganisms, which is approximately ten times the number of human cells. Many factors influence the microbiota composition over the course of a person's life, mainly diet, route of birth delivery, and the use of medications, especially antibiotics[20].

Out of more than 50 described phyla, four predominate: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*[21]. Recent studies have shown that an HFD increases the *Firmicutes* and *Proteobacteria* proportions concomitant with decreased *Bacteroidetes*. The abundance of *Proteobacteria* usually increases in obese individuals, and *Proteobacteria* is

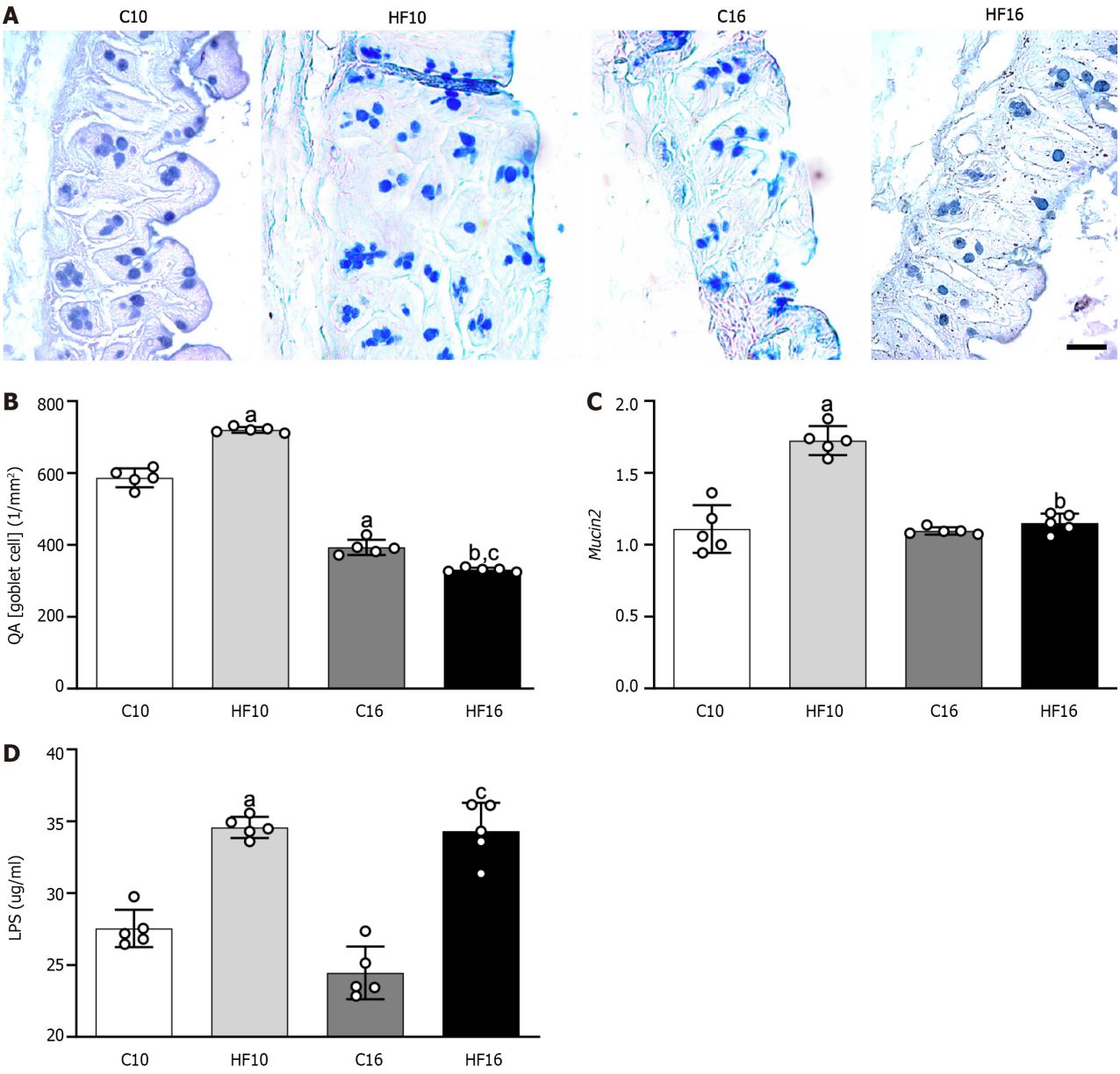

Figure 2 Staining of goblet cells with alcian blue, Q_A (goblet cells), relative *Mucin2* mRNA expression, and plasma lipopolysaccharide concentrations. A: Large intestine tissue stained with alcian blue and periodic acid-Schiff (PAS), revealing that the goblet cells were stained blue; B: Numerical density of goblet cells per area [Q_A (goblet cells)]. Alcian blue and PAS stained glycoproteins produced by goblet cells (scale bar = 40 μm) were markedly increased in the high-fat diet for 10 wk group according to stereology [Q_A (goblet cells)]. ; C: Relative *Mucin2* mRNA expression in different groups; D: Plasma lipopolysaccharide concentrations in different groups. Brown-Forsythe and Welch one-way ANOVA and Dunnett T3 *post hoc* test were used (mean ± SD, $n = 5$). $^{a}P < 0.01$, compared with control; $^{b}P < 0.01$, compared with high-fat diet for 10 wk; $^{c}P < 0.01$ compared with control diet for 16 wk. C10: Control diet for 10 wk; C16: Control diet for 16 wk; HF10: High-fat diet for 10 wk; HF16: High-fat diet for 16 wk; LPS: Lipopolysaccharide.

emerging as a possible marker of microbiota variability and metabolic constraints[22]. However, in mice, the proportion of *Proteobacteria* increases more in response to high-carbohydrate conditions than to HF conditions[1,23]. Notably, both the HF10 and HF16 populations exhibited a marked decrease in *Actinobacteria*, which strongly suggested impaired gut homeostasis. *Bifidobacteria*, a class of *Actinobacteria*, are widely used as probiotics to treat many diseases[24]. In addition to gut dysbiosis, the increase in plasma LPS in the HF10 and HF16 groups suggested intestinal paracellular permeability changes, characterizing metabolic endotoxemia[23].

TJ proteins are responsible for sealing the intestinal mucosa and maintaining barrier function by regulating the permeability of the intestinal mucosa[25]. The role of occludin in TJs seems to encompass the macromolecular permeability barrier, although this phenomenon is still under debate[9]. Our investigation revealed that occludin expression decreased in a time-dependent manner in HFD-fed mice, with a negative immunoreaction in the HF16 group. When large molecules are abnormally translocated from the lumen to the villi, these molecules are absorbed; this process is called leaky gut, which was presented by HF10 and HF16 mice, in agreement with the literature[26].

Within this context, altered intestinal permeability is highly detrimental, as it loses its ability to block the systemic entry of harmful agents, whether food-derived microorganisms or antigens. An increase in the endotoxin LPS in systemic

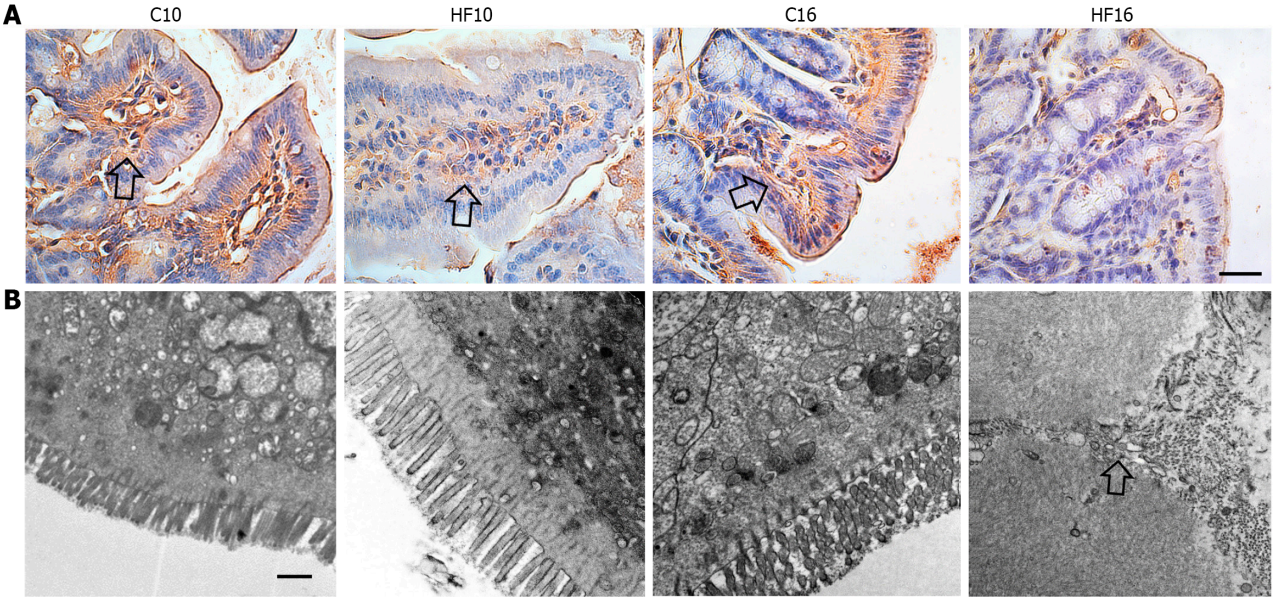

Figure 3 Immunohistochemical staining for occludin in the ileum and transmission electron microscopy of the ileum. A: Occludin immunostaining is shown in light brown (open arrow) and was decreased in a time-dependent manner in the high-fat (HF)-fed groups. B: Transmission electron microscopy images showing normal ileal ultrastructures in the control diet for 10 wk and control diet for 16 wk groups, with preserved tight junctions (asterisk). In contrast, the HF diet for 10 wk and HF diet for 16 wk groups exhibited disrupted tight junctions (open arrow) in addition to a damaged ultrastructure. C10: Control diet for 10 wk; C16: Control diet for 16 wk; HF10: High-fat diet for 10 wk; HF16: High-fat diet for 16 wk.

circulation culminates in the release of proinflammatory cytokines, triggering tissue damage in several systems[27].

Goblet cells are ubiquitously present in the intestinal epithelium and secrete vesicles composed mainly of mucin that are found between enterocytes. Its mucus-producing characteristic confers the ability to lubricate and protect the intestinal epithelium[28]. The histological analysis of this study revealed the presence of numerous goblet cells in the HF10 group, suggesting an attempt to compensate for the alterations caused by bacterial products and toxins by stimulating the thickening of the mucus layer of the intestinal epithelium in response to tissue damage, such as LPS, which also had high plasma levels in this group[13]. On the other hand, the progression of dietary intake in the HF16 group caused a reduction in the Q_A (goblet cells). This time-dependent reduction in the Q_A (goblet cells) was related to *Mucin2* gene expression and was consistent with the adverse ultrastructural remodeling observed in the HF16 group.

Secretory mucins, such as Mucin2, which are present in the small and large intestines, are the chief mucins synthesized and secreted by goblet cells. Mucin2 secretion is stimulated by a wide range of bioactive factors, including microbial products, toxins, and inflammatory cytokines, which are reportedly involved in the up- or downregulation of *Mucin2* transcription. The microbiota and microbial products can modulate the synthesis and secretion of Mucin2, either through several signaling cascades or through bioactive factors generated by epithelial and lamina propria cells[29]. The Q_A (goblet cells) indirectly reflects the ability to secrete Mucin2. Thus, *Mucin2* gene expression followed that of the Q_A (goblet cells), indicating time-dependent damage caused by the HFD to the mucus layer of the intestinal mucosa[12].

A dysfunctional intestinal mucus system and altered goblet cell profile are associated with maturity and inflammatory conditions in mice, resulting in a thin mucus layer and increased vulnerability to infections[30]. A previous study revealed the occurrence of an infection process that progresses through the dysregulation of the microvillus and the presence of cavities on the epithelial surface[31]. In this sense, transmission electron microscopy revealed that HFD intake led to time-dependent changes in the mucous layer and impairment of microvilli, a reduction in mitochondria within the EICs, and damaged TJs in the HF16 group, consistent with the negative occludin immunostaining in the HF16 group, metabolic endotoxemia, and aggravation of obesity from 10 to 16 wk of HFD feeding. Figure 4 summarizes our main findings.

CONCLUSION

In conclusion, chronic HF intake causes overweight, gut dysbiosis, and morphological and functional alterations in the intestinal barrier after 10 or 16 wk. The increased plasma LPS concentrations in the HF groups occurred after progressive damage to mucus-producing cells and occludin (TJ protein) expression. Our results provided original evidence of enhanced Q_A (goblet cells) in the HF10 group as compensatory hyperplasia triggered by lipotoxicity followed by a reduced Q_A (goblet cells) in the HF16 group with maximization of ultrastructural intestinal damage and obesity. The present results highlight the exacerbation of obesity-driven intestinal alterations from 10 to 16 wk of the dietary protocol, suggesting new targets for further studies aimed at obesity management.

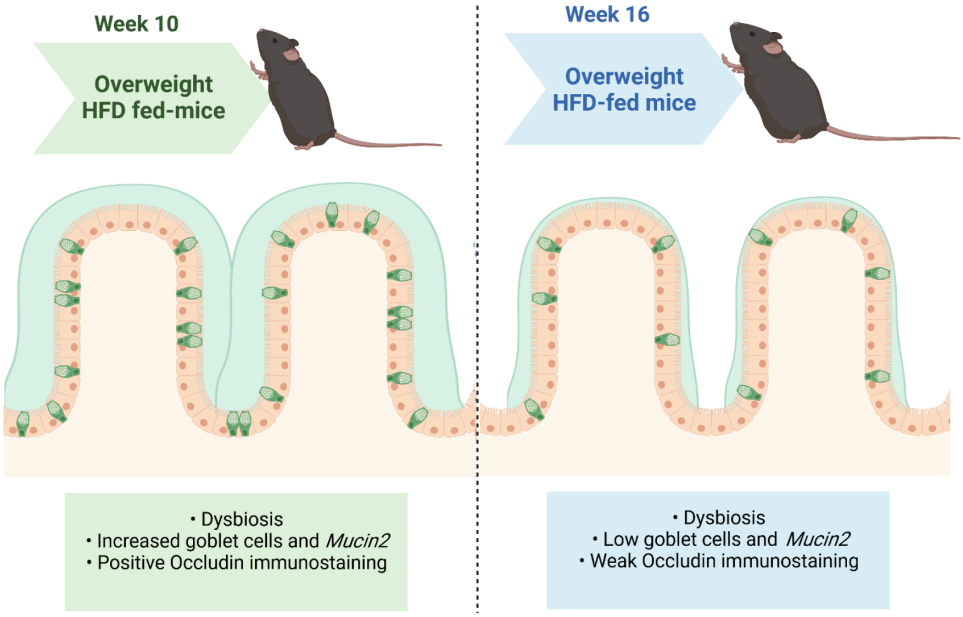

Figure 4 Summary of the main results. The animals fed the high-fat diet for 10 wk (HF10) had overweight, increased goblet cell numerical density, and *Mucin2* expression as a compensatory mechanism to prevent injury from the HFD. However, after 16 wk, HFD-fed mice remained overweight and had a greater body mass than the HF10 group but had a decreased goblet cell number and *Mucin2* expression. Therefore, HF16 mice exhibited a completely disarranged intestinal ultrastructure, with damaged tight junctions and negative occludin immunostaining. Created with Biorender (www.biorender.com). HFD: High-fat diet.

ARTICLE HIGHLIGHTS

Research background

There is great interest in the scientific community in the role of gut dysbiosis in obesity and metabolic impairments. However, there is little evidence in the literature on the progressive impact of a high-fat diet (HFD) on mouse intestinal barrier structure and microbiota composition.

Research motivation

Recently, different dietary models and durations of diet administration have yielded controversial results regarding the Q_A (goblet cells), mucus layer, and microbiota composition in mice with diet-induced obesity. Considering the importance of understanding the progressive changes in intestinal structure and microbiota composition that occur during obesity onset, we examined these changes after 10 or 16 wk of HFD feeding to establish tools to characterize obesity-related intestinal impairments.

Research objectives

We further studied the chronic effects (at 10 and 16 wk) of an HFD (with 50% energy as fat) on the phylogenetic gut microbiota distribution and intestinal barrier structure and protection in C57BL/6 mice. The study considered the mouse model, diet composition, and duration of intervention that were most prevalent in the literature.

Research methods

Mice were fed *ad libitum*, and food intake and body mass gain were monitored during the experiment. Intestinal samples were subjected to light and electron microscopy, and plasma lipopolysaccharide concentrations were determined through ELISA. After 16S rRNA gene amplification by qPCR was performed on the frozen cecal feces to determine the phylogenetic microbiota distribution, the ileum samples were subjected to qPCR analysis for *Occludin* expression.

Research results

Our results confirmed that body mass increased gradually with HFD feeding without altering food intake. Dysbiosis in the HFD model involved an increase in *Firmicutes* and a decrease in *Bacteroidetes* concomitant with an increase in plasma lipopolysaccharide concentrations, so-called endotoxemia. The original findings were compensatory goblet cell hyperplasia and increased *Mucin2* expression in the tenth week, followed by a drastic reduction in both parameters after 16 wk of HFD feeding. These structural alterations were consistent with the progressive damage to the intestinal ultrastructure in obese mice.

Research conclusions

Chronic HFD intake causes gut dysbiosis and endotoxemia, with time-dependent overweight, and morphological and functional alterations of the intestinal barrier after 10 or 16 wk. We identified Q_A (goblet cells) and mucin expression as

viable tools to address the progressive damage caused by obesity in the intestine.

Research perspectives

The evaluation of QA (goblet cells), functionality, and ultrastructure has led to the identification of potential targets for addressing the impact of excessive saturated fatty acid intake on the intestine.

ACKNOWLEDGEMENTS

The authors thank Aline Penna and Andrea Bertoldo for their technical assistance and "Unidade de Microscopia Multiusuário Padrón-Lins (UNIMICRO)" for obtaining and preparing the transmission electron microscopy grids.

FOOTNOTES

Author contributions: Miranda CS, Santana-Oliveira DA, Vasques-Monteiro IL, Silva-Veiga FM, and Souza-Mello V designed and coordinated the study, and interpreted the data; Miranda CS, Santana-Oliveira DA, Vasques-Monteiro IL, Dantas-Miranda NS, and Glauser JSO performed the experiments, and acquired and analyzed the data; Miranda CS, Santana-Oliveira DA, Vasques-Monteiro IL, and Silva-Veiga FM wrote the manuscript; and all authors approved the final version of the article.

Institutional animal care and use committee statement: All animal experiments conformed to the internationally accepted principles for the care and use of laboratory animals (the local Ethics Committee, Institute of Biology, State University of Rio de Janeiro, CEUA Nº 017/2021).

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

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Country/Territory of origin: Brazil

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S-Editor: Wang JJ
L-Editor: Wang TQ
P-Editor: Zhang XD

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World Journal of Methodology

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

World J Methodol 2024 March 20; 14(1): 88619

DOI: 10.5662/wjm.v14.i1.88619

ISSN 2222-0682 (online)

SYSTEMATIC REVIEWS

Duodenal Crohn's disease: Case report and systematic review

Muniratu Amadu, Jonathan Soldera

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Hariyanto TI, Indonesia; Nasa P, United Arab Emirates; Zhang F, China

Received: October 1, 2023
Peer-review started: October 1, 2023
First decision: December 11, 2023
Revised: December 16, 2023
Accepted: January 18, 2024
Article in press: January 18, 2024
Published online: March 20, 2024

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Abstract

BACKGROUND

Inflammatory bowel disease, including ulcerative colitis, microscopic colitis, and Crohn's disease (CD), has a global impact. This review focuses on duodenal CD (DCD), a rare subtype affecting the duodenum. DCD's rarity and asymptomatic nature create diagnostic challenges, impacting prognosis and patient well-being. Delayed diagnosis can worsen DCD outcomes.

AIM

To report a rare case of DCD and to discuss the diagnostic challenges and its implications on prognosis.

METHODS

A systematic literature search, following the PRISMA statement, was conducted. Relevant studies were identified and analysed using specific Medical Subject Terms (MeSH) from PubMed/MEDLINE, American Journal of Gastroenterology, and the University of South Wales database. Data collection included information from radiology scans, endoscopy procedures, biopsies, and histopathology results.

RESULTS

The review considered 8 case reports and 1 observational study, involving 44 participants diagnosed with DCD, some of whom developed complications due to delayed diagnosis. Various diagnostic methods were employed, as there is no gold standard workup for DCD. Radiology scans [magnetic resonance imaging (MRI), computed tomography (CT), and upper gastrointestinal X-ray], endoscopy procedures (colonoscopy and esophagogastroduodenoscopy), biopsies, and clinical suspicions were utilized.

CONCLUSION

This review discusses DCD diagnosis challenges and the roles of CT, MRI, and fluoroscopy. It notes their limitations and compares findings with endoscopy and histopathology studies. Further research is needed to improve diagnosis, emphasizing scan interpretation, endoscopy procedures, and biopsies, especially

in high-risk patients during routine endoscopy.

Key Words: Inflammatory bowel diseases; Crohn's disease; Duodenum; Diagnostic challenges; Prognosis

Core Tip: This systematic review explores the diagnostic challenges and implications of duodenal Crohn's disease (DCD), a rare subtype. Delayed diagnosis can worsen DCD outcomes, emphasizing the need for improved diagnostic methods. The study considers various diagnostic approaches, including radiology scans and endoscopy procedures, highlighting their limitations and the importance of biopsy and histopathology. Further research is essential to enhance DCD diagnosis, particularly in high-risk patients during routine endoscopy.

Citation: Amadu M, Soldera J. Duodenal Crohn's disease: Case report and systematic review. *World J Methodol* 2024; 14(1): 88619
URL: https://www.wjgnet.com/2222-0682/full/v14/i1/88619.htm
DOI: https://dx.doi.org/10.5662/wjm.v14.i1.88619

INTRODUCTION

Inflammatory bowel disease (IBD) comprises various chronic inflammatory gut conditions, including Crohn's disease (CD), Microscopic Colitis, and ulcerative colitis, imposing a longstanding challenge for those affected. Among these, CD and ulcerative colitis have emerged as global health concerns, impacting millions, with significant prevalence in Europe (3.2 million) and North America (2 million)[1,2].

CD is an autoimmune inflammatory condition affecting the gastrointestinal tract, presenting inflammation from the mouth to the anus, with the ileum and colon being commonly affected[3]. Distinct types of CD include ileocolitis, ileitis (ileum inflammation), gastroduodenal Crohn's (stomach and duodenum inflammation), jejunoileitis (jejunum inflammation), and Crohn's colitis (granulomatous). It can manifest from childhood to adulthood, affecting both genders equally[4]. The pathogenesis involves gene susceptibility, immune system vulnerability, environmental factors, and microbiome alterations, disrupting intestinal mucosa[5].

Despite variations in reported figures, the exact prevalence and incidence of DCD worldwide remain uncertain[6]. Estimates reach millions globally, with the United Kingdom showing a prevalence of 10.6 per 100000[7]. In the United States and Europe, prevalence ranges from 1.6 million to 3 million, predominantly affecting younger individuals[8]. Incidence patterns vary across regions and age groups. China reports a peak incidence at 32.3 years, while Western studies identify peaks between 20-39 years and 50-79 years, with Asian studies displaying different patterns[9,10]. Factors contributing to these disparities, such as smoking initiation age and infection sensitivity, are still unclear[10,11].

The duodenum, situated between the stomach and the jejunum, marks the initial section of the small intestine. Duodenal Crohn's disease (DCD), though rare, carries the potential for significant complications if not promptly identified. First documented by Gottlieb *et al*[12] in 1937[13], DCD's prevalence is estimated at 0.5% to 4% among DCD patients[14], constituting less than 0.07% of all CD cases[15,16]. However, these figures may underestimate the actual occurrence due to the asymptomatic nature of many cases and the absence of routine endoscopy in initial evaluations[17].

While some DCD cases remain asymptomatic, symptomatic presentations also occur, either concurrently or subsequent to related bowel symptoms[17]. Symptoms of DCD encompass weight loss, early satiation, nausea, occasional vomiting, dyspepsia, and anorexia. Epigastric pain, typically postprandial and non-radiating, often responds to antacids and specific foods and is the most frequently reported symptom[16,18]. Rarely, melena and haematemesis are observed, and chronic anaemia may result from upper gastrointestinal bleeding[19]. Lossing *et al*[20] study noted abdominal cramp pain and diarrhoea as common presenting complaints among DCD patients, with some displaying additional symptoms like haematemesis, postprandial vomiting, epigastric pain, and upper intestinal bloating, especially in those with pre-existing intestinal disease.

DCD is associated with various complications discussed in multiple articles, impacting patients' health. These complications include strictures causing gastric outlet obstruction, acute or chronic pancreatitis, and stenosis leading to obstruction. Fistulas, such as duodenopancreatic, duodenobiliary, duodenocolic, and duodenocutaneous, may develop in active or inactive DCD regions[21]. The diverse complications contribute to variations in patient symptoms, posing challenges for diagnosis. Differential diagnoses for DCD encompass peptic ulcer, pancreatic cancer, lymphoma, pancreatitis, and carcinoma. The complexity of diagnosing DCD can be attributed to its variable presentation, subtle nature, lack of a definitive diagnostic standard, and propensity to remain asymptomatic.

Additionally, DCD poses multifaceted challenges affecting various aspects of patients' lives, including dietary, financial, physical, psychological, sexual, and social dimensions, ultimately impacting their overall quality of life[22]. Physically, patients grapple with unattractiveness, debilitating cramp-like pain, urgency, increased bowel movements, fatigue, and sleep disruptions. Excessive flatulence is also a distressing symptom reported by patients[23].

Furthermore, DCD can trigger feelings of isolation and depression, stemming from a lack of understanding and support from others, making it challenging for patients to discuss their condition, especially with those less familiar or

misunderstood by their family and friends[24]. Socially, some patients may withdraw from social gatherings, offering excuses to avoid specific foods and frequent restroom trips. Their interests and activities may need adjustment due to the disease's limitations[23]. Moreover, DCD-related complications significantly worsen patients' quality of life, imposing a substantial financial burden on both patients and the healthcare system[25].

Patients often modify their diets post-diagnosis in an attempt to extend remission periods and alleviate symptoms, but this can inadvertently result in adopting restrictive diets, leading to malnutrition and diminished quality of life. Foods commonly avoided include spicy items, alcoholic beverages, dairy products, and fried foods. Some of these restricted foods contain essential nutrients like calcium, protein, vitamins, and minerals necessary for bodily functions. Historically, patients have regarded food as playing a pivotal role in managing IBD symptoms, akin to medication[26,27]. Furthermore, Limdi *et al*[28] reported that approximately two-thirds of patients are eager to receive dietary advice, with half having never received such guidance.

Research indicates that IBD conditions, including DCD, decrease sexual activity frequency in patients experiencing inadequate disease control, thereby affecting their overall quality of life. Some patients may perceive themselves as less attractive, impacting their desirability. Rates of sexual dysfunction in this chronic disease surpass those in the general population[29]. This issue may manifest before diagnosis and worsen as the disease progresses, with multiple factors contributing, including disease activity, surgery due to complications, and psychosocial and biological factors[30]. Some authors argue that patients with this chronic condition and the general population exhibit similar sexual activities but with lower sexual satisfaction.

Diagnosing DCD is a complex process due to variations in presentation, subtle symptoms, lack of a definitive diagnostic standard, and its asymptomatic nature. The asymptomatic nature can cause delays in diagnosis and low clinical suspicion[31]. Diagnostic delays are also linked to vague symptoms and diagnostic challenges[32]. A comprehensive approach involving various diagnostic methods is necessary for accurate diagnosis[33]. The European Crohn's and Colitis Organisation [ECCO] recommends endoscopy, radiology evaluation, clinical suspicion, and histology for diagnosing DCD, even without colonoscopy findings[17]. Biopsy often reveals granulomas, mucosal erosion, and active inflammation[34]. The choice of imaging modality varies, with some preferring computer tomography initially and others upper gastrointestinal X-rays. Computer tomography enterography (CTE) and Magnetic resonance enterography (MRE) provide valuable information, with MRE being advantageous due to its radiation-free nature[35]. Endoscopy is a relevant standard for a definite diagnosis[35].

Timely and accurate diagnosis is crucial. DCD diagnosis can be delayed for weeks, months, or even years, leading to a poor prognosis. However, DCD generally has a good prognosis[24]. Further research and awareness are needed to improve understanding and support for DCD patients.

This systematic review aims to report a rare case of DCD and to discuss the diagnostic challenges and its implications on prognosis.

Case report

A 53-year-old female patient presented with upper abdominal pain. She had a previous diagnosis of long-term serum-negative peripheral symmetric arthritis and was undergoing daily treatment with leflunomide, along with dipirone on an as-needed basis, effectively controlling the disease. The patient denied the use of non-steroidal anti-inflammatory drugs. An upper digestive endoscopy revealed small ulcers in the second portion of the duodenum. Biopsies yielded non-specific results, and testing for Helicobacter pylori was negative. Immunohistochemistry for cytomegalovirus and herpes virus, as well as special colorations for fungi, acid-resistant bacilli, and PAS-positive pathogens, all returned negative results. Laboratory data showed an elevated C-reactive protein level of 2.7 mg/L, gastrin at 10 pg/mL, erythrocyte sedimentation rate at 18 mm/h, and negative results for ASCA, ANCA, and syphilis.

Despite initiating proton pump inhibitors, there was no improvement in symptoms, and a subsequent upper digestive endoscopy confirmed the persistence of the duodenal lesions (Figure 1). On the same day, a colonoscopy was performed, revealing aphthous ulcers in the terminal ileum (Figure 1). Enteric magnetic resonance imaging (MRI) indicated mild enteritis in the terminal ileum. With a diagnosis of DCD, azathioprine treatment was initiated but did not lead to endoscopic improvement within 6 months. Due to worsening arthritis, azathioprine was replaced with methotrexate, and adalimumab was introduced, resulting in complete healing of the ulcers (Figure 2). After 24 months of continuous adalimumab and methotrexate use, the patient remains in remission for both arthritis and DCD.

MATERIALS AND METHODS

Method

This systematic review strictly adhered to the guidelines provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 checklist[36].

Eligible studies

Included in this review were studies that met the following criteria: participants diagnosed with DCD within healthcare settings and published in English. The study designs considered encompassed observational and retrospective studies that specifically focused on the diagnosis and prognosis of DCD. Studies falling under systematic reviews, randomized control trials, editorials, and meta-analyses were excluded.

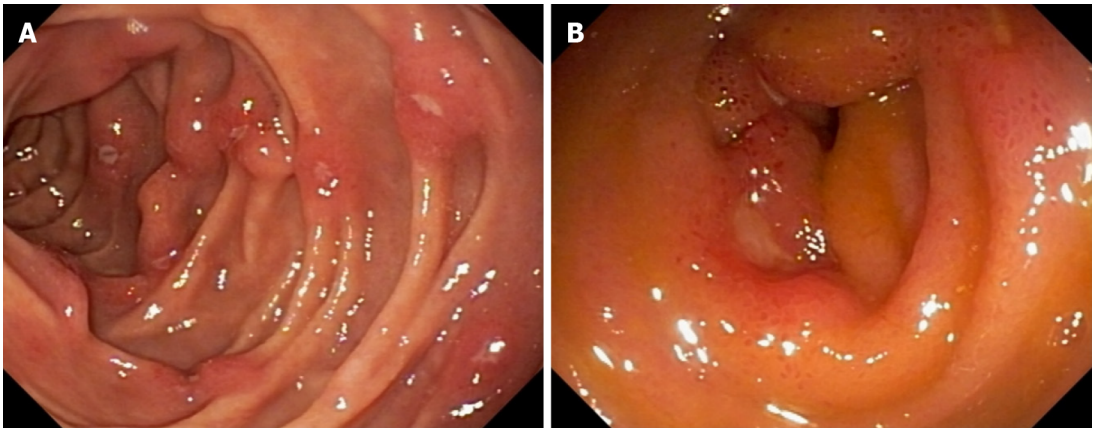

Figure 1 Endoscopy and colonoscopy. A: Upper digestive endoscopy, duodenum, second portion. Small aphthous ulcers, diffuse in the involved region; B: Colonoscopy, terminal ileum. Aphthous ulcers in involved region.

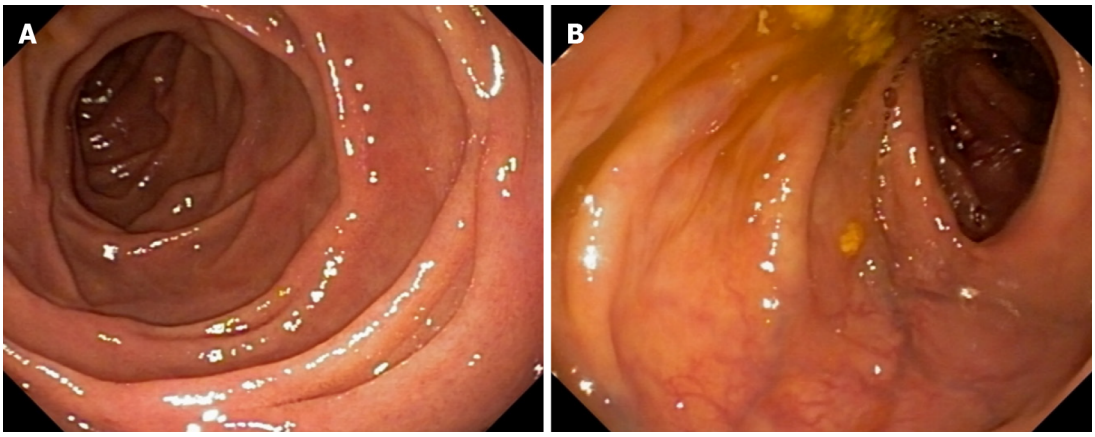

Figure 2 Endoscopy and colonoscopy. A: Upper digestive endoscopy, duodenum, second portion; B: Colonoscopy, terminal ileum. Normal mucosa after six months of methotrexate and adalimumab therapy.

Study selection and search strategy

The initial search was performed on Google, followed by subsequent searches on PubMed/MEDLINE, American Journal of Gastroenterology, and the University of South Wales database using the search terms "duodenal crohn's disease"[tiab:~0]. The study selection process involved three phases. Phase 1 involved searching titles and screening abstracts. Phase 2 entailed obtaining full-text articles for meticulous examination based on the selected abstracts. In phase 3, relevant papers were chosen for further review and data collection.

Inclusion and exclusion criteria

All selected studies included adult patients diagnosed with DCD who had undergone investigations within a healthcare setting. There were no restrictions on the publication timeframe or year, considering the limited research available on this specific area of review. Papers that matched the search terms and criteria were included, while those related to complications, surgery, treatment, management, and other types of DCD were excluded. The geographic location of the research and publication was not a limiting factor, as DCD is a global concern.

Limitations and additions

This review can provide valuable insights into selecting the appropriate investigation process or imaging for DCD cases. However, discrepancies in the results of these investigations may lead to challenges in definitively confirming whether a presented case is indeed DCD or another differential disease. As with any systematic review, the limitations of available data and potential bias in the included studies must also be considered.

RESULTS

A total of 2046 studies were retrieved, and following a review of titles and abstracts, 1022 studies were excluded. Among the remaining studies related to DCD, 1024 were identified for further analysis. Subsequent examination led to the

selection of 50 full-length papers. After careful scrutiny, 9 relevant studies were identified, with a combined sample size of 44 participants diagnosed with DCD. These chosen studies were included in the review for comprehensive reading and analysis. The search strategy is illustrated in Figure 3, and detailed information on the selected studies is presented in Table 1.

Concerning the participants, their ages ranged from 25 to late 70s, with the majority consisting of 30 males and 14 females. Among these participants, 8 were observed individually at various healthcare facilities in different countries and time periods, while retrospective data extraction from medical files was conducted for 36 participants.

All 9 studies included in this review exclusively focused on DCD. Notably, the work conducted by Plerhoples *et al*[37] stood out. The participant in this study exhibited symptoms persisting for over 25 years, leading to diverse diagnoses during each visit to healthcare facilities. Initially diagnosed with celiac sprue, the participant was subsequently diagnosed with follicular hyperplasia, despite presenting with consistent symptoms throughout the duration.

Among the 44 participants, 11 had a prior history of DCD affecting other parts of the digestive system, occurring between 4 to 40 years before the involvement of the duodenum. While one of the studies specifically reported dyspepsia as a symptom of DCD, none of the studies employed a standardized scale, such as the Crohn's Disease Activity Index, to assess the severity of DCD.

DISCUSSION

The studies employed various investigative methods to diagnose DCD, with esophagogastroduodenoscopy (EGD) and biopsy commonly utilized. Furthermore, X-ray, MRI, blood tests, and computer tomography (CT) were used as diagnostic tools to facilitate the identification of DCD. The following paragraphs provide detailed findings obtained through these investigative approaches.

X-ray

Among the studies reviewed, five included X-ray examinations as part of the initial assessment. Song *et al*[38] and Ehwarieme *et al*[39] reported normal chest X-ray findings in their patients. In contrast, Nugent *et al*[40] identified a duodenal stricture in the upper gastrointestinal (GI) X-ray, similar to Odashima *et al*[41]. Plerhoples *et al*[37] reported findings of mild inflammation and delayed proximal duodenal and gastric emptying during upper GI fluoroscopy. A summary of these results is provided in Table 2.

Although chest X-rays are not typically employed for diagnosing DCD, research indicates that nearly half of DCD patients may exhibit subclinical alterations, suggesting underlying bronchial inflammation[42,43]. Importantly, this subclinical inflammation in DCD appears unrelated to its asymptomatic nature[44]. During DCD exacerbations, pulmonary function abnormalities and reduced diffusing capacity may lead to pulmonary inflammation, possibly correlated with small bowel inflammation[45].

Fluoroscopy plays a crucial role in assessing mucosal disease, peristalsis abnormalities, and postoperative complications like extraluminal leaks and obstructions. It allows real-time visualization of duodenal motility and mucosa[46]. Fluoroscopy can be conducted *via* small bowel follow-through (SBFT), which involves oral ingestion of a barium solution, with the progression monitored during the examination to record multiple images. The presence of intestinal strictures and peristalsis can influence the examination's success[47]. Alternatively, enteroclysis administers a water contrast solution with or without methylcellulose through a nasoduodenal tube.

Historically, SBFT and small bowel enteroclysis (SBE) have been the standard investigative methods for suspected or confirmed DCD. However, controversies exist regarding the appropriateness and accuracy of these procedures in diagnosing DCD. Ott *et al*'s prospective study indicated that patients prefer fluoroscopy due to its safety compared to SBE, which is also less likely to miss gastroduodenal disease[48]. Conversely, Bernstein *et al*[49] suggested that SBE is more effective in detecting early mucosal lesions than fluoroscopy. Notably, SBE demonstrates high accuracy in diagnosing small bowel diseases, with reported sensitivity of 100% and specificity of 98.3%[49]. This was reaffirmed by Panes *et al*[51] with a sensitivity of 95% and specificity of 96.5%, whereas SBFT reported sensitivity ranging from 67% to 72%[51,52]. However, Wills *et al*[53] and Maglinte *et al*[50] argue that both SBE and fluoroscopy provide limited and varied information regarding the bowel wall and extraluminal extension of DCD.

In conclusion, upper GI X-rays can visualize duodenal abnormalities like strictures and inflammation, and existing literature has explored their utility in diagnosing DCD with positive outcomes. Nevertheless, further research is necessary to determine the effectiveness of chest X-rays in diagnosing DCD and its complications. Additionally, SBFT may have limitations in visualizing the intestine and its structures, while SBE's drawback lies in providing limited information at the disease onset and regarding extraintestinal involvement[50,52].

CT

CT scans are crucial in diagnosing DCD. They reveal various findings, such as normal results[54,55], a distended stomach leading to the proximal duodenum, diffuse duodenal distention, and duodenojejunal junction narrowing[56], an antral mass suggesting malignancy[40], and marked duodenal wall thickness[41]. Conventional CT, with or without contrast, is used for previously unknown Crohn's cases or acute complications like perforation and abscess[57,58]. Table 3 contains the CT findings from the aforementioned studies. Accurate evaluation of DCD on CT scan requires the administration of enteric contrast before the examination and a fasting period of 4-6 h[57,58]. Enteric contrast can be administered orally or *via* a nasoenteric tube. On the other hand, CTE enhances visualization by distending the small bowel, making it increasingly preferred as the first-line imaging modality for patients with existing DCD and those with suspected DCD

Table 1 Information from the studies reviewed

Ref.	Study population	Study design	Presentation/investigation	Result	Treatment
Song *et al* [38], 2016	A single case	Observational study (case report)	Presentation: Constant epigastric soreness with a month of dyspepsia. Investigations: EGD, colonoscopy, physical examination, biopsy, histology, chest X-ray, blood works and vital signs checks	Vital signs were normal. Physical examination showed no epigastric tenderness. Chest X-ray was unremarkable. Blood works showed no alarming result. Colonoscopy showed unremarkable terminal ileum and entire colon. EGD showed multiple progressive ulcers and erosion in the duodenal bulb and second portion of the duodenum with mucosal oedematous. Biopsy and histology showed ulceration of the duodenal bulb indicating erosion of the infiltration of inflammatory cell	20 mg of prednisolone was given for two week and reduced to 10 mg for 1 wk and reduced to 5 mg after a week to take for a week
Lightner *et al*[68], 2018	A single patient	Observational study (case report)	Presentation: Early satiety, distention, recent weight loss, nausea, microcytic anaemia, and vomiting. Investigations are: MRE, biopsy, EGD and histology	MRE showed high grade stricture in the distal portion of the abnormal segment resulting in dilatation of both the stomach and the second part of the duodenum. EGD showed ulceration with stenosis in D2 and stricture in D3 and oedema in the surrounding the area which confirmed the finding of MRE to be highly suspicious of duodenal Crohn's disease. Histology confirmed the suspicion of EGD	Laparoscopic gastroduodenal bypass with ongoing proton pump inhibitor
Ashraf *et al* [56], 2022	A single case	Observational study (case reports)	Presentation: 6 mo of 60 pounds weight loss, intractable nausea, mild epigastric pain, and vomiting. Investigations: CT, biopsies and EGD	CT result showed distention of the stomach with tapering into the proximal duodenum followed by a marked diffuse distention of the rest of the duodenum and narrowing of the duodenojejunal junction. Biopsy showed duodenitis consistence with duodenal Crohn's disease. EGD showed stricture at proximal of the duodenum, distended duodenum, and stricture at the duodenojejunal junction coupled with severe stenosis and inflamed mucosa	PEG J tube was inserted for feeding but the patient did not tolerate. A duodenal resection was done
Plerhoples *et al*[37], 2012	A single case	Observation (case reports)	Presentation: 25 yr of intermittent nausea, precipitous with loss of 50 pound in last 7 mo, bloating and vomiting. Investigations: EGD, colonoscopy, biopsy, fluoroscopy, and exploratory laparotomy	EGD showed ulcerating inflammation at the distal duodenum associated with stricture, dilation in the oesophagus and pyloric lumina and retained food in the proximal megaduodenum and antrum. Biopsy showed active acute inflammation. Fluoroscopy showed mild inflammation and delayed proximal duodenal and gastric emptying	Exploratory laparotomy with mobilization and duodenal bypass using antecolic roux-en-Y duodeno-jejunostomy bypas
Helms *et al* [55], 2016	A single case	Observational study (case report)	Presentation: Unintentional weight loss, abdominal pain, bright red blood in stool, vomiting and diarrhoea. Investigations: CT, colonoscopy, biopsy, EGD, and upper endoscopy	EDG showed persistence duodenal Crohn's. Unremarkable CT. Colonoscopy showed ileocecal valve stenosis preventing intubation. Upper endoscopy showed partially gastric outlet obstruction, duodenitis couple with edematous stenosis. Biopsy showed duodinitis with granulating tissue and ulceration	Protonix, Amoxil and Biaxin were used to treat *H. pylori*. Asacol was used to treat Crohn's flare up
Ehwarieme *et al*[39], 2015	A single case	Observational study (case report)	Presentation: Intermittent epigastric pain, post prandial nausea and vomiting for more than 9 yr and unintentional weight loss. Investigation: Chest X-ray, colonoscopy, endoscopic ultrasound, abdominal examination, biopsy, CT and upper endoscopy	CT showed antral mass which suggest malignancy. Initial endoscopy showed atypical cell. Chest X-ray is normal. Colonoscopy is unremarkable. Endoscopic ultrasound. Showed diffused thickening of the antral wall. Upper endoscopy showed oedematous, abnormal granular, ulcerated mucosa, friable affecting the duodenal bulb, pylorus, second part of the duodenum, antrum, pre pyloric area accompanied by a mild gastric outlet obstruction. Biopsy showed multinucleated cell and granulating tissue in addition to chronic inflammation	Patient was treated with proton pump inhibitor, prednisolone and sucralfate
Odashima	A single	Observational	Presentation: Abdominal pain,	CT showed thickness of the duodenum	Patient was treated

et al[41], 2006	case	study (case report)	abdominal distention, vomiting and nausea. Investigation: CT, upper GI X-ray, endoscopy and biopsy	wall. Upper GI X-ray showed duodenal stricture. Endoscopy revealed mucosal oedema, large ulceration and stricture in the duodenal bulb	with inflixamab
Nugent *et al* [14], 1977	36 patients	Retrospective study (case report)	Presentation: Abdominal pain, abdominal distention, vomiting and nausea significant weight loss. Investigation: Endoscopy, pathology features, radilogy features and abdominal examination	Endoscopy features showed superficial ulceration in duodenum and antrum with granular appearance in addition to stenosis. Radiology feature showed irregular thickness, oedema and cobblestone pattern of the mucosa. Pathology findings showed mesentric lymph node enlargement with oedema and thickness. Duodenal wall fibrosis with chronic inflammation was noted	Vagotomy, gastroenterostomy amd Billroth
Karateke *et al*[54], 2013	A single case	Observational study (case report)	Presentation: 6 mo of abdominal pain with progressive nausea, weight loss and bilious emesis. Investigation: Physical examination, CT routine blood work, biopsy, EGD and colonoscopy	Blood works showed mild normocytic anaemia. CT and colonoscopy are normal. Physical examination shows fulness and slight tenderness in the epigastric region. EGD showed tight stricture and oedema in the mucosal, long ulceration of the duodenal bulb and nearly complete obstruction. Biopsy showed cryptitis and severe inflammation, mixed chronic inflammatory infiltration of the laminal propria evidencing duodenal Crohn's disease	Gastrojejunostomy without vagotmoy and subsequent proton pump inhibitor

CT: Computer tomography; EGD: Esophagogastroduodenoscopy; GI: Gastrointestinal; MRE: Magnetic resonance enterography.

Table 2 X-rays used by the studies in the review and their results

Type of X-ray	Number of studies	Results
Chest X-ray	2	Unremarkable
Upper GI X-ray	2	Duodenal stricture
Upper GI fluoroscopy	1	Delayed emptying in both proximal duodenal and gastric

GI: Gastrointestinal.

Table 3 Computed tomography features reported in the review

Features	Number of studies
Distention of stomach and duodenum including narrowing of the duodenojejunal junction	1
Antral mass	1
Wall thickness	1
Nothing unusual found	2

[59].

In a study of non-neoplastic duodenal diseases, CT revealed findings such as perforation of the duodenum, thickening, hyperemia of the wall, and fibrotic bulbar stenosis[60]. CT can also detect bowel wall thickening, strictures, free fluid, fistulas, bowel obstruction, and abscesses, consistent with magnetic resonance enterography[61]. CT images can be reconstructed three-dimensionally, facilitating the assessment of mucosal abnormalities, extraintestinal complications, bowel wall thickening, and intestinal loops. Multidetector CT is now a preferred and readily available modality for evaluating peri-duodenal and duodenal abnormalities, offering less invasiveness than traditional barium studies[60].

CTE accurately assesses small bowel damage, including the duodenum, with a sensitivity of 77.8% and specificity of 86.8% for detecting fistulas compared to surgery findings[62]. It has identified penetrating disease in Crohn's patients, highlighting the limitation of relying solely on clinical symptoms[63,64]. CTE is also useful in detecting asymptomatic stenosis associated with small bowel Crohn's, with a specificity of 38.9% and sensitivity of 92.3%[62]. However, CTE may struggle to differentiate intramural conditions and duodenal wall layers[60].

While multidetector CT is highly sensitive and specific in detecting DCD, it may face challenges in differentiating intramural conditions and duodenal wall layers[60]. Inflammatory processes in the duodenum are rarely diagnosed using CT due to nonspecific findings like luminal dilation, peri-duodenal fat stranding, and duodenal wall thickening.

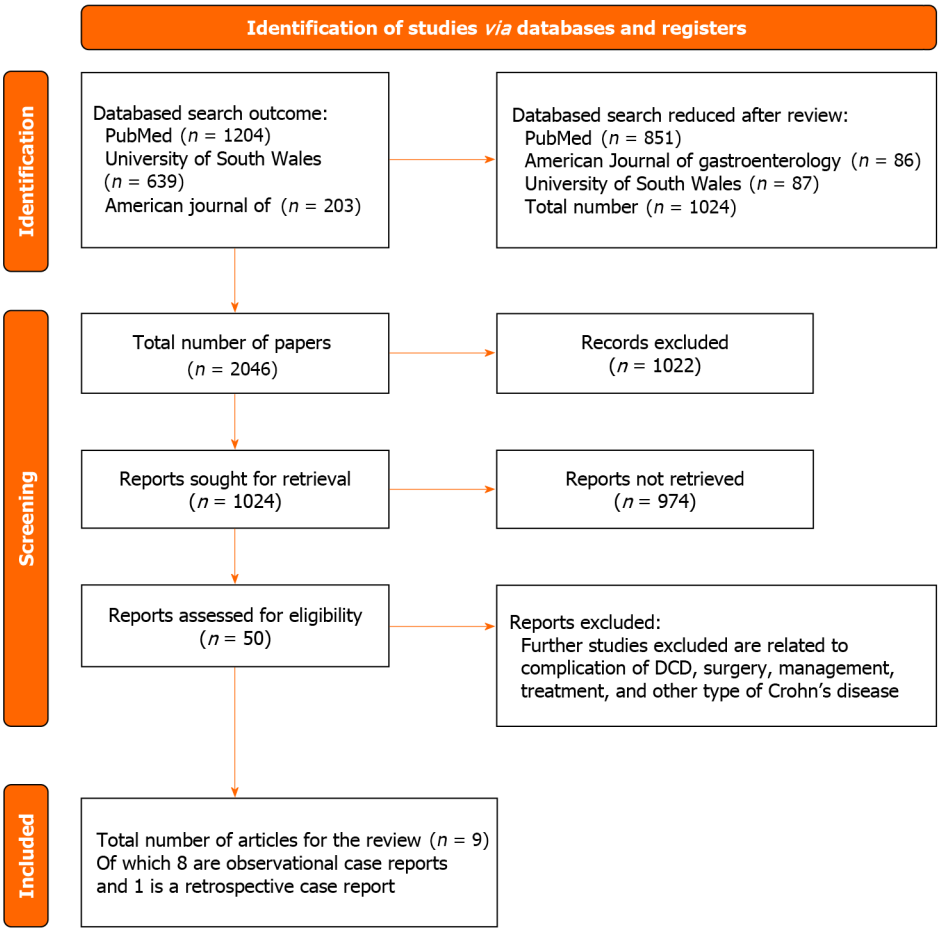

Figure 3 PRISMA- P protocol flow chart for systematic review. DCD: Crohn's disease.

CT and CTE have the disadvantage of exposing patients to radiation, which can be harmful, especially in younger and elderly patients. This risk is amplified when patients require frequent CT or CTE for follow-up examinations, potentially increasing the risk of GI cancer[65,66]. The ECCO consensus suggests considering radiation exposure when choosing scanning techniques for Crohn's patients and recommends MRI as the preferable scan during follow-up[67]. The ECCO consensus also highlights the value of CT in diagnosing acute DCD complications, such as abscesses and obstructions, making it a definitive choice for managing such complications.

In conclusion, CT and CTE are essential in diagnosing DCD and assessing duodenal and small bowel abnormalities. However, their limitations in differentiating intramural conditions and potential radiation exposure risk should guide personalized imaging choices for Crohn's patients. Further research is necessary to enhance the accuracy and specificity of CT-based DCD diagnosis.

MRI

MRI has been investigated for its utility in diagnosing DCD in a limited number of studies. Lightner *et al*[68] conducted MRE as part of their investigations and reported that MRE revealed a high-grade stricture in the distal portion of the abnormal segment, resulting in duodenal and stomach dilation. MRE is valued for its impartial and comprehensive evaluation of the intestine[63,69]. These findings align with a study by Tsai *et al*[70], which identified features like dilation, strictures, and abnormal segments in their MRE, consistent with standard DCD features.

While many of the selected studies did not utilize MRE/MRI, other relevant research by Ram *et al*[71], Gourtsoyiannis *et al*[72], and Sinha *et al*[73] explored the relevance of MRE in DCD investigations. Ram *et al*[71] observed small aphthous ulcers and deep transmural ulcers in the bowel wall, corresponding with Sinha *et al*[73], who linked deep ulceration to cobblestone-like mucosa and the potential for fistula and disease penetration. Additionally, Sinha *et al*[73] and Gourtsoyiannis *et al*[72] reported findings such as intestinal ulcers, bowel wall thickness, and lymph node enhancement, indicating active DCD.

MRE is a radiation-free technique gaining popularity as the preferred choice for evaluating inflammation in DCD, especially in younger and older patients[67]. It excels in detecting penetrating DCD complications, with a reported sensitivity and specificity exceeding 93% for small bowel CD diagnosis[74,75].

However, MRE's sensitivity and specificity in detecting strictures are reported to be suboptimal. While it may identify strictures in symptomatic patients, it can miss partial or incipient strictures[76]. This limitation may stem from enterography techniques that provide insufficient bowel distention to highlight partial or early-stage strictures. In terms of penetrating and fistulizing disease, MRE is reported to have high specificity (100%) and varying sensitivity (83.3% to

84.4%)[77]. Nevertheless, partial volume averaging effects can lead to the overlooking of small interloop fistulas on MRE. Patients with DCD often face challenges in drinking and retaining contrast for MRE scans, potentially resulting in inadequate bowel distention and false appearances of bowel wall thickening and superficial enhancement[71].

In conclusion, further research is necessary to establish the role and effectiveness of MRI, particularly MRE, in assessing duodenal abnormalities associated with DCD.

Endoscopic studies

Endoscopy, encompassing colonoscopy and EGD, is integral in the diagnosis and management of duodenal DCD and other IBD. The studies under analysis underscore the efficacy of combining endoscopy and biopsy as a diagnostic approach for DCD[4,19].

Colonoscopy

Song *et al*[38] reported unremarkable findings in the terminal ileum and entire colon during colonoscopy. Similarly, Karateke *et al*[54] and Plerhoples *et al*[37] documented normal colonoscopy outcomes in their patients. Conversely, Helms *et al*[55] encountered ileocecal valve stenosis hindering colonoscopy intubation. Odashima *et al*[41] noted a stricture with extensive ulceration and mucosal edema in the duodenal bulb during endoscopy. Nugent *et al*[40] observed diffuse granularity with nodularity, varying stenosis degrees, and superficial ulceration in the duodenum and antrum, obstructing duodenal and pyloric canal traversal in 17 out of 36 participants. Helms *et al*[55] described inflammation and ulceration with stricture in the distal duodenum during endoscopy. Ehwarieme *et al*[39] reported oedematous, granular, ulcerated mucosa with mild gastric outlet obstruction in upper endoscopy.

Despite varied findings, these studies collectively underscore the diagnostic complexity of DCD. The recommendation is to employ endoscopy in conjunction with biopsy as an effective diagnostic approach[4,19]. Endoscopic features of DCD encompass friable mucosa, irregular erythema, aphthous ulcers, gastric outlet narrowing, and mucosal thickness[19,78]. Duodenal manifestations may include polypoid lesions, a cobblestone appearance, and Kerckring's folds, considered pathognomonic[79]. Serpiginous or linear ulcers distinguish DCD ulcers from peptic ulcers[80]. Graca-Pakulsa *et al*[81] reported a higher likelihood of duodenal abnormalities in DCD patients through endoscopy, including aphthous lesions, duodenal bulb deformation, duodenal ulceration, and mucosal swelling.

In summary, colonoscopy, coupled with biopsy, remains a valuable diagnostic tool for identifying and managing ileal CD. Nonetheless, the variability in endoscopic findings across studies underscores the need for further research and standardization in DCD diagnosis.

EGD

EGD, featured in 5 of 9 reviewed studies, proved valuable in diagnosing DCD. Lightner *et al*[68] identified ulceration with stenosis in D2, stricture in D3, and surrounding edema, aligning with MRE findings. Song *et al*[38] reported multiple duodenal bulb ulcers, gastric erosion, and fundal hemorrhage during initial EGD, with progressive ulcers and erosions in follow-up. Karateke *et al*[54] noted a tight duodenal stricture, mucosal edema, and extensive ulceration. Ashraf *et al*[56] found duodenal distention, strictures, mucosal inflammation, and severe stenosis. Plerhoples *et al*[37] documented ulcerating inflammation, strictures, dilation, and food retention. Helms *et al*[55] confirmed persistent DCD during EGD.

A two-decade-old prospective study found abnormalities like mucosal thickening, ulcers, and aphthoid erosion in 56% of Crohn's patients undergoing EGD[82]. Kefalas *et al*[18] associated granuloma presence on EGD with existing endoscopic abnormalities. Other Crohn's EGD findings include erythematous mucosa, fistulas, thickened folds, erosions, bamboo joint-like stomach appearance, cobblestone appearance, villous patterns, nodular lymphoid hyperplasia, and notch-like or longitudinal protrusions in the second part of the duodenum[21,83-85]. Notably, the mucosal DCD feature on EGD is non-specific, but a notch-like or longitudinal protrusion in the second part of the duodenum may serve as a reliable inflammatory marker[86].

In conclusion, endoscopy, encompassing colonoscopy and EGD, is pivotal for diagnosing, monitoring, and managing DCD. It facilitates visual assessment, treatment confirmation, disease activity evaluation, and complication detection. Regular endoscopic evaluation using the Simple Endoscopic Score for Crohn's Disease (SES-CD) is recommended. However, expertise is essential for interpreting DCD endoscopic findings due to potential overlap with other gastrointestinal conditions.

Biopsy and histology

Biopsy and histology serve as vital components in diagnosing and understanding DCD. Biopsy procedures aim to acquire tissue samples for pathological examination, often playing a role in surveillance or disease detection in the duodenum [87]. However, certain contraindications, including perforation, varices, bleeding, and coagulation disorders, can limit the use of duodenal biopsies[87].

Histological findings in DCD encompass various markers, including transmural inflammation, granulomas, mucosal erosion, and more. Studies reviewed revealed a range of histological observations. These findings included erosion and inflammatory cell infiltration, especially plasma cells, in the ulcerated duodenal bulb[38]. Lightner *et al*[68] identified foveolar and pyloric metaplasia, crypt abscess, and inceptive granulomas. Karateke *et al*[54] noted severe inflammation, villous blunting, mixed chronic inflammation, and cryptitis. Ashraf *et al*[56] reported duodenitis consistent with DCD in their biopsy samples. Plerhoples *et al*[37] found active acute inflammation in the biopsy from the stricture and mild inflammation in colonoscopy biopsy, along with an increase in intraepithelial lymphocytes and other histological changes. Helms *et al*[55] observed duodenitis with granulating tissue and ulceration. Ehwarieme *et al*[39] found giant Polynuclear cells and granulating tissue. Nugent *et al*[40] reported fibrosis and chronic inflammation in some

participants, with a few showing granulomas in capsule biopsies. Odashima *et al*[41] reported active chronic inflammation in the biopsy and histology of the duodenal mucosa.

Histological findings in DCD demonstrated a variety of markers, with fibrosis, chronic inflammation, and duodenitis emerging as prominent features. There is some debate about which histological features are the most reliable for diagnosing DCD. While some studies emphasize the significance of granulomas, others suggest the presence of granuloma and another feature, such as architectural abnormalities or focal inflammation, for confirming the diagnosis of DCD[88]. Discrepancies in the significance of granulomas in diagnosing DCD highlight the challenges faced by clinicians in reaching a conclusive diagnosis.

Fibrosis in DCD remains poorly understood, with emerging evidence suggesting it may result from adaptive immune responses regulated by noncoding RNA molecules[89]. Early diagnosis of fibrosis is crucial, as it currently lacks pharmaceutical treatment, making surgery or endoscopic balloon dilatation the primary options. Potential biomarkers for fibrosis require further research to develop reliable and cost-effective diagnostic tools.

Villous blunting or atrophy in the duodenum can take various forms, including fused or branched villi. Diagnosis of duodenal bulb villous atrophy can be challenging due to the short and thick villi in this region, often leading to interpretation difficulties[90].

Duodenitis is characterized by inflammatory cell infiltration, changes in crypt epithelium, and villous atrophy[91]. Other diagnostic features for DCD include focal chronic inflammation without crypt atrophy, increased intraepithelial lymphocytes, submucosal inflammation, focal cryptitis, proximal ulceration, hyperplasia, and aphthoid ulcers[92,93]. Table 4 provides a summary of the histology and biopsy findings from the reviewed studies.

Common presentation

Common presentations of DCD were reported in various studies. Epigastric pain and unintentional weight loss were observed by several studies[38-41,54-56,68]. Additionally, vomiting was reported by various studies[38-41,54-56,68]. Abdominal distention was also noted by another few studies[37,41,68]. Upper gastrointestinal bleeding was observed by two studies[40,55], while microcytic anemia[68], dyspepsia[38] and diarrhea[55] was identified by only one study[68].

These symptoms, including nausea, vomiting, epigastric pain, weight loss, gastrointestinal bleeding, and microcytic anemia, are common in DCD. They are associated with the disease's severity, particularly obstruction[15]. Some symptoms, such as gastrointestinal bleeding and microcytic anemia, are interconnected and result from chronic blood loss and impaired iron absorption due to inflammation related to DCD[94]. However, the similarity of these symptoms with those of other conditions poses challenges in diagnosing DCD. Table 5 presents the count of patients experiencing the aforementioned symptoms in the reviewed studies.

Laboratory workup

In the reviewed studies, only 2 out of the 9 investigations included blood tests as part of their initial assessment. Karateke *et al*[54] conducted blood work, revealing mild normocytic anemia in their subjects, while Song *et al*[38] reported blood work with no alarming results. Historically, serology, particularly blood tests, has been undervalued in the diagnosis of DCD due to its low specificity. However, recent developments in the field, especially the introduction of biological drugs, have prompted a reevaluation of the role of serological markers.

One of the key serological biomarkers used in assessing responses to drugs in DCD patients is C-reactive protein (CRP). Recent biologics trials often include raised CRP levels as an inclusion criterion to determine the efficacy of drugs. An increasing CRP level after drug administration is interpreted as a sign of treatment failure, whereas a decrease in CRP indicates the drug's effectiveness. Furthermore, CRP levels are used to monitor disease activity, particularly in patients with severe disease, where elevated CRP levels are more common compared to patients in mild or remission states.

A prospective study conducted by Brignola *et al*[95] investigated inflammation markers in the blood results of 41 DCD patients who were in remission for 6 months. This study found that CRP levels remained elevated after 2 years in remission patients who initially had high CRP levels. Despite efforts to include serology in the criteria for diagnosing IBD, the diagnostic benefits of serology remain limited and lack sensitivity. However, certain antibodies tested for DCD, such as perinuclear antineutrophil cytoplasmic antibody negative and anti-Saccharomyces cerevisiae antibody immunoglobulin G, or a positive immunoglobulin A, have shown a sensitivity of 55% and specificity of 93%[96].

In conclusion, blood tests, particularly serological markers like CRP, have gained importance in assessing drug responses and monitoring disease activity in DCD. However, their role in the diagnosis of IBD remains limited and less sensitive, with the use of specific antibodies showing varying levels of diagnostic accuracy.

Other diagnostic challenges

The presentation of DCD is highly variable, presenting a considerable challenge to physicians. Studies have indicated that this diversity in symptoms can lead to delayed diagnoses, ranging from a minimum of 5 months to several years[97]. Such delays can result in additional complications and irreversible damage to the small bowel over time[98], which may occur rapidly[99]. Diseases with more common symptoms are typically easier to identify and diagnose, enabling early intervention and improved prognoses. Research has shown that the risk of complications in DCD increases with delayed diagnosis, ranging from 18.2% within 90 d to 22% within a year[100].

Furthermore, observations from studies comparing DCD in the Chinese population to Western countries have revealed significant differences in disease characteristics, such as location, age of onset, extraintestinal manifestations, disease behavior, treatment approaches, and gender distribution[101].

In addition to the variability in symptoms, challenges arise from the endoscopic techniques used for DCD diagnosis. Standard upper gastrointestinal endoscopy may encounter difficulties in reaching the distal duodenum, necessitating a

Table 4 Histology results from the biopsies

Duodenal biopsy and histology	Number of cases
Chronic inflammation	18
Erosion	1
Infiltration of inflammatory cells.	1
Granulomas	4
Foveolar metaplasia	2
Duodenitis	12
Increased intraepithelial lymphocytes	1
Fibrosis	17
Villous blunting	1
Ulceration	2
Acute active inflammation	2
Polynuclear cells	1

Table 5 Symptoms of Crohn's disease in the studies reviewed

Symptoms	Cases
Epigastric pain	7
Weight loss	7
Nausea and vomiting	6
Vomiting	1
Dyspepsia	1
Abdominal distention	3
Upper GI bleeding	2
Microcytic anaemia	1
Diarrhoea	1

GI: Gastrointestinal.

greater reliance on imaging techniques. MRE and CTE are currently employed to visualize the small bowel and detect complications like fistulas and strictures[102]. However, capsule endoscopy is favored over CTE, despite the potential risk of capsule retention in patients with strictures.

Performing biopsy tissue sampling during endoscopy can also be challenging when attempting to intubate an inflamed site, further complicating the diagnostic process. Moreover, endoscopy tends to be primarily conducted in symptomatic patients, potentially overlooking a significant number of asymptomatic DCD cases.

The variations in symptoms and subtle presentation of DCD underscore the need to address these diagnostic challenges. Physicians must navigate the complexities of reaching the distal duodenum during endoscopy, with imaging modalities playing a critical role in achieving accurate diagnoses. Addressing these challenges is crucial for achieving early diagnosis and effective management of DCD, ultimately improving patient outcomes. Further research into the clinical behavior of the disease in different populations can contribute to more tailored and effective management strategies.

It is paramount to consider many differentials when DCD is suspected. For example, celiac disease[103,104], enteric neoplasms and metastasis[105,106], foreign body ingestion[107], pellagra[108], enteric infections[109-111], sprue-like enteropathy associated with the angiotensin II receptor blocker[112], extra-intestinal manifestations[113] and hemophagocytic lymphohistiocytosis[114,115].

Prognosis of DCD

Generally, the prognosis for DCD is favorable, even in patients who require surgery due to medical refractoriness. The ability to manage DCD symptoms is also generally good. However, DCD can lead to complications that may necessitate surgical or medical intervention. These complications include pancreatitis, stenosis, fistulas, and strictures. Pancreatitis

can occur due to inflammation damaging the duodenal bulb or compression of the pancreatic head, leading to fibrosis [116]. Stenosis, which occurs in about 1 in 10 DCD patients within ten years of diagnosis, results from chronic inflammation, tissue remodeling, and mesenchymal cell hypertrophy[117,118]. DCD strictures develop due to repeated submucosal injury and chronic inflammation, leading to the accumulation of extracellular components like smooth muscle cells and collagen, causing scar tissue formation and narrowing[119,120]. Strictures can put patients at risk of abscesses and fistulas, which may require surgery[121].

In cases of suspected stricture, radiologic investigation, such as CT scans, is essential to assess the severity and nature of the obstruction[122]. Another severe complication is the development of duodenal fistulas, which can lead to the leakage of duodenal contents into other body parts or organs. Duodenal fistulas are challenging to treat and have a high mortality rate, often requiring complex surgical interventions[123].

Initial management of DCD typically involves medications like 6-mercaptopurine or sulfasalazine[124]. However, the management of DCD-related ulceration frequently involves proton pump inhibitors, corticosteroids, or histamine 2 receptor antagonists. It's worth noting that previous studies[40,125] have indicated that these pharmacological treatments may be ineffective for some patients, leading to surgery due to controllable pain and complications. In fact, one-third of patients with refractory DCD end up requiring surgery[125].

Various pharmacological treatments, including Infliximab and Adalimumab, have been effective in managing complications of DCD. Infliximab has shown positive outcomes in treating fistulas and refractory DCD[126]. Studies have also indicated that Infliximab is beneficial in addressing issues like duodenal stenosis, fistulas, and ulcers[127,128]. The ACCENT I study reported a 56% success rate within a week of Infliximab therapy for gastroduodenal DCD[129]. Adalimumab is another pharmacological option, particularly effective in severe cases of gastroduodenal DCD[130,131]. A prospective study by Annunziata *et al*[132] found that 72.7% of patients treated with Adalimumab or Infliximab achieved mucosal healing within 12 wk, compared to only 12.5% with conventional therapy.

Anti-inflammatory therapy, often involving tumor necrosis factor (TNF) inhibitors, is increasingly used in acute settings to manage fibrotic strictures in DCD patients. TNF inhibitors are used alone or in combination with steroids as a first-line treatment and for maintaining DCD[119,133]. Effective therapy can help prevent or delay long-term complications associated with strictures[134,135]. Surgical procedures, including strictureplasty, resection, bypass, and endoscopic balloon dilation, are available for DCD complications. However, their efficacy varies, and further research is needed to refine their management[136].

The treatment of DCD-induced pancreatitis involves analgesia, intravenous fluid resuscitation, and nutritional support in acute stages. In chronic or severe cases, treatment may include antibiotic therapy, pancreatic necrosis drainage, and necrosectomy. Despite available management options, treating DCD remains challenging due to its heterogeneous presentations. Therapy aims for deep remission and mucosal healing, encompassing symptom relief, endoscopic improvements, and histological changes. There is limited evidence regarding patient assessments of medication tolerability before treatment initiation[137].

However, managing DCD remains intricate due to its heterogeneous presentations. Recent studies, such as one investigating the efficacy of rapamycin in Crohn's-related strictures, provide insights into potential therapeutic avenues. Rapamycin's effectiveness in upper gastrointestinal strictures but not in lower gastrointestinal tract lesions has been reported[138]. As we navigate the challenges of DCD treatment, it becomes imperative to explore emerging strategies that go beyond conventional approaches, considering location-specific treatments and tailoring interventions based on the anatomical site of involvement[139].

CONCLUSION

In conclusion, this review has focused on the challenges associated with diagnosing DCD and its profound impact on prognosis. The diagnosis of DCD necessitates a comprehensive approach that involves histopathology, endoscopy, clinical evaluation, and radiological imaging. The prognosis of DCD is intricately linked to early diagnosis and is contingent on the specific type of the disease and the extent of its complications.

Imaging, notably radiological techniques, plays a pivotal role in the identification and management of DCD by providing critical insights into the disease's location and severity. Radiologists and endoscopists should familiarize themselves with the common sites of DCD and potential areas of complications. This knowledge, coupled with histopathological and clinical findings, enhances the ability to diagnose symptomatic patients accurately. Moreover, conducting endoscopic examinations in individuals at risk of developing DCD can facilitate early detection in asymptomatic cases, ultimately leading to more favorable prognoses.

Significant delays in diagnosis can have detrimental effects on patients, affecting their well-being, quality of life, and overall disease outcomes. While there is no universally accepted gold standard for DCD workup, EGD stands out as the preferred endoscopic method for investigation. Other radiological modalities, such as CT and MRI, may be employed initially to assess small bowel damage, with considerations for radiation exposure and the adaptability of subsequent scans. Endoscopy with biopsy aids in excluding alternative diagnoses and associated complications, thereby reducing the risks of comorbidities and mortality linked to a poor prognosis.

Nonetheless, the challenges inherent in diagnosing DCD necessitate further research to comprehensively understand their implications on prognosis. Additionally, the complications, prevalence, therapeutic implications, and the interpretation of histological findings related to DCD remain subjects of ongoing investigation. A more profound comprehension of DCD's characteristics is essential for gastroenterologists to effectively differentiate it from conditions that mimic its presentation and enable timely and accurate diagnoses. As such, further research is imperative to unravel the

full significance of diagnosing and managing DCD.

ARTICLE HIGHLIGHTS

Research background

Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis, presents a global health challenge affecting millions. While duodenal CD (DCD) manifests throughout the gastrointestinal tract, duodenal DCD specifically involves the initial section of the small intestine. Despite its rarity (0.5%-4% prevalence among Crohn's patients), DCD poses significant complications, including strictures, fistulas, and varied symptoms. Diagnostic challenges arise from its asymptomatic nature, subtle presentation, and lack of a definitive standard, leading to delayed diagnosis and potential health consequences. The prevalence and incidence of DCD vary globally, with factors contributing to these disparities still unclear. Additionally, the multifaceted impact of DCD on patients' lives extends beyond physical symptoms, affecting dietary, financial, psychological, and social dimensions, highlighting the need for comprehensive research and awareness to enhance patient support.

Research motivation

This study is motivated by the need to address critical gaps in understanding and managing DCD. The rare yet impactful nature of DCD necessitates a focused exploration of its complications, varied presentations, and their implications on patients' quality of life. The diagnostic challenges associated with DCD demand attention to improve clinical suspicion, reduce delays in diagnosis, and enhance prognosis. By investigating the multifaceted challenges patients face, including the physical, psychological, and social dimensions, this research seeks to contribute valuable insights for future studies in IBD research. Understanding the significance of dietary modifications and their impact on malnutrition, as well as the complexities of sexual dysfunction in IBD patients, can inform tailored interventions and improve overall patient care.

Research objectives

The primary objectives of this systematic review include reporting a rare case of DCD, elucidating the diagnostic challenges associated with the condition, and exploring its implications on prognosis. The study aims to provide a comprehensive overview of DCD's clinical manifestations, emphasizing the necessity for timely and accurate diagnosis. Realizing these objectives will contribute to enhancing awareness and support for DCD patients, paving the way for future research addressing critical gaps in understanding the global prevalence, diagnostic standards, and comprehensive management strategies for this rare but impactful facet of IBD.

Research methods

The research employed a rigorous methodology, adhering to the PRISMA 2020 guidelines, ensuring transparency in its systematic review. Utilizing Google, PubMed/MEDLINE, American Journal of Gastroenterology, and the University of South Wales database, a focused search with the term "duodenal Crohn's disease" emphasized the specificity of investigation. The three-phase study selection involved meticulous screening of titles and abstracts, followed by a thorough examination of full-text articles based on selected abstracts. Eligible studies, comprising observational and retrospective research in healthcare settings, addressed the diagnosis and prognosis of DCD in adults. Exclusion criteria targeted systematic reviews, randomized control trials, editorials, and meta-analyses, along with studies on complications, surgery, treatment, management, and other DCD types. No restrictions on publication timeframe or geographic location were imposed, acknowledging DCD as a global concern. The review recognizes limitations, including potential discrepancies in investigation results impacting definitive DCD confirmation and inherent biases within available data.

Research results

The systematic review identified and scrutinized 9 relevant studies encompassing 44 participants diagnosed with DCD. From an initial pool of 2046 studies, careful screening led to the exclusion of 1022, with 50 full-length papers selected for further analysis. The participant ages ranged from 25 to late 70s, predominantly comprising 30 males and 14 females. Remarkably, Plerhoples *et al*'s work highlighted a case with persistent symptoms spanning over 25 years, undergoing diverse diagnoses, initially as celiac sprue and later as follicular hyperplasia. Of the 44 participants, 11 had a history of DCD affecting other digestive system parts, occurring 4 to 40 years prior to duodenal involvement. Notably, none of the studies utilized standardized scales, like the Crohn's Disease Activity Index, to assess DCD severity, indicating a gap in consistent measurement. While shedding light on the clinical aspects of DCD, these findings underscore the need for standardized assessment tools and further exploration of the condition's long-term impact and diagnostic challenges.

Research conclusions

This study proposes novel insights into the diagnostic approaches for DCD. The research underscores the effectiveness of upper gastrointestinal X-rays, computed tomography, magnetic resonance imaging, endoscopy, and histology in diagnosing DCD. The study critically evaluates the strengths and limitations of each method, highlighting the need for personalized imaging choices. The significance of endoscopic techniques, particularly EGD, is underscored, emphasizing its combination with biopsy for effective DCD diagnosis and acknowledges the evolving diagnostic landscape, considering the influence of biological drugs on serology's reevaluation in DCD diagnosis.

Research perspectives

The direction of future research should focus on enhancing the diagnostic accuracy and specificity of investigative methods for DCD. Prospective research could delve deeper into histological features, unraveling the significance of granulomas, fibrosis, and villous blunting. Further research should explore emerging strategies beyond conventional approaches, considering anatomical site-specific interventions and the potential role of rapamycin in managing strictures. Overall, future research should aim for a comprehensive understanding of DCD characteristics, encompassing prevalence, complications, therapeutic implications, and histological interpretations. This knowledge is vital for gastroenterologists to accurately diagnose and manage DCD, ultimately improving patient outcomes.

ACKNOWLEDGEMENTS

We would like to extend our sincere appreciation to the Acute Medicine MSc program at the University of South Wales for their invaluable assistance in our work. We acknowledge and commend the University of South Wales for their commitment to providing advanced problem-solving skills and life-long learning opportunities for healthcare professionals.

FOOTNOTES

Author contributions: Amadu M and Soldera J participated in the concept and design research, drafted the manuscript and contributed to data acquisition, analysis and interpretation; Soldera J contributed to study supervision; all authors contributed to critical revision of the manuscript for important intellectual content.

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.

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S-Editor: Liu JH
L-Editor: A
P-Editor: Yuan YY

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