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EDITORIAL

Impact of vitamin D on COVID-19 and other viral diseases

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Abstract

This editorial aims to elucidate the intricate relationship between vitamin D and viral pathogenesis. It explores the anticipated role of vitamin D as a modulator in the immune response against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other viral pathogens. The editorial comments are based on the review article by Engin *et al*. The potential role of vitamin D in modulating immune responses has been highlighted by several studies, suggesting that it may influence both the risk and severity of infections. Vitamin D receptors are present in immunocompetent cells, which indicates that vitamin D can potentially modulate innate and adaptive immune responses. This context is relevant in the pathophysiology of coronavirus disease 2019 (COVID-19), where the immune response to the virus can significantly impact the disease progression and outcome. The immunomodulatory effects of vitamin D can protect against SARS-CoV-2 infection by enhancing innate and adaptive immune responses. It also maintains the integrity of the body's physical barriers and modulates inflammatory responses, thereby preventing entry and replication of the virus. Many studies have suggested that adequate vitamin D levels help alleviate morbidity and mortality associated with COVID-19. Furthermore, vitamin D supplementation has been linked with a lower risk of severe disease and mortality in COVID-19 patients, particularly in those with a deficiency during seasons with less sunlight exposure.

Key Words: COVID-19; Immunomodulation; Pandemic; Severe acute respiratory syndrome coronavirus 2; Vitamin D

Core Tip: Amidst the global health crisis, where coronavirus disease 2019 has claimed millions of lives and disrupted the very fabric of society, the quest for effective prophylactic and therapeutic strategies has become paramount. Vitamin D, a secosteroid with profound immunomodulatory effects, emerges as a molecule of consequence in this narrative. However, the scientific discourse must be unanimous, as current literature presents conflicting evidence and viewpoints reflecting the dynamic nature of scientific inquiry.

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INTRODUCTION

The editorial comments are based on the review article by Engin *et al*[1], which emphasizes role of vitamin D and its immunological influence by promoting the release of antimicrobial peptides, fine-tuning the responses of the immune system, modulating renin-angiotensin system thereby improving the morbidity and mortality in coronavirus disease 2019 (COVID-19) and other viral infections. In the relentless pursuit of understanding the intricacies of viral infections, the role of vitamin D, often called the "sunshine hormone," has surfaced as a beacon of interest, particularly in the context of the COVID-19 pandemic[2]. The potential role of vitamin D in modulating immune responses has been highlighted by several studies, suggesting that it may influence both the risk and severity of infections. Vitamin D receptors are present in immune cells, which indicate that vitamin D can potentially modulate innate and adaptive immune responses. This context is relevant in the pathophysiology of COVID-19, where the immune response to the virus can significantly impact the disease progression and outcome. Amidst the global health crisis, where COVID-19 has claimed millions of lives and disrupted the very fabric of society, the quest for effective prophylactic and therapeutic strategies has become paramount. Vitamin D, a secosteroid with profound immunomodulatory effects, emerges as a molecule of consequence in this narrative. Epidemiological data suggest a correlation between vitamin D deficiency and the severity of COVID-19 cases [3]. The resultant propelling wave of research now aims at deciphering its role in mediating respiratory infections. The immunological mechanisms by which vitamin D exerts its influence are complex, involving the modulation of both innate and adaptive immune responses, suggesting the potential to alter the course of viral infections[4]. However, the scientific discourse must be unanimous, as current literature presents conflicting evidence and viewpoints reflecting the dynamic nature of scientific inquiry.

COVID-19 OVERVIEW

In late 2019, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19 disease, emerged in Wuhan, China. Its rapid escalation into a global pandemic led to unprecedented health crises and economic disruptions worldwide. As of 2024, the pandemic has claimed nearly 7 million lives globally, with a significant impact on healthcare systems and day-to-day life[5]. Understanding the immune response to COVID-19 is crucial for several reasons. (1) It aids in the development of effective vaccines and treatments. Studies have identified unpredicted T-cell responses to adenoviral vaccines, highlighting the complexity of the immune reaction against the virus; (2) Immune response data can guide public health strategies to manage outbreaks and mitigate transmission. Research has shown that specific genes may confer resistance to the virus, offering insights into potential protective mechanisms; and (3) Analyzing the immune responses helps identify risk factors for severe disease, enabling targeted interventions for vulnerable populations.

In short, a comprehensive understanding of the immune responses to COVID-19 is essential for controlling the pandemic and improving global health outcomes[6].

VITAMIN D AND COVID-19 CORRELATION

The relationship between vitamin D levels and COVID-19 outcomes has been the subject of extensive research since the onset of the pandemic. A systematic review and meta-analysis up to April 2021 summarized the data from 38 eligible studies, including 2 randomized controlled trials and 27 cohort studies involving over 205000 patients[7]. This comprehensive analysis revealed a significantly lower risk of severe COVID-19 disease and associated mortality with supple-

mentation of vitamin D. Specifically, the supplementation reduced severe disease risk by 62% and mortality risk by 65%. This observation highlights the possible protective role of vitamin D against the worsening of COVID-19, particularly in seasons characterized by vitamin D deficiency and in patients with non-severe cases.

Further supporting this, an observational cross-sectional study on 80 COVID-19 patients concluded that lower vitamin D levels were associated with more severe disease. Patients presenting with cough and fever had significantly lower vitamin D levels than those without these symptoms. Moreover, the study observed that patients with the most severe COVID-19 symptoms, as indicated by chest computed tomography scans, had the lowest vitamin D levels, alongside significantly higher blood levels of inflammatory markers such as C-reactive protein and ferritin[8]. Another study reported that individuals with lower vitamin D levels were more likely to test positive for SARS-CoV-2 and experience severe outcomes[9].

These studies collectively suggest a significant association between vitamin D levels and the incidence, severity, and mortality rates of COVID-19. While the exact mechanisms by which vitamin D influences COVID-19 outcomes are still being investigated, the current evidence points out that immune response may be enhanced, potentially reducing the risk of severe disease[10]. It is important to note, however, that these findings should not be interpreted as a direct cause-and-effect relationship, and further research, particularly randomized clinical trials, is encouraged to confirm these results and fully understand the implications of vitamin D on COVID-19. The potential for vitamin D supplementation to serve as a complementary approach to managing COVID-19, especially for individuals at risk of deficiency, is a promising area for future investigation.

VITAMIN D AND IMMUNE FUNCTION

Vitamin D plays a crucial role in maintaining various physiological processes, including the modulation of the immune system. It is well documented that vitamin D influences innate and adaptive immune responses, which are critical in defense against pathogens. The active form of vitamin D, calcitriol, has been shown to enhance the function of immune cells such as T cells and macrophages. Furthermore, the presence of vitamin D receptors on immune cells indicates the importance of vitamin D in immune regulation.

The potential mechanisms by which vitamin D may influence the immune response to COVID-19 and other viral infections can be explained as follows: It enhances monocyte and macrophage phagocytosis and facilitates immunocompetence, thereby enhancing innate immunity and modulating adaptive immunity. Vitamin D induces the transcription of antimicrobial peptides, such as cathelicidin and defensins, which can lower viral replication rates and reduce concentrations of pro-inflammatory cytokines that produce the inflammation responsible for damaging lung tissue. Vitamin D may also lower the risk of developing specific conditions, such as respiratory tract infections, through various mechanisms, including maintaining epithelial barriers and modulating the inflammatory response to viral infections. Furthermore, vitamin D has a role in preventing the cytokine storm. This is particularly important to decrease the severity of inflammation, acute respiratory distress syndrome, and other critical complications[7].

CONTROVERSIES AND DEBATES

Recent clinical trials and studies have provided valuable insights into the effects of vitamin D supplementation on COVID-19 outcomes. A Phase 3 randomized controlled trial, CORONAVIT, investigated the impact of a test-and-treat approach to vitamin D supplementation on the risk of acute respiratory tract infections, including COVID-19. The study involved participants who were not taking vitamin D supplements at baseline and provided them with either a lower dose (800 IU/day) or a higher dose (3200 IU/day) of vitamin D based on their blood 25-hydroxyvitamin D levels. The primary outcome measured was the proportion of participants with at least one swab test or doctor-confirmed acute respiratory tract infection. The results did not support the hypothesis that vitamin D regimens offer significant protection against acute respiratory tract infections or COVID-19[11].

Further research published in *Inflammopharmacology* highlighted that some randomized controlled trials found vitamin D supplementation beneficial for reducing SARS-CoV-2 RNA positivity but not for reducing intensive care unit admission or all-cause mortality in patients with moderate-to-severe COVID-19[12]. Similarly, a meta-analysis by Varikasuvu *et al*[13] indicated that COVID-19 patients supplemented with vitamin D showed fewer intensive care unit admission and mortality rates, although the differences were not statistically significant.

A Cochrane database systematic review emphasized the need of high-quality evidence to determine whether vitamin D is an effective and safe treatment for adults with COVID-19. The review called for more well-designed studies with robust methods to explore this potential relationship further. It also mentioned ongoing research, with 21 studies on the topic at the time of the review, promising future updates as more evidence becomes available[14]. A rapid review from Oxford University's Centre for Evidence-Based Medicine referenced by FactCheck.org found no clinical evidence that vitamin D could prevent or treat COVID-19[15]. Additionally, a review published by nutrition experts in BMJ Nutrition, Prevention & Health recommended against high doses of vitamin D, instead advising to avoid deficiency[16]. The Mayo Clinic also weighed in, stating that while researchers are examining the effect of vitamin D levels on infection with the COVID-19 virus, there is need for a clear consensus on preventive or curative intent of prescribing vitamin D for COVID-19[17].

These findings suggest that vitamin D supplementation may benefit COVID-19 outcomes, particularly in reducing the severity of the disease and mortality; but the evidence is inconclusive. These findings must be interpretated in the context of individual patient needs and the broader spectrum of COVID-19 treatment and prevention strategies.

Moreover, the role of vitamin D in enhancing the immune response to vaccines is still unclear. Despite its immune-modulatory effects, no solid evidence supports the claim that vitamin D supplementation can improve vaccine efficacy [18]. This highlights the complexity of the immune system and the multifaceted nature of viral infections. Each virus interacts with the host's immune system differently; thus, vitamin D's role may vary across different viral infections.

VITAMIN D AND OTHER VIRAL INFECTIONS

The active form of vitamin D helps to reduce the inflammatory response, which often cause severe symptoms in viral infections. It decreases pro-inflammatory cytokines such as interleukin 6 (IL-6), IL-8, IL-12, interferon gamma, tumor necrosis factor alpha, and IL-17, while increasing anti-inflammatory cytokines (*e.g.*, IL-4, IL-5, IL-17, and IL-10). Moreover, it also regulates the recognition of viral double-stranded RNA (dsRNA) through toll-like receptor 3, thereby playing a pivotal role in the immune response against viral infections. For instance, in the case of influenza, vitamin D is thought to reduce the risk of infection by modulating the response to viral antigens[19]. Vitamin D plays a pro-apoptotic role causing proliferation of plasma cells and immunoglobulin production, triggering recruitment of neutrophils, monocyte-macrophages, and dendritic cells, thereby increasing their intracellular pathogen-killing capabilities. This is especially critical in combating infections not only by viral pathogens such as dengue and rotavirus but also by bacterial pathogens such as gram-positive and gram-negative bacteria, and even *Mycobacterium tuberculosis*[3,20].

In conclusion, while vitamin D plays a crucial role in the immune system and may influence the course of viral infections, more research is needed to understand its effects fully. The ongoing research into its preventive and therapeutic effects on viral infections, including COVID-19, influenza, and human immunodeficiency virus, underscores the importance of nutrients and the need for a nuanced understanding of its role in immune function.

PUBLIC HEALTH IMPLICATIONS

Vitamin D plays a critical role in maintaining optimal health, and the potential consequences of deficiency include increased risk of chronic diseases such as osteoporosis, diabetes, cancer, cardiovascular disease, and certain autoimmune and neurological disorders[21]. The growing body of evidence suggests that ensuring adequate vitamin D levels in the population could be a cost-effective strategy to reduce the incidence of these conditions, potentially saving billions in healthcare costs[22].

Public health policies must evolve to address the widespread issue of vitamin D deficiency. The strategies should involve food fortification, public education campaigns to promote safe sun exposure, and targeted supplementation programs, especially for at-risk populations such as the elderly, individuals with limited sun exposure, and those with pre-existing health conditions. As research continues to evolve, it will be essential to refine these recommendations to ensure that they are based on solid evidence and are tailored to the needs of different populations. Moreover, standardizing vitamin D assays and agreeing on threshold values for deficiency and sufficiency are crucial for accurately assessing vitamin D status, which is a prerequisite for effective public health interventions[23]. Finally, the role of vitamin D in the modulation of the innate and adaptive immune systems and its potential synergistic effects with other treatments are promising research directions that could yield significant benefits for public health.

Furthermore, the research underscores the necessity for randomized controlled trials to determine the optimal dosage and formulation of vitamin D supplements that would confer the most significant health benefits without the risk of toxicity. It also calls for a nuanced approach to public health recommendations that considers individual variability in vitamin D metabolism, influenced by genetics, skin pigmentation, latitude, and lifestyle.

EDITORIAL STANCE

The editorial stance, grounded in the latest scientific evidence, posits that vitamin D plays a multifaceted role in the immune response to viral infections. Observational studies have consistently demonstrated a correlation between low vitamin D levels and a higher incidence of respiratory infections. It is important to note that while vitamin D's immune-modulatory effects are promising, they should not be viewed as a standalone solution but rather as a complementary measure within a broader treatment and prevention strategy. The editorial advocates for a balanced perspective that recognizes the potential benefits of vitamin D, further acknowledging the need for further research to understand its role fully in the context of viral infections. This stance is supported by a body of research that, while indicative of vitamin D's positive impact on immune health, also underscores the complexity of viral diseases and the many factors that influence their progression and outcome.

CONCLUSION

The consensus leans towards the necessity for more rigorous and dedicated research to conclusively determine the role of vitamin D in COVID-19 outcomes. These findings underscore the complexity of nutritional science and its intersection with infectious diseases, where definitive answers often require extensive, collaborative, and meticulously conducted research.

Another area that requires further investigation is the potential for vitamin D to modulate the immune response in chronic respiratory diseases, where viral infections can exacerbate symptoms. Understanding the full range of implications that vitamin D may have on lung inflammation, infection, and disease severity could lead to more effective management strategies for chronic respiratory conditions. Moreover, the interplay between vitamin D levels and the risk of recurrent infections is an important topic that needs more attention. With the ongoing COVID-19 pandemic, there is an urgent need to explore how vitamin D supplementation could affect the outcomes of those infected with the virus. The seasonality of certain viral infections and the corresponding fluctuations in vitamin D levels due to changes in sunlight exposure also present an exciting area for research.

In conclusion, the current perspective on vitamin D research advocates for a proactive stance in public health nutrition, emphasizing the importance of vitamin D status in preventing disease and maintaining overall health. As such, public health policies must align with the emerging evidence to effectively address the challenges posed by vitamin D deficiency on a community level.

FOOTNOTES

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REFERENCES

1 **Engin MMN**, Özdemir Ö. Role of vitamin D in COVID-19 and other viral infections. *World J Virol* 2024; **13**: 95349 [PMID: 39323448 DOI: 10.5501/wjv.v13.i3.95349]
2 **Ben-Eltriki M**, Hopefl R, Wright JM, Deb S. Association between Vitamin D Status and Risk of Developing Severe COVID-19 Infection: A Meta-Analysis of Observational Studies. *J Am Nutr Assoc* 2022; **41**: 679-689 [PMID: 34464543 DOI: 10.1080/07315724.2021.1951891]
3 **Grant WB**, Lahore H, McDonnell SL, Baggerly CA, French CB, Aliano JL, Bhattoa HP. Evidence that Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Deaths. *Nutrients* 2020; **12** [PMID: 32252338 DOI: 10.3390/nu12040988]
4 **Sapir T**, Averch Z, Lerman B, Bodzin A, Fishman Y, Maitra R. COVID-19 and the Immune Response: A Multi-Phasic Approach to the Treatment of COVID-19. *Int J Mol Sci* 2022; **23** [PMID: 35955740 DOI: 10.3390/ijms23158606]
5 **Saxena P**, Nigam K, Mukherjee S, Chadha S, Sanyal S. Relation of vitamin D to COVID-19. *J Virol Methods* 2022; **301**: 114418 [PMID: 34919979 DOI: 10.1016/j.jviromet.2021.114418]
6 **Gibbons JB**, Norton EC, McCullough JS, Meltzer DO, Lavigne J, Fiedler VC, Gibbons RD. Association between vitamin D supplementation and COVID-19 infection and mortality. *Sci Rep* 2022; **12**: 19397 [PMID: 36371591 DOI: 10.1038/s41598-022-24053-4]
7 **D'Ecclesiis O**, Gavioli C, Martinoli C, Raimondi S, Chiocca S, Miccolo C, Bossi P, Cortinovis D, Chiaradonna F, Palorini R, Faciotti F, Bellerba F, Canova S, Jemos C, Salé EO, Gaeta A, Zerbato B, Gnagnarella P, Gandini S. Vitamin D and SARS-CoV2 infection, severity and mortality: A systematic review and meta-analysis. *PLoS One* 2022; **17**: e0268396 [PMID: 35793346 DOI: 10.1371/journal.pone.0268396]
8 **Lordan R**. Notable Developments for Vitamin D Amid the COVID-19 Pandemic, but Caution Warranted Overall: A Narrative Review. *Nutrients* 2021; **13** [PMID: 33652653 DOI: 10.3390/nu13030740]
9 **Argano C**, Mallaci Bocchio R, Natoli G, Scibetta S, Lo Monaco M, Corrao S. Protective Effect of Vitamin D Supplementation on COVID-19-Related Intensive Care Hospitalization and Mortality: Definitive Evidence from Meta-Analysis and Trial Sequential Analysis. *Pharmaceuticals*

(Basel) 2023; **16** [PMID: 36678627 DOI: 10.3390/ph16010130]

10 Sunshine for Immunity: New Health Products' Vitamin D Supplements - Just Perfect Health. [cited August 13, 2024]. Available from: https://www.jp-health.com/sunshine-for-immunity-new-health-products-vitamin-d-supplements

11 **Jolliffe DA**, Holt H, Greenig M, Talaei M, Perdek N, Pfeffer P, Vivaldi G, Maltby S, Symons J, Barlow NL, Normandale A, Garcha R, Richter AG, Faustini SE, Orton C, Ford D, Lyons RA, Davies GA, Kee F, Griffiths CJ, Norrie J, Sheikh A, Shaheen SO, Relton C, Martineau AR. Effect of a test-and-treat approach to vitamin D supplementation on risk of all cause acute respiratory tract infection and covid-19: phase 3 randomised controlled trial (CORONAVIT). *BMJ* 2022; **378**: e071230 [PMID: 36215226 DOI: 10.1136/bmj-2022-071230]

12 **Gomaa AA**, Abdel-Wadood YA, Thabet RH, Gomaa GA. Pharmacological evaluation of vitamin D in COVID-19 and long COVID-19: recent studies confirm clinical validation and highlight metformin to improve VDR sensitivity and efficacy. *Inflammopharmacology* 2024; **32**: 249-271 [PMID: 37957515 DOI: 10.1007/s10787-023-01383-x]

13 **Varikasuvu SR**, Thangappazham B, Vykunta A, Duggina P, Manne M, Raj H, Aloori S. COVID-19 and vitamin D (Co-VIVID study): a systematic review and meta-analysis of randomized controlled trials. *Expert Rev Anti Infect Ther* 2022; **20**: 907-913 [PMID: 35086394 DOI: 10.1080/14787210.2022.2035217]

14 **Stroehlein JK**, Wallqvist J, Iannizzi C, Mikolajewska A, Metzendorf MI, Benstoem C, Meybohm P, Becker M, Skoetz N, Stegemann M, Piechotta V. Vitamin D supplementation for the treatment of COVID-19: a living systematic review. *Cochrane Database Syst Rev* 2021; **5**: CD015043 [PMID: 34029377 DOI: 10.1002/14651858.CD015043]

15 **Raisi-Estabragh Z**, Martineau AR, Curtis EM, Moon RJ, Darling A, Lanham-New S, Ward KA, Cooper C, Munroe PB, Petersen SE, Harvey NC. Vitamin D and coronavirus disease 2019 (COVID-19): rapid evidence review. *Aging Clin Exp Res* 2021; **33**: 2031-2041 [PMID: 34118024 DOI: 10.1007/s40520-021-01894-z]

16 **Lanham-New SA**, Webb AR, Cashman KD, Buttriss JL, Fallowfield JL, Masud T, Hewison M, Mathers JC, Kiely M, Welch AA, Ward KA, Magee P, Darling AL, Hill TR, Greig C, Smith CP, Murphy R, Leyland S, Bouillon R, Ray S, Kohlmeier M. Vitamin D and SARS-CoV-2 virus/COVID-19 disease. *BMJ Nutr Prev Health* 2020; **3**: 106-110 [PMID: 33230499 DOI: 10.1136/bmjnph-2020-000089]

17 **DeSimone DC**. Reply to Can taking a vitamin D supplement prevent infection with the virus that causes coronavirus disease 2019 (COVID-19)? [Cited on August 13, 2024] Available from: https://www.mayoclinic.org/diseases-conditions/coronavirus/expert-answers/coronavirus-and-vitamin-d/faq-20493088

18 **Sadarangani SP**, Whitaker JA, Poland GA. "Let there be light": the role of vitamin D in the immune response to vaccines. *Expert Rev Vaccines* 2015; **14**: 1427-1440 [PMID: 26325349 DOI: 10.1586/14760584.2015.1082426]

19 **Martineau AR**, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, Dubnov-Raz G, Esposito S, Ganmaa D, Ginde AA, Goodall EC, Grant CC, Griffiths CJ, Janssens W, Laaksi I, Manaseki-Holland S, Mauger D, Murdoch DR, Neale R, Rees JR, Simpson S Jr, Stelmach I, Kumar GT, Urashima M, Camargo CA Jr. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ* 2017; **356**: i6583 [PMID: 28202713 DOI: 10.1136/bmj.i6583]

20 **L Bishop E**, Ismailova A, Dimeloe S, Hewison M, White JH. Vitamin D and Immune Regulation: Antibacterial, Antiviral, Anti-Inflammatory. *JBMR Plus* 2021; **5**: e10405 [PMID: 32904944 DOI: 10.1002/jbm4.10405]

21 **Sizar O**, Khare S, Goyal A, Givler A. Vitamin D Deficiency. 2023 Jul 17. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan- [PMID: 30335299]

22 **Hiligsmann M**, Ben Sedrine W, Bruyère O, Evers SM, Rabenda V, Reginster JY. Cost-effectiveness of vitamin D and calcium supplementation in the treatment of elderly women and men with osteoporosis. *Eur J Public Health* 2015; **25**: 20-25 [PMID: 25096255 DOI: 10.1093/eurpub/cku119]

23 **Holick MF**. The Vitamin D Deficiency Pandemic: a Forgotten Hormone Important for Health. *Public Health Rev* 2010; **32**: 267-283 [DOI: 10.1007/bf03391602]

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REVIEW

Role of viruses in periodontitis: An extensive review of herpesviruses, human immunodeficiency virus, coronavirus-19, papillomavirus and hepatitis viruses

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Abstract

Periodontitis is the inflammation of the supporting structures around the dentition. Several microbial agents, mostly bacteria, have been identified as causative factors for periodontal disease. On the other hand, oral cavity is a rich reservoir for viruses since it contains a wide variety of cell types that can be targeted by viruses. Traditionally, the focus of research about the oral flora has been on bacteria because the most widespread oral diseases, like periodontitis and dental caries, are outcomes of bacterial infection. However, recently and especially after the emergence of coronavirus disease 2019, there is a growing tendency toward including viruses also into the scope of oral microbiome investigations. The global high prevalence of periodontitis and viral infections may point out to a concomitant or synergistic effect between the two. Although the exact nature of the mechanism still is not clearly understood, this could be speculated through the manipulation of the immune system by viruses; hence facilitating the furthermore colonization of the oral tissues by bacteria. This review provides an extensive and detailed update on the role of the most common viruses including herpes family (herpes simplex, varicella-zoster, Epstein-Barr, cytomegalovirus), Human papillomaviruses, Human immunodeficiency virus and severe acute respiratory syndrome coronavirus 2 in the initiation, progression and prognosis of periodontitis.

Key Words: Virus; Periodontitis; Bacteria; Herpesvirus; Herpes simplex virus; Varicella-zoster virus; Epstein-Barr virus; Cytomegalovirus; Human papillomaviruses; SARS-CoV-2; Human immunodeficiency virus

Core Tip: Periodontitis affects millions of people worldwide. It has been connected to several systemic inflammations and infections. Periodontitis is a complex and multifactorial disease. The main microorganisms involved in periodontitis are bacteria. However, viruses may have a contribution in the etio-pathogenesis of periodontitis also. In this article we extensively reviewed the role of some of the most common viruses in the initiation and progression of periodontitis.

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INTRODUCTION

Periodontitis which is an immune-inflammatory disease is characterized by the host's response to the presence of subgingival polymicrobial biofilms, eventually leading to clinical attachment loss of periodontium and alveolar bone degeneration. Gingivitis, or gum inflammation, usually does not cause any serious problems at first. If it spreads further the soft tissue and bone that firmly anchors the teeth, called the periodontium, could be harmed also. Inflammation of this periodontium is known as periodontitis in medicine[1]. Twenty percent of adults globally are thought to suffer from severe periodontal diseases, with over 1 billion incidences believed to exist[2].

Periodontitis has multiple contributing factors. One of the key components is a person's genetic susceptibility to the disease. Numerous bacteria have been identified in the field of microbiology. *Porphyromonas gingivalis (P. gingivalis)*, *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), and *Treponema denticola* (*T. denticola*) are among the most crucial bacteria that have been linked to the disease's onset and progression[3]. Subgingival calculus was revealed to be a local component connected with the beginning of the condition, and dental plaque was linked to its advancement. Therefore, the etio-pathogenesis of periodontitis involves a variety of bacteria, both beneficial and detrimental host immune responses, environmental variables that are modifiable and those that are not, as well as genetic and epigenetic factors[4,5].

Oral cavity, on the other hand, is a significant source of viruses since it has a diverse range of cell types that viruses can target. Because bacterial infections are the cause of the most common oral diseases, such as dental caries and periodontitis, research on the oral flora has traditionally focused on bacteria[6]. However, conditions have changed with the development of molecular biology technologies.

Detecting viruses in periodontitis is crucial for understanding their role in the disease. Two advanced molecular techniques, polymerase chain reaction (PCR) and next-generation sequencing (NGS), have significantly improved the sensitivity and specificity of viral detection in periodontal tissues. PCR is a widely used method for detecting viral DNA or RNA in subgingival plaque samples. PCR and its quantitative variant, qPCR, are highly sensitive and specific techniques that can amplify and quantify viral genetic material. For example, Chalabi *et al*[6] used PCR to detect periodontopathic bacteria and herpesviruses in chronic periodontitis, demonstrating the presence of these pathogens in periodontal pockets[6]. Similarly, Thomasini and Pereira utilized PCR-based assays to identify herpesviral infections in

the oral cavity, highlighting the relevance of viral detection in periodontal disease[7]. NGS is another powerful technique that allows comprehensive analysis of the viral genome in periodontal samples. NGS can identify and quantify a broad range of viral species, providing a detailed profile of the viral community present in periodontal tissues. This method offers several advantages over traditional techniques, including high throughput, deep sequencing depth, and the ability to detect low-abundance viruses. Carrozzo and Scally employed NGS to study the oral microbiome and its association with hepatitis C virus (HCV) infection, providing insights into the complex interactions between viruses and periodontal disease[8]. Both PCR and NGS have significantly advanced our ability to detect and analyze viruses in periodontitis. PCR is particularly useful for targeted detection and quantification of specific viral pathogens, while NGS provides a comprehensive overview of the viral community and its diversity. The integration of these techniques in periodontal research has led to a better understanding of the viral contributions to periodontal inflammation and disease progression.

There has been an increasing trend recently, particularly with the rise of coronavirus disease 2019 (COVID-19), to include viruses in the scope of studies on the oral microbiota. While some viruses are systemic and involve secondarily the oral cavity, others have a relative predilection for the oral tissues. Only a small percentage of the viruses that live in the oral cavity are pathogenic and have the potential to cause symptoms. The majority of viruses are commensals. The significant frequency of viral infections and oral diseases worldwide may indicate a concurrent or synergistic influence between the two[8]. Even though the precise mechanism is yet unknown, it may be related to viruses' ability to influence the immune system, which in turn allows bacteria to colonize oral and dental tissue. This has led to viral-bacterial plaque hypothesis. Moreover, a similar correlation between several viral infections and oral cancer can be proposed[9]. This review provides an extensive and detailed update on the role of the most common viruses including herpes family (herpes simplex, varicella-zoster, Epstein-Barr, cytomegalovirus), human papillomaviruses (HPV), human immunodeficiency virus (HIV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the initiation, progression and prognosis of periodontitis.

HUMAN HERPESVIRUS

Human herpesvirus (HHV) refers to a broad family of DNA viruses that includes the varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and, more recently, the herpes simplex virus (HSV), which comes in two kinds, HSV-1 and HSV-2. The only known natural reservoir for these endemic viruses is humans[10]. These viruses are prone to latency, repeated infections, and dissemination. All eight varieties result in a primary infection and remain dormant within particular cell types for the duration of the host's life. These viruses can reactivate and induce asymptomatic or symptomatic reactivation. Saliva and vaginal fluids carry the viruses, which can spread to new hosts[7].

In 2016, 491 million persons worldwide or 13% of the population between the ages of 15 and 49- were projected to have HSV-2 infection. In the same year, an estimated 3.7 billion persons worldwide, or 66% of those between the ages of 0 and 49, contracted HSV-1[11]. Table 1 provides some key information on the main target cells of the HHV types, their oral and systemic pathologies.

Numerous causes served as inspiration for periodontal HHV research. The 1960s periodontal model was centered on dental biofilm and plaque. However, the low frequency of periodontitis in some patients with large biofilm buildup and the quick progression of juvenile (aggressive) periodontitis lesions with modest biofilms, cannot be explained by the presence of biofilm production alone[7]. Results of periodontal biofilm eradication techniques have been inconsistent. In the late 1970s, due to these clinical circumstances, the etiology of severe periodontitis shifted from a broad microbiologic disease to a specific infection involving different anaerobic bacteria. However, bacterial infections alone cannot explain periodontitis' site-specificity, especially its bilateral symmetrical distribution around the mouth's midline. The site-specificity of periodontitis pointed to herpesviruses, which are the prototype agents for tissue tropism infectious (*e.g.*, herpes labialis)[12]. The pattern of HHV infection may account for a number of characteristic features of periodontal diseases, including: (1) The localized pattern of tissue destruction, which may be caused by viral tissue tropism; (2) The disease's episodic, progressive nature, which may be caused by temporary local immunosuppression resulting from either an active or latent viral infection; and (3) The fact that some people have periodontopathogenic bacteria but still have healthy gums maybe due to lack of viral infection[13].

Many studies have found a significant incidence of HHVs in periodontal disease beginning in the mid-1990s. The presence of HHVs in gingival tissue, gingival cervicular fluid (GCF), and subgingival plaque in periodontal disease suggests that HHVs might be involved in the periodontal disease etiology. First of all, it has been demonstrated that human gingiva from clinically healthy individuals can get infected with HSV, indicating that these cells may serve as a reservoir for the latent virus[14]. Eventually, it was shown that the gingival tissue of periodontitis sites had a higher frequency of viral detection than did healthy locations[15]. Furthermore, also in the in GCF, it was observed that the frequency of HHV detection from periodontally diseased sites was higher than that from gingivitis/healthy sites[16]. Again, it was shown that subgingival plaque from periodontally diseased locations had a higher frequency of viral detection than plaque from healthy sites[17]. Moreover, it was found that the GCF of periodontal lesions contained active HHVs[18]. Additionally, the relationship between HHVs and periodontal bacterial infections was examined in a number of investigations[19]. Lastly, extensive populations were the subject of epidemiological investigations. For instance, a recent study that examined periodontitis and HSV co-infection used extensive cross-sectional data from the National Health and Nutrition Examination Survey (NHANES), which included 4733 adults aged 30-50. Both HSV-1 [odds ratio (OR) = 1.09, $P < 0.001$] and HSV-2 (OR = 1.06, $P = 0.030$) infections were substantially linked to periodontitis after controlling for variables. Those with HSV-1 (+) and HSV-2 (+) and HSV-1 (+) and HSV-2 (-) infection demonstrated greater incidence of periodontitis in all subgroups (OR = 1.15, OR = 1.09, $P < 0.001$) in subgroup analyses compared to

Table 1 Key information on the human herpes virus types, the main target cells, their oral and systemic pathologies

Human herpes virus	Main target cells	Oral pathology	Systemic pathology
HHV-1 and 2 (HSV 1 and 2)	Mucoepithelial cells (orofacial and genital tract)	Primary herpetic gingivostomatitis. Recurrent herpetic gingivostomatitis. Chronic herpetic gingivostomatitis. Herpes labialis. Increased risk of periodontitis	Genital ulcers
HHV-3 (VZV)	Mucoepithelial cells and T cells (orofacial and any skin or mucosa of the body)	Possible oral vesicles and ulcers, increased risk of periodontitis	Primary infection: Varicella (chicken pocks). Recurrent infection: Herpes zoster (shingles)
HHV-4 (EBV)	Mucoepithelial cells and B cells	Hairy leukoplakia, leukoplakia, nasopharyngeal carcinoma, ulcerations and palatal petechiae, oral lymphoma, increased risk of periodontitis	Infectious mononucleosis, lymphoma
HHV-5 (CMV)	Monocytes, fibroblasts, lymphocytes and epithelial cells	Oral vesicles and ulcers, increased risk of periodontitis	Infectious mononucleosis
HHV-6	T cells, epithelial cells, monocytes, fibroblasts and more	Unknown	
HHV-7	T cells, epithelial cells, monocytes, fibroblasts and more	Unknown	
HHV-8	Not identified	Ulcers and tumors	Kaposi's sarcoma

EBV: Epstein-Barr virus; CMV: Cytomegalovirus; HHV: human herpes virus, HSV: Herpes simplex virus; VZV: Varicella-zoster virus.

those without HSV infection[20].

These preliminary studies accumulated through time to form a considerable amount of literature body on the same association studied globally. Starting from the last decade, this series of investigations led to several systematic reviews and meta-analyses on the available data. Table 2 collects a list of selected systematic reviews and meta-analyses on the association between HHV and periodontitis. Findings from these reviews show a pattern and a potential consistency. For the EBV, which is the most studied HHV, a strong correlation with chronic, aggressive and advanced periodontitis was seen. This may reflect the high prevalence of EBV in most of the oral cavity tissues, its virulence and aggressive pathogenicity. However, when it comes to the apical periodontitis and peri-implantitis, negative association was reported [21-23]. The same pattern is true for human CMV (HCMV) since most of the data shows a strong association between its detected prevalence and chronic, advanced and aggressive periodontitis but not with apical periodontitis[24]. Regarding the HSV, statistically significant relation with chronic, advanced and aggressive periodontitis were reported[25]; although in one meta-analysis including twelve studies no significant association was seen[26]. Finally, the same result was repeated in a recent umbrella review including six meta-analyses. The association between HHVs and marginal periodontitis was significant in contrast to the apical periodontitis of endodontic origin[27].

The exact mechanism by which HHVs affect the initiation and progression of periodontitis is still to be uncovered. However a HHV-bacterial model for periodontitis have been proposed briefly as follows: Important components of periodontal pathosis in the herpesviral-bacterial model of periodontitis include immunopathogenicity of the virus, its escaping capability from immune system, latency, reactivation after latency, and tissue/site tropism[28-31]. First, bacteria in the dental biofilm cause gingivitis, which opens the door for latent HHV to reach the periodontium through macrophages, T-lymphocytes, and B-lymphocytes. Latent HHV can reactivate on its own or during times when the host's defenses are weakened, as in the case of concurrent infections, drug-induced immunosuppression, unusually high levels of emotional stress, hormonal fluctuations, physical trauma, *etc*. Interestingly enough, the majority of conditions that activate herpesviruses are also thought to be risk factors or markers of periodontitis. As previously mentioned, the herpesvirus infection can release pro-inflammatory cytokines that may activate osteoclasts and matrix metalloproteinases, so compromising the immune system's ability to combat periodontopathic bacteria[32].

In conclusion, based on the mentioned data, an association between HHVs (especially EBV, HCMV and HSV) and marginal periodontitis is highly plausible; however all the data shows no such relation with apical periodontitis. This con-clusion may support the hypothesis that HHVs have an affinity toward reaching and infecting the gingiva and periodontium but not the pulpal and apical periodontal tissues.

HIV

HIV is a single stranded RNA virus that belongs to the family of Retroviridae. Two main species have been identified: HIV-1 and HIV-2. The former is exhibited globally and responsible for most of the cases, while the latter predominates in West Africa and is related with a relatively decreased risk of transmission and slower progression of the disease. By the end of 2022, there were an anticipated 39 million HIV-positive individuals worldwide, with the World Health Organization (WHO) African Region housing two thirds of them. In 2022, 1.3 million new cases of HIV infection and 630000 deaths from HIV-related causes were reported[33].

Table 2 A list of systematic reviews and meta-analyses on the association between human herpesviruses and periodontitis

Ref.	Study design	Findings	Conclusion	Reported statistical significance
Jakovljevic *et al*[27], 2022	Umbrella review including six meta-analyses on HHVs detection in MP and apical periodontitis of endodontic origin (APEO)	MP risk increased with subgingival HHVs. The connection was robust (OR > 3.0), although confidence intervals were broad, heterogeneity was high, and studies were small. However, systematic reviews of APEO and HHVs found no significant relationships	There was a substantial correlation between HHVs and MP, but not APEO, according to low-quality, highly unclear research	MP: Yes; APEO: No
Arduino *et al*[26], 2023	Meta-analysis of eight observational studies on HSV-1 in endodontic peri-apical lesions including 194 adult patients	Pooled HSV-1 prevalence was 4.8% (95%CI: 2.0%–11.4%; adjusted for small-study effect); 8.1% (95%CI: 4.4%–14.5%, quality-adjusted); and 6.8% (95%CI: 3.6%–11.0%, random-effects)	3%–11% of periapical disease patients had HSV-1 colonization. Such data do not suggest HSV-1 causes the onset and progression of periodontitis	No
Arduino *et al*[25], 2022	Meta-analysis of twelve case–control and cross- sectional studies (738 cases, 551 controls), investigating HSV-1 in subgingival plaque/crevicular fluid and periodontitis	For any type of periodontitis, the pooled ORs were 44 (95%CI: 1.9–10.2); for chronic periodontitis, they were 28 (95%CI: 1.0–8.3); and for aggressive periodontitis, they were 118 (95%CI: 5.4–25.8)	HSV-1 was associated with periodontitis	Yes
Maulani *et al*[21], 2021	Meta-analysis of studies on EBV, involving 1354 periodontitis patients and 819 healthy controls	When subgingival EBV was found, there was an increased incidence of periodontitis: OR = 7.069 (95%CI: 4.197–11.905, $P < 0.001$)	An elevated risk of periodontitis is linked to a high frequency of EBV detection	Yes
Roca-Millan *et al*[22], 2021	Meta-analysis of five researches on EBV and peri-implantitis. The study included 274 patients (125 men and 149 women) and 388 implants (197 healthy, 166 peri-implantitis, and 25 mucositis)	In the peri-implant sulcus, there was no significant difference in EBV presence between peri-implantitis and healthy implant groups (OR = 4.14; 95%CI: 0.93-18.37; $P = 0.06$)	EBV prevalence in the sulcus was not statistically different between peri-implantitis and normal implant groups	No
Li *et al*[28], 2017	Meta-analysis of twelve case-control studies on the presence of HHVs in AgP involving 322 patients and 342 controls	EBV showed substantial connection with AgP, however publication bias was present (10 studies: OR = 6.11, 95%CI: 2.13–17.51, $P = 0.0008$). HCMV and HSV-1 also showed significant associations (12 studies: OR = 3.63, 95%CI: 2.15–6.13 $P = 0.009$; 4 studies: OR = 19.19, 95%CI: 4.16–79.06, $P < 0.001$). Relation between HSV-2 and AgP was inconclusive (2 studies: OR = 3.46, 95%CI: 0.51–23.51, $P = 0.20$)	AgP showed strong associations with EBV, HCMV, and HSV-1. But there was a lot of heterogeneity among the studies	Yes
Zhu *et al*[29], 2015	Meta-analysis of 12 studies (including 552 cases and 371 controls) investigated the association between HHVs and chronic periodontitis	EBV: 12 studies (OR = 5.74, 95%CI: 2.53–13.00, $P < 0.001$). HCMV: 10 studies (OR = 3.59, 95%CI: 1.41–9.16, $P = 0.007$). HSV: 2 studies (OR = 2.81 95%CI: 0.95–8.27, $P = 0.06$). HHV-7: 1 study (OR = 1.00, 95%CI: 0.21–4.86)	Chronic periodontitis was highly correlated with both HCMV and EBV. Inadequate evidence was found for HHV-7 and HSV	EBV: Yes; HCMV: Yes; HSV: No; HHV-7: No
Botero *et al*[24], 2020	Meta-analysis of 32 studies on HCMV in periodontitis (26 studies involving periodontitis and 6 involving apical periodontitis)	Significantly elevated periodontitis risks with subgingival HCMV (OR = 5.31; 95%CI: 3.15-8.97). HCMV was not linked to apical periodontitis (OR 3.65; 95%CI: 0.49-27.10)	HCMV was significantly associated with periodontitis but not with apical periodontitis	Periodontitis: Yes; Apical periodontitis: No
Gao *et al*[23], 2017	Meta-analysis of 21 case–control studies (including 995 patients and 564 healthy people) on the association between EBV and periodontitis	Significant differences were found in the odds of periodontitis and EBV detection (OR = 6.199, 95%CI: 3.119–12.319, $P < 0.001$)	An elevated risk of periodontal diseases was connected with a high prevalence of EBV	Yes
Alzahrani [19], 2016	Systematic review of 12 studies on the association between HHVs and risk of AgP and AP		In contrast to healthy individuals, HHVs (HSV, CMV, and EBV) levels were elevated and linked to AgP and AP	Yes
Jakovljevic *et al*[30], 2014	Meta-analysis of 17 cross-sectional studies on the association of HCMV and EBV with apical periodontitis	No statistically significant relationship between the presence of HCMV and EBV messenger RNA transcripts ($P = 0.083$ and $P = 0.306$, respectively) and the clinical features of apical periodontitis	HCMV and EBV were common in symptomatic and large-size periapical lesions, but not statistically significant	No

HSV: Herpes simplex virus; AgP: Aggressive periodontitis; MP: Marginal periodontitis; APEO: Apical periodontitis of endodontic origin; EBV: Epstein-Barr virus; HCMV: Human cytomegalovirus; AP: Advance periodontitis; HHV: Human herpesvirus; OR: Odds ratio.

It's critical to distinguish between two things when discussing HIV/acquired immunodeficiency syndrome (AIDS): First, the infection pattern both prior to and following contemporary antiretroviral therapy (ART). Due to the introduction and widespread use of ART since 1996, AIDS-which was defined by a fast advancing immunodeficiency course that ultimately results in death- has recently been reduced to a chronic condition that could be managed[33]. Therefore, those living with HIV experience longer life expectancies, lower rates of death and morbidity, and roughly the same rates of development of chronic non-HIV-related illnesses as the general population[34]. These patients also saw improvements in their quality of life. The second point is that industrialized and developing countries have different illness distribution and types. Unfortunately, socioeconomic factors like poverty, prejudice, stigma, and inadequate healthcare systems continue to be major obstacles to treatment and prevention initiatives in underdeveloped nations[35].

A series of oral manifestations of HIV infection have been identified including oral hairy leukoplakia, oral candidiasis, oral warts, salivary gland diseases, Kaposi sarcoma, linear gingival erythema, necrotizing ulcerative gingivitis, necrotizing ulcerative periodontitis and chronic periodontitis[36]. In a recent meta-analysis, the overall prevalence of the manifestations were reported as follows: Oral candidiasis (35%), pseudomembranous candidiasis (19%), oral hairy leukoplakia (12%), Kaposi sarcoma (5%) and erythematous candidiasis (18%)[37]. Atypical lesions affecting the periodontal tissues, such as necrotizing ulcerative gingivitis, necrotizing ulcerative periodontitis, and linear gingival erythema, were seen prior to the development of ART[38]. However, there has been a noticeable decrease in the incidence of oral candidiasis, hairy leukoplakia, and destructive and atypical periodontal disease in HIV patients since the introduction of combined ART[39]. HIV-positive patients on ART had a statistically significant reduction in the prevalence of angular cheilitis, erythematous candidiasis, oral herpes, pseudomembranous candidiasis, Kaposi sarcoma, and oral hairy leukoplakia when compared to those who were not on ART[40].

The most prevalent kind of periodontal disease, chronic periodontitis, has been the subject of epidemiological research. A variety of potential correlations between HIV and the frequency and severity of periodontal illnesses were documented. Prior to the development of ART, certain research found that HIV patients who also had chronic periodontal disease experienced higher levels of attachment loss than non-infected patients, and this was associated with decreasing CD4 counts[41] and a greater extent of gingival recession, with or without greater alveolar bone loss[42]. However, in the combined ART era, studies have reported reductions in the prevalence of periodontal diseases in adults with HIV[40,43]. Moreover, studies carried out during the era of combined ART have not discovered any discernible variations in the frequency or severity of periodontal disease between HIV-positive patients receiving these therapies and non-infected individuals[43]. Furthermore, a large longitudinal study conducted on a cohort of women from 1995 to 2002 found no significant differences between HIV-positive and HIV-negative women in terms of baseline mean probing depths, clinical attachment levels, the advancement of attachment loss, or pocket depths[44]. When comparing HIV patients receiving ART *vs* those not, it seemed that patients on ART had a significantly lower prevalence of necrotizing gingivitis, and there was no statistically significant difference in the prevalence of either chronic or necrotizing periodontitis between the two groups[45]. Periodontal inflammation was common in HIV patients regardless of ART status, and in participants with virological suppression, the periodontal treatment reduced periodontitis along with a concurrent decrease in IL-6 and an increase in CD4. These findings highlight the impact of periodontal treatment on local inflammation and periodontitis in HIV patients[46]. A recent systematic review showed that HIV infection does not seriously threaten implant survival in the short term, but the data was of high quality[47].

In the combined ART era, as in the pre-combined ART era, there have been conflicting results regarding the relationship between periodontal attachment loss or pocket depth and CD4 counts and/or HIV viral load. Even though HIV can be found and measured in the subgingival biofilm of HIV-positive people[48], some research found no significant differences in periodontal parameters[49,50] or in tooth loss patterns[51], or the stages of HIV infection particularly for patients with a CD4 count higher than 500 cells/mm^3[52]. However, patients on combined ARTs who showed resistance to the therapy or a lack of compliance did show a slight increase in tooth loss along with a 10-fold increase in viral load[44]. Meanwhile, a different study found that patients on combined ART who had a CD4 count of less than 200 cells/mm^3 were more likely to develop periodontal disease[53]. Lastly, in HIV-positive individuals, the severity of periodontitis was linked to greater frequencies of circulating CD8+ senescent cells, raised CRP levels, and an increased prevalence of *P. gingivalis*[54].

In children, early research conducted in the pre-ART era indicated high incidence of gingivitis and early periodontitis in many different nations worldwide. Clinical markers such as bleeding on probing, increasing depths of probing, and/or loss of clinical attachment were used in these studies[55-57]. More recently, in the age of ART, extensive cross-sectional and prospective epidemiological studies have been conducted to examine the impact of these medications on the prevalence and severity of periodontal problems in children and adolescents. A large multicenter prospective cohort research included 2767 HIV-positive children from United States and Puerto Rico indicated that children on combined ART had significantly decreased occurrences of oral candidiasis and herpes zoster when compared to children on pre-combined ART[58]. In addition, a recent meta-analysis reported that HIV-positive children taking ART had a significantly higher prevalence of periodontal diseases (three-fold), oral ulcer (6-fold), oral candidiasis (17-fold) and mucosal hyperpigmentation (20-fold) than the healthy controls[59].

The periodontal microbiota of HIV-positive patients has been the subject of numerous studies. According to the findings of certain studies, the subgingival microbiota of people with HIV infection and those without it is similar in composition[60,61]. Other research found that HIV-positive individuals had higher prevalence of periodontal pathogens, including *Prevotella intermedia*, *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *Fusobacterium nucleatum* and *T. denticola*, as well as a mixture of these species[62-64].

HIV targets the immune system, namely CD4+ T cells, which causes widespread viremia and leading to a status known as AIDS. Most bodily fluids, including blood, saliva, urine, tears, breast milk, ear secretions, semen, and vaginal secretions can contain the HIV virus in people who are positive for the infection[65]. HIV is typically found in saliva at

much lower concentrations in patients than in blood. Saliva has been found to contain RNA and DNA of HIV[66,67]. Serum and HIV-positive macrophages and lymphocytes from gingival crevicular fluid, which the latter is elevated during periodontal disease, are potential sources of infectious HIV DNA in saliva[68]. It's unclear how exactly the HIV enters the periodontium. However, it is well known that the presence of inflammation causes the surface of the oral epithelium to express higher number of receptors, including HIV receptors, which may increase the epithelium's permeability[69].

In conclusion, ART has decreased aggressive forms of atypical periodontitis, while the results on chronic periodontitis are inconclusive. A higher prevalence of traditional periodontal pathogens, which are typically also found in non-infected persons, is present in the subgingival microbiota of HIV-positive patients with chronic periodontitis. Moreover, the extreme immunosuppression appears to encourage the colonization of these species and other species that are uncommon in the subgingival microbiota. This mandates a closer and personalized monitoring and follow-up of HIV patients concerning their periodontal health status.

SARS-COV-2

The coronavirus is a common single-stranded RNA virus that is extremely infectious among humans and animals and can cause respiratory infections ranging from mild to severe. Late in 2019, a novel strain of the β-coronavirus family, known as SARS-CoV-2, surfaced in Wuhan, China. It quickly spread throughout the globe, forcing the WHO to designate the disease a global pandemic on March, 2020[70].

The virus enters the body and generates viremia, which initiates the early stage of the sickness, which typically lasts three to seven days. The illness may then progress into a second stage where the virus enters the bloodstream and targets a variety of tissues and organs[71]. Critical organ damage, including damage to the heart, lungs, nervous system, gastrointestinal tract, and kidneys, causes the patient's condition to worsen. Furthermore, a pathological inflammatory reaction labeled the "cytokine storm" raises the COVID-19 mortality rate. This cytokine storm is indicative of an overreaction of the immune system, as indicated by increased levels of inflammatory mediators in the blood[72]. One of the main ways that SARS-CoV-2 is contagious is because of a spike of protein on its surface that can bind to the angiotensin-converting enzyme 2 (ACE 2), which is found on the membrane of several human body cells[73]. Actually, the fact that ACE 2 is highly expressed in the lower respiratory tract, namely on alveolar pneumocytes, is one of the main mechanisms that SARS-CoV-2 infection can harm the lungs and cause acute respiratory distress syndrome[74].

Severe COVID-19 disease has been linked to a number of risk factors. These include comorbidities like obesity and the existence of underlying disorders, as well as factors like advanced age and sex (male), as well as diabetes, hypertension, cerebrovascular disease, cardiovascular disease and chronic renal disease[73].

It appears that the oral cavity contributes significantly to the pathogenicity of COVID-19. The oral cavity exhibits high expression levels of membrane proteins such as transmembrane protease serine 2 and ACE 2, which are utilized by SARS-CoV-2 to infect cells[75]. Similar to those in the lungs and tonsils, these membrane proteins have been detected at similar concentrations on the epithelial cells of the oral mucosa, tongue, gingiva, salivary glands, and periodontal pockets[76]. Furthermore, oral fluids and saliva may contain SARS-CoV-2; the oral viral load has been linked to the severity of the disease[77].

There have been some nonspecific oral lesions linked to COVID-19 infection. Lip necrosis, dry mouth, fissured or depapillated tongue, oral vesiculobullous or pustulous lesions, and hemorrhagic or erythematous mucosal lesions are a few of these[75]. Patients with systemic diseases that include some degree of immunosuppression are more likely to have these lesions[78]. The oral lesions can affect both keratinized and nonkeratinized mucosa, and they resemble a number of viral diseases[79]. The degree of oral lesions is mostly influenced by an older age and the severity of COVID-19 infection. The beginning of oral lesions typically corresponds with taste and smell chemosensory dysfunctions[80].

Several epidemiological studies have been performed to investigate the association between periodontitis and COVID-19 infection, severity, complications and mortality. A comprehensive investigation involving 1299010 people examined the genotyping of single nucleotide variants linked to periodontitis. Higher susceptibility to COVID-19 infection (OR = 1.029; 95%CI: 1.003–1.055, P = 0.024) and severity of COVID-19 (OR = 1.030; 95%CI: 1.003–1.058, P = 0.027) was significantly linked to periodontitis. The authors came to the conclusion that there may be a causal relationship between periodontitis and COVID-19 severity and susceptibility to infection[81]. Larvin *et al*[82] investigated self-reported oral health indicators (bleeding gums, loose teeth and painful gums) in 13253 individuals (1616 positive COVID-19 patients and 11637 negative participants) during the course of a 6-month study. Those with loose teeth did not show an increased risk of death or hospital admission (OR = 1.85; 95%CI: 0.92–2.72), in comparison to the control group. On the other hand, persons who tested positive for COVID-19 and experienced bleeding or sore gums were more likely to die (OR = 1.71; 95%CI: 1.05–2.72). Insufficient evidence was available to determine a link between periodontitis and an increased risk of COVID-19 infection. However, among those who tested positive for COVID-19, those with periodontal disease had a significantly higher death rate[82]. The same researchers looked at the self-reported oral health indicators (loose teeth, bleeding and painful gums) in 58897 participants who were followed up for nearly a year, this time with a focus on body weight. Hospital admission rates for patients with periodontal disease were 57% higher [hazard ratio (HR) = 1.57; 95%CI: 1.25-1.97] than for the obese group without the illness. Obesity-related death rates were substantially higher in people with periodontal disease (HR = 3.11; 95%CI: 1.91-5.06) than in people without the illness. The authors speculated that the relationship between obesity and higher hospitalization and death rates could be exacerbated by the effects of periodontal disease[83]. A study conducted in Qatar with 568 participants examined the connection between COVID-19 issues and periodontitis. In the study, dental panoramic radiographs were utilized to assess the condition of the periodontal tissue. After adjusting for potential confounders like age, gender, smoking, body mass index, and other chronic diseases, the

study's findings showed a significant correlation between moderate to severe periodontitis and a higher risk of COVID-19 complications, including death (OR = 8.81, 95%CI: 1.00-77.7), admissions to an intensive care unit (OR = 3.54, 95%CI: 1.39-9.05), and the need for assisted ventilation (OR = 4.57, 95%CI: 1.19-17.4)[84]. A similar study with 137 COVID-19 patients found that patients with signs of oral disease (radiographic alveolar bone loss, dental caries, and apical periodontitis) had a significantly higher risk of developing COVID-19 complications compared to those without oral diseases. These complications included positive symptomatology, hospitalizations, and mortality. The study used oral examination records and panoramic X-rays. However, a positive specific correlation with periodontitis could not be observed[85]. Regarding systematic reviews, a study found a reciprocal relationship between COVID-19 and periodontitis; but for peri-implantitis, the authors came to the conclusion that there was still a lack of information[86]. According to a mendelian randomization research there was no correlation between periodontitis and hospitalization for COVID-19 (OR = 0.97, 95%CI: 0.78–1.20; P = 0.76), vulnerability to COVID-19 (OR = 1.04, 95%CI: 0.88–1.21; P = 0.65), or severity of COVID-19 disease (OR = 1.01, 95%CI: 0.92–1.11; P = 0.81)[87]. A meta-analysis, on the other hand, clearly suggested that COVID-19 patients with periodontal disease have a 4-fold increased risk of hospitalization, a 6-fold increased risk of assisted ventilation, and a 7-fold increased risk of death[88]. However, a systematic review stated that there is no evidence to support or contradict a strong relationship between periodontitis and the likelihood of COVID-19 complications and death[89].

Investigations are ongoing to determine the biological mechanism directing a possible association between COVID-19 and periodontitis. It is commonly known that the linkages between systemic disorders including diabetes, cardiovascular disease, and rheumatoid arthritis and periodontitis are caused by the translocation of periodontal bacteria to blood and consequent systemic inflammation[90,91]. Furthermore, a number of risk factors, such as smoking, old age, obesity, diabetes, hypertension, and cardiovascular disease, are shared by both the severity of COVID-19 disease and periodontitis[1,73,83,92]. A probable correlation between periodontitis and greater severity of COVID-19 infection has been indicated by several investigations due to the increased systemic risk of periodontitis. Certain researchers have questioned if COVID-19 risk factors for periodontal illnesses should be given the same weight as cardiovascular, diabetes, and other conditions[93]. It is unclear, nonetheless, if there are particular processes and pathological pathways connecting periodontitis and greater COVID-19 severity, or if these factors could simply operate as comorbidities. Researchers have suggested a number of mechanisms to explain this association and provide evidence for the connection between COVID-19 infection and periodontitis: (1) SARS-CoV-2 can be detected in periodontal pockets. In COVID-positive cadaver biopsies, SARS-CoV-2 was found in periodontal tissues[94]. This pathogenic environment may allow SARS-CoV-2 to enter through damaged epithelia or by upregulating ACE 2 receptor expression by periodontal bacterial pathogens[95]. These suggest that periodontal pockets may harbor SARS-CoV-2 and allow it to enter the bloodstream; (2) COVID-19 patients can aspirate pathogens from periodontitis. The well-established association between oral microbiome and respiratory illnesses may also relate periodontitis to COVID-19 severity. Poor dental hygiene and periodontitis may collect respiratory microorganisms in the mouth. These pathogens may be eventually aspirated into the lung. For instance, hospital-acquired pneumonia and pneumonia deaths are more common in older adults with periodontal pockets[96]. In a systematic review, respiratory disease and periodontitis were linked to higher prevalence of chronic obstructive pulmonary disease, obstructive sleep apnea, and COVID-19 infection[97]. Respiratory diseases related to periodontitis may worsen COVID-19 and result in a higher mortality[98]. Some periodontopathic bacteria increase ACE 2 and respiratory tract inflammatory cytokines. Bronchoalveolar fluid from COVID-19 patients contained oral opportunistic infections[99]. Lung hypoxia in COVID-19 patients may further encourage the growth of oral microbiota anaerobes; and (3) Periodontitis causes systemic inflammation. High C-reactive protein and proinflammatory cytokines in periodontitis promote systemic inflammation that might aggravate conditions like diabetes and cardiovascular disease [90,91]. Periodontitis synergistically stimulates peripheral leukocytes to local and remote inflammatory stimuli, preparing the immune system for a stronger innate response[100]. COVID-19 infection can trigger a "cytokine storm" an uncontrollable hyperinflammatory reaction. The condition is marked by increased serum levels of interleukin family, C-reactive protein, and tumor necrosis factor-α, and decreased T-lymphocyte numbers[101]. In COVID-19 hospital records, patients with periodontitis had far higher blood levels of inflammatory markers such C-reactive protein than those without periodontitis[84]. Based on this premise, inflammatory upregulation may link periodontitis to COVID-19 severity. Figure 1 shows how periodontitis and COVID-19 may interact (Figure 1). (1) Periodontal pocket act like a reservoir for SARS-CoV-2 and an entrance into the blood stream; (2) Periodontitis as a source for increased direct inoculation of periodontopathic bacteria into the respiratory system in COVID-19 patients; and (3) Periodontitis as a source for increased systemic inflammation and priming the immune system in COVID-19 patients.

Given these connections between periodontitis and COVID-19 severity, periodontal treatment may help prevent and manage COVID-19 problems[102,103]. Impaired immune response, medicines, and reduced diet intake make COVID-19 patients more susceptible to oral dysbiosis. Due to illness and hospitalization, critically ill individuals have poor oral hygiene, which worsens dysbiosis[104]. Periodontitis can be prevented by everyday oral hygiene and professional biofilm removal. Periodontal therapy have decreased the severity of pneumonia and other systemic infections, improves the gingival epithelial barrier, preventing oral harmful viruses and bacteria from entering the bloodstream. Thus, periodontal care may lessen COVID-19's systemic effects and, regular oral hygiene may reduce aspiration pneumonia and COVID-19 complications[98].

To sum up, there are a number of similarities between COVID-19 and periodontitis, such as their effects on systemic inflammation and shared comorbidities. In fact, a few preliminary investigations have suggested a potential link between periodontitis and the likelihood of contracting COVID-19 and its consequences. These correlations may be caused by a priming effect on systemic inflammation, but periodontal bacteria in the lungs may also play a role. In this case, reducing COVID-19 infections and consequences may be aided by preventative dental hygiene practices and periodontal treatment. Nevertheless, more investigation would be required to validate these theories.

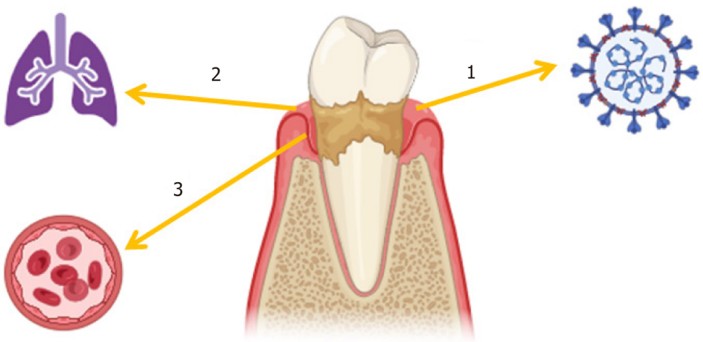

Figure 1 Possible mechanisms of interaction between periodontitis and coronavirus disease 2019 infection. (1) Periodontal pocket act like a reservoir for severe acute respiratory syndrome coronavirus 2 and an entrance into the blood stream; (2) Periodontitis as a source for increased direct inoculation of periodontopathic bacteria into the respiratory system in coronavirus disease 2019 (COVID-19) patients; and (3) Periodontitis as a source for increased systemic inflammation and priming the immune system in COVID-19 patients. (Created at BioRender.com)

HPV

HPV is a double-stranded DNA virus. HPV is one of the three most prevalent sexually transmitted infections (STIs) in both genders and the most prevalent viral STI, which is primarily sexually transmitted. Currently, HPV is the most prevalent infection linked to cancer in women; it has been found in over 90% of cases of cervical cancer, the fourth most deadly type of cancer in women[105,106].

Multiple symptoms, including benign warty or potentially malignant lesions, intraepithelial neoplasia, and invasive carcinomas, can result from HPV infections[107]. There are currently over 200 known HPV genotypes, about 40 of which are mucosal HPV types, meaning they affect the mucosa. The alpha genus of HPV is primarily clinically significant because it harbors the majority of mucosal HPVs, both high-risk and low-risk varieties[108]. High-risk mucosal HPVs, such HPV-16 and HPV-18, produce squamous intraepithelial lesions that can proceed to squamous cell carcinoma in the head and neck area and/or anogenital tract, while low-risk mucosal HPVs, like HPV-6 and HPV-11, cause benign papilloma/condyloma[109]. The global pooled prevalence for genital HPV in men, derived from 35 countries, was 21% for high-risk-HPV and 31% for any HPV[110]. The estimated global prevalence of genital HPV in women was 11.7%[111]. The global incidence of oral HPV in healthy individuals is believed to be between 4 to 7.5%[112,113].

HPV has been detected in the oral cavity especially in the gingival crevicular fluid, gingival tissue, oral swab and oral rinse samples[114-116]. It's possible that HPV enters the human body through the oral cavity first. The main oral manifestations of HPV include oral papillomas/condylomas, focal epithelial hyperplasia and squamous cell carcinoma of the oropharyngeal region[117].

The oral mucosa can harbor HPV infection asymptomatically. The mouth's border, oropharynx, tonsil cryptal epithelium, salivary gland ductal epithelium, and inflammatory gingival pocket are among the potential reservoirs. Another possible reservoir is a latent HPV infection in epithelial basal cells, where a local irritation might trigger a transition from stable to vegetative viral DNA replication[117].

The majority of research on oral HPV has been on its association with oropharyngeal cancer. Nonetheless, a number of research also investigated the connection between periodontitis and HPV found in the gingival/periodontal tissue. For instance, data from the United States NHANES from 2009 to 2012 allowed for the analysis of almost 6000 people (30–69 years old) with clinically determined HPV and periodontal status. After controlling for confounding variables, the adjusted OR for the presence of HPV in oral rinse specimens of participants with periodontal disease was 1.04 (95%CI: 0.63–1.73)[115]. In another study, 223 patients with known periodontal disease status, oral hygiene practices, and HPV-positive oral rinse samples were included. Ten (4.5%) of these individuals tested positive for HPV-16 DNA. Among the participants who tested positive for HPV-16 DNA, periodontal disease was linked to three (30%) and poor oral hygiene to seven (70%) of them[118]. Moreover, in a multivariable analysis, adults with severe periodontitis had higher odds of oral HPV infection than adults with none or mild periodontal disease (OR = 2.9, 95%CI: 1.0-8.4, $P < 0.05$). Adults with pocket depth > 6 mm and clinical attachment loss ≥ 7 mm exhibited 2- to 3-fold increased risks of HPV infection[119]. Furthermore, PCR analysis of gingival biopsies obtained from patients with periodontal disease who had a clinical diagnosis of periodontitis revealed the presence of high-risk HPV strains in 26% (8/31) of the samples[116]. Again, when eight participants with widespread chronic periodontitis had their periodontal pockets scraped, HPV E6/E7 mRNA was found in four of the eight samples[120]. In a study with 822 patients, McDaniel *et al*[121] found that those without a vaccination against HPV and periodontitis had a higher incidence of oral HPV[121].

Furthermore, a higher number of oral bacteria was linked to higher HPV16 E6 viral copy numbers in hospital patients, indicating a possible link between oral HPV infection and viral replication and inadequate oral hygiene[122]. As a result, there was evidence for a tendency toward a positive connection between oral HPV-16 infection and clinical oral health. Furthermore, there was a strong correlation found between the prevalence of *Fusobacterium nucleatum* and *T. denticola* and the HPV16 DNA positive in gingival crevicular fluid[123]. Therefore, it is thought that the prevalence of oral HPV and periodontal microorganisms are connected. It is hypothesized that whereas periodontal disease provide an environment in which oral HPV infections can thrive, oral HPV infections may also make periodontal diseases worse.

However, some negative findings also reported in the literature about the correlation between periodontitis and identified HPV in periodontium. For instance, HPV-16 was not found in any of the 104 gingival samples examined in a case-control study that included gingivitis, periodontitis, and healthy periodontium[114]. PCR was used to screen 74 oral biopsies from kidney transplant recipients and non-recipients with gingivitis and/or periodontitis for the presence of HPV and EBV viruses. In transplant recipients, EBV was substantially linked to periodontitis and/or gingivitis (P = 0.011) but not HPV (P = 0.766)[124]. There was no correlation found between the periodontal state and the presence of HPV in the oral cavity in an investigation from Argentine involving women with gynecological infections[125]. Finally, a very recent meta-analysis found that among people with confirmed periodontitis, there was no significant increase in the likelihood of high-risk oral HPV infection (OR = 4.71, 95%CI: 0.57–38.97). However, compared to people without periodontitis, individuals with the disease had a 3.65-fold increased risk of developing an oral HPV infection of any kind (95%CI: 1.67–8.01)[126].

The precise nature and degree of the link between HPV and the onset and progression of periodontal disease remains unclear. In addition, the mechanism is also under investigation. Nonetheless, it is well known that HPV has a tropism for squamous epithelium. Viral particles cause micro-abrasions or epithelial injury, which expose the basal cells of the epithelium. There is still some mystery about HPV receptors and the way the virus enters cells. Briefly, HPV-16 attaches to laminin in the extracellular matrix or basement membrane, which causes epithelial cells to die.

In conclusion, people with periodontal disease may have HPV in their periodontal epithelium, and these individuals often have a higher risk of developing periodontitis. It is believed that basal keratinocytes in the ulcerated gingival sulcus epithelium are infected by HPV in the inflammatory periodontal tissue. A distinct oral microbiome may be linked to oral HPV infection, even if the precise connections between periodontopathic bacteria and HPV are still unknown. Using clinical definitions of oral HPV infection and periodontitis and concentrating on high-risk populations for oral HPV infection, future longitudinal research should assess this association in more detail. Examining this correlation is crucial since periodontitis may indicate who is more susceptible to oral HPV infection and maybe oropharyngeal malignancies linked to HPV. As a result, treating patients with chronic periodontitis is essential to maintaining dental health and hygiene and preventing potentially fatal conditions like oral cancer.

HEPATITIS B AND C VIRUSES

HCV is an RNA virus that is a member of the flaviviridae family of viruses. Hepatitis B virus (HBV), on the other hand, is a DNA virus that only infects humans. These viruses are not immediately cytopathic; rather, they multiply in the cytoplasm of hepatocytes[127]. Fast viral replication, ongoing cell-to-cell transmission, and a weak T-cell immune response to viral antigens appear to be necessary for persistent infection[128].

An estimated 50 million people worldwide suffer from a chronic HCV infection, and one million new cases are reported each year. According to WHO estimates, hepatitis C killed about 242 thousand individuals in 2022[129]. According to WHO predictions, 1.2 million new cases of chronic hepatitis B infection occur annually, impacting 254 million people worldwide in 2022. Hepatitis B is expected to have killed 1.1 million people in 2022. Millions of people are afflicted with viral hepatitis and its aftereffects despite the availability of an efficient treatment and vaccine[130].

Acute and chronic hepatitis, non-alcoholic fatty liver disease (NAFLD), liver fibrosis and cirrhosis, hepatocellular cancer, and extra-hepatic symptoms are among the sequelae and effects of HCV and HBV. Regarding the extra-hepatic symptoms, glomerulonephritis and polyarteritis nodosa have been reported in cases of persistent HBV infection[131]. Mixed cryoglobulinemia, along with the accompanying systemic vasculitis, is a common extra-hepatic symptom of chronic HCV infection[132]. Possible clinical manifestations include weakness, arthralgias, purpura, and renal dysfunction. Primary oral manifestations of HBV and HBV include lichen planus, Sjögren-like sialadenitis, and oral squamous cell carcinoma[133,134].

Periodontitis have been studied in relation to various aspects of liver disease, such as NAFLD, liver cirrhosis (LC), hepatocellular carcinoma and liver transplantation. For instance, in a Finnish cohort study comprised of 6165 individuals without baseline liver diseases were followed up for 12 years. During the follow-up, 79 patients had a serious liver incident. In the general population, periodontitis was linked to incidence of liver disease, regardless of other factors[135]. Periodontal disease is a risk factor for NAFLD, according to numerous cross-sectional and epidemiological studies. According to certain *in vivo* animal models, periodontopathic bacterial infection quickens the course of NAFLD and is associated with increased steatosis[136]. Furthermore, the presence of periodontopathic bacteria in the liver may indicate a direct correlation between these bacteria and NAFLD. Additionally, the lipopolysaccharide produced by *P. gingivalis* and *A. actinomycetemcomitans* induces inflammation and intracellular lipid buildup in hepatocytes[136,137]. Out of four epidemiological systematic review and meta-analyses on the association between NAFLD and periodontitis, three of them found significant correlation[138-141]. Grønkjaer[142] conducted a systematic review of the literature from 1981 to 2014 and found that, according to multiple different periodontal indicators, patients with LC had a higher incidence of periodontal disease. But the type and degree of the relationship were still unknown, particularly in terms of whether periodontal disease and the severity and etiology of cirrhosis are associated[142]. In a recent meta-analysis, cirrhotic patients presented a greater and significant mean of clinical attachment loss, probing depth and alveolar bone loss than those without LC. Authors concluded that LC patients have poor periodontal conditions and a higher prevalence of periodontitis[143]. Concerning the risk of hepatocellular, Al-Maweri *et al*[144] systematically reviewed the published literature on the link between tooth loss/periodontitis and the risk of liver cancer. Researchers stated that available evidence suggests a possibility; however, the evidence was not conclusive enough[144]. In most of these researches, the clinical liver condition is investigated, rather than the exact presence and effect of HBV/HCV. Therefore, it is important

to bear in mind that the studied liver conditions may have been caused by other non-viral causes.

Some other research examined periodontitis in individuals with confirmed HCV and HBV infections. Hepatitis virus infection and periodontitis were positively correlated in a large cross-sectional study from the NHANES spanning the years 2003–2018, with 5755 individuals (OR = 2.60; 95%CI: 1.51-4.49). Additionally, there was a high correlation (OR = 2.13; 95%CI: 1.19-3.82) found between moderate periodontitis and hepatitis virus infection. This correlation was even more pronounced for severe periodontitis (OR = 3.58; 95%CI: 1.77-7.21). Significantly, there was a constant positive correlation between periodontitis and hepatitis virus infection in several subgroups[145]. In patients with chronic hepatitis C who had periodontitis, positive associations were seen between the levels of IL-1β and IL-1α in gingival fluid and specific clinical periodontal criteria; and this was also related with the age of the viral hepatitis C diagnosis[146]. Salivary occult blood test was used in a retrospective research to screen for periodontal disease in 351 individuals with liver disorders due to HBV, HCV, or both. Multivariate research revealed that five factors—being 65 years of age or older, tooth brushing just once a day, having a platelet count below 80000, and being obese—were linked to periodontal disease. The authors mentioned that the development of viral liver disease may be linked to periodontitis[147]. Using the CPITN index (Community Periodontal Index of Treatment Needs), Coates *et al*[148] examined the periodontal condition in patients with hepatitis C. Despite no discernible variation in CPITN categories across the persons examined, there was a clear indication of poor periodontal health in the hepatitis patients[148]. Aspartate amino-transferase levels in the gingival tissue of patients with periodontal disease have been demonstrated to be elevated and to be correlated with the activity of the disease[149]. Additionally, non-surgical periodontal therapy has been shown to improve periodontal inflammation in patients with chronic viral hepatitis[150]. Finally, in animal model study, authors concluded that periodontitis may be an independent risk factor for liver fibrosis in rats when periodontitis was induced by ligatures[151].

Based on these and the similar findings, the concept of oral-intestine-liver axis has been proposed. This is explained through certain premises: The etiology of periodontitis is largely influenced by bacteria of the red complex, which are abundant in deep periodontal pockets and active periodontal lesions[152]. These particular periodontal infectious agents are easily swallowed and go from the oral cavity to the stomach, where they may significantly alter the gut microbiota. It has recently proposed that the cause of some systemic disease, particularly liver disease, might be the disruption of the composition of the intestinal microbiota by orally derived periodontal pathogenic bacteria[153]. Due to the possibility of liver-related consequences and the translocation of oral bacteria and their toxins into the intestine, periodontitis may be especially concerning in patients with liver disease. Systemic endotoxemia, usually intestinal in origin, is linked to liver damage, liver disease development, and decompensation of cirrhosis[154,155]. Hence, the mechanisms linking periodontitis to viral hepatitis include periodontopathogenic bacteria, pro-inflammatory mediators and oxidative stress.

To sum up, there seems to be a bi-directional relation between periodontitis and certain liver diseases of viral origin. Additional research is necessary to determine whether there is a connection between the viral infection and other inflammatory markers found in the gingival fluid. This will allow us to better understand if patients with viral hepatitis who are already at risk of developing periodontal disease or those who are already at risk of developing periodontitis may express other biomarkers specific to the liver more frequently.

CONCLUSION

Although periodontitis is mainly a bacterial inflammation, several other modifiable and non-modifiable risk factors may play role in its initiation and progression. Viruses may take part directly through attacking the periodontal tissue and indirectly through increasing the vulnerability of the immune system and providing a higher chance for the colonization of the periodontopathic bacteria in the oral cavity. Further clinical, translational, experimental, epigenetic and epidemiological research is needed to clarify the exact nature and extent of the role of viruses in periodontitis. This will positively affect the life of millions among healthy population and virally infected people with periodontitis.

FOOTNOTES

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REFERENCES

1 **Ioannou AL**, Kotsakis GA, Hinrichs JE. Prognostic factors in periodontal therapy and their association with treatment outcomes. *World J Clin Cases* 2014; **2**: 822-827 [PMID: 25516855 DOI: 10.12998/wjcc.v2.i12.822]
2 **Bhansali RS**. Non-surgical periodontal therapy: An update on current evidence. *World J Stomatol* 2014; **3**: 38 [DOI: 10.5321/wjs.v3.i4.38]
3 **Albandar JM**. Epidemiology and risk factors of periodontal diseases. *Dent Clin North Am* 2005; **49**: 517-532, v-vi [PMID: 15978239 DOI: 10.1016/j.cden.2005.03.003]
4 **Xu HM**, Shen XJ, Liu J. Establishment of models to predict factors influencing periodontitis in patients with type 2 diabetes mellitus. *World J Diabetes* 2023; **14**: 1793-1802 [PMID: 38222787 DOI: 10.4239/wjd.v14.i12.1793]
5 **Mahmood MK**, Kurda HA, Qadir BH, Tassery H, Lan R, Tardivo D, Abdulghafor MA. Implication of serum and salivary albumin tests in the recent oral health related epidemiological studies: A narrative review. *Saudi Dent J* 2024; **36**: 698-707 [PMID: 38766281 DOI: 10.1016/j.sdentj.2024.02.019]
6 **Chalabi M**, Rezaie F, Moghim S, Mogharehabed A, Rezaei M, Mehraban B. Periodontopathic bacteria and herpesviruses in chronic periodontitis. *Mol Oral Microbiol* 2010; **25**: 236-240 [PMID: 20536751 DOI: 10.1111/j.2041-1014.2010.00571.x]
7 **Thomasini RL**, Pereira FSM. Impact of different types of herpesviral infections in the oral cavity. *World J Stomatol* 2016; **5**: 22 [DOI: 10.5321/wjs.v5.i2.22]
8 **Carrozzo M**, Scally K. Oral manifestations of hepatitis C virus infection. *World J Gastroenterol* 2014; **20**: 7534-7543 [PMID: 24976694 DOI: 10.3748/wjg.v20.i24.7534]
9 **Markopoulos AK**. Role of human papillomavirus in the pathogenesis of oral squamous cell carcinoma. *World J Exp Med* 2012; **2**: 65-69 [PMID: 24520535 DOI: 10.5493/wjem.v2.i4.65]
10 **Abdel Massih RC**, Razonable RR. Human herpesvirus 6 infections after liver transplantation. *World J Gastroenterol* 2009; **15**: 2561-2569 [PMID: 19496184 DOI: 10.3748/wjg.15.2561]
11 **Looker KJ**, Welton NJ, Sabin KM, Dalal S, Vickerman P, Turner KME, Boily MC, Gottlieb SL. Global and regional estimates of the contribution of herpes simplex virus type 2 infection to HIV incidence: a population attributable fraction analysis using published epidemiological data. *Lancet Infect Dis* 2020; **20**: 240-249 [PMID: 31753763 DOI: 10.1016/S1473-3099(19)30470-0]
12 **Slots J**, Rams TE. Herpesvirus-Bacteria pathogenic interaction in juvenile (aggressive) periodontitis. A novel etiologic concept of the disease. *Periodontol 2000* 2024; **94**: 532-538 [PMID: 37345343 DOI: 10.1111/prd.12501]
13 **Slots J**. Periodontal herpesviruses: prevalence, pathogenicity, systemic risk. *Periodontol 2000* 2015; **69**: 28-45 [PMID: 26252400 DOI: 10.1111/prd.12085]
14 **Zakay-Rones Z**, Hochman N, Rones Y. Immunological response to herpes simplex virus in human gingival fluid. *J Periodontol* 1982; **53**: 42-45 [PMID: 6276526 DOI: 10.1902/jop.1982.53.1.42]
15 **Contreras A**, Nowzari H, Slots J. Herpesviruses in periodontal pocket and gingival tissue specimens. *Oral Microbiol Immunol* 2000; **15**: 15-18 [PMID: 11155159 DOI: 10.1034/j.1399-302x.2000.150103.x]
16 **Kamma JJ**, Contreras A, Slots J. Herpes viruses and periodontopathic bacteria in early-onset periodontitis. *J Clin Periodontol* 2001; **28**: 879-885 [PMID: 11493359 DOI: 10.1034/j.1600-051x.2001.028009879.x]
17 **Yapar M**, Saygun I, Ozdemir A, Kubar A, Sahin S. Prevalence of human herpesviruses in patients with aggressive periodontitis. *J Periodontol* 2003; **74**: 1634-1640 [PMID: 14682660 DOI: 10.1902/jop.2003.74.11.1634]
18 **Contreras A**, Slots J. Active cytomegalovirus infection in human periodontitis. *Oral Microbiol Immunol* 1998; **13**: 225-230 [PMID: 10093537 DOI: 10.1111/j.1399-302x.1998.tb00700.x]
19 **Alzahrani AA**. Association between human herpes virus and aggressive periodontitis: A systematic review. *Saudi J Dent Res* 2017; **8**: 97-104 [DOI: 10.1016/j.sjdr.2016.06.004]
20 **Song Y**, Liu N, Gao L, Yang D, Liu J, Xie L, Dan H, Chen Q. Association between human herpes simplex virus and periodontitis: results from the continuous National Health and Nutrition Examination Survey 2009-2014. *BMC Oral Health* 2023; **23**: 675 [PMID: 37723536 DOI: 10.1186/s12903-023-03416-x]
21 **Maulani C**, Auerkari EI, C Masulili SL, Soeroso Y, Djoko Santoso W, S Kusdhany L. Association between Epstein-Barr virus and periodontitis: A meta-analysis. *PLoS One* 2021; **16**: e0258109 [PMID: 34618843 DOI: 10.1371/journal.pone.0258109]
22 **Roca-Millan E**, Domínguez-Mínger J, Schemel-Suárez M, Estrugo-Devesa A, Marí-Roig A, López-López J. Epstein-Barr Virus and Peri-Implantitis: A Systematic Review and Meta-Analysis. *Viruses* 2021; **13** [PMID: 33562820 DOI: 10.3390/v13020250]
23 **Gao Z**, Lv J, Wang M. Epstein-Barr virus is associated with periodontal diseases: A meta-analysis based on 21 case-control studies. *Medicine (Baltimore)* 2017; **96**: e5980 [PMID: 28178139 DOI: 10.1097/MD.0000000000005980]
24 **Botero JE**, Rodríguez-Medina C, Jaramillo-Echeverry A, Contreras A. Association between human cytomegalovirus and periodontitis: A systematic review and meta-analysis. *J Periodontal Res* 2020; **55**: 551-558 [PMID: 32167179 DOI: 10.1111/jre.12742]
25 **Arduino PG**, Cabras M, Lodi G, Petti S. Herpes simplex virus type 1 in subgingival plaque and periodontal diseases. Meta-analysis of observational studies. *J Periodontal Res* 2022; **57**: 256-268 [PMID: 34978079 DOI: 10.1111/jre.12968]
26 **Arduino PG**, Alovisi M, Petti S. Herpes simplex virus type 1 in periapical pathoses: Systematic review and meta-analysis. *Oral Dis* 2024; **30**: 865-876 [PMID: 37338057 DOI: 10.1111/odi.14645]
27 **Jakovljevic A**, Andric M, Jacimovic J, Milasin J, Botero JE. Herpesviruses in Periodontitis: An Umbrella Review. *Adv Exp Med Biol* 2022; **1373**: 139-155 [PMID: 35612796 DOI: 10.1007/978-3-030-96881-6_7]
28 **Li F**, Zhu C, Deng FY, Wong MCM, Lu HX, Feng XP. Herpesviruses in etiopathogenesis of aggressive periodontitis: A meta-analysis based on case-control studies. *PLoS One* 2017; **12**: e0186373 [PMID: 29036216 DOI: 10.1371/journal.pone.0186373]
29 **Zhu C**, Li F, Wong MC, Feng XP, Lu HX, Xu W. Association between Herpesviruses and Chronic Periodontitis: A Meta-Analysis Based on Case-Control Studies. *PLoS One* 2015; **10**: e0144319 [PMID: 26666412 DOI: 10.1371/journal.pone.0144319]

30 **Jakovljevic A**, Andric M. Human cytomegalovirus and Epstein-Barr virus in etiopathogenesis of apical periodontitis: a systematic review. *J Endod* 2014; **40**: 6-15 [PMID: 24331984 DOI: 10.1016/j.joen.2013.10.001]

31 **Contreras A**, Mardirossian A, Slots J. Herpesviruses in HIV-periodontitis. *J Clin Periodontol* 2001; **28**: 96-102 [PMID: 11142675 DOI: 10.1034/j.1600-051x.2001.280115.x]

32 **Slots J**. Herpesvirus periodontitis: infection beyond biofilm. *J Calif Dent Assoc* 2011; **39**: 393-399 [PMID: 21823497 DOI: 10.1080/19424396.2011.12221912]

33 **Carpenter CC**, Fischl MA, Hammer SM, Hirsch MS, Jacobsen DM, Katzenstein DA, Montaner JS, Richman DD, Saag MS, Schooley RT, Thompson MA, Vella S, Yeni PG, Volberding PA. Antiretroviral therapy for HIV infection in 1998: updated recommendations of the International AIDS Society-USA Panel. *JAMA* 1998; **280**: 78-86 [PMID: 9660368 DOI: 10.1001/jama.280.1.78]

34 **Ball SC**. Increased longevity in HIV: caring for older HIV-infected adults. *Care Manag J* 2014; **15**: 76-82 [PMID: 25118513 DOI: 10.1891/1521-0987.15.2.76]

35 **Tran BX**, Phan HT, Latkin CA, Nguyen HLT, Hoang CL, Ho CSH, Ho RCM. Understanding Global HIV Stigma and Discrimination: Are Contextual Factors Sufficiently Studied? (GAP(RESEARCH)). *Int J Environ Res Public Health* 2019; **16** [PMID: 31146379 DOI: 10.3390/ijerph16111899]

36 **Tappuni AR**. The global changing pattern of the oral manifestations of HIV. *Oral Dis* 2020; **26** Suppl 1: 22-27 [PMID: 32862536 DOI: 10.1111/odi.13469]

37 **Moosazadeh M**, Shafaroudi AM, Gorji NE, Barzegari S, Nasiri P. Prevalence of oral lesions in patients with AIDS: a systematic review and meta-analysis. *Evid Based Dent* 2021 [PMID: 34795396 DOI: 10.1038/s41432-021-0209-8]

38 **Itin PH**, Lautenschlager S. Viral lesions of the mouth in HIV-infected patients. *Dermatology* 1997; **194**: 1-7 [PMID: 9031782 DOI: 10.1159/000246047]

39 **Ryder MI**, Shiboski C, Yao TJ, Moscicki AB. Current trends and new developments in HIV research and periodontal diseases. *Periodontol 2000* 2020; **82**: 65-77 [PMID: 31850628 DOI: 10.1111/prd.12321]

40 **de Almeida VL**, Lima IFP, Ziegelmann PK, Paranhos LR, de Matos FR. Impact of highly active antiretroviral therapy on the prevalence of oral lesions in HIV-positive patients: a systematic review and meta-analysis. *Int J Oral Maxillofac Surg* 2017; **46**: 1497-1504 [PMID: 28684301 DOI: 10.1016/j.ijom.2017.06.008]

41 **Ndiaye CF**, Critchlow CW, Leggott PJ, Kiviat NB, Ndoye I, Robertson PB, Georgas KN. Periodontal status of HIV-1 and HIV-2 seropositive and HIV seronegative female commercial sex workers in Senegal. *J Periodontol* 1997; **68**: 827-831 [PMID: 9379325 DOI: 10.1902/jop.1997.68.9.827]

42 **McKaig RG**, Thomas JC, Patton LL, Strauss RP, Slade GD, Beck JD. Prevalence of HIV-associated periodontitis and chronic periodontitis in a southeastern US study group. *J Public Health Dent* 1998; **58**: 294-300 [PMID: 10390712 DOI: 10.1111/j.1752-7325.1998.tb03012.x]

43 **Kroidl A**, Schaeben A, Oette M, Wettstein M, Herfordt A, Häussinger D. Prevalence of oral lesions and periodontal diseases in HIV-infected patients on antiretroviral therapy. *Eur J Med Res* 2005; **10**: 448-453 [PMID: 16287607]

44 **Alves M**, Mulligan R, Passaro D, Gawell S, Navazesh M, Phelan J, Greenspan D, Greenspan JS. Longitudinal evaluation of loss of attachment in HIV-infected women compared to HIV-uninfected women. *J Periodontol* 2006; **77**: 773-779 [PMID: 16671868 DOI: 10.1902/jop.2006.P04039]

45 **Ntolou P**, Pani P, Panis V, Madianos P, Vassilopoulos S. The effect of antiretroviral therapyon the periodontal conditions of patients with HIV infection: A systematic review and meta-analysis. *J Clin Periodontol* 2023; **50**: 170-182 [PMID: 36261851 DOI: 10.1111/jcpe.13735]

46 **Valentine J**, Saladyanant T, Ramsey K, Blake J, Morelli T, Southerland J, Quinlivan EB, Phillips C, Nelson J, DeParis K, Webster-Cyriaque J. Impact of periodontal intervention on local inflammation, periodontitis, and HIV outcomes. *Oral Dis* 2016; **22** Suppl 1: 87-97 [PMID: 27109277 DOI: 10.1111/odi.12419]

47 **Sivakumar I**, Arunachalam S, Choudhary S, Buzayan MM. Does HIV infection affect the survival of dental implants? A systematic review and meta-analysis. *J Prosthet Dent* 2021; **125**: 862-869 [PMID: 32694022 DOI: 10.1016/j.prosdent.2020.04.001]

48 **Pavan P**, Pereira VT, Souza RC, Souza CO, Torres SR, Colombo AP, da Costa LJ, Sansone C, de Uzeda M, Gonçalves LS. Levels of HIV-1 in subgingival biofilm of HIV-infected patients. *J Clin Periodontol* 2014; **41**: 1061-1068 [PMID: 25197037 DOI: 10.1111/jcpe.12306]

49 **Gonçalves LS**, Ferreira SM, Silva A Jr, Villoria GE, Costinha LH, Colombo AP. Association of T CD4 lymphocyte levels and chronic periodontitis in HIV-infected brazilian patients undergoing highly active anti-retroviral therapy: clinical results. *J Periodontol* 2005; **76**: 915-922 [PMID: 15948685 DOI: 10.1902/jop.2005.76.6.915]

50 **Brito A**, Escalona LA, Correnti M, Perrone M, Bravo IM, Tovar V. Periodontal conditions and distribution of Prevotella intermedia, Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans in HIV-infected patients undergoing anti-retroviral therapy and in an HIV-seronegative group of the Venezuelan population. *Acta Odontol Latinoam* 2008; **21**: 89-96 [PMID: 18841752]

51 **Engeland CG**, Jang P, Alves M, Marucha PT, Califano J. HIV infection and tooth loss. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008; **105**: 321-326 [PMID: 18280966 DOI: 10.1016/j.tripleo.2007.10.012]

52 **Vastardis SA**, Yukna RA, Fidel PL Jr, Leigh JE, Mercante DE. Periodontal disease in HIV-positive individuals: association of periodontal indices with stages of HIV disease. *J Periodontol* 2003; **74**: 1336-1341 [PMID: 14584867 DOI: 10.1902/jop.2003.74.9.1336]

53 **Vernon LT**, Demko CA, Whalen CC, Lederman MM, Toossi Z, Wu M, Han YW, Weinberg A. Characterizing traditionally defined periodontal disease in HIV+ adults. *Community Dent Oral Epidemiol* 2009; **37**: 427-437 [PMID: 19624697 DOI: 10.1111/j.1600-0528.2009.00485.x]

54 **Groenewegen H**, Delli K, Vissink A, Spijkervet FKL, Bierman WFW. Immune markers and microbial factors are related with periodontitis severity in people with HIV. *Clin Oral Investig* 2023; **27**: 1255-1263 [PMID: 36316604 DOI: 10.1007/s00784-022-04758-6]

55 **Howell RB**, Jandinski JJ, Palumbo P, Shey Z, Houpt MI. Oral soft tissue manifestations and CD4 lymphocyte counts in HIV-infected children. *Pediatr Dent* 1996; **18**: 117-120 [PMID: 8710712]

56 **Santos LC**, Castro GF, de Souza IP, Oliveira RH. Oral manifestations related to immunosuppression degree in HIV-positive children. *Braz Dent J* 2001; **12**: 135-138 [PMID: 11450684]

57 **Ranganathan K**, Geethalakshmi E, Krishna Mohan Rao U, Vidya KM, Kumarasamy N, Solomon S. Orofacial and systemic manifestations in 212 paediatric HIV patients from Chennai, South India. *Int J Paediatr Dent* 2010; **20**: 276-282 [PMID: 20536589 DOI: 10.1111/j.1365-263X.2010.01050.x]

58 **Gona P**, Van Dyke RB, Williams PL, Dankner WM, Chernoff MC, Nachman SA, Seage GR 3rd. Incidence of opportunistic and other infections in HIV-infected children in the HAART era. *JAMA* 2006; **296**: 292-300 [PMID: 16849662 DOI: 10.1001/jama.296.3.292]

59 **Lam PPY**, Zhou N, Yiu CKY, Wong HM. Impact of Antiretroviral Therapy on Oral Health among Children Living with HIV: A Systematic

Review and Meta-Analysis. *Int J Environ Res Public Health* 2022; **19** [PMID: 36231240 DOI: 10.3390/ijerph191911943]
60 **Brady LJ**, Walker C, Oxford GE, Stewart C, Magnusson I, McArthur W. Oral diseases, mycology and periodontal microbiology of HIV-1-infected women. *Oral Microbiol Immunol* 1996; **11**: 371-380 [PMID: 9467369 DOI: 10.1111/j.1399-302x.1996.tb00198.x]
61 **Tsang CS**, Samaranayake LP. Predominant cultivable subgingival microbiota of healthy and HIV-infected ethnic Chinese. *APMIS* 2001; **109**: 117-126 [PMID: 11398993 DOI: 10.1034/j.1600-0463.2001.d01-113.x]
62 **Valian NK**, houshmand B, Ardakani MT, Mahmoudi S. Microbiological study of periodontal disease in populations with HIV: a systematic review and meta-analysis. 2021 preprint [DOI: 10.21203/rs.3.rs-538808/v1]
63 **Patel M**, Coogan M, Galpin JS. Periodontal pathogens in subgingival plaque of HIV-positive subjects with chronic periodontitis. *Oral Microbiol Immunol* 2003; **18**: 199-201 [PMID: 12753474 DOI: 10.1034/j.1399-302x.2003.00064.x]
64 **Ramos MP**, Ferreira SM, Silva-Boghossian CM, Souto R, Colombo AP, Noce CW, Gonçalves LS. Necrotizing periodontal diseases in HIV-infected Brazilian patients: a clinical and microbiologic descriptive study. *Quintessence Int* 2012; **43**: 71-82 [PMID: 22259811]
65 **Lackner AA**, Lederman MM, Rodriguez B. HIV pathogenesis: the host. *Cold Spring Harb Perspect Med* 2012; **2**: a007005 [PMID: 22951442 DOI: 10.1101/cshperspect.a007005]
66 **Spear GT**, Alves ME, Cohen MH, Bremer J, Landay AL. Relationship of HIV RNA and cytokines in saliva from HIV-infected individuals. *FEMS Immunol Med Microbiol* 2005; **45**: 129-136 [PMID: 16051064 DOI: 10.1016/j.femsim.2005.03.002]
67 **Yeung SC**, Kazazi F, Randle CG, Howard RC, Rizvi N, Downie JC, Donovan BJ, Cooper DA, Sekine H, Dwyer DE. Patients infected with human immunodeficiency virus type 1 have low levels of virus in saliva even in the presence of periodontal disease. *J Infect Dis* 1993; **167**: 803-809 [PMID: 8450244 DOI: 10.1093/infdis/167.4.803]
68 **Mataftsi M**, Skoura L, Sakellari D. HIV infection and periodontal diseases: an overview of the post-HAART era. *Oral Dis* 2011; **17**: 13-25 [PMID: 21029260 DOI: 10.1111/j.1601-0825.2010.01727.x]
69 **Challacombe SJ**, Naglik JR. The effects of HIV infection on oral mucosal immunity. *Adv Dent Res* 2006; **19**: 29-35 [PMID: 16672546 DOI: 10.1177/154407370601900107]
70 **SeyedAlinaghi S**, Oliaei S, Kianzad S, Afsahi AM, MohsseniPour M, Barzegary A, Mirzapour P, Behnezhad F, Noori T, Mehraeen E, Dadras O, Voltarelli F, Sabatier JM. Reinfection risk of novel coronavirus (COVID-19): A systematic review of current evidence. *World J Virol* 2020; **9**: 79-90 [PMID: 33363000 DOI: 10.5501/wjv.v9.i5.79]
71 **Taherkhani R**, Taherkhani S, Farshadpour F. Dynamics of host immune responses to SARS-CoV-2. *World J Clin Cases* 2021; **9**: 4480-4490 [PMID: 34222416 DOI: 10.12998/wjcc.v9.i18.4480]
72 **Bahmani M**, Chegini R, Ghanbari E, Sheykhsaran E, Shiri Aghbash P, Leylabadlo HE, Moradian E, Kazemzadeh Houjaghan AM, Bannazadeh Baghi H. Severe acute respiratory syndrome coronavirus 2 infection: Role of interleukin-6 and the inflammatory cascade. *World J Virol* 2022; **11**: 113-128 [PMID: 35665236 DOI: 10.5501/wjv.v11.i3.113]
73 **Nassar M**, Nso N, Alfishawy M, Novikov A, Yaghi S, Medina L, Toz B, Lakhdar S, Idrees Z, Kim Y, Gurung DO, Siddiqui RS, Zheng D, Agladze M, Sumbly V, Sandhu J, Castillo FC, Chowdhury N, Kondaveeti R, Bhuiyan S, Perez LG, Ranat R, Gonzalez C, Bhangoo H, Williams J, Osman AE, Kong J, Ariyaratnam J, Mohamed M, Omran I, Lopez M, Nyabera A, Landry I, Iqbal S, Gondal AZ, Hassan S, Daoud A, Baraka B, Trandafirescu T, Rizzo V. Current systematic reviews and meta-analyses of COVID-19. *World J Virol* 2021; **10**: 182-208 [PMID: 34367933 DOI: 10.5501/wjv.v10.i4.182]
74 **Rezaei M**, Ziai SA, Fakhri S, Pouriran R. ACE2: Its potential role and regulation in severe acute respiratory syndrome and COVID-19. *J Cell Physiol* 2021; **236**: 2430-2442 [PMID: 32901940 DOI: 10.1002/jcp.30041]
75 **Iranmanesh B**, Khalili M, Amiri R, Zartab H, Aflatoonian M. Oral manifestations of COVID-19 disease: A review article. *Dermatol Ther* 2021; **34**: e14578 [PMID: 33236823 DOI: 10.1111/dth.14578]
76 **Şehirli AÖ**, Aksoy U, Koca-Ünsal RB, Sayıner S. Role of NLRP3 inflammasome in COVID-19 and periodontitis: Possible protective effect of melatonin. *Med Hypotheses* 2021; **151**: 110588 [PMID: 33848919 DOI: 10.1016/j.mehy.2021.110588]
77 **Herrera D**, Serrano J, Roldán S, Sanz M. Is the oral cavity relevant in SARS-CoV-2 pandemic? *Clin Oral Investig* 2020; **24**: 2925-2930 [PMID: 32577830 DOI: 10.1007/s00784-020-03413-2]
78 **Dziedzic A**, Wojtyczka R. The impact of coronavirus infectious disease 19 (COVID-19) on oral health. *Oral Dis* 2021; **27** Suppl 3: 703-706 [PMID: 32304276 DOI: 10.1111/odi.13359]
79 **Katz J**, Yue S, Xue W. Dental diseases are associated with increased odds ratio for coronavirus disease 19. *Oral Dis* 2022; **28** Suppl 1: 991-993 [PMID: 32989904 DOI: 10.1111/odi.13653]
80 **Farid H**, Khan M, Jamal S, Ghafoor R. Oral manifestations of Covid-19-A literature review. *Rev Med Virol* 2022; **32**: e2248 [PMID: 34028129 DOI: 10.1002/rmv.2248]
81 **Wang Y**, Deng H, Pan Y, Jin L, Hu R, Lu Y, Deng W, Sun W, Chen C, Shen X, Huang XF. Periodontal disease increases the host susceptibility to COVID-19 and its severity: a Mendelian randomization study. *J Transl Med* 2021; **19**: 528 [PMID: 34952598 DOI: 10.1186/s12967-021-03198-2]
82 **Larvin H**, Wilmott S, Wu J, Kang J. The Impact of Periodontal Disease on Hospital Admission and Mortality During COVID-19 Pandemic. *Front Med (Lausanne)* 2020; **7**: 604980 [PMID: 33330570 DOI: 10.3389/fmed.2020.604980]
83 **Larvin H**, Wilmott S, Kang J, Aggarwal VR, Pavitt S, Wu J. Additive Effect of Periodontal Disease and Obesity on COVID-19 Outcomes. *J Dent Res* 2021; **100**: 1228-1235 [PMID: 34271846 DOI: 10.1177/00220345211029638]
84 **Marouf N**, Cai W, Said KN, Daas H, Diab H, Chinta VR, Hssain AA, Nicolau B, Sanz M, Tamimi F. Association between periodontitis and severity of COVID-19 infection: A case-control study. *J Clin Periodontol* 2021; **48**: 483-491 [PMID: 33527378 DOI: 10.1111/jcpe.13435]
85 **Sirin DA**, Ozcelik F. The relationship between COVID-19 and the dental damage stage determined by radiological examination. *Oral Radiol* 2021; **37**: 600-609 [PMID: 33389600 DOI: 10.1007/s11282-020-00497-0]
86 **Mancini L**, Americo LM, Pizzolante T, Donati R, Marchetti E. Impact of COVID-19 on Periodontitis and Peri-Implantitis: A Narrative Review. *Front Oral Health* 2022; **3**: 822824 [PMID: 35224542 DOI: 10.3389/froh.2022.822824]
87 **Song J**, Wu Y, Yin X, Zhang J. Relationship between periodontitis and COVID-19: A bidirectional two-sample Mendelian randomization study. *Health Sci Rep* 2023; **6**: e1413 [PMID: 37564397 DOI: 10.1002/hsr2.1413]
88 **Baima G**, Marruganti C, Sanz M, Aimetti M, Romandini M. Periodontitis and COVID-19: Biological Mechanisms and Meta-analyses of Epidemiological Evidence. *J Dent Res* 2022; **101**: 1430-1440 [PMID: 35774019 DOI: 10.1177/00220345221104725]
89 **Espinoza-Espinoza DAK**, Dulanto-Vargas JA, Cáceres-LaTorre OA, Lamas-Castillo FE, Flores-Mir C, Cervantes-Ganoza LA, López-Gurreonero C, Ladera-Castañeda MI, Cayo-Rojas CF. Association Between Periodontal Disease and the Risk of COVID-19 Complications and

Mortality: A Systematic Review. *J Int Soc Prev Community Dent* 2021; **11**: 626-638 [PMID: 35036371 DOI: 10.4103/jispcd.JISPCD_189_21]
90 **Zardawi F**, Gul S, Abdulkareem A, Sha A, Yates J. Association Between Periodontal Disease and Atherosclerotic Cardiovascular Diseases: Revisited. *Front Cardiovasc Med* 2020; **7**: 625579 [PMID: 33521070 DOI: 10.3389/fcvm.2020.625579]
91 **Llambés F**, Arias-Herrera S, Caffesse R. Relationship between diabetes and periodontal infection. *World J Diabetes* 2015; **6**: 927-935 [PMID: 26185600 DOI: 10.4239/wjd.v6.i7.927]
92 **Liu H**, Chen S, Liu M, Nie H, Lu H. Comorbid Chronic Diseases are Strongly Correlated with Disease Severity among COVID-19 Patients: A Systematic Review and Meta-Analysis. *Aging Dis* 2020; **11**: 668-678 [PMID: 32489711 DOI: 10.14336/AD.2020.0502]
93 **Basso L**, Chacun D, Sy K, Grosgogeat B, Gritsch K. Periodontal Diseases and COVID-19: A Scoping Review. *Eur J Dent* 2021; **15**: 768-775 [PMID: 34500484 DOI: 10.1055/s-0041-1729139]
94 **Fernandes Matuck B**, Dolhnikoff M, Maia GVA, Isaac Sendyk D, Zarpellon A, Costa Gomes S, Duarte-Neto AN, Rebello Pinho JR, Gomes-Gouvêa MS, Sousa SCOM, Mauad T, Saldiva PHDN, Braz-Silva PH, da Silva LFF. Periodontal tissues are targets for Sars-Cov-2: a post-mortem study. *J Oral Microbiol* 2020; **13**: 1848135 [PMID: 33391625 DOI: 10.1080/20002297.2020.1848135]
95 **Mancini L**, Quinzi V, Mummolo S, Marzo G, Marchetti E. Angiotensin-Converting Enzyme 2 as a Possible Correlation between COVID-19 and Periodontal Disease. *Appl Sci* 2020; **10**: 6224 [DOI: 10.3390/app10186224]
96 **Awano S**, Ansai T, Takata Y, Soh I, Akifusa S, Hamasaki T, Yoshida A, Sonoki K, Fujisawa K, Takehara T. Oral health and mortality risk from pneumonia in the elderly. *J Dent Res* 2008; **87**: 334-339 [PMID: 18362314 DOI: 10.1177/154405910808700418]
97 **Molina A**, Huck O, Herrera D, Montero E. The association between respiratory diseases and periodontitis: A systematic review and meta-analysis. *J Clin Periodontol* 2023; **50**: 842-887 [PMID: 36606394 DOI: 10.1111/jcpe.13767]
98 **Brock M**, Bahammam S, Sima C. The Relationships Among Periodontitis, Pneumonia and COVID-19. *Front Oral Health* 2021; **2**: 801815 [PMID: 35128525 DOI: 10.3389/froh.2021.801815]
99 **Alfaifi AA**, Holm JB, Wang TW, Lim J, Meiller TF, Rock P, Sultan AS, Jabra-Rizk MA. Oral Microbiota Alterations in Subjects with SARS-CoV-2 Displaying Prevalence of the Opportunistic Fungal Pathogen Candida albicans. *Microorganisms* 2024; **12** [PMID: 39065125 DOI: 10.3390/microorganisms12071356]
100 **Fine N**, Chadwick JW, Sun C, Parbhakar KK, Khoury N, Barbour A, Goldberg M, Tenenbaum HC, Glogauer M. Periodontal Inflammation Primes the Systemic Innate Immune Response. *J Dent Res* 2021; **100**: 318-325 [PMID: 33078669 DOI: 10.1177/0022034520963710]
101 **Ragab D**, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 Cytokine Storm; What We Know So Far. *Front Immunol* 2020; **11**: 1446 [PMID: 32612617 DOI: 10.3389/fimmu.2020.01446]
102 **Said KN**, Al-Momani AM, Almaseeh JA, Marouf N, Shatta A, Al-Abdulla J, Alaji S, Daas H, Tharupeedikayil SS, Chinta VR, Hssain AA, Abusamak M, Salih S, Barhom N, Cai W, Sanz M, Tamimi F. Association of periodontal therapy, with inflammatory biomarkers and complications in COVID-19 patients: a case control study. *Clin Oral Investig* 2022; **26**: 6721-6732 [PMID: 35906340 DOI: 10.1007/s00784-022-04631-6]
103 **Hosoglu S**, Mahmood MK. COVID-19 infection among dentists in Iraqi Kurdistan Region. *J Infect Dev Ctries* 2022; **16**: 1439-1444 [PMID: 36223619 DOI: 10.3855/jidc.15962]
104 **Darestani MN**, Akbari A, Yaghobee S, Taheri M, Akbari S. COVID-19 Pandemic and Periodontal Practice: The Immunological, Clinical, and Economic Points of View. *Biomed Res Int* 2022; **2022**: 3918980 [PMID: 35047633 DOI: 10.1155/2022/3918980]
105 **Bae JM**. Human papillomavirus infection and gastric cancer risk: A meta-epidemiological review. *World J Virol* 2021; **10**: 209-216 [PMID: 34631472 DOI: 10.5501/wjv.v10.i5.209]
106 **Sigaroodi A**, Nadji SA, Naghshvar F, Nategh R, Emami H, Velayati AA. Human papillomavirus is associated with breast cancer in the north part of Iran. *ScientificWorldJournal* 2012; **2012**: 837191 [PMID: 22566779 DOI: 10.1100/2012/837191]
107 **Shigeishi H**, Sugiyama M, Ohta K. Relationship between the prevalence of oral human papillomavirus DNA and periodontal disease (Review). *Biomed Rep* 2021; **14**: 40 [PMID: 33728046 DOI: 10.3892/br.2021.1416]
108 **Soheili M**, Keyvani H, Soheili M, Nasseri S. Human papilloma virus: A review study of epidemiology, carcinogenesis, diagnostic methods, and treatment of all HPV-related cancers. *Med J Islam Repub Iran* 2021; **35**: 65 [PMID: 34277502 DOI: 10.47176/mjiri.35.65]
109 **World Health Organization**. Human papillomavirus vaccines: WHO position paper, May 2017-Recommendations. *Vaccine* 2017; **35**: 5753-5755 [PMID: 28596091 DOI: 10.1016/j.vaccine.2017.05.069]
110 **Bruni L**, Albero G, Rowley J, Alemany L, Arbyn M, Giuliano AR, Markowitz LE, Broutet N, Taylor M. Global and regional estimates of genital human papillomavirus prevalence among men: a systematic review and meta-analysis. *Lancet Glob Health* 2023; **11**: e1345-e1362 [PMID: 37591583 DOI: 10.1016/S2214-109X(23)00305-4]
111 **Bruni L**, Diaz M, Castellsagué X, Ferrer E, Bosch FX, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J Infect Dis* 2010; **202**: 1789-1799 [PMID: 21067372 DOI: 10.1086/657321]
112 **Kreimer AR**, Bhatia RK, Messeguer AL, González P, Herrero R, Giuliano AR. Oral human papillomavirus in healthy individuals: a systematic review of the literature. *Sex Transm Dis* 2010; **37**: 386-391 [PMID: 20081557 DOI: 10.1097/OLQ.0b013e3181c94a3b]
113 **Tam S**, Fu S, Xu L, Krause KJ, Lairson DR, Miao H, Sturgis EM, Dahlstrom KR. The epidemiology of oral human papillomavirus infection in healthy populations: A systematic review and meta-analysis. *Oral Oncol* 2018; **82**: 91-99 [PMID: 29909908 DOI: 10.1016/j.oraloncology.2018.04.005]
114 **Horewicz VV**, Feres M, Rapp GE, Yasuda V, Cury PR. Human papillomavirus-16 prevalence in gingival tissue and its association with periodontal destruction: a case-control study. *J Periodontol* 2010; **81**: 562-568 [PMID: 20367098 DOI: 10.1902/jop.2009.090571]
115 **Wiener RC**, Sambamoorthi U, Jurevic RJ. Association of periodontitis and human papillomavirus in oral rinse specimens: Results from the National Health and Nutrition Survey 2009-2012. *J Am Dent Assoc* 2015; **146**: 382-389 [PMID: 26025825 DOI: 10.1016/j.adaj.2015.01.019]
116 **Hormia M**, Willberg J, Ruokonen H, Syrjänen S. Marginal periodontium as a potential reservoir of human papillomavirus in oral mucosa. *J Periodontol* 2005; **76**: 358-363 [PMID: 15857068 DOI: 10.1902/jop.2005.76.3.358]
117 **Syrjänen S**. Oral manifestations of human papillomavirus infections. *Eur J Oral Sci* 2018; **126** Suppl 1: 49-66 [PMID: 30178562 DOI: 10.1111/eos.12538]
118 **Sun CX**, Bennett N, Tran P, Tang KD, Lim Y, Frazer I, Samaranayake L, Punyadeera C. A Pilot Study into the Association between Oral Health Status and Human Papillomavirus-16 Infection. *Diagnostics (Basel)* 2017; **7** [PMID: 28257064 DOI: 10.3390/diagnostics7010011]
119 **Ortiz AP**, González D, Vivaldi-Oliver J, Castañeda M, Rivera V, Díaz E, Centeno H, Muñoz C, Palefsky J, Joshipura K, Pérez CM. Periodontitis and oral human papillomavirus infection among Hispanic adults. *Papillomavirus Res* 2018; **5**: 128-133 [PMID: 29555599 DOI: 10.1016/j.pvr.2018.03.003]

120 **Dayakar MM**, Shipilova A, Gupta D. Periodontal pocket as a potential reservoir of high risk human papilloma virus: A pilot study. *J Indian Soc Periodontol* 2016; **20**: 136-140 [PMID: 27143823 DOI: 10.4103/0972-124X.170815]

121 **McDaniel JT**, Davis JM, McDermott RJ, Maxfield I, Kapatamoyo K. Predicted prevalence of oral human papillomavirus (HPV) by periodontitis status and HPV vaccination status. *J Public Health Dent* 2020; **80**: 132-139 [PMID: 31991496 DOI: 10.1111/jphd.12357]

122 **Shigeishi H**, Sugiyama M, Ohta K, Yokoyama S, Sakuma M, Murozumi H, Kato H, Takechi M. High HPV16 E6 viral load in the oral cavity is associated with an increased number of bacteria: A preliminary study. *Biomed Rep* 2018; **8**: 59-64 [PMID: 29399339 DOI: 10.3892/br.2017.1025]

123 **Shigeishi H**, Murodumi H, Ohta K, Sugiyama M. Detection of HPV16 E6 DNA in periodontal pockets of middle-aged and older people. *Oral Sci Int* 2021; **18**: 50-55 [DOI: 10.1002/osi2.1079]

124 **Baez CF**, Savassi-Ribas F, Rocha WM, Almeida SG, Gonçalves MT, Guimarães MA, Cavalcanti SM, Varella RB. Association of Epstein-Barr virus (EBV) but not human papillomavirus (HPV) with gingivitis and/or periodontitis in transplant patients. *Rev Inst Med Trop Sao Paulo* 2016; **58**: 58 [PMID: 27410918 DOI: 10.1590/S1678-9946201658058]

125 **Fuster-Rossello L**, Ribotta E, Cuffini C, Fuster-Juan M. Human papilloma virus in oral mucosa and its association with periodontal status of gynecologically infected women. *Acta Odontol Latinoam* 2014; **27**: 82-88 [PMID: 25523960 DOI: 10.1590/S1852-48342014000200007]

126 **Ali A**, Lassi ZS, Kapellas K, Jamieson L, Rumbold AR. A systematic review and meta-analysis of the association between periodontitis and oral high-risk human papillomavirus infection. *J Public Health (Oxf)* 2021; **43**: e610-e619 [PMID: 32915228 DOI: 10.1093/pubmed/fdaa156]

127 **Schaefer S**. Hepatitis B virus taxonomy and hepatitis B virus genotypes. *World J Gastroenterol* 2007; **13**: 14-21 [PMID: 17206751 DOI: 10.3748/wjg.v13.i1.14]

128 **Caccamo G**, Saffioti F, Raimondo G. Hepatitis B virus and hepatitis C virus dual infection. *World J Gastroenterol* 2014; **20**: 14559-14567 [PMID: 25356020 DOI: 10.3748/wjg.v20.i40.14559]

129 Updated Recommendations on Treatment of Adolescents and Children with Chronic HCV Infection, and HCV Simplified Service Delivery and Diagnostics [Internet]. Geneva: World Health Organization; 2022– [PMID: 37410875]

130 **Vittal A**, Ghany MG. WHO Guidelines for Prevention, Care and Treatment of Individuals Infected with HBV: A US Perspective. *Clin Liver Dis* 2019; **23**: 417-432 [PMID: 31266617 DOI: 10.1016/j.cld.2019.04.008]

131 **Irshad M**, Mankotia DS, Irshad K. An insight into the diagnosis and pathogenesis of hepatitis C virus infection. *World J Gastroenterol* 2013; **19**: 7896-7909 [PMID: 24307784 DOI: 10.3748/wjg.v19.i44.7896]

132 **Aslan AT**, Balaban HY. Hepatitis E virus: Epidemiology, diagnosis, clinical manifestations, and treatment. *World J Gastroenterol* 2020; **26**: 5543-5560 [PMID: 33071523 DOI: 10.3748/wjg.v26.i37.5543]

133 **Utsumi T**, Lusida MI. Viral hepatitis and human immunodeficiency virus co-infections in Asia. *World J Virol* 2015; **4**: 96-104 [PMID: 25964874 DOI: 10.5501/wjv.v4.i2.96]

134 **Salama II**, Raslan HM, Abdel-Latif GA, Salama SI, Sami SM, Shaaban FA, Abdelmohsen AM, Fouad WA. Impact of direct-acting antiviral regimens on hepatic and extrahepatic manifestations of hepatitis C virus infection. *World J Hepatol* 2022; **14**: 1053-1073 [PMID: 35978668 DOI: 10.4254/wjh.v14.i6.1053]

135 **Helenius-Hietala J**, Suominen AL, Ruokonen H, Knuuttila M, Puukka P, Jula A, Meurman JH, Åberg F. Periodontitis is associated with incident chronic liver disease-A population-based cohort study. *Liver Int* 2019; **39**: 583-591 [PMID: 30300961 DOI: 10.1111/liv.13985]

136 **Mei EH**, Yao C, Chen YN, Nan SX, Qi SC. Multifunctional role of oral bacteria in the progression of non-alcoholic fatty liver disease. *World J Hepatol* 2024; **16**: 688-702 [PMID: 38818294 DOI: 10.4254/wjh.v16.i5.688]

137 **Komazaki R**, Katagiri S, Takahashi H, Maekawa S, Shiba T, Takeuchi Y, Kitajima Y, Ohtsu A, Udagawa S, Sasaki N, Watanabe K, Sato N, Miyasaka N, Eguchi Y, Anzai K, Izumi Y. Periodontal pathogenic bacteria, Aggregatibacter actinomycetemcomitans affect non-alcoholic fatty liver disease by altering gut microbiota and glucose metabolism. *Sci Rep* 2017; **7**: 13950 [PMID: 29066788 DOI: 10.1038/s41598-017-14260-9]

138 **Alakhali MS**, Al-Maweri SA, Al-Shamiri HM, Al-Haddad K, Halboub E. The potential association between periodontitis and non-alcoholic fatty liver disease: a systematic review. *Clin Oral Investig* 2018; **22**: 2965-2974 [PMID: 30357481 DOI: 10.1007/s00784-018-2726-1]

139 **Chen Y**, Yang YC, Zhu BL, Wu CC, Lin RF, Zhang X. Association between periodontal disease, tooth loss and liver diseases risk. *J Clin Periodontol* 2020; **47**: 1053-1063 [PMID: 32621350 DOI: 10.1111/jcpe.13341]

140 **Wijarnpreecha K**, Panjawatanan P, Cheungpasitporn W, Lukens FJ, Harnois DM, Pungpapong S, Ungprasert P. The Association between Periodontitis and Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-analysis. *J Gastrointestin Liver Dis* 2020; **29**: 211-217 [PMID: 32530988 DOI: 10.15403/jgld-841]

141 **Alazawi W**, Bernabe E, Tai D, Janicki T, Kemos P, Samsuddin S, Syn WK, Gillam D, Turner W. Periodontitis is associated with significant hepatic fibrosis in patients with non-alcoholic fatty liver disease. *PLoS One* 2017; **12**: e0185902 [PMID: 29220367 DOI: 10.1371/journal.pone.0185902]

142 **Grønkjær LL**. Periodontal disease and liver cirrhosis: A systematic review. *SAGE Open Med* 2015; **3**: 2050312115601122 [PMID: 26770799 DOI: 10.1177/2050312115601122]

143 **Bie M**, Wu P, Zhou J, Li Y, Zhao L. Periodontal health status in cirrhotic patients: a systematic review and meta-analysis. *BMC Oral Health* 2023; **23**: 362 [PMID: 37277748 DOI: 10.1186/s12903-023-03052-5]

144 **Al-maweri SA**, Ibraheem WI, Al-akhali MS, Shamala A, Halboub E, Alhajj M. Periodontal disease, tooth loss, and the risk of liver cancer: A systematic review. 2021 preprint [DOI: 10.21203/rs.2.18708/v1]

145 **Chen X**, Zeng Z, Xiao L. The association between periodontitis and hepatitis virus infection: a cross-sectional study utilizing data from the NHANES database (2003-2018). *Public Health* 2024; **226**: 114-121 [PMID: 38056398 DOI: 10.1016/j.puhe.2023.11.005]

146 **Surlin P**, Gheorghe DN, Popescu DM, Martu AM, Solomon S, Roman A, Lazar L, Stratul SI, Rusu D, Foia L, Boldeanu MV, Boldeanu L, Danilescu M, Rogoveanu I. Interleukin-1α and -1β assessment in the gingival crevicular fluid of periodontal patients with chronic hepatitis C. *Exp Ther Med* 2020; **20**: 2381-2386 [PMID: 32765719 DOI: 10.3892/etm.2020.8906]

147 **Nagao Y**, Kawahigashi Y, Sata M. Association of Periodontal Diseases and Liver Fibrosis in Patients With HCV and/or HBV infection. *Hepat Mon* 2014; **14**: e23264 [PMID: 25737729 DOI: 10.5812/hepatmon.23264]

148 **Coates EA**, Brennan D, Logan RM, Goss AN, Scopacasa B, Spencer AJ, Gorkic E. Hepatitis C infection and associated oral health problems. *Aust Dent J* 2000; **45**: 108-114 [PMID: 10925506 DOI: 10.1111/j.1834-7819.2000.tb00249.x]

149 **Yucekal-Tuncer B**, Uygur C, Firatli E. Gingival crevicular fluid levels of aspartate amino transferase, sulfide ions and N-benzoyl-DL-arginine-2-naphthylamide in diabetic patients with chronic periodontitis. *J Clin Periodontol* 2003; **30**: 1053-1060 [PMID: 15002891 DOI: 10.1046/j.0303-6979.2003.00426.x]

150 **Gheorghe DN**, Popescu DM, Salan A, Boldeanu MV, Ionele CM, Pitru A, Turcu-Stiolica A, Camen A, Florescu C, Rogoveanu I, Surlin P. Non-Surgical Periodontal Therapy Could Improve the Periodontal Inflammatory Status in Patients with Periodontitis and Chronic Hepatitis C. *J Clin Med* 2021; **10** [PMID: 34830557 DOI: 10.3390/jcm10225275]

151 **Mester A**, Ciobanu L, Taulescu M, Apostu D, Lucaciu O, Filip GA, Feldrihan V, Licarete E, Ilea A, Piciu A, Oltean-Dan D, Scurtu I, Berce C, Campian RS. Periodontal disease may induce liver fibrosis in an experimental study on Wistar rats. *J Periodontol* 2019; **90**: 911-919 [PMID: 30689209 DOI: 10.1002/JPER.18-0585]

152 **Nagao Y**, Tanigawa T. Red complex periodontal pathogens are risk factors for liver cirrhosis. *Biomed Rep* 2019; **11**: 199-206 [PMID: 31632667 DOI: 10.3892/br.2019.1245]

153 **Arimatsu K**, Yamada H, Miyazawa H, Minagawa T, Nakajima M, Ryder MI, Gotoh K, Motooka D, Nakamura S, Iida T, Yamazaki K. Oral pathobiont induces systemic inflammation and metabolic changes associated with alteration of gut microbiota. *Sci Rep* 2014; **4**: 4828 [PMID: 24797416 DOI: 10.1038/srep04828]

154 **Åberg F**, Helenius-Hietala J. Oro-hepatic link, endotoxemia, and systemic inflammation: The role of chronic periodontitis. *Hepatology* 2016; **63**: 1736 [PMID: 26105750 DOI: 10.1002/hep.27953]

155 **Bajaj JS**, Betrapally NS, Hylemon PB, Heuman DM, Daita K, White MB, Unser A, Thacker LR, Sanyal AJ, Kang DJ, Sikaroodi M, Gillevet PM. Salivary microbiota reflects changes in gut microbiota in cirrhosis with hepatic encephalopathy. *Hepatology* 2015; **62**: 1260-1271 [PMID: 25820757 DOI: 10.1002/hep.27819]

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MINIREVIEWS

Climate change and the emergence and exacerbation of infectious diseases: A review

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Abstract

Experts expressed severe concerns over the possibility of increasing burden of infectious diseases as the planet's climate began to change years ago. There have been increased rates of climate-related catastrophes and as global temperatures rise, emergence of certain viruses has become a serious concern. Vectors are susceptible to changing temperatures as they exhibit innate responses to thermal stress to increase survivability. Climate change impacts virus reservoirs, increasing transmission rates of vectors. Vector-borne diseases have already witnessed increasing numbers compared to before. Certain non-endemic areas are encountering their first-ever infectious disease cases due to increasing temperatures. Tick-borne diseases are undergoing transformations provoking a heightened prevalence. Food-borne illnesses are expected to increase owing to warmer temperatures. It is important to recognize that climate change has a multivariable impact on the transmission of viruses. With climate change comes the potential of increasing interspecies interactions promoting jumps. These factors must be considered, and an informed strategy must be formulated. Adaptation and mitigation strategies are required to curb these diseases from spreading. Despite significant evidence that climate change affects infectious diseases, gaps in research exist. We conducted this review to identify the potential role climate change plays in the emergence of new viruses.

Key Words: Climate change; Infectious disease; Emergence; Catastrophe; Vector-borne disease

Core Tip: Changing core temperatures and increased incidence of climate change catastrophes have led to certain infectious disease outbreaks across the globe. As the climate continues to change and leads to the destruction of local biodiversity, the consequent spread of vectors is predicted to lead to further escalation in vector-borne diseases. The global community has also expressed significant concern regarding the spread of known lethal pathogens such as malaria and the West Nile viruses. Development and implementation of National Health Adaptation Plans is recommended to predict and tackle these emerging threats effectively.

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INTRODUCTION

Climate change is one of the most predictable and detrimental global hazards with significant long-term consequences on human health and well-being. Health impacts are visible on every continent as temperatures increase, sea levels rise, and a greater incidence of flooding and drought is observed[1,2]. These changes are significant as they threaten individual health crises and present a prominent threat to the public health sector. While a myriad of adverse health complaints, such as respiratory diseases, malnutrition, heat-related morbidity, and mortality, are escalated due to climate change, the emergence and dissemination of infectious diseases is of particular concern[2].

The relationship between climate change and infectious diseases is intricate, requiring views from multiple standpoints. As the climate warms and biodiversity is lost, the spread of vectors and animal species contributes to the emergence of diseases[3]. While the direct effects of the changing climate on circulation and incidence of infectious diseases are evident, one needs to recognize the indirect effects. Increased flooding in certain areas of the world prompts climate-driven migration into overcrowded shelters, increasing the burden of communicable diseases[4]. Similarly, harsher climates induce food and water insecurity, contributing to malnutrition and predisposing individuals to infectious diseases. Keeping in mind recent global health crises, especially the coronavirus disease 2019 (COVID-19) pandemic, it is imperative to have a broader understanding of the emergence of infectious diseases influenced by climate change worldwide.

This review aims to explore climate change's role in surfacing of infectious diseases through direct effects. By exploring this multifaceted relationship between climate change and infectious disease, this review aims to identify significant trends, highlight the ramifications, provide adaptation and mitigation strategies, and analyze the existing gaps. Through this, effective policy change decisions can be taken to prevent public health crises related to climate change.

IMPACT OF CLIMATE CHANGE ON INFECTIOUS DISEASES

Vector-borne disease

Diseases transmitted through infected arthropod species, such as mosquitoes, sandflies, and ticks, are classified as vector-borne diseases. Climate readily affects the biological traits of both the vector and pathogen. Temperature variations can reshape the emergence of zoonotic diseases through changes in the number of vectors, transmission cycles, and the contact between species[5]. Water availability is a determinant in oviposition patterns of vectors. As precipitation increases, vectors are more likely to find suitable habitats and mature faster with increased breeding.

Mosquito-borne disease

Dengue: Dengue, transmitted by Aedes mosquitoes, is among the most important vector-borne diseases worldwide with a high disease burden. Increasing temperatures exhibit an almost exponential pattern on the species' physiological traits, such as biting and development rates for vectors up to a certain level before tapering off. Similarly, increased rainfall facilitates the development of mosquito breeding sites up to a certain threshold, after which flooding demolishes them [6]. The primary vectors for dengue Aedes aegyptii and Ae. Albopictus has increased in transmission by 10% and 15% since 1950 due to greater climate suitability[7]. Increased precipitation and temperatures due to climate change are projected to intensify dengue by 2050 in various parts of the world, including western Africa, the southeastern United States, and inland areas of Australia. These favorable climate conditions for the vector-borne virus will also prolong the transmission period by four months, specifically in the Eastern Mediterranean and Western Pacific regions[6,8].

Moreover, long-term weather and population estimations predict 50%-60% of the population to be at threat of dengue virus by 2085 compared to 35% of the population at risk if climate change did not ensue[9]. These projections are alarming as they emphasize the ramifications of unmitigated climate change on disease burden. Moreover, it highlights how dengue fever may not be limited to specific parts of the world but become a global health issue if necessary actions are not taken to minimize climate change.

Malaria: Malaria is a life-threatening disease caused by the Plasmodium parasites transmitted by the female Anopheles mosquitoes. Although the past five years have witnessed a decrease in the incidence of this disease owing to a range of human interventions, climate change can still cause increased transmission[6]. Spread of malaria is affected by ecological factors such as temperatures, precipitation, and topography[10]. As a result, equatorial regions with lower altitudes, increased rainfall, warmer temperatures, and higher humidity are favorable for the vector's reproduction and longevity. Studies demonstrate that based on the projected climate change scenarios of longer monsoon periods and increased temperatures in tropical areas of Africa, the Anopheles vector is expected to widen its distribution area[8]. Similarly, geographical expansion of the virus is predicted in various other parts of the world, such as in Southern Europe, South Africa, China, and the highland areas of Columbia and Ethiopia[8,9]. These predicted projections underscore the global impact of climate change on public health and serve as a reminder to adopt enhanced policies for vector control and slowing down climate change to mitigate the risks of infectious diseases.

West Nile virus: West Nile virus (WNV), primarily transmitted by the Culex species of mosquitoes, can cause serious health infections ranging from mild febrile illness to severe meningitis or encephalitis. The principal hosts for these mosquitoes are wild migratory birds, but there have been cases of human-to-human transmission (through blood or transplacental transfusions)[10]. Temperature appears to be the predominant factor in WNV transmission. Optimum temperatures regulate vector growth rate, mosquito survival, and external incubation time period[11]. As temperatures increase (up to a certain threshold), the virus develops within vectors, and their transmission becomes increasingly efficient. These findings are particularly important as vectors can circulate WNV to further geographical regions. Germany, previously considered a non-endemic region for WNV, witnessed its first appearance of the vector-borne virus in 2018, which was the country's warmest year[12]. As a result, human infections have been recorded in all the following years demonstrating constant dissemination in the transformed environment of Central-East Germany[13]. Furthermore, in areas with previously established presence of the virus, studies demonstrate an increasing likelihood of WNV infections by 2025 based on climate change projections[14]. This phenomenon not only highlights the potential role climate change plays in expanding suitable habitats for vectors to grow and transmit but also the strengthening of the virus in already existing territorial areas. As a result, regions already susceptible to the disease are at risk of increasing their disease burden.

Tick-borne

Lyme disease is the most common vector-borne disease in North America and Europe. It is caused by the Borrelia burgdorferi spherocyte bacteria transmitted mainly by Ixodes pacificus and I. scapularis ticks[15]. Like mosquitoes, ticks are affected by weather conditions, and the changing climate can be responsible for the frequency and severity of Lyme disease. Elevated temperatures affect egg development, population density, growth cycle, and ticks' dispersal. Studies demonstrate that temperature is the most important factor of tick colony establishment in Canada, considering the increase in disease incidence in the past few years and a predicted increase of 213% of suitable habitat by 2080s[9,16]. Presuming a 2°C increase in the average temperature, a 20% increase in disease prevalence is estimated in the coming decades. Increased suitable habitat directly influences the prevalence of the disease as enhanced environmental suitability encourages tick breeding, consequently increasing the tick population and the likelihood of disease transmission. In the United States, a 2-4°C anticipatory increase in the average temperature is speculated to cause a 20% increase in Lyme disease cases in the coming decades[17].

Similarly, a 10% increase in infection susceptibility at the end of the century is predicted for Slovenia, a country that is already vulnerable to Lyme disease[17]. These alarming projections raise concerns 1about the pervasive influence of climate change on disease proliferation. Without proper mitigation strategies to minimize climate change, increasing suitable habitats and disease prevalence run the risk of aggravating public health catastrophes.

Food-borne diseases

Food-borne diseases are those transmitted by eating food infected with pathogens or toxins. The risk of foodborne illness can be directly modified through high temperatures, causing amplification of replication cycles and growth, survival, and easy transfer of the pathogens. Drastic heat and precipitation can influence pathogens, worsening the risk of gastrointestinal and diarrheal diseases. The likelihood of mean weekly temperatures is directly associated with increased suscepti -bility to campylobacteriosis in the European Union[9]. Climate change projections indicate an increase of 6000 cases of Campylobacter per year in Norway, Sweden, Denmark, and Finland by the end of the century, translating to a 200% rise [18]. Massachusetts witnessed an increase in the incidence of the disease, with a lag of 2-14 days following a climax in annual temperature. Similarly, Canada detected a growth in cases of Campylobacter (along with Salmonella and pathogenic Escherichia coli) by 2.2% for every degree of increase in temperature[19].

Salmonella is another food-borne pathogen causing global outbreaks. Increase in temperatures directly influences Salmonella's reproduction rates as an upsurge in ambient temperature correlates with incidence spikes. In Maryland, the likelihood of infection was related to extreme heat events between 2002-2012[20]. According to studies, a 5.6% and 8.8% increase in the incidence of cases was anticipated with a 1°C increase in mean weekly minimum and maximum temperatures, respectively[21].

Air-borne diseases

Meningococcal meningitis is an infection of meninges, caused by the bacteria Neisseria *meningitidis*, that causes high death rates in developing countries. It is believed that increased concentration of dust, high winds, elevated temperatures, and low humidity may cause damage to nasopharyngeal mucosa leading to increased susceptibility to meningitis[22].

COVID-19 cases and temperature have also shown a positive correlation[23]. It is possible that extreme heat forces people indoors which can increase the risk of virus transmission.

IMPLICATED MECHANISMS AND TRENDS IN INFECTIOUS DISEASES AS INFLUENCED BY CLIMATE CHANGE

A detailed review published in Nature expanded upon how often, and through which mechanisms, climate change aggravates infectious diseases. It found that more than 50% of pathogenic diseases have, at some point, been exacerbated directly by climate change with most cases being impacted by warming, precipitation, and flooding leading to the aggravation in vector-borne, waterborne and airborne diseases, respectively[24].

Some pragmatic mechanisms that have been identified include increase in proximity of pathogens and human beings through these changes in climate. Wildlife migration due to changing temperatures and habitability of regions[25] coupled with directly changing human societal behavior, such as being involved in more recreational activities involving water bodies and the outdoors in general, are two common mechanisms identified. Additionally, specific changes such as decreased salination levels of certain water bodies, increased stagnation of water, changing season length prolonging pathogen and vector lifecycles are common mechanisms which have increased disease transmissibility and strengthened pathogen adaptability and resilience[26,27]. Interestingly, in a detailed statistical analysis, temperature itself was found to positively correlate with the number of patients with infectious diseases[28].

In addition to this, the World Health Organization, more than a decade ago, found that climate change will cause an additional burden of certain diseases, such as diarrheal disease, directly due to the impact of climate change. It also suggested increased rates of malaria by up to 5% due to temperature surges[29].

The Center for Disease Control reports that infectious diseases have been on an upward trajectory. For example, cases of tick-borne diseases have increased from 10000 cases per year to 60000 cases per year from 2001 to 2019 in the United States[30]. Furthermore, currently it is assumed that 25% of the global population consumes fecally contaminated water. As droughts worsen due to increased temperatures in certain parts of the world and access to clean drinking water is further restricted, these rates will likely rise in certain vulnerable regions leading to worsening of water-borne diseases in these geographies[31]. Certain viruses such as Zika and Chikungunya have also been demonstrating increased geographical and seasonal range[32,33].

ADAPTATION AND MITIGATION STRATEGIES

Adaption and mitigation strategies are crucial in diminishing climate change's ramifications on infectious diseases. Adaptation strategies focus on tackling climate change[33]. Key strategies include modeling climate resilient health systems that include a (1) Well-informed health workforce equipped with the necessary mechanisms to assist climate resilience; (2) Health information systems that facilitate skillful handling of health risks arising from extreme events caused by climate change; (3) Efficient service distribution including necessary foundations in cases of emergencies; and (4) Sufficient financing[34] and improving education[35]. However, in order to build these climate-resilient health systems, comprehensive knowledge about current consequences and expected risks is required in the form of a Health National Adaptation Plan (HNAP). Developing an HNAP helps certify that the consequences of climate change on human health are taken into account from community to national levels[36]. An important component of establishing a HNAP is administering vulnerability and adaptation assessments.

Vulnerability and adaptation assessments should inspect the capabilities of health systems and services to resist extreme events caused by climate change. Stress tests can provide scenario-based evaluations to complement these assessments through recognition of circumstances under which health systems would struggle to manage climate-dependent health outcomes[37,38]. A summary of these key adaptation strategies is illustrated below in Figure 1.

Adaptation strategies go in junction with mitigation measures that are also important in controlling the ramifications of climate change on health systems. Mitigation denotes to pursuits that curb greenhouse gas emissions or remove them from the atmosphere[33]. Afforestation and tree-planting programs, intensifying energy effectiveness in operations, and greening vehicle fleets are all helpful mitigation strategies for reducing the impact of climate change[33,37]. A multidisciplinary collaboration is required between multiple organizations, including healthcare workers, environmental scientists, researchers, policy-makers, and government bodies, to effectively address the complex challenges climate change poses on public health.

GAPS IN RESEARCH AND FUTURE DIRECTIONS

Despite the strong relationship between climate change and infectious diseases, significant limitations persist in research.

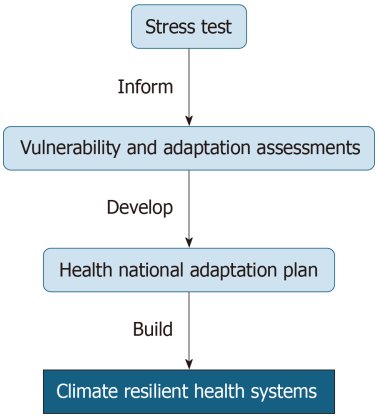

Figure 1 Health system adaptation to climate change.

While the effects of climate change and infectious diseases are commonly studied in developed countries, underdeveloped countries, which often face stronger consequences of increased disease burden, need to be considered. There is a growing need to study the intricate interlinkage between other climate variables and the different vectors and pathogens in relation to their effect on human health. Longitudinal studies are required to better understand climate change's sustained impact on human health. Additionally, research is particularly lacking in evaluating the effectiveness of adaption and mitigation strategies that can help alleviate the burden of diseases greatly impacted by climate change. Current studies have focused on trends and have attempted to predict how the burden of infectious disease will escalate in the future owing to climate change. However, very few studies the intricate impact and evolutionary force exerted on pathogens to adapt. Laboratory studies evaluating how these pressure force pathogens to adapt could be very useful. Furthermore, evaluating pragmatic approaches such as increased access to antibiotics and antiviral medications, role of socio-economic status, and precisely evaluating how a shift in human behavior makes us vulnerable to pathogens is also very important. By incorporating these limitations, evidence-based interventions can be integrated to curb the effects climate change potentially has on infectious diseases.

CONCLUSION

Infectious diseases related to climate change can be prevented. This review highlights sufficient evidence to emphasize climate change's role in exacerbating and developing infectious diseases, particularly vector-borne and food-borne, in regions where they did not exist previously. Without proper adaptations and mitigation strategies, the projections provided are likely to worsen over time, causing frequent worldwide pandemics.

FOOTNOTES

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REFERENCES

1 **Watts N**, Amann M, Arnell N, Ayeb-Karlsson S, Beagley J, Belesova K, Boykoff M, Byass P, Cai W, Campbell-Lendrum D, Capstick S, Chambers J, Coleman S, Dalin C, Daly M, Dasandi N, Dasgupta S, Davies M, Di Napoli C, Dominguez-Salas P, Drummond P, Dubrow R, Ebi KL, Eckelman M, Ekins P, Escobar LE, Georgeson L, Golder S, Grace D, Graham H, Haggar P, Hamilton I, Hartinger S, Hess J, Hsu SC, Hughes N, Jankin Mikhaylov S, Jimenez MP, Kelman I, Kennard H, Kiesewetter G, Kinney PL, Kjellstrom T, Kniveton D, Lampard P, Lemke B, Liu Y, Liu Z, Lott M, Lowe R, Martinez-Urtaza J, Maslin M, McAllister L, McGushin A, McMichael C, Milner J, Moradi-Lakeh M, Morrissey K, Munzert S, Murray KA, Neville T, Nilsson M, Sewe MO, Oreszczyn T, Otto M, Owfi F, Pearman O, Pencheon D, Quinn R, Rabbaniha M, Robinson E, Rocklöv J, Romanello M, Semenza JC, Sherman J, Shi L, Springmann M, Tabatabaei M, Taylor J, Triñanes J, Shumake-Guillemot J, Vu B, Wilkinson P, Winning M, Gong P, Montgomery H, Costello A. The 2020 report of The Lancet Countdown on health and climate change: responding to converging crises. *Lancet* 2021; **397**: 129-170 [PMID: 33278353 DOI: 10.1016/S0140-6736(20)32290-X]

2 **Rocque RJ**, Beaudoin C, Ndjaboue R, Cameron L, Poirier-Bergeron L, Poulin-Rheault RA, Fallon C, Tricco AC, Witteman HO. Health effects of climate change: an overview of systematic reviews. *BMJ Open* 2021; **11**: e046333 [PMID: 34108165 DOI: 10.1136/bmjopen-2020-046333]

3 **Tajudeen YA**, Oladunjoye IO, Adebayo AO, Adebisi YA. The need to adopt planetary health approach in understanding the potential influence of climate change and biodiversity loss on zoonotic diseases outbreaks. *Public Health Pract (Oxf)* 2021; **2**: 100095 [PMID: 36101577 DOI: 10.1016/j.puhip.2021.100095]

4 **Coates SJ**, Enbiale W, Davis MDP, Andersen LK. The effects of climate change on human health in Africa, a dermatologic perspective: a report from the International Society of Dermatology Climate Change Committee. *Int J Dermatol* 2020; **59**: 265-278 [PMID: 31970754 DOI: 10.1111/ijd.14759]

5 **Esposito MM**, Turku S, Lehrfield L, Shoman A. The Impact of Human Activities on Zoonotic Infection Transmissions. *Animals (Basel)* 2023; **13** [PMID: 37238075 DOI: 10.3390/ani13101646]

6 **Colón-González FJ**, Sewe MO, Tompkins AM, Sjödin H, Casallas A, Rocklöv J, Caminade C, Lowe R. Projecting the risk of mosquito-borne diseases in a warmer and more populated world: a multi-model, multi-scenario intercomparison modelling study. *Lancet Planet Health* 2021; **5**: e404-e414 [PMID: 34245711 DOI: 10.1016/S2542-5196(21)00132-7]

7 **Kulkarni MA**, Duguay C, Ost K. Charting the evidence for climate change impacts on the global spread of malaria and dengue and adaptive responses: a scoping review of reviews. *Global Health* 2022; **18**: 1 [PMID: 34980187 DOI: 10.1186/s12992-021-00793-2]

8 **Semenza JC**, Rocklöv J, Ebi KL. Climate Change and Cascading Risks from Infectious Disease. *Infect Dis Ther* 2022; **11**: 1371-1390 [PMID: 35585385 DOI: 10.1007/s40121-022-00647-3]

9 **Rupasinghe R**, Chomel BB, Martínez-López B. Climate change and zoonoses: A review of the current status, knowledge gaps, and future trends. *Acta Trop* 2022; **226**: 106225 [PMID: 34758355 DOI: 10.1016/j.actatropica.2021.106225]

10 **Chala B**, Hamde F. Emerging and Re-emerging Vector-Borne Infectious Diseases and the Challenges for Control: A Review. *Front Public Health* 2021; **9**: 715759 [PMID: 34676194 DOI: 10.3389/fpubh.2021.715759]

11 **D'Amore C**, Grimaldi P, Ascione T, Conti V, Sellitto C, Franci G, Kafil SH, Pagliano P. West Nile Virus diffusion in temperate regions and climate change. A systematic review. *Infez Med* 2022; **31**: 20-30 [PMID: 36908379 DOI: 10.53854/liim-3101-4]

12 **Farooq Z**, Sjödin H, Semenza JC, Tozan Y, Sewe MO, Wallin J, Rocklöv J. European projections of West Nile virus transmission under climate change scenarios. *One Health* 2023; **16**: 100509 [PMID: 37363233 DOI: 10.1016/j.onehlt.2023.100509]

13 **Beermann S**, Dobler G, Faber M, Frank C, Habedank B, Hagedorn P, Kampen H, Kuhn C, Nygren T, Schmidt-Chanasit J, Schmolz E, Stark K, Ulrich RG, Weiss S, Wilking H. Impact of climate change on vector- and rodent-borne infectious diseases. *J Health Monit* 2023; **8**: 33-61 [PMID: 37342429 DOI: 10.25646/11401]

14 **Frank C**, Schmidt-Chanasit J, Ziegler U, Lachmann R, Preußel K, Offergeld R. West Nile Virus in Germany: An Emerging Infection and Its Relevance for Transfusion Safety. *Transfus Med Hemother* 2022; **49**: 192-204 [PMID: 36159956 DOI: 10.1159/000525167]

15 **Alkishe A**, Raghavan RK, Peterson AT. Likely Geographic Distributional Shifts among Medically Important Tick Species and Tick-Associated Diseases under Climate Change in North America: A Review. *Insects* 2021; **12** [PMID: 33807736 DOI: 10.3390/insects12030225]

16 **Carignan A**, Valiquette L, Laupland KB. Impact of climate change on emerging infectious diseases: Implications for Canada. *J Assoc Med Microbiol Infect Dis Can* 2019; **4**: 55-59 [PMID: 36337740 DOI: 10.3138/jammi.2018-12-10]

17 **Deshpande G**, Beetch JE, Heller JG, Naqvi OH, Kuhn KG. Assessing the Influence of Climate Change and Environmental Factors on the Top Tick-Borne Diseases in the United States: A Systematic Review. *Microorganisms* 2023; **12** [PMID: 38257877 DOI: 10.3390/microorganisms12010050]

18 **Kuhn KG**, Nygård KM, Guzman-Herrador B, Sunde LS, Rimhanen-Finne R, Trönnberg L, Jepsen MR, Ruuhela R, Wong WK, Ethelberg S. Campylobacter infections expected to increase due to climate change in Northern Europe. *Sci Rep* 2020; **10**: 13874 [PMID: 32807810 DOI: 10.1038/s41598-020-70593-y]

19 **Djennad A**, Lo Iacono G, Sarran C, Lane C, Elson R, Höser C, Lake IR, Colón-González FJ, Kovats S, Semenza JC, Bailey TC, Kessel A, Fleming LE, Nichols GL. Seasonality and the effects of weather on Campylobacter infections. *BMC Infect Dis* 2019; **19**: 255 [PMID: 30866826 DOI: 10.1186/s12879-019-3840-7]

20 **Welch K**, Shipp-Hilts A, Eidson M, Saha S, Zansky S. Salmonella and the changing environment: systematic review using New York State as a model. *J Water Health* 2019; **17**: 179-195 [PMID: 30942769 DOI: 10.2166/wh.2018.224]

21 **Dietrich J**, Hammerl JA, Johne A, Kappenstein O, Loeffler C, Nöckler K, Rosner B, Spielmeyer A, Szabo I, Richter MH. Impact of climate change on foodborne infections and intoxications. *J Health Monit* 2023; **8**: 78-92 [PMID: 37342431 DOI: 10.25646/11403]

22 **World Bank Group**. Data: Nigeria. [Cited August 2024]. Available from: http://data.worldbank.org/country/nigeria

23 **Shenoy A**, Sharma B, Xu G, Kapoor R, Rho HA, Sangha K. God is in the rain: The impact of rainfall-induced early social distancing on COVID-19 outbreaks. *J Health Econ* 2022; **81**: 102575 [PMID: 34923344 DOI: 10.1016/j.jhealeco.2021.102575]

24 **Mora C**, McKenzie T, Gaw IM, Dean JM, von Hammerstein H, Knudson TA, Setter RO, Smith CZ, Webster KM, Patz JA, Franklin EC. Over half of known human pathogenic diseases can be aggravated by climate change. *Nat Clim Chang* 2022; **12**: 869-875 [PMID: 35968032 DOI: 10.1038/s41558-022-01426-1]

25 **Butler CD**, Harley D. Primary, secondary and tertiary effects of eco-climatic change: the medical response. *Postgrad Med J* 2010; **86**: 230-234 [PMID: 20354046 DOI: 10.1136/pgmj.2009.082727]

26 **Guzman Herrador BR**, de Blasio BF, MacDonald E, Nichols G, Sudre B, Vold L, Semenza JC, Nygård K. Analytical studies assessing the

association between extreme precipitation or temperature and drinking water-related waterborne infections: a review. *Environ Health* 2015; **14**: 29 [PMID: 25885050 DOI: 10.1186/s12940-015-0014-y]

27 **Gubler DJ**, Reiter P, Ebi KL, Yap W, Nasci R, Patz JA. Climate variability and change in the United States: potential impacts on vector- and rodent-borne diseases. *Environ Health Perspect* 2001; **109** Suppl 2: 223-233 [PMID: 11359689 DOI: 10.1289/ehp.109-1240669]

28 **Anwar A**, Anwar S, Ayub M, Nawaz F, Hyder S, Khan N, Malik I. Climate Change and Infectious Diseases: Evidence from Highly Vulnerable Countries. *Iran J Public Health* 2019; **48**: 2187-2195 [PMID: 31993386]

29 **Shuman EK**. Global climate change and infectious diseases. *N Engl J Med* 2010; **362**: 1061-1063 [PMID: 20335580 DOI: 10.1056/NEJMp0912931]

30 **NETEC**. Climate Change and Infectious Diseases. Cited: 2024 September. Available from: https://netec.org/2024/03/25/climate-change-and-infectious-diseases/#:~:text=Climate%20change%20trends%20such%20as,the%20spread%20of%20infectious%20diseases

31 **Gall AM**, Mariñas BJ, Lu Y, Shisler JL. Waterborne Viruses: A Barrier to Safe Drinking Water. *PLoS Pathog* 2015; **11**: e1004867 [PMID: 26110535 DOI: 10.1371/journal.ppat.1004867]

32 **Taylor L**. Dengue and chikungunya cases surge as climate change spreads arboviral diseases to new regions. *BMJ* 2023; **380**: 717 [PMID: 36972905 DOI: 10.1136/bmj.p717]

33 **Ngonghala CN**, Ryan SJ, Tesla B, Demakovsky LR, Mordecai EA, Murdock CC, Bonds MH. Effects of changes in temperature on Zika dynamics and control. *J R Soc Interface* 2021; **18**: 20210165 [PMID: 33947225 DOI: 10.1098/rsif.2021.0165]

34 **Buse CG**, Patrick R. Climate change glossary for public health practice: from vulnerability to climate justice. *J Epidemiol Community Health* 2020; **74**: 867-871 [PMID: 32620579 DOI: 10.1136/jech-2020-213889]

35 **Ebi KL**, Vanos J, Baldwin JW, Bell JE, Hondula DM, Errett NA, Hayes K, Reid CE, Saha S, Spector J, Berry P. Extreme Weather and Climate Change: Population Health and Health System Implications. *Annu Rev Public Health* 2021; **42**: 293-315 [PMID: 33406378 DOI: 10.1146/annurev-publhealth-012420-105026]

36 **World Health Organization**. Operational framework for building climate resilient health systems [Internet]. Geneva: World Health Organization; 2015. [Cited: March 2024]. Available from: https://iris.who.int/handle/10665/189951

37 **Ebi KL**, Berry P, Hayes K, Boyer C, Sellers S, Enright PM, Hess JJ. Stress Testing the Capacity of Health Systems to Manage Climate Change-Related Shocks and Stresses. *Int J Environ Res Public Health* 2018; **15** [PMID: 30373158 DOI: 10.3390/ijerph15112370]

38 **Jacobsen AP**, Khiew YC, Duffy E, O'Connell J, Brown E, Auwaerter PG, Blumenthal RS, Schwartz BS, McEvoy JW. Climate change and the prevention of cardiovascular disease. *Am J Prev Cardiol* 2022; **12**: 100391 [PMID: 36164332 DOI: 10.1016/j.ajpc.2022.100391]

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Insights from respiratory virus co-infections

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Abstract

Respiratory viral co-infections present significant challenges in clinical settings due to their impact on disease severity and patient outcomes. Current diagnostic methods often miss these co-infections, complicating the epidemiology and management of these cases. Research, primarily conducted *in vitro* and *in vivo*, suggests that co-infections can lead to more severe illnesses, increased hospitalization rates, and greater healthcare utilization, especially in high-risk groups such as children, the elderly, and immunocompromised individuals. Common co-infection patterns, risk factors, and their impact on disease dynamics highlight the need for advanced diagnostic techniques and tailored therapeutic strategies. Understanding the virological interactions and immune response modulation during co-infections is crucial for developing effective public health interventions and improving patient outcomes. Future research should focus on the molecular mechanisms of co-infection and the development of specific therapies to mitigate the adverse effects of these complex infections.

Key Words: Respiratory viral co-infections; Disease severity; Diagnostic challenges; Immune response modulation; Public health strategies

Core Tip: Respiratory viral co-infections significantly complicate clinical management due to their impact on disease severity and patient outcomes. Current diagnostic techniques often miss these co-infections, making it difficult to accurately define their epidemiology and management strategies. Research suggests that co-infections can exacerbate illnesses, leading to higher hospitalization rates and increased healthcare utilization, particularly among high-risk groups such as children, the elderly, and immunocompromised individuals. Thus, advancing diagnostic methods and developing targeted therapeutic strategies are essential for improving public health interventions and patient outcomes. Understanding the virological interactions and immune response during co-infections is crucial for these advancements.

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INTRODUCTION

While viral-viral co-infections can be studied in the clinical setting, it is difficult to ascertain the true incidence of specific co-infections and almost impossible to assess the effects of co-infection on disease severity and patient outcomes. Due to the insensitivity of current diagnosis methods and the non-specific clinical presentation of viral respiratory tract infections, many co-infections go unnoticed or receive incorrect diagnoses[1]. These limitations make the epidemiology of co-infection difficult to define, even in controlled prospective studies[2]. As a result, a significant portion of current research on respiratory viral co-infections takes place *in vitro* using cell culture and *in vivo* using animal models. These methods enable controlled infection and identification of pathogens, resulting in a wealth of data. However, extrapolating these findings to humans is challenging, and the data have raised more questions than answers[3].

Respiratory viruses are important causes of morbidity and mortality worldwide[4]. In addition to the threat of emerging infections, there is concern about the implications of co-infections between respiratory viruses in certain groups in the population, such as young children, the elderly, and the immunocompromised[5]. Co-infection is defined as the simultaneous infection of a single host by two or more pathogens, and because of shared routes of transmission, it is a common occurrence with both respiratory viruses and bacteria[6]. The availability of molecular diagnostics has unveiled the greater than anticipated incidence of viral-viral co-infections[7]. A recent study identified an etiological viral agent in 85% of children with lower respiratory tract infections[7]. Researchers have estimated the average detection rates at 20%–40% using viral culture and antigen detection methods[8]. Similarly, in asymptomatic individuals and individuals with infections not involving the respiratory tract, molecular methods have revealed a high incidence of respiratory viral infections[9]. This increased awareness has raised many questions about the epidemiology, pathogenesis, and management of viral co-infections[10].

CO-INFECTION PATTERNS

Researchers have described respiratory virus co-infection as an independent risk factor for severe illness. Several studies have shown that co-infection leads to increased disease severity, with more severe symptoms and acute respiratory tract infections being the most common outcomes[11]. The admission rate for lower respiratory tract illness is 70.5% for cases of single viral infection compared to 92% for viral-viral co-infection and 95% for viral-bacterial co-infection[12]. Eighty-six percent of people who had more than one respiratory virus infection needed extra oxygen, while only 52% of people who had a single respiratory virus infection did[13]. Another study revealed that hospital admission rates for children with single virus detections were lower than those for co-infection cases (30% *vs* 57%)[14]. According to these studies, people who have more than one infection were more likely to be hospitalized and need oxygen therapy. This may be because the symptoms get worse when different respiratory pathogens interact with each other[15]. This is of particular significance for respiratory virus co-infections among the elderly and other high-risk groups who already have underlying health conditions. In these cases, co-infection may lead to rapid health deterioration with added complications, greater healthcare utilization, and increased morbidity and mortality[16]. High-risk groups are also more susceptible to respiratory virus co-infection due to the increased prevalence of these viruses among them and high-risk factors such as immunocompromised states[17]. Studies have linked influenza virus co-infection to the exacerbation of chronic diseases, often resulting in hospitalization and death[18].

Impact on disease severity

While co-infections affect disease severity, more research is necessary to determine the exact nature of this relationship and the underlying mechanisms[19]. There are several factors that may influence the observed effect of co-infections on disease severity. These include the study population's age and nature, case definition and outcome measures, as well as the spectrum of pathogens and methods used for pathogen detection[20]. The relationship between co-infection and exacerbation of pre-existing cardiopulmonary disease is also difficult to assess. It may be that viruses are particularly associated with exacerbations, but it is also possible that patients with chronic lung disease are simply more susceptible to

viral infections[21].

Several recent epidemiological studies have investigated this issue in a more organized way by comparing the severity of disease in people with a single virus infection *vs* people with dual virus infections using standard measures of severity, such as hospitalization, certain clinical parameters, and sometimes death[22]. Whereas some studies have found an association between dual infections and increased disease severity, others have found no association, and a few have found that co-infected patients have milder disease[23].

In humans, co-infection patterns can have an impact on disease presentation and outcome. However, deciphering the precise contribution of individual pathogens to the clinical syndrome in co-infected patients is often difficult[24]. Concurrent respiratory virus infections have long been considered a cause of increased disease severity, particularly in the pediatric population. Evidence to support this contention, however, has been largely anecdotal, derived from small case studies or series, and is thus inconclusive[25].

Co-infection combinations

Co-infection of a single population with different disease-causing agents can occur by a variety of methods. A study elegantly demonstrated this for human metapneumovirus (hMPV) and respiratory syncytial virus (RSV), using sequence information to establish molecular clocks and the likely R0 for each virus. The study suggested that the R0 of HMPV was insufficient for the virus to co-evolve with humans separately from RSV. Instead, researchers believed that hMPV spread to humans through zoonoses from RSV, and it only survived by co-infecting populations already infected with RSV[26].

Animal models may provide more detailed virological insights. Mouse studies have shown that a mild respiratory viral infection can lead to more severe disease upon secondary infection with RSV or other respiratory viruses[27]. However, models of RSV and hMPV in cotton rats showed no obvious increase in pathogenicity or virus load upon simultaneous infection with both viruses[28]. Common co-infection combinations may occur by chance, but they are more likely the result of complex immune responses to different infections[23]. A thorough understanding of why certain viruses tend to co-infect will help in designing preventative and treatment strategies. Molecular epidemiological studies suggest that RSV co-infection with hMPV or influenza is more common than it would occur by chance[29].

Risk factors for co-infections

The types and number of pathogens infecting an individual host are typically a result of the host's exposure to those pathogens balanced against the defenses mounted against them. Environmental factors have a major role in determining infection risks, an example being the seasonality and climate in temperate regions that result in marked annual peaks of viral respiratory infections but with different pathogens predominant in the winter and summer months[30]. Exposure to pollutants, cigarette smoke, and other harmful particulates impairs mucociliary clearance and alveolar macrophage function while also irritating the airways and increasing the susceptibility to infection[31]. Animal models and, in some cases, following controlled human infections, have demonstrated their ability to increase susceptibility to and severity of respiratory viral infections[32]. There is a significant association between smoking and viral respiratory infections[33]. Institutions, schools, military establishments, and households have long been associated with increased risks of respiratory infections such as the common cold, primarily due to increased opportunities for interpersonal contact and fomite transmission[34]. This is also true of increased social contact, which is a likely reason why school-aged children are known to be major transmitters of many infections to household contacts[35]. Globalization and international travel have made the world a smaller place, increasing the risk of emerging infections and the spread of infections to new areas and host populations[36]. The disease burden of RSV infection in infants in the United States is significantly higher than the hospitalization rate for RSV infection in infants in England, despite consistent monitoring of National Health Service hospitalization data indicating similar hospital admission criteria[37]. This may be due to differences in social and housing conditions between the two countries or unspecified differences in the use of health care services. Travel has also resulted in an increasing number of reports of travel-associated pneumonia due to a variety of pathogens, which can be difficult to precisely diagnose due to co-infection possibilities[38]. An example of Avian influenza infections in humans has demonstrated that higher intensity and nature of contact with animal populations can increase the risk of zoonotic infections[39]. Some viral infections can have indirect effects on immune system function. For instance, influenza virus or RSV infection of respiratory epithelial cells triggers the production of pro-inflammatory cytokines, which can predispose people to bacterial infections by up-regulating adhesion molecule expression and promoting the influx of inflammatory cells[40]. Chronic infections or the transmission of certain viruses can increase the risk of co-infections for a long time[41]. Immune suppression is a well-recognized risk factor for many infections and has led to greatly increased rates of various co-infections, particularly in the context of the HIV pandemic[42].

VIROLOGICAL INTERACTIONS

A study on dengue virus co-infection revealed an unfavorable effect on viral clearance, primarily due to a competitive interaction. This interaction showed that having two infections with similar viral loads increased the risk of getting dengue hemorrhagic fever and dengue shock syndrome for people who already had another infection[43]. This has an underlying significance. A study of competitive and cooperative interactions between RSV and influenza in a mouse model demonstrated their unfavorable effects and potential prolongation of the disease[44]. In this case, both viruses caused more illness and made it last longer in some cases. This was true even though they only slightly affected the second virus's ability to replicate, and only one infection was found to speed up viral clearance and disease resolution. With little concrete evidence regarding the effect of co-infection on viral interactions, it is important to first establish

theoretical scenarios based on known immunological and virological principles[45]. Despite a potential interaction period during the initial co-infection of both viruses, it is likely that the overall dominance of one virus over the other would lead to a primarily competitive interaction. When the second virus introduces itself during the first virus's infection, it perpetuates the co-infection's effects on viral clearance, prolonging the period of incomplete immunity against one of the viruses[46].

Dynamics of viral replication

Due to its higher prevalence rate in the community and the greater knowledge of *in vitro* studies already conducted, influenza is another likely candidate for RSV co-infection. RSV replication primarily occurs in the respiratory epithelium, but influenza can infect both the upper respiratory tract and type II pneumocytes in the lungs[47]. Because influenza tropism is so complicated, there are many ways that co-infection can happen. It is important to think about how co-infection with influenza, which can be mild or severe, might affect the spread of RSV replication. An example of this is the impact of influenza infection on RSV replication, which may not hinder the migration of RSV-infected cells from the lower respiratory tract to the upper mucosal areas. Additionally, the co-infection of RSV and influenza on type II pneumocytes can significantly influence RSV replication, though it is challenging to evaluate in an *in vivo* setting[48].

Extrinsic and intrinsic factors combine to determine the viral replication dynamics. It's important to know how to spread target cells and how cell tropism affects the overall viral load in an infected person[27]. It is also important to know how likely it is for cells to become co-infected. As RSV is well known to infect ciliated and goblet cells within the respiratory tract, it is highly likely for RSV to be involved in a co-infection scenario with either itself in the form of re-infection or with another virus. Researchers have extensively studied co-infection *in vivo* between RSV and hMPV[23]. Because the symptoms of both viruses are similar and the rates of infection in similar age groups are known, hMPV is a great candidate to study in the context of RSV co-infection to identify new subtle ways that they interact[49].

Immune response modulation

Scientists showed that infecting a BALB/c mouse model with a recombinant RSV strain activated a T-helper 2 (Th2) response, leading to eosinophilia and the airway becoming more reactive[50]. Researchers have obtained similar results from hMPV infections[28]. It was found that using an inactivated RSV vaccine to treat the infection improved eosinophilia, T-helper 1 (Th1)-biased cytokine and antibody responses. It was found that this vaccine-related disease was caused by immune complex formation. The researchers inhibited disease in mice by removing their complement[24]. Vaccine-enhanced disease has hindered the successful development of both RSV and hMPV vaccines[32].

Researchers have found that increasing a Th1 immune response helps stop the spread of viruses like RSV and hMPV in lab experiments[45]. However, increasing a Th2 immune response makes the disease worse, as shown by higher eosinophilia, mucus production in the airways, and disease severity[51].

The local response to a respiratory virus infection is quite varied, depending on the infecting virus. Antiviral and pro-inflammatory activities classify the immune response. The immune response starts with the production of interferon and virus-specific cytotoxic T lymphocytes[28]. Th1 cytokine and IgG2a antibody production is also indicative of a protective immune response[52]. Table 1 summarizes the virological interaction in co-infections.

CLINICAL IMPLICATIONS

For patients presenting to the hospital with respiratory viral infections, one study found that patients positive for influenza have similar characteristics to those with community-acquired pneumonia (CAP)[53]. Another study specifically on patients with CAP requiring intensive care unit admission produced some interesting findings. In the multivariate analysis, influenza was the only independent factor associated with death when comparing the viral etiology of pneumonia[54]. Therefore, finding influenza in respiratory viral infection patients is even more important, and finding a specific respiratory virus instead of just a clinical diagnosis could change how different lung infections are treated and how well they do. An observational study on cellular viral loads in immunosuppressed patients diagnosed with pneumonia found that patients with a lower respiratory illness and a positive respiratory virus PCR had an attributable mortality of 26% for respiratory viral infections and improved antivirals could reduce mortality outcomes[55]. In the United States, influenza virus infects 20% of the population annually, and during a circulating influenza outbreak, many clinicians diagnose influenza-like illness (ILI) as influenza and initiate treatment with oseltamivir or zanamivir in a hospital or community setting[56]. A recent systematic Cochrane review, encompassing 20 trials of oseltamivir and zanamivir treatment in adult and pediatric outpatients, determined the clinical effectiveness of ILI treatment to be modestly beneficial, at best reducing the duration of illness by 1 day[57]. Initiating treatment within the first 48 hours of symptoms enhances the clinical effectiveness of these antivirals. A specific diagnosis of influenza and a high level of illness severity yield the best results. Data on hospitalized adult patients who can have very severe outcomes from respiratory viral infections are lacking. High costs for individual patients, particularly in third-world countries, are problematic. For instance, respiratory viral infections exacerbate heart failure exacerbations and chronic obstructive pulmonary disease, frequently leading to hospital admissions for ILI patients. The clinical effectiveness of antivirals, particularly in high-risk groups such as the elderly and those with co-morbidities, remains an important issue[58]. Because ILI is highly non-specific, many respiratory virus infections cluster around this case definition, making a specific diagnosis and a decision on how to manage the patient frequently difficult. Without virological testing, a specific diagnosis remains rare, even in the best of clinical settings[34].

Table 1 Virological interactions in co-infections

Co-infection combination	Interaction type	Impact on disease progression
Dengue virus + secondary virus	Competitive	Increased risk of severe dengue outcomes (DHF, DSS)
RSV + influenza	Cooperative/competitive	Prolongs disease, increases severity and duration in some cases
RSV + other respiratory viruses (*e.g.*, hMPV)	Varies; neutral in some models	Little to no increase in pathogenicity in some animal models

DHF: Dengue hemorrhagic fever; DSS: Dengue shock syndrome; hMPV: Human metapneumovirus; RSV: Respiratory syncytial virus.

Diagnosis challenges

The lack of compelling evidence for the clinical implications of viral interactions on disease severity is likely to reflect the methodological limitations impacting the success of future virus-virus interaction studies. Observational studies or traditional statistical methods in existing datasets pose many difficulties in definitively diagnosing the viruses involved, specifying the timing, order, and type of infection, establishing a causative association between infection and disease, and controlling for confounding factors[59].

While rapid progress in diagnostic technology has been a major contributor to the recent discoveries of viral prevalence, incidence, and etiology in the population, it has also generated a wealth of information describing simple viral shedding or presence. An example of this is the frequency of detection of all viruses within a diagnostic test, even when multiple specimens are not available from every patient at the time of disease incidence. Epidemiologists have interpreted this to mean a surrogation of clinical disease, with the most severe symptoms being attributable to the virus. However, tests detect all levels of virus RNA or antigen, including asymptomatic shedding, and detection of viruses often continues well after cessation of symptoms[60].

Viral vaccination studies have convincingly demonstrated the important implications of identifying virus-specific etiologies of disease for public health and global health issues[61].

Treatment considerations

The lack of specific antiviral therapies for many viruses is a limitation, and more broad-spectrum anti-infective agents have increased potential for drug-drug interactions and may have unknown effects on a second virus. These points highlight the complex issues regarding the treatment of co-infections and the need for further research to determine the optimum management strategies[45]. At present, many treatment regimens are based on specific etiologies. However, for many viral pathogens, effective therapies are lacking and management is often supportive. If co-infections occur, it may be unclear which virus is the causative agent, and treatment regimens may overlap, potentially exacerbating a treatment-induced adverse event[48].

TRANSMISSION DYNAMICS

High co-infection rates can sustain an epidemic or lead to an outbreak of a particular virus if transmission rates between co-infections and single infections are significantly different. This was the case in RSV outbreak in a pediatric ward, where nosocomial transmission of multiple virus types, including RSV itself, caused prolonged ward closure[34]. In terms of acute respiratory infection, it is extremely rare for just one type of virus to be the sole cause, as exemplified by a study that showed 61% of cases tested negative for all virus types. However, prolonged viral shedding can complicate the classification of individuals as co-infected by PCR testing methods, potentially leading to the emergence of a second infection before clearing the first[38].

Co-infection transmission rates

This information is not yet available to respiratory virus agents. Co-infection is likely to increase viral transmission, as secondary strains are unlikely to evolve efficient transmission between humans without first increasing their prevalence. On the other hand, some viruses are known to speed up the spread of others through immunological or other means. For example, RSV speeds up the spread of *Streptococcus pneumoniae* in an animal model. This could be more important than any competitive effect between co-infecting strains[62].

Increasing transmission could also occur simply because of co-infection, increasing the overall disease burden on the host and making them more infectious in terms of contact rates with other individuals. An intriguing but neglected issue is the potential for complex interactions between different respiratory viruses in communal settings to affect each other's transmission. Schools play a crucial role in this context, as research indicates that children frequently contract multiple viruses simultaneously[63].

Mathematical models of infection for one specific virus often include a large number of simplifying assumptions about the host population, and it can be difficult to integrate multiple models for different viruses in a consistent way[64].

But adding information to these models from virological or epidemiological studies on how certain virus types can infect each other could help us understand how respiratory viruses spread in communities.

Role of co-infections in outbreaks

Large amounts of evidence from experiments with both animal models and *in vitro* systems with model viral infections show that how viruses interact with each other can have a big impact on how bad the disease is. It can be predicted that the potential net effect of virus-virus interaction can range from increased pathogenesis due to the reactivation of latent infection, where one or more viruses interact at the level of viral gene expression, to complete interference between two or more viruses in an attempt to establish a productive infection[65]. This can mean that one virus effectively outcompetes another virus for a susceptible host cell, resulting in the loss of a viral genetic lineage (extinction of one of the interacting viruses in a defined ecological niche). We now recognize these outcomes as potentially important in the natural ecology of virus infections, and it is challenging to study how they facilitate some of these events in humans, like viral reactivation and extinction. From a public health perspective, one of the most important consequences of virus-virus interactions is the exacerbation of acute disease manifestations. The most extreme example of this is the increased mortality seen with certain respiratory virus infections in the very young and/or elderly due to a variety of interacting infections.

PUBLIC HEALTH STRATEGIES

The majority of global morbidity from respiratory viruses occurs in developing countries, where infectious diseases significantly impact malnutrition and chronic diseases. Efforts here focus on identifying broad syndromes and developing interventions. Recent strategies using novel PCR techniques to identify viral causes of syndromes like fever in immunocompromised patients or acute respiratory infections in bone marrow transplant units show promise in understanding these infections' multifactorial impacts. In developed countries, public health surveillance emphasizes epidemiology and identifying etiological agents. Systems in the US and Canada linking pneumonia admissions to pathogen identification exemplify these efforts, which inform prevention and control strategies. Sentinel hospital networks monitoring pediatric respiratory virus infections correlate trends with broader morbidity data, proving effective in countries with good healthcare access. Advances in identification techniques highlight the global significance of respiratory viruses and the need for tailored public health strategies to address healthcare system disparities[66-68].

RNA-based molecular testing methods enhance the ability to define and characterize respiratory viral infections, improving recognition within communities and among patients. Despite effective antiviral therapy for influenza, presumptive diagnoses can be inaccurate, necessitating distinct management approaches. PCR testing and modern pneumococcal vaccines are expected to clarify the roles of respiratory viral infections and bacterial coinfections. Point-of-care PCR testing will facilitate accurate diagnoses similar to chest X-rays, aiding community physicians in recognizing respiratory viral infections. Real-time (RT)-PCR testing has high sensitivity and specificity, and it enables prompt diagnosis and detailed studies of disease burden and seasonality, crucial for understanding respiratory viral infections' impact on chronic disease exacerbations. Effective containment and treatment of respiratory viral infections rely on detecting the infecting agent and understanding its community impact. Traditional viral diagnosis methods like culture and serology are slow and often provide insufficient information[69-71].

Future molecular diagnostics will likely identify the ways in which respiratory virus exacerbate chronic lung disease, guiding prevention and treatment strategies. Effective public health strategies must be specific and practical, particularly for co-infections, which are under-researched. Evidence-based interventions can significantly improve health outcomes and reduce healthcare costs[72].

Therapeutic development

Developing therapies specific to co-infections can limit adverse outcomes for patients. One promising approach is altering the host cellular environment to prevent viral replication by modifying host cell proteins necessary for viral entry and replication. These findings can translate into drug therapies tested in animal models and clinical trials. Another strategy involves altering the ability of cells to produce proteins crucial for virus replication or modulating the immune response to prevent disease exacerbation. Further studies on host immune responses to virus-virus interactions, followed by animal models and human trials, can lead to the development of vaccines preventing respiratory virus co-infections[7,73].

Host immune response

While the immune response to single respiratory virus infections is well-studied, the effects of multiple viral infections are less understood. To comprehend the immune system's response to virus interactions, it is crucial to map the immune response sequence using animal models, followed by human clinical studies. Understanding these mechanisms can help develop therapies to prevent or limit co-infections, improving patient outcomes[74].

Current co-infection studies

Most studies use nucleic acid amplification to identify viruses present in a host, but they do not consider multiple strains of a single virus. Determining the *in vivo* significance of co-infection requires animal models to track the effects on viral replication, virulence, and host immune responses. These findings can then be applied to human clinical trials.

Understanding co-infection mechanisms

Co-infection mechanisms are poorly understood and often modeled in animals with viruses that do not naturally co-infect. For instance, RSV does not replicate well in mice, which can confound results[31]. Identifying replicated viruses *vs*

Table 2 Public health strategies and recommendations

Strategy	Recommendation
Enhanced surveillance systems	Focus on high-risk groups for better identification and control of co-infections
Targeted vaccination campaigns	Develop vaccines considering common co-infecting viruses, especially for high-risk groups
Multiplex PCR testing implementation	Implement in clinical settings for simultaneous diagnosis of multiple respiratory viruses
Education on infection control measures	Increase awareness among public and healthcare workers regarding transmission risks
Development of broad-spectrum antivirals	Encourage R&D for antivirals effective against multiple viruses to address co-infections

R&D: Research and Development.

marker viruses in these models is challenging, as highlighted in a study with murine gastroenteritis virus and transmissible gastroenteritis virus in pigs, where the data became blurred, complicating the understanding of co-infections on a molecular level[75].

Co-infection-specific therapies

Few antiviral agents have well-defined molecular mechanisms, and *in vivo* outcomes can vary based on administration timing and the host's immune status. While co-infection-tailored therapies are complex, they may be feasible. For instance, if a co-infection exacerbates disease by inhibiting IFN production, using IFN as a therapeutic agent might be effective. However, simplistic approaches, such as administering all antivirals simultaneously, should be avoided due to potential drug competition or pathway inhibition[76,77]. Table 2 summarizes public health strategies and recommendations.

CONCLUSION

Conclusion and future perspectives

In summary, respiratory viral co-infections pose significant challenges to clinical management due to their complex impact on disease severity and patient outcomes. Current diagnostic methods often fail to detect these co-infections, leading to underestimation of their prevalence and complicating the development of effective treatment strategies. There is an urgent need for advanced diagnostic techniques that can accurately identify co-infections and their specific viral combinations.

Future research should focus on elucidating the molecular mechanisms that underpin viral interactions during co-infections. A deeper understanding of these interactions will be crucial in developing targeted therapeutic strategies that can mitigate the exacerbation of symptoms caused by co-infections. Additionally, there is a pressing need to explore the role of the immune system in co-infections, particularly how it can be modulated to improve patient outcomes.

Public health strategies must also be adapted to address the challenges posed by respiratory viral co-infections. This includes improving surveillance systems to better capture data on co-infections and integrating this information into the development of vaccines and antiviral treatments. Ultimately, a multidisciplinary approach that combines clinical, virological, and epidemiological expertise will be essential to advance our understanding and management of respiratory viral co-infections, thereby reducing their burden on global health.

FOOTNOTES

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REFERENCES

1 **Goka EA**, Vallely PJ, Mutton KJ, Klapper PE. Single, dual and multiple respiratory virus infections and risk of hospitalization and mortality. *Epidemiol Infect* 2015; **143**: 37-47 [PMID: 24568719 DOI: 10.1017/S0950268814000302]

2 **Chamseddine S**, Chmaisse A, Akel I, Zein ZE, Khalil S, Raad SA, Khati A, Ghandour H, Khafaja S, Haj M, Abboud M, Mahfouz R, Araj G, Zaraket H, Hanna-Wakim R, Muwakkit S, Dbaibo G. Epidemiology and clinical characteristics of viral infections in hospitalized children and adolescents with cancer in Lebanon. *PLoS One* 2020; **15**: e0239258 [PMID: 32961548 DOI: 10.1371/journal.pone.0239258]

3 **Rijsbergen LC**, van Dijk LLA, Engel MFM, de Vries RD, de Swart RL. In Vitro Modelling of Respiratory Virus Infections in Human Airway Epithelial Cells - A Systematic Review. *Front Immunol* 2021; **12**: 683002 [PMID: 34489934 DOI: 10.3389/fimmu.2021.683002]

4 **Iuliano AD**, Roguski KM, Chang HH, Muscatello DJ, Palekar R, Tempia S, Cohen C, Gran JM, Schanzer D, Cowling BJ, Wu P, Kyncl J, Ang LW, Park M, Redlberger-Fritz M, Yu H, Espenhain L, Krishnan A, Emukule G, van Asten L, Pereira da Silva S, Aungkulanon S, Buchholz U, Widdowson MA, Bresee JS; Global Seasonal Influenza-associated Mortality Collaborator Network. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. *Lancet* 2018; **391**: 1285-1300 [PMID: 29248255 DOI: 10.1016/S0140-6736(17)33293-2]

5 **GBD 2016 Lower Respiratory Infections Collaborators**. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis* 2018; **18**: 1191-1210 [PMID: 30243584 DOI: 10.1016/S1473-3099(18)30310-4]

6 **Du Y**, Wang C, Zhang Y. Viral Coinfections. *Viruses* 2022; **14** [PMID: 36560647 DOI: 10.3390/v14122645]

7 **Jain S**, Williams DJ, Arnold SR, Ampofo K, Bramley AM, Reed C, Stockmann C, Anderson EJ, Grijalva CG, Self WH, Zhu Y, Patel A, Hymas W, Chappell JD, Kaufman RA, Kan JH, Dansie D, Lenny N, Hillyard DR, Haynes LM, Levine M, Lindstrom S, Winchell JM, Katz JM, Erdman D, Schneider E, Hicks LA, Wunderink RG, Edwards KM, Pavia AT, McCullers JA, Finelli L; CDC EPIC Study Team. Community-acquired pneumonia requiring hospitalization among U.S. children. *N Engl J Med* 2015; **372**: 835-845 [PMID: 25714161 DOI: 10.1056/NEJMoa1405870]

8 **Schütze H**, Weigl J, Puppe W, Forster J, Berner R. Diagnostic performance of a rapid antigen test for RSV in comparison with a 19-valent multiplex RT-PCR ELISA in children with acute respiratory tract infections. *Eur J Pediatr* 2008; **167**: 745-749 [PMID: 17764017 DOI: 10.1007/s00431-007-0581-1]

9 **Pretorius MA**, Madhi SA, Cohen C, Naidoo D, Groome M, Moyes J, Buys A, Walaza S, Dawood H, Chhagan M, Haffjee S, Kahn K, Puren A, Venter M. Respiratory viral coinfections identified by a 10-plex real-time reverse-transcription polymerase chain reaction assay in patients hospitalized with severe acute respiratory illness--South Africa, 2009-2010. *J Infect Dis* 2012; **206** Suppl 1: S159-S165 [PMID: 23169964 DOI: 10.1093/infdis/jis538]

10 **Crotty MP**, Meyers S, Hampton N, Bledsoe S, Ritchie DJ, Buller RS, Storch GA, Micek ST, Kollef MH. Epidemiology, Co-Infections, and Outcomes of Viral Pneumonia in Adults: An Observational Cohort Study. *Medicine (Baltimore)* 2015; **94**: e2332 [PMID: 26683973 DOI: 10.1097/MD.0000000000002332]

11 **Cilla G**, Oñate E, Perez-Yarza EG, Montes M, Vicente D, Perez-Trallero E. Viruses in community-acquired pneumonia in children aged less than 3 years old: High rate of viral coinfection. *J Med Virol* 2008; **80**: 1843-1849 [PMID: 18712820 DOI: 10.1002/jmv.21271]

12 **Martin ET**, Kuypers J, Wald A, Englund JA. Multiple versus single virus respiratory infections: viral load and clinical disease severity in hospitalized children. *Influenza Other Respir Viruses* 2012; **6**: 71-77 [PMID: 21668660 DOI: 10.1111/j.1750-2659.2011.00265.x]

13 **Scotta MC**, Chakr VC, de Moura A, Becker RG, de Souza AP, Jones MH, Pinto LA, Sarria EE, Pitrez PM, Stein RT, Mattiello R. Respiratory viral coinfection and disease severity in children: A systematic review and meta-analysis. *J Clin Virol* 2016; **80**: 45-56 [PMID: 27155055 DOI: 10.1016/j.jcv.2016.04.019]

14 **Calvo C**, García-García ML, Blanco C, Vázquez MC, Frías ME, Pérez-Breña P, Casas I. Multiple simultaneous viral infections in infants with acute respiratory tract infections in Spain. *J Clin Virol* 2008; **42**: 268-272 [PMID: 18455958 DOI: 10.1016/j.jcv.2008.03.012]

15 **Greer RM**, McErlean P, Arden KE, Faux CE, Nitsche A, Lambert SB, Nissen MD, Sloots TP, Mackay IM. Do rhinoviruses reduce the probability of viral co-detection during acute respiratory tract infections? *J Clin Virol* 2009; **45**: 10-15 [PMID: 19376742 DOI: 10.1016/j.jcv.2009.03.008]

16 **Morris DE**, Cleary DW, Clarke SC. Secondary Bacterial Infections Associated with Influenza Pandemics. *Front Microbiol* 2017; **8**: 1041 [PMID: 28690590 DOI: 10.3389/fmicb.2017.01041]

17 **Wark PA**, Tooze M, Cheese L, Whitehead B, Gibson PG, Wark KF, McDonald VM. Viral infections trigger exacerbations of cystic fibrosis in adults and children. *Eur Respir J* 2012; **40**: 510-512 [PMID: 22855475 DOI: 10.1183/09031936.00202311]

18 **Uyeki TM**, Bernstein HH, Bradley JS, Englund JA, File TM, Fry AM, Gravenstein S, Hayden FG, Harper SA, Hirshon JM, Ison MG, Johnston BL, Knight SL, McGeer A, Riley LE, Wolfe CR, Alexander PE, Pavia AT. Clinical Practice Guidelines by the Infectious Diseases Society of America: 2018 Update on Diagnosis, Treatment, Chemoprophylaxis, and Institutional Outbreak Management of Seasonal Influenzaa. *Clin Infect Dis* 2019; **68**: e1-e47 [PMID: 30566567 DOI: 10.1093/cid/ciy866]

19 **Moreno-Valencia Y**, Hernandez-Hernandez VA, Romero-Espinoza JAI, Coronel-Tellez RH, Castillejos-Lopez M, Hernandez A, Perez-Padilla R, Alejandre-Garcia A, de la Rosa-Zamboni D, Ormsby CE, Vazquez-Perez JA. Detection and characterization of respiratory viruses causing acute respiratory illness and asthma exacerbation in children during three different seasons (2011-2014) in Mexico City. *Influenza Other Respir Viruses* 2015; **9**: 287-292 [PMID: 26289993 DOI: 10.1111/irv.12346]

20 **Branche AR**, Walsh EE, Formica MA, Falsey AR. Detection of respiratory viruses in sputum from adults by use of automated multiplex PCR. *J Clin Microbiol* 2014; **52**: 3590-3596 [PMID: 25056335 DOI: 10.1128/JCM.01523-14]

21 **Linden D**, Guo-Parke H, Coyle PV, Fairley D, McAuley DF, Taggart CC, Kidney J. Respiratory viral infection: a potential "missing link" in the pathogenesis of COPD. *Eur Respir Rev* 2019; **28** [PMID: 30872396 DOI: 10.1183/16000617.0063-2018]

22 **Marguet C**, Lubrano M, Gueudin M, Le Roux P, Deschildre A, Forget C, Couderc L, Siret D, Donnou MD, Bubenheim M, Vabret A, Freymuth F. In very young infants severity of acute bronchiolitis depends on carried viruses. *PLoS One* 2009; **4**: e4596 [PMID: 19240806 DOI: 10.1371/journal.pone.0004596]

23 **Semple MG**, Cowell A, Dove W, Greensill J, McNamara PS, Halfhide C, Shears P, Smyth RL, Hart CA. Dual infection of infants by human

metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. *J Infect Dis* 2005; **191**: 382-386 [PMID: 15633097 DOI: 10.1086/426457]

24 **Liu YN**, Zhang YF, Xu Q, Qiu Y, Lu QB, Wang T, Zhang XA, Lin SH, Lv CL, Jiang BG, Li H, Li ZJ, Gao GF, Yang WZ, Hay SI, Wang LP, Fang LQ, Liu W; Chinese Center for Disease Control and Prevention Etiology Surveillance Study Team of Acute Respiratory Infections. Infection and co-infection patterns of community-acquired pneumonia in patients of different ages in China from 2009 to 2020: a national surveillance study. *Lancet Microbe* 2023; **4**: e330-e339 [PMID: 37001538 DOI: 10.1016/S2666-5247(23)00031-9]

25 **Wishaupt JO**, van der Ploeg T, de Groot R, Versteegh FG, Hartwig NG. Single- and multiple viral respiratory infections in children: disease and management cannot be related to a specific pathogen. *BMC Infect Dis* 2017; **17**: 62 [PMID: 28077074 DOI: 10.1186/s12879-016-2118-6]

26 **Gaunt ER**, Jansen RR, Poovorawan Y, Templeton KE, Toms GL, Simmonds P. Molecular epidemiology and evolution of human respiratory syncytial virus and human metapneumovirus. *PLoS One* 2011; **6**: e17427 [PMID: 21390255 DOI: 10.1371/journal.pone.0017427]

27 **Hall CB**, Walsh EE, Long CE, Schnabel KC. Immunity to and frequency of reinfection with respiratory syncytial virus. *J Infect Dis* 1991; **163**: 693-698 [PMID: 2010624 DOI: 10.1093/infdis/163.4.693]

28 **Hamelin ME**, Yim K, Kuhn KH, Cragin RP, Boukhvalova M, Blanco JC, Prince GA, Boivin G. Pathogenesis of human metapneumovirus lung infection in BALB/c mice and cotton rats. *J Virol* 2005; **79**: 8894-8903 [PMID: 15994783 DOI: 10.1128/JVI.79.14.8894-8903.2005]

29 **Cilla G**, Oñate E, Perez-Yarza EG, Montes M, Vicente D, Perez-Trallero E. Hospitalization rates for human metapneumovirus infection among 0- to 3-year-olds in Gipuzkoa (Basque Country), Spain. *Epidemiol Infect* 2009; **137**: 66-72 [PMID: 18419854 DOI: 10.1017/S0950268808000666]

30 **Lofgren E**, Fefferman NH, Naumov YN, Gorski J, Naumova EN. Influenza seasonality: underlying causes and modeling theories. *J Virol* 2007; **81**: 5429-5436 [PMID: 17182688 DOI: 10.1128/JVI.01680-06]

31 **Morris DR**, Qu Y, Thomason KS, de Mello AH, Preble R, Menachery VD, Casola A, Garofalo RP. The impact of RSV/SARS-CoV-2 co-infection on clinical disease and viral replication: insights from a BALB/c mouse model. *bioRxiv* 2023 [PMID: 37292863 DOI: 10.1101/2023.05.24.542043]

32 **Mallia P**, Johnston SL. How viral infections cause exacerbation of airway diseases. *Chest* 2006; **130**: 1203-1210 [PMID: 17035457 DOI: 10.1378/chest.130.4.1203]

33 **Arcavi L**, Benowitz NL. Cigarette smoking and infection. *Arch Intern Med* 2004; **164**: 2206-2216 [PMID: 15534156 DOI: 10.1001/archinte.164.20.2206]

34 **Monto AS**. Epidemiology of viral respiratory infections. *Am J Med* 2002; **112** Suppl 6A: 4S-12S [PMID: 11955454 DOI: 10.1016/s0002-9343(01)01058-0]

35 **Musher DM**. How contagious are common respiratory tract infections? *N Engl J Med* 2003; **348**: 1256-1266 [PMID: 12660390 DOI: 10.1056/NEJMra021771]

36 **Wilson ME**. Travel and the emergence of infectious diseases. *Emerg Infect Dis* 1995; **1**: 39-46 [PMID: 8903157 DOI: 10.3201/eid0102.950201]

37 **Akinboyo IC**, Young RR, Smith MJ, Lewis SS, Smith BA, Anderson DJ. Burden of healthcare-associated infections among hospitalized children within community hospitals participating in an infection control network. *Infect Control Hosp Epidemiol* 2022; **43**: 510-512 [PMID: 33685533 DOI: 10.1017/ice.2021.67]

38 **Farr BM**, Bartlett CL, Wadsworth J, Miller DL. Risk factors for community-acquired pneumonia diagnosed upon hospital admission. British Thoracic Society Pneumonia Study Group. *Respir Med* 2000; **94**: 954-963 [PMID: 11059948 DOI: 10.1053/rmed.2000.0865]

39 **Hatta M**, Kawaoka Y. The continued pandemic threat posed by avian influenza viruses in Hong Kong. *Trends Microbiol* 2002; **10**: 340-344 [PMID: 12110213 DOI: 10.1016/s0966-842x(02)02388-0]

40 **de Jong MD**, Simmons CP, Thanh TT, Hien VM, Smith GJ, Chau TN, Hoang DM, Chau NV, Khanh TH, Dong VC, Qui PT, Cam BV, Ha do Q, Guan Y, Peiris JS, Chinh NT, Hien TT, Farrar J. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med* 2006; **12**: 1203-1207 [PMID: 16964257 DOI: 10.1038/nm1477]

41 **Lok AS**, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662 [PMID: 19714720 DOI: 10.1002/hep.23190]

42 **Nomah DK**, Reyes-Urueña J, Llibre JM, Ambrosioni J, Ganem FS, Miró JM, Casabona J. HIV and SARS-CoV-2 Co-infection: Epidemiological, Clinical Features, and Future Implications for Clinical Care and Public Health for People Living with HIV (PLWH) and HIV Most-at-Risk Groups. *Curr HIV/AIDS Rep* 2022; **19**: 17-25 [PMID: 35113346 DOI: 10.1007/s11904-021-00596-5]

43 **Rothman AL**. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. *Nat Rev Immunol* 2011; **11**: 532-543 [PMID: 21760609 DOI: 10.1038/nri3014]

44 **Waterlow NR**, Flasche S, Minter A, Eggo RM. Competition between RSV and influenza: Limits of modelling inference from surveillance data. *Epidemics* 2021; **35**: 100460 [PMID: 33838587 DOI: 10.1016/j.epidem.2021.100460]

45 **Graham BS**. Biological challenges and technological opportunities for respiratory syncytial virus vaccine development. *Immunol Rev* 2011; **239**: 149-166 [PMID: 21198670 DOI: 10.1111/j.1600-065X.2010.00972.x]

46 **Oldstone MB**. Viral persistence: parameters, mechanisms and future predictions. *Virology* 2006; **344**: 111-118 [PMID: 16364742 DOI: 10.1016/j.virol.2005.09.028]

47 **Uyeki TM**, Hui DS, Zambon M, Wentworth DE, Monto AS. Influenza. *Lancet* 2022; **400**: 693-706 [PMID: 36030813 DOI: 10.1016/S0140-6736(22)00982-5]

48 **Glezen WP**, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. *Am J Dis Child* 1986; **140**: 543-546 [PMID: 3706232 DOI: 10.1001/archpedi.1986.02140200053026]

49 **Boivin G**, De Serres G, Hamelin ME, Côté S, Argouin M, Tremblay G, Maranda-Aubut R, Sauvageau C, Ouakki M, Boulianne N, Couture C. An outbreak of severe respiratory tract infection due to human metapneumovirus in a long-term care facility. *Clin Infect Dis* 2007; **44**: 1152-1158 [PMID: 17407031 DOI: 10.1086/513204]

50 **Haeberle HA**, Takizawa R, Casola A, Brasier AR, Dieterich HJ, Van Rooijen N, Gatalica Z, Garofalo RP. Respiratory syncytial virus-induced activation of nuclear factor-kappaB in the lung involves alveolar macrophages and toll-like receptor 4-dependent pathways. *J Infect Dis* 2002; **186**: 1199-1206 [PMID: 12402188 DOI: 10.1086/344644]

51 **Manohar P**, Loh B, Athira S, Nachimuthu R, Hua X, Welburn SC, Leptihn S. Secondary Bacterial Infections During Pulmonary Viral Disease: Phage Therapeutics as Alternatives to Antibiotics? *Front Microbiol* 2020; **11**: 1434 [PMID: 32733404 DOI: 10.3389/fmicb.2020.01434]

52 **van den Hoogen BG**, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, Osterhaus AD. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 2001; **7**: 719-724 [PMID: 11385510 DOI: 10.1038/89098]

53 **Falsey AR**, Becker KL, Swinburne AJ, Nylen ES, Formica MA, Hennessey PA, Criddle MM, Peterson DR, Baran A, Walsh EE. Bacterial

complications of respiratory tract viral illness: a comprehensive evaluation. *J Infect Dis* 2013; **208**: 432-441 [PMID: 23661797 DOI: 10.1093/infdis/jit190]

54 **Kumar A**, Zarychanski R, Pinto R, Cook DJ, Marshall J, Lacroix J, Stelfox T, Bagshaw S, Choong K, Lamontagne F, Turgeon AF, Lapinsky S, Ahern SP, Smith O, Siddiqui F, Jouvet P, Khwaja K, McIntyre L, Menon K, Hutchison J, Hornstein D, Joffe A, Lauzier F, Singh J, Karachi T, Wiebe K, Olafson K, Ramsey C, Sharma S, Dodek P, Meade M, Hall R, Fowler RA; Canadian Critical Care Trials Group H1N1 Collaborative. Critically ill patients with 2009 influenza A(H1N1) infection in Canada. *JAMA* 2009; **302**: 1872-1879 [PMID: 19822627 DOI: 10.1001/jama.2009.1496]

55 **Couch RB**, Englund JA, Whimbey E. Respiratory viral infections in immunocompetent and immunocompromised persons. *Am J Med* 1997; **102**: 2-9; discussion 25 [PMID: 10868136 DOI: 10.1016/s0002-9343(97)00003-x]

56 **Flight WG**, Bright-Thomas RJ, Tilston P, Mutton KJ, Guiver M, Morris J, Webb AK, Jones AM. Incidence and clinical impact of respiratory viruses in adults with cystic fibrosis. *Thorax* 2014; **69**: 247-253 [PMID: 24127019 DOI: 10.1136/thoraxjnl-2013-204000]

57 **Jefferson T**, Jones MA, Doshi P, Del Mar CB, Hama R, Thompson MJ, Spencer EA, Onakpoya I, Mahtani KR, Nunan D, Howick J, Heneghan CJ. Neuraminidase inhibitors for preventing and treating influenza in adults and children. *Cochrane Database Syst Rev* 2014; **2014**: CD008965 [PMID: 24718923 DOI: 10.1002/14651858.CD008965.pub4]

58 **McCullers JA**. Preventing and treating secondary bacterial infections with antiviral agents. *Antivir Ther* 2011; **16**: 123-135 [PMID: 21447860 DOI: 10.3851/IMP1730]

59 **Yadav SK**, Akhter Y. Statistical Modeling for the Prediction of Infectious Disease Dissemination With Special Reference to COVID-19 Spread. *Front Public Health* 2021; **9**: 645405 [PMID: 34222166 DOI: 10.3389/fpubh.2021.645405]

60 **Lin YC**, Malott RJ, Ward L, Kiplagat L, Pabbaraju K, Gill K, Berenger BM, Hu J, Fonseca K, Noyce RS, Louie T, Evans DH, Conly JM. Detection and quantification of infectious severe acute respiratory coronavirus-2 in diverse clinical and environmental samples. *Sci Rep* 2022; **12**: 5418 [PMID: 35354854 DOI: 10.1038/s41598-022-09218-5]

61 **Jamrozik E**, Heriot G, Bull S, Parker M; Oxford-Johns Hopkins Global Infectious Disease Ethics (GLIDE). Vaccine-enhanced disease: case studies and ethical implications for research and public health. *Wellcome Open Res* 2021; **6**: 154 [PMID: 34235275 DOI: 10.12688/wellcomeopenres.16849.1]

62 **Hament JM**, Aerts PC, Fleer A, van Dijk H, Harmsen T, Kimpen JL, Wolfs TF. Direct binding of respiratory syncytial virus to pneumococci: a phenomenon that enhances both pneumococcal adherence to human epithelial cells and pneumococcal invasiveness in a murine model. *Pediatr Res* 2005; **58**: 1198-1203 [PMID: 16306193 DOI: 10.1203/01.pdr.0000188699.55279.1b]

63 **Tupper P**, Colijn C. COVID-19 in schools: Mitigating classroom clusters in the context of variable transmission. *PLoS Comput Biol* 2021; **17**: e1009120 [PMID: 34237051 DOI: 10.1371/journal.pcbi.1009120]

64 **White PJ**. Mathematical Models in Infectious Disease Epidemiology. *Infect Dis* 2017 [DOI: 10.1016/b978-0-7020-6285-8.00005-8]

65 **Pinky L**, DeAguero JR, Remien CH, Smith AM. How Interactions during Viral-Viral Coinfection Can Shape Infection Kinetics. *Viruses* 2023; **15** [PMID: 37376603 DOI: 10.3390/v15061303]

66 **Emukule GO**, Paget J, van der Velden K, Mott JA. Influenza-Associated Disease Burden in Kenya: A Systematic Review of Literature. *PLoS One* 2015; **10**: e0138708 [PMID: 26398196 DOI: 10.1371/journal.pone.0138708]

67 **Brooks WA**, Goswami D, Rahman M, Nahar K, Fry AM, Balish A, Iftekharuddin N, Azim T, Xu X, Klimov A, Bresee J, Bridges C, Luby S. Influenza is a major contributor to childhood pneumonia in a tropical developing country. *Pediatr Infect Dis J* 2010; **29**: 216-221 [PMID: 20190613 DOI: 10.1097/INF.0b013e3181bc23fd]

68 **Fontana L**, Strasfeld L. Respiratory Virus Infections of the Stem Cell Transplant Recipient and the Hematologic Malignancy Patient. *Infect Dis Clin North Am* 2019; **33**: 523-544 [PMID: 30940462 DOI: 10.1016/j.idc.2019.02.004]

69 **Stamm BD**, Tamerius J, Reddy S, Barlow S, Hamer C, Kempken A, Goss M, He C, Bell C, Arnold M, Checovich M, Temte E, Norton D, Chen G, Baltus J, Gurley ES, Temte JL. The Influence of Rapid Influenza Diagnostic Testing on Clinician Decision-Making for Patients With Acute Respiratory Infection in Urgent Care. *Clin Infect Dis* 2023; **76**: 1942-1948 [PMID: 36723863 DOI: 10.1093/cid/ciad038]

70 **Ferguson NM**, Cummings DA, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic. *Nature* 2006; **442**: 448-452 [PMID: 16642006 DOI: 10.1038/nature04795]

71 **Fry AM**, Chittaganpitch M, Baggett HC, Peret TC, Dare RK, Sawatwong P, Thamthitiwat S, Areerat P, Sanasuttipun W, Fischer J, Maloney SA, Erdman DD, Olsen SJ. The burden of hospitalized lower respiratory tract infection due to respiratory syncytial virus in rural Thailand. *PLoS One* 2010; **5**: e15098 [PMID: 21152047 DOI: 10.1371/journal.pone.0015098]

72 **Frieden TR**. Six components necessary for effective public health program implementation. *Am J Public Health* 2014; **104**: 17-22 [PMID: 24228653 DOI: 10.2105/AJPH.2013.301608]

73 **Hayden FG**, Sugaya N, Hirotsu N, Lee N, de Jong MD, Hurt AC, Ishida T, Sekino H, Yamada K, Portsmouth S, Kawaguchi K, Shishido T, Arai M, Tsuchiya K, Uehara T, Watanabe A; Baloxavir Marboxil Investigators Group. Baloxavir Marboxil for Uncomplicated Influenza in Adults and Adolescents. *N Engl J Med* 2018; **379**: 913-923 [PMID: 30184455 DOI: 10.1056/NEJMoa1716197]

74 **Newton AH**, Cardani A, Braciale TJ. The host immune response in respiratory virus infection: balancing virus clearance and immunopathology. *Semin Immunopathol* 2016; **38**: 471-482 [PMID: 26965109 DOI: 10.1007/s00281-016-0558-0]

75 **Dulac GC**, Ruckerbauer GM, Boulanger P. Transmissible gastroenteritis: demonstration of the virus from field specimens by means of cell culture and pig inoculation. *Can J Comp Med* 1977; **41**: 357-363 [PMID: 200316]

76 **Strannegård Ö**. Interferons and their Therapeutic Applications. *EJIFCC* 1999; **11**: 52-58 [PMID: 30707527]

77 **Žigrayová D**, Mikušová V, Mikuš P. Advances in Antiviral Delivery Systems and Chitosan-Based Polymeric and Nanoparticulate Antivirals and Antiviral Carriers. *Viruses* 2023; **15** [PMID: 36992356 DOI: 10.3390/v15030647]

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MINIREVIEWS

Viral-host molecular interactions and metabolic modulation: Strategies to inhibit flaviviruses pathogenesis

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Abstract

Flaviviruses, which include globally impactful pathogens, such as West Nile virus, yellow fever virus, Zika virus, Japanese encephalitis virus, and dengue virus, contribute significantly to human infections. Despite the ongoing emergence and resurgence of flavivirus-mediated pathogenesis, the absence of specific therapeutic options remains a challenge in the prevention and treatment of flaviviral infections. Through the intricate processes of fusion, transcription, replication, and maturation, the complex interplay of viral and host metabolic interactions affects pathophysiology. Crucial interactions involve metabolic molecules, such as amino acids, glucose, fatty acids, and nucleotides, each playing a pivotal role in the replication and maturation of flaviviruses. These viral-host metabolic molecular interactions hijack and modulate the molecular mechanisms of host metabolism. A comprehensive understanding of these intricate metabolic

pathways offers valuable insights, potentially unveiling novel targets for therapeutic interventions against flaviviral pathogenesis. This review emphasizes promising avenues for the development of therapeutic agents that target specific metabolic molecules, such as amino acids, glucose, fatty acids, and nucleotides, which interact with flavivirus replication and are closely linked to the modulation of host metabolism. The clinical limitations of current drugs have prompted the development of new inhibitory strategies for flaviviruses based on an understanding of the molecular interactions between the virus and the host.

Key Words: Flavivirus; Nonstructural proteins; Virus-host interaction; Metabolism; Inhibitors; Vaccines

Core Tip: Targeting host metabolic molecules and interactions shows promise for combating flavivirus infections but has limitations such as potential off-target effects, disruption of essential cellular functions, and virus resistance. A viral-host interactome can elucidate complex interactions, guiding anti-flavivirus drug and vaccine development. By inhibiting metabolic signaling, researchers can disrupt viral replication, entry, and assembly, increasing the likelihood of effective antiviral agents. This approach is key for developing treatments despite its challenges.

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INTRODUCTION

Flavivirus, now designated as the genus Orthoflavivirus, exhibits global distribution, and its transmission over the past few decades has been remarkable[1]. The genus Flavivirus comprises more than 50 viruses, including West Nile virus (WNV), yellow fever virus (YFV), Zika virus (ZIKV), Japanese encephalitis virus, and dengue virus (DENV), all of which are highly infectious to humans[2]. According to the World Health Organization, half of the world's population is at risk of DENV infection, which carries a higher mortality rate compared with that of other flaviviral infections[3]. Flaviviruses cause up to 400 million infections per year and lead to critical forms of disease, including fatal hemorrhage, encephalitis, and death[3]. Despite the considerable impact of flaviviral infections on human health, only a limited number of vaccines are available, and no specific antiviral therapies have been identified for any flavivirus[4]. Thus, there is an urgent need to understand host-virus interactions to develop specific anti-flavivirus therapeutics.

Typically, flaviviruses interact with host cells by manipulating cellular and molecular mechanisms to create a favorable environment for replication. Following infection, flaviviruses can significantly modulate all classes of host metabolic pathways[5]. They initially engage through protein-protein interactions, manipulating the host's crucial cellular and molecular metabolic pathways, especially those involved in carbohydrate (glucose and glutamine), lipid (fatty acids), and nucleotide metabolism, to enhance their replication and maturation[5-9]. Targeting the interface between flaviviruses and host cellular metabolic interactions represents a potential strategy for antiviral interventions, elucidating viral pathology and fostering the development of therapeutic agents. Specifically, the inactivation of targeted metabolites by inhibitors holds promise for the development of antiviral agents. Consequently, there is a pressing need to develop therapeutics that effectively target and inhibit the essential metabolic mechanisms underlying viral replication and maturation.

Here, we explore flavivirus-host interactions to highlight potential inhibitors targeting metabolic molecules at the cellular and molecular levels, aiming to advance flavivirus therapeutics. For this purpose, we discuss potential strategies targeting viral-host metabolic interactions to inhibit flavivirus pathogenesis.

VIRUS-HOST MOLECULAR INTERACTIONS AND METABOLISM

Flavivirus structural features

Flaviviruses are icosahedral and characterized by a positive-sense, single-stranded RNA (approximately 11 kb), enclosed within a nucleocapsid. The nucleocapsid is further surrounded by the envelope glycoprotein E (53 kDa) and membrane protein M (8 kDa). The M protein is a small proteolytic fragment of its precursor form, prM (approximately 21 kDa), which is anchored to the viral membrane *via* two transmembrane helices. The genome contains a 5′ untranslated region and a singular open reading frame, which includes signals necessary for viral translation and replication, and concludes with a type 1 cap structure. Notably, the 3′ untranslated region harbors signals for replication and RNA synthesis but lacks a poly(A) tail. The single open reading frame undergoes translation into a polyprotein, which is subsequently cleaved to generate 10 viral proteins. The capsid, pre-membrane, and envelope proteins constitute the viral capsid shell that encapsulates the viral RNA genome, while seven nonstructural (NS) proteins-NS1, NS2A, NS2B, NS3, NS4A, NS4B,

and NS5—play critical roles in viral genome replication and polyprotein processing (Figure 1).

NS1 exists in diverse oligomeric forms, including an endoplasmic reticulum (ER) membrane-bound form within the larger viral replication complex, and a soluble secreted hexametric form implicated in viral immune evasion[10]. Membrane-associated proteins, such as NS2A, NS2B, NS4A, and NS4B, likely serve as scaffolds for assembling the viral replication complex on the ER membrane. NS2A contributes to the assembly of infectious virions, whereas NS2B acts as a cofactor for NS3 protease activity[11]. NS4A participates in membrane rearrangement during viral replication, and NS4B modulates host responses to facilitate viral replication. Notably, NS3 and NS5, which exist in a free form, exhibit enzymatic activities that are crucial for viral RNA synthesis. NS3, a multifunctional protein, comprises an N-terminal protease and a C-terminal helicase domain with 5′ RNA triphosphatase, nucleoside triphosphatase, and helicase activities [12]. NS5, the largest flaviviral protein, contains an RNA-dependent RNA polymerase, which is vital for viral replication and transcription[13]. NS1 has the highest number of interactions with host proteins, followed by NS3 and NS5, making them promising candidates for anti-flaviviral therapies. The aldehyde form of the tripeptide phenacetyl-Lys-Lys-Arg-CHO has demonstrated inhibitory activity against the WNV NS2B-NS3 protease[14]. Flaviviruses can significantly modulate all classes of host metabolites, including proteins, carbohydrates, lipids, and nucleotides, during entry, transcription, replication, and maturation, as discussed in the upcoming section[15].

Lifecycle of flavivirus

The flaviviral lifecycle begins when the virus enters cells through receptor-mediated endocytosis. Inside the cell, in a slightly acidic environment, the viral membrane fuses with the cell membrane, releasing its genetic material. This genetic material is then utilized by the cells to produce various viral proteins through a series of steps (Figure 2).

In the cytoplasm, the viral genetic material is translated into a polyprotein, which is then inserted into the cell membrane. Both host and viral proteases cleave this polyprotein into structural and NS proteins. The NS proteins form a complex responsible for viral replication. As the virus replicates, it creates structures called replication organelles (ROs) and vesicle packets (VPs) within the ER[13]. These structures shield the virus from the cytoplasm. The viral genetic material is then transported to an assembly site, where the viral capsid protein accumulates on lipid droplets and combines with the genetic material. Other viral proteins are recruited to the assembly site, where the entire structure buds into the ER, forming an immature virion. These virions accumulate in the ER before being transported through the host secretory pathway[16]. Mature viruses can be released as free virions or enclosed within membranes derived from cellular structures called autophagosomes. These membrane-enclosed forms may contain a lipid bilayer derived from the host cell and represent variations in the virus.

Following fusion, the positive-stranded RNA genome is released into the cytoplasm and used to produce a viral polyprotein[17]. This polyprotein is then cleaved into structural and NS proteins by both host and viral proteases. The polyprotein is synthesized in the ER and relies on the ER membrane protein complex for proper folding and stable expression[4]. NS viral proteins, along with host proteins, cause significant changes in the ER, forming ROs and VPs. Within these ROs, the positive-sense RNA serves as a template for the viral polymerase NS5 to generate an intermediate negative-sense RNA. This negative-sense RNA is then used to produce positive-sense progeny RNA, either for incorporation into new viral particles or for further translation. Progeny viral RNA is encapsulated in immature virions that form within VPs in the rough ER, adjacent to the ROs. Recent findings suggest that specific ER proteins, known as atlastins, play a central role in inducing membrane remodeling for RO formation, viral replication, and viral assembly. The newly synthesized capsid protein, along with the structural prM and E proteins, is recruited to VPs. These proteins assist in the budding of the nucleocapsid into the ER, and viral NS3 recruits host endosomal sorting complexes required for transport machinery to release immature virions into the ER[4]. These immature virions are then transported and secreted into the extracellular space (Figure 2).

The biogenesis of ROs and VPs is tightly regulated by NS viral proteins to prevent innate immune activation and ER stress while securing the necessary energy and membrane resources. Viruses exploit cellular lipid metabolism, with viral proteins influencing processes, such as fatty acid and cholesterol synthesis. These pathways are crucial for efficient viral replication. The final steps of flaviviral assembly and secretion involve multiple exit strategies. Immature viral progeny accumulate in the ER cisternae before undergoing maturation, which includes the cleavage of prM, glycosylation, and ubiquitylation of the E protein. Mature viral progeny exit the cell through various routes, including the host secretory pathway and the secretory arm of autophagy. Although the exact mechanisms are not fully understood, recent studies have provided insights into alternative exit routes and their potential implications for tissue tropism and immune evasion [2,4,17]. Several questions regarding the viral lifecycle remain unanswered, such as the mechanisms of RO biogenesis. *In vitro* reconstitution using liposomes and a combination of host and viral factors is needed to gain further insights into the lifecycle of other positive-sense RNA viruses.

Resistance mechanisms of flaviviruses

Flaviviruses exhibit several resistance mechanisms that enable them to evade the effects of antiviral drugs and vaccines, thereby complicating their treatment. One primary mechanism is the mutation of viral targets, such as RNA polymerase, which reduces the efficacy of antiviral drugs, including ribavirin and favipiravir[18]. Alterations in viral entry mechanisms due to mutations in viral proteins can render entry inhibitors less effective, thereby challenging the prevention of viral infection in its initial stages[19]. Flaviviruses can also upregulate their replication pathways by increasing the expression or activity of proteins involved in replication to counteract the inhibitory effects of antiviral drugs. Evasion of the host immune response is another critical resistance mechanism. Flaviviruses can evolve to avoid detection and destruction by immune cells, thereby diminishing the effectiveness of immunomodulatory therapies. Furthermore, continuous exposure to antiviral agents can lead to the selection of viral strains that evade adaptive immune responses, such as those induced by vaccines[20]. These adaptive immune escape mechanisms highlight the

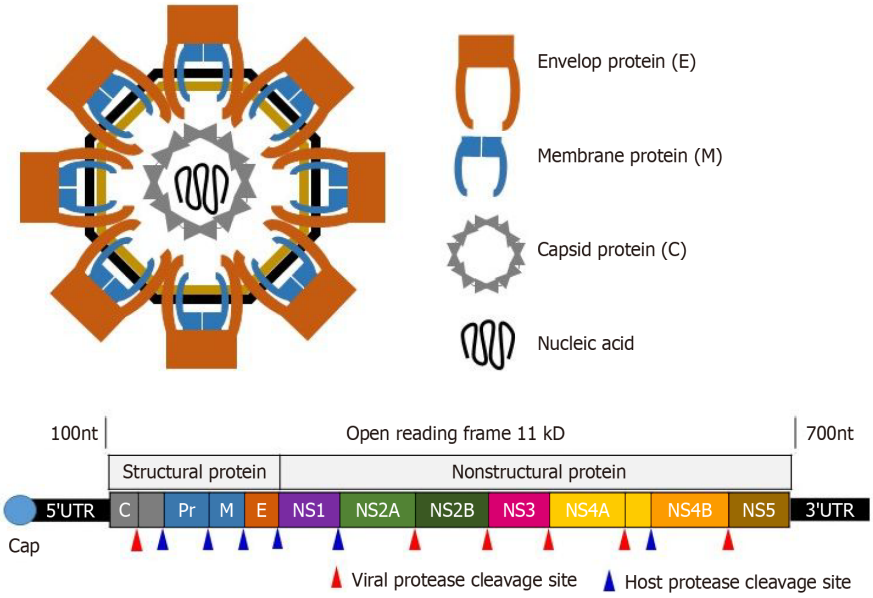

Figure 1 Flavivirus structural features. NS: Nonstructural.

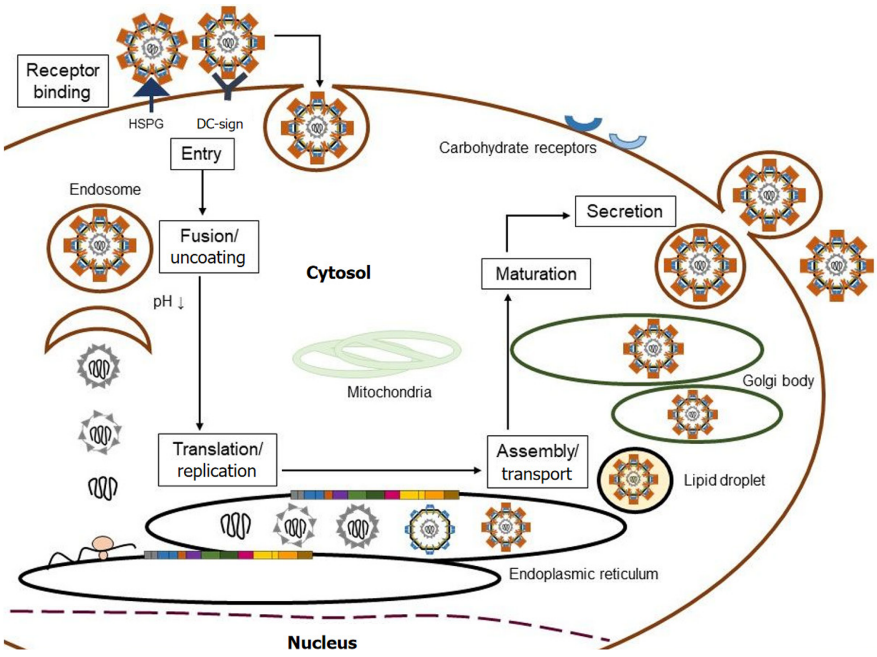

Figure 2 Flavivirus mechanism of infection to host cell. PH: Pondus hydrogenii; DC-Sign: Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin.

dynamic nature of flaviviral evolution and the ongoing challenges in developing effective, long-lasting therapeutic and preventive measures.

STRATEGIES TO INHIBIT FLAVIVIRUSES PATHOGENESIS

Flavivirus-host protein-protein metabolism interactions and associated therapeutics targets

Flaviviral infection begins with receptor-mediated interactions with host cells. The interaction between the flavivirus and the host involves protein-protein interactions, which facilitate the fusion of the viral envelope with the host cell membrane. For instance, WNV and ZIKV interact through the heparan sulfate proteoglycan (HSP) receptor, YFV interacts with the dendritic cell (DC)-specific intercellular adhesion molecule-3-grabbing non-integrin receptor, and DENV interacts with both DC-specific intercellular adhesion molecule-3-grabbing non-integrin and HSP receptors[21]. In addition, each flavivirus interacts with specific host cells, allowing it to enter and replicate. WNV, ZIKV, and Japanese

encephalitis virus interact with neuronal cells, YFV interacts with macrophages and DC, and DENV interacts with monocytes, macrophages, and DC[15]. Understanding cellular tropism—the affinity for specific cell types and receptor interactions of flaviviruses—is a crucial step in developing antiviral therapies.

The molecular mechanisms underlying viral fusion triggers are not yet fully understood, but it is known that histidine residues on the E glycoprotein interact with host cell protein receptors and have been proposed as prime candidates to act as pondus hydrogenii (pH) sensors (at pH 6.0) to initiate the fusion process[6,15]. The E protein contains five histidine residues that are conserved among all flaviviruses: two in domain I, H146 and H323; two in domain II, H248 and H287; and one in the stem region, H438[22]. At low pH, these histidine residues trigger conformational changes that expose the amino acid residue of the E protein, allowing fusion of the virus with the host cell. This fusion permits the viral genome to enter the cytoplasm of the host cell and initiate infection. Mutational analysis of the conserved histidine residues in domains I and III among all flavivirus E proteins has provided evidence that H323 is an important residue for initiating the low-pH-dependent multistep fusion process[17]. In addition, the host receptor HSP, identified as an attachment factor for several flaviviruses, has been shown to inhibit flavivirus replication (Figure 3)[21].

Flavivirus-host carbohydrate metabolism interactions and associated therapeutic targets

Flaviviral infection modulates carbohydrate metabolism, particularly glucose and glutamine utilization, to meet the increased energy demands required for optimal viral replication. Following successful entry into host cells, viruses raise their energy requirements. Infection leads to elevated cellular glucose concentrations, likely resulting in increased expression of glucose transporter 1 and hexokinase 2, the initial enzymes involved in glycolysis[22]. Limited glucose availability significantly impedes viral replication, whereas restricted glutamine exerts only a modest effect on viral replication, and glutaminase inhibition disrupts glutamine conversion. Glycolytic stimulation induced by flaviviruses may enhance various processes, including the production of glutamine, to increase adenosine triphosphate and nucleotide pools. Additionally, the production of citrate, a precursor of fatty acid synthesis, is enhanced. Inhibiting the key enzymes involved in glycolysis or disrupting glucose uptake by virus-infected cells can hinder viral replication.

Many viruses utilize host cell-surface carbohydrates as receptors for entry, where viral envelope proteins interact with specific carbohydrates on the cell surface to initiate the infection process. Carbohydrate inhibitors disrupt various stages of the viral lifecycle, including entry, attachment, and fusion. Carbohydrate-receptor interactions often play a crucial role in the docking of viruses to host cells, representing a necessary step in the viral lifecycle that precedes infection and, ultimately, replication. Understanding these interactions provides insight into druggable targets. In this context, the role of carbohydrate-receptor interactions in flavivirus entry and their potential to prevent viral infection should be investigated. To date, several molecules have been identified as potential targets for therapeutic intervention at carbohydrate receptors in flaviviruses, including the mannose receptor[23], glucose-regulating protein 78[24], heparan sulfate[25], and glycosphingolipids[26]. The mannose receptor is a type 1 transmembrane protein that recognizes and binds to pathogens, particularly glycoproteins containing mannose. This recognition initiates phagocytosis by immune cells, including macrophages and DC. These carbohydrate-receptor interactions provide potential targets for intervention in the flavivirus lifecycle, making them promising candidates for developing antiviral strategies. Flaviviruses often interact with cell surface glycosaminoglycans during the initial stages of infection[27]. Carbohydrate moieties on the surfaces of viral glycoproteins can disrupt viral entry and infection[28]. Inhibitors that interfere with this interaction may hinder viral entry (Figure 3).

Flavivirus-host lipid metabolism interaction and associated therapeutic targets

Flaviviruses manipulate host lipid metabolism by hijacking viral replication complexes during their assembly and maturation. Flaviviruses have been observed to strategically alter the composition of lipid rafts to evade host immune responses. Lipid rafts, which are specialized, cholesterol-rich microdomains in cellular membranes, play a pivotal role in signaling pathways that are crucial for antiviral immune responses. These microdomains are integral to the efficient assembly of viruses. During the assembly phase, the lipid composition of the viral envelope is influenced by the host ER membrane from which the virus buds. The orchestrated modulation of lipid rafts by flaviviruses highlights the intricate strategies employed by these pathogens to navigate the host immune system and ensure successful viral assembly. Cholesterol plays a pivotal role in the viral envelope, influencing membrane fluidity and stability and profoundly impacting flaviviral infectivity. Disruption of the formation of these complexes, which are crucial for viral replication, can be achieved by inhibiting enzymes involved in lipid synthesis or transport. Viruses manipulate host cell lipid metabolism to create an optimal replication environment. This manipulation may entail modifications to the lipid composition of cellular membranes or the induction of specialized membrane structures. Fatty acid synthesis begins with the carboxylation of acetyl-coenzyme A (CoA) to malonyl-CoA, catalyzed by the enzymatic activity of acetyl-CoA carboxylase (ACC). ACC is a rate-limiting enzyme in the intricate process of lipid biosynthesis. Notably, cells infected with flaviviruses exhibit pronounced upregulation of fatty acid synthesis, as documented by Heaton *et al*[29] in 2010. Flaviviral infection exhibits heightened sensitivity to the inhibition of fatty acid synthesis, particularly through the targeted inhibition of ACC or fatty acid synthase (FASN)[30]. Understanding the intricate interplay between flaviviruses and lipids is pivotal for the formulation of antiviral strategies. Targeting lipid-centric processes, including membrane fusion, lipid raft formation, and viral envelope biogenesis, is a promising approach for the development of antiviral drugs. Viruses may modulate fatty acid synthesis and oxidation in host cells to meet their lipid requirements. This modulation can affect the lipid composition of cellular membranes and viral replication. Notably, hypolipidemic drugs, such as 5-(tetradecyloxyl)-2-furoic acid and MEDICA, which are designed to target ACC, have demonstrated efficacy in inhibiting fatty acid synthesis during WNV infection[31]. Furthermore, compounds, such as C75 and cerulenin, act as inhibitors of DENV and YFV, thereby contributing to the inhibition of fatty acid synthesis.

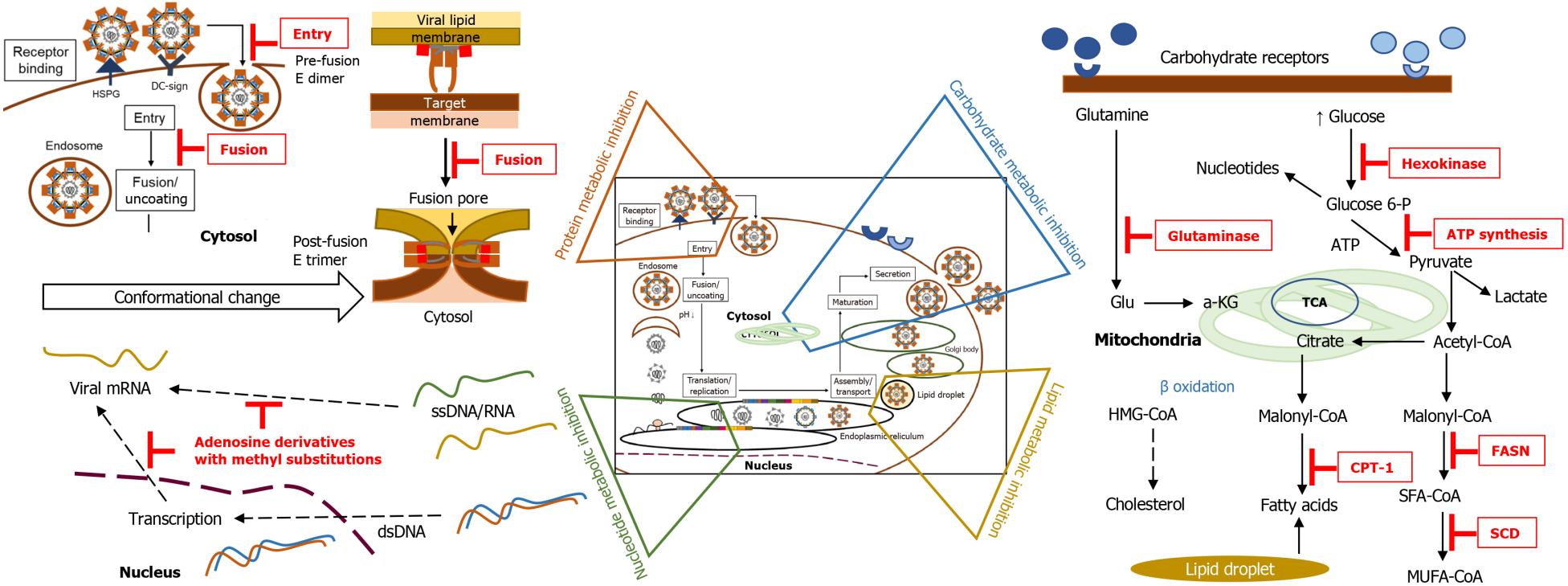

Figure 3 Flavivirus-host metabolic interaction and associated therapeutics targets. Protein-protein metabolic interaction, carbohydrate metabolic interaction, lipid metabolic interaction, nucleotide metabolic interaction associated therapeutic strategy. PH: Pondus hydrogenii; DC-Sign: Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; FASN: Fatty acid synthase; ATP: Adenosine triphosphate; COA: Coenzyme A; TCA: Tricarboxylic acid; SCD: Sperm chromatin dispersion; SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; CPT: Camptothecin; HMG: 3-hydroxy-3-methylglutaryl.

The DENV NS3 protein establishes a direct link between fatty acid synthesis and flaviviral infection. Through its interaction with FASN, DENV NS3 redirects the enzymatic complex to the viral replication sites, thereby stimulating its functional activity. Intriguingly, NS1 is secreted and associates with the cholesterol transporter caveolin chaperone complex within cells, highlighting a unique aspect of flavivirus biology and suggesting potential implications for viral pathogenesis and host-cell interactions. In patients with dengue, NS1 is detected in complex with high-density and low-density lipoproteins and accumulates over time[32]. Current in-depth protein interaction studies have revealed that NS1 is a focal point in flaviviral protein research for therapeutic endeavors[33]. Researchers continue to investigate the specific mechanisms and pathways through which flaviviruses manipulate host lipids, shedding light on potential targets for therapeutic interventions (Figure 3).

Flavivirus-host nucleotide metabolism interaction and associated therapeutic targets

Flaviviruses, similar to other viruses, exhibit a pronounced reliance on host cell nucleotide pools to facilitate their

Table 1 Summary of therapeutic options for flaviviruses and vaccines

Antiviral drug	Therapy name	Development stage	Target action site	Available data	Ongoing trials	Classification	Ref.
Favipiravir	Avigan	Clinical trials	Viral RNA polymerase	Some efficacies in clinical trials; used in Japan for influenza and Ebola	Ongoing trials for dengue and Zika	RNA-based therapy	[18]
Ribavirin	Virazole	Completed/discontinued	Viral RNA polymerase	Mixed results; used for HCV, not widely effective for flaviviruses	Not actively pursued for flavivirus	RNA-based therapy	[18]
Sofosbuvir	Sovaldi	Completed/discontinued	Viral RNA polymerase	Highly effective for HCV; no data for flavivirus	No ongoing trials for flavivirus	RNA-based therapy	[42]
Interferon-alpha	Intron A	Clinical trials	Immune modulation	Used for hepatitis B and C; some efficacy in flavivirus treatment	Trials for dengue and West Nile	Immunotherapy	[38]
Dengvaxia	Dengue vaccine	Approved	Immune response	Approved for dengue; mixed safety and efficacy profiles	Post-marketing surveillance ongoing	Immunotherapy	[50]
Chimeric yellow fever 17D-tetravalent dengue vaccine	Dengue vaccine	Approved	Immune response	Approved for dengue; mixed safety and efficacy profiles	Post-marketing surveillance ongoing	Immunotherapy	[50]

HCV: Hepatitis C virus.

replication, making targeting this intricate process a promising strategy for antiviral interventions. Disruption of enzymes integral to nucleotide biosynthesis is a potent means of impeding viral RNA and DNA synthesis. The modulation of nucleotide metabolism by viruses is carefully orchestrated to ensure an ample supply for the replication of their genomic material. Pathogenic organisms possess a genomic architecture characterized by an ordered sequence of nucleotides. This sequence not only contains essential information for synthesizing and expressing proteins necessary for growth and survival, but also holds critical details that shape the organism's evolutionary trajectory. Changes in the precise arrangement of nucleotides can lead to the emergence of novel species or strains, thereby underscoring the pivotal role of genetic variation in evolutionary processes. Nucleosides can be integrated into cellular RNA. Interestingly, nucleoside analogs, particularly adenosine derivatives with methyl substitutions at the 2´-C position, exhibit potent inhibitory effects against DENV, WNV, and YFV. This inhibition occurs through the disruption of RNA synthesis *via* chain termination, indicating their potential as effective antiviral compounds[34]. Notably, a single point mutation within the active site of hepatitis C virus NS5B polymerase confers resistance to the antiviral effects of these nucleosides[35]. These approaches affect flaviviral replication at the nucleoside level (Figure 3).

Ribavirin, initially approved for treating other viral infections, targets RNA polymerase inhibition and is currently in combination trials for flavivirus infections, with results expected within 1–2 years[36]. Favipiravir, originally developed for influenza, has shown promising results in preclinical studies for dengue and Zika and is now undergoing multiple phase II/III trials, with results anticipated within the next 2 years[37]. Interferons, long used to treat various viral infections, work by modulating the immune response and are being tested in combination therapies for their efficacy against flaviviruses. Tilorone, an experimental antiviral agent that induces interferon production, is in the early phase of clinical trials for flaviviral infections, with preliminary results expected within 1–2 years. NITD008, a preclinical-stage drug, inhibits viral RNA synthesis but faces significant toxicity concerns that must be addressed before further development can proceed[38]. Balapiravir, another investigational drug, has shown mixed results in early trials for flaviviral infections, leading to a temporary hold on future trials pending further data and analysis[39]. Remdesivir, initially approved for the coronavirus disease treatment, targets RNA polymerase and is currently in phase II/III trials for dengue and Zika, with results expected within 1–2 years[40]. Ivermectin, a repurposed antiparasitic drug, inhibits viral replication and is undergoing multiple phase II trials for dengue and Zika, with results anticipated in the coming years [41]. These therapeutic options reflect a multifaceted approach to tackling flaviviral infections by targeting various metabolic pathways to inhibit viral replication and pathogenesis. The following Table 1 highlights the diverse treatment options currently under investigation and provides a snapshot of the therapeutic landscape and future directions. Ongoing trials and research efforts have underscored the complexity of developing effective therapies against flaviviruses, emphasizing the need for continued innovation and clinical testing to bring these potential treatments to fruition[18,38,42,43].

Side effects of drugs and vaccines targeting host metabolic pathways

The therapeutic options and vaccines developed to combat flavivirus pathogenesis comes with a range of potential side effects, reflecting the complexity of targeting host metabolic pathways. For example, ribavirin can induce anemia and has teratogenic effects, limiting its use, particularly in pregnant individuals[44]. Favipiravir, while promising, also presents a risk of teratogenicity and mild gastrointestinal symptoms[45]. Drugs, such as sofosbuvir, are generally well-tolerated but can cause fatigue and headaches[42]. Chloroquine, although initially promising, has been associated with retinal toxicity

and gastrointestinal issues[46]. Balapiravir has shown potential hepatotoxicity, adding a layer of caution to its application [47]. Remdesivir, a widely used antiviral agent, can lead to kidney and liver function abnormalities alongside gastrointestinal symptoms[48]. NITD008, though effective in preclinical studies, poses long-term toxicity risks[38]. Immunomodulators, such as tilorone and seliciclib, may cause mild gastrointestinal symptoms and potential hepatotoxicity, respectively. Ivermectin is usually well-tolerated but can cause mild gastrointestinal disturbances[49]. Mycophenolic acid, an inhibitor of nucleotide synthesis, can lead to immunosuppression and gastrointestinal symptoms [50]. Vaccines, such as Dengvaxia and YF-VAX, have shown efficacy but come with risks, such as severe dengue in seronegative individuals and rare severe reactions, respectively[50]. These side effects highlight the need for careful monitoring and tailored treatment strategies.

CONCLUSION

Targeting host metabolic molecules and their interactions to inhibit flaviviral pathogenesis presents several challenges. These include potential off-target effects, disruption of essential host functions, and the risk of unintended side effects during antiviral drug or vaccine development. The dynamic virus-host interactions and the potential for viral resistance complicate the sustained effectiveness of this approach. Despite these challenges, manipulating host metabolic pathways remains a promising strategy owing to its impact on viral replication and pathogenesis.

Developing a detailed viral-host interactome is crucial for understanding the virus-host interplay, identifying key targets for intervention, and designing effective antiviral agents. Targeting the metabolic signaling pathways that the virus exploits can disrupt multiple stages of the viral lifecycle, including entry, replication, and assembly. This approach has the potential to hinder the ability of the virus to establish and propagate infections, making it a valuable avenue for anti-flavivirus drug and vaccine development.

FOOTNOTES

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REFERENCES

1 **Postler TS**, Beer M, Blitvich BJ, Bukh J, de Lamballerie X, Drexler JF, Imrie A, Kapoor A, Karganova GG, Lemey P, Lohmann V, Simmonds P, Smith DB, Stapleton JT, Kuhn JH. Renaming of the genus Flavivirus to Orthoflavivirus and extension of binomial species names within the family Flaviviridae. *Arch Virol* 2023; **168**: 224 [PMID: 37561168 DOI: 10.1007/s00705-023-05835-1]

2 **Mukhopadhyay S**, Kuhn RJ, Rossmann MG. A structural perspective of the flavivirus life cycle. *Nat Rev Microbiol* 2005; **3**: 13-22 [PMID: 15608696 DOI: 10.1038/nrmicro1067]

3 **Messina JP**, Brady OJ, Golding N, Kraemer MUG, Wint GRW, Ray SE, Pigott DM, Shearer FM, Johnson K, Earl L, Marczak LB, Shirude S, Davis Weaver N, Gilbert M, Velayudhan R, Jones P, Jaenisch T, Scott TW, Reiner RC Jr, Hay SI. The current and future global distribution and population at risk of dengue. *Nat Microbiol* 2019; **4**: 1508-1515 [PMID: 31182801 DOI: 10.1038/s41564-019-0476-8]

4 **Zhao R**, Wang M, Cao J, Shen J, Zhou X, Wang D, Cao J. Flavivirus: From Structure to Therapeutics Development. *Life (Basel)* 2021; **11**: 615 [PMID: 34202239 DOI: 10.3390/life11070615]

5 **Fontaine KA**, Sanchez EL, Camarda R, Lagunoff M. Dengue virus induces and requires glycolysis for optimal replication. *J Virol* 2015; **89**: 2358-2366 [PMID: 25505078 DOI: 10.1128/JVI.02309-14]

6 **Modis Y**, Ogata S, Clements D, Harrison SC. Structure of the dengue virus envelope protein after membrane fusion. *Nature* 2004; **427**: 313-

319 [PMID: 14737159 DOI: 10.1038/nature02165]
7 **Tian H**, Sun Z, Faria NR, Yang J, Cazelles B, Huang S, Xu B, Yang Q, Pybus OG, Xu B. Increasing airline travel may facilitate co-circulation of multiple dengue virus serotypes in Asia. *PLoS Negl Trop Dis* 2017; **11**: e0005694 [PMID: 28771468 DOI: 10.1371/journal.pntd.0005694]
8 **Medin CL**, Fitzgerald KA, Rothman AL. Dengue virus nonstructural protein NS5 induces interleukin-8 transcription and secretion. *J Virol* 2005; **79**: 11053-11061 [PMID: 16103156 DOI: 10.1128/JVI.79.17.11053-11061.2005]
9 **Goodwin CM**, Xu S, Munger J. Stealing the Keys to the Kitchen: Viral Manipulation of the Host Cell Metabolic Network. *Trends Microbiol* 2015; **23**: 789-798 [PMID: 26439298 DOI: 10.1016/j.tim.2015.08.007]
10 **Muller DA**, Young PR. The flavivirus NS1 protein: molecular and structural biology, immunology, role in pathogenesis and application as a diagnostic biomarker. *Antiviral Res* 2013; **98**: 192-208 [PMID: 23523765 DOI: 10.1016/j.antiviral.2013.03.008]
11 **Falgout B**, Pethel M, Zhang YM, Lai CJ. Both nonstructural proteins NS2B and NS3 are required for the proteolytic processing of dengue virus nonstructural proteins. *J Virol* 1991; **65**: 2467-2475 [PMID: 2016768 DOI: 10.1128/JVI.65.5.2467-2475.1991]
12 **Wengler G**, Wengler G. The NS 3 nonstructural protein of flaviviruses contains an RNA triphosphatase activity. *Virology* 1993; **197**: 265-273 [PMID: 8212562 DOI: 10.1006/viro.1993.1587]
13 **Issur M**, Geiss BJ, Bougie I, Picard-Jean F, Despins S, Mayette J, Hobdey SE, Bisaillon M. The flavivirus NS5 protein is a true RNA guanylyltransferase that catalyzes a two-step reaction to form the RNA cap structure. *RNA* 2009; **15**: 2340-2350 [PMID: 19850911 DOI: 10.1261/rna.1609709]
14 **Stoermer MJ**, Chappell KJ, Liebscher S, Jensen CM, Gan CH, Gupta PK, Xu WJ, Young PR, Fairlie DP. Potent cationic inhibitors of West Nile virus NS2B/NS3 protease with serum stability, cell permeability and antiviral activity. *J Med Chem* 2008; **51**: 5714-5721 [PMID: 18729351 DOI: 10.1021/jm800503y]
15 **Jordan TX**, Randall G. Flavivirus modulation of cellular metabolism. *Curr Opin Virol* 2016; **19**: 7-10 [PMID: 27280383 DOI: 10.1016/j.coviro.2016.05.007]
16 **Benarroch D**, Selisko B, Locatelli GA, Maga G, Romette JL, Canard B. The RNA helicase, nucleotide 5'-triphosphatase, and RNA 5'-triphosphatase activities of Dengue virus protein NS3 are Mg2+-dependent and require a functional Walker B motif in the helicase catalytic core. *Virology* 2004; **328**: 208-218 [PMID: 15464841 DOI: 10.1016/j.virol.2004.07.004]
17 **Sánchez-San Martín C**, Liu CY, Kielian M. Dealing with low pH: entry and exit of alphaviruses and flaviviruses. *Trends Microbiol* 2009; **17**: 514-521 [PMID: 19796949 DOI: 10.1016/j.tim.2009.08.002]
18 **Veit EC**, Salim MS, Jung MJ, Richardson RB, Boys IN, Quinlan M, Barrall EA, Bednarski E, Hamilton RE, Kikawa C, Elde NC, García-Sastre A, Evans MJ. Evolution of STAT2 resistance to flavivirus NS5 occurred multiple times despite genetic constraints. *Nat Commun* 2024; **15**: 5426 [PMID: 38926343 DOI: 10.1038/s41467-024-49758-0]
19 **Kaufmann B**, Rossmann MG. Molecular mechanisms involved in the early steps of flavivirus cell entry. *Microbes Infect* 2011; **13**: 1-9 [PMID: 20869460 DOI: 10.1016/j.micinf.2010.09.005]
20 **Strasfeld L**, Chou S. Antiviral drug resistance: mechanisms and clinical implications. *Infect Dis Clin North Am* 2010; **24**: 809-833 [PMID: 20674805 DOI: 10.1016/j.idc.2010.07.001]
21 **Gao H**, Lin Y, He J, Zhou S, Liang M, Huang C, Li X, Liu C, Zhang P. Role of heparan sulfate in the Zika virus entry, replication, and cell death. *Virology* 2019; **529**: 91-100 [PMID: 30684694 DOI: 10.1016/j.virol.2019.01.019]
22 **Stiasny K**, Fritz R, Pangerl K, Heinz FX. Molecular mechanisms of flavivirus membrane fusion. *Amino Acids* 2011; **41**: 1159-1163 [PMID: 19882217 DOI: 10.1007/s00726-009-0370-4]
23 **Miller JL**, de Wet BJ, Martinez-Pomares L, Radcliffe CM, Dwek RA, Rudd PM, Gordon S. The mannose receptor mediates dengue virus infection of macrophages. *PLoS Pathog* 2008; **4**: e17 [PMID: 18266465 DOI: 10.1371/journal.ppat.0040017]
24 **Jindadamrongwech S**, Thepparit C, Smith DR. Identification of GRP 78 (BiP) as a liver cell expressed receptor element for dengue virus serotype 2. *Arch Virol* 2004; **149**: 915-927 [PMID: 15098107 DOI: 10.1007/s00705-003-0263-x]
25 **Chen Y**, Maguire T, Hileman RE, Fromm JR, Esko JD, Linhardt RJ, Marks RM. Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. *Nat Med* 1997; **3**: 866-871 [PMID: 9256277 DOI: 10.1038/nm0897-866]
26 **Wichit S**, Jittmittraphap A, Hidari KI, Thaisomboonsuk B, Petmitr S, Ubol S, Aoki C, Itonori S, Morita K, Suzuki T, Suzuki Y, Jampangern W. Dengue virus type 2 recognizes the carbohydrate moiety of neutral glycosphingolipids in mammalian and mosquito cells. *Microbiol Immunol* 2011; **55**: 135-140 [PMID: 21265875 DOI: 10.1111/j.1348-0421.2010.00293.x]
27 **Aquino RS**, Park PW. Glycosaminoglycans and infection. *Front Biosci (Landmark Ed)* 2016; **21**: 1260-1277 [PMID: 27100505 DOI: 10.2741/4455]
28 **Leikina E**, Delanoe-Ayari H, Melikov K, Cho MS, Chen A, Waring AJ, Wang W, Xie Y, Loo JA, Lehrer RI, Chernomordik LV. Carbohydrate-binding molecules inhibit viral fusion and entry by crosslinking membrane glycoproteins. *Nat Immunol* 2005; **6**: 995-1001 [PMID: 16155572 DOI: 10.1038/ni1248]
29 **Heaton NS**, Perera R, Berger KL, Khadka S, Lacount DJ, Kuhn RJ, Randall G. Dengue virus nonstructural protein 3 redistributes fatty acid synthase to sites of viral replication and increases cellular fatty acid synthesis. *Proc Natl Acad Sci USA* 2010; **107**: 17345-17350 [PMID: 20855599 DOI: 10.1073/pnas.1010811107]
30 **Perera R**, Riley C, Isaac G, Hopf-Jannasch AS, Moore RJ, Weitz KW, Pasa-Tolic L, Metz TO, Adamec J, Kuhn RJ. Dengue virus infection perturbs lipid homeostasis in infected mosquito cells. *PLoS Pathog* 2012; **8**: e1002584 [PMID: 22457619 DOI: 10.1371/journal.ppat.1002584]
31 **Merino-Ramos T**, Vázquez-Calvo Á, Casas J, Sobrino F, Saiz JC, Martín-Acebes MA. Modification of the Host Cell Lipid Metabolism Induced by Hypolipidemic Drugs Targeting the Acetyl Coenzyme A Carboxylase Impairs West Nile Virus Replication. *Antimicrob Agents Chemother* 2016; **60**: 307-315 [PMID: 26503654 DOI: 10.1128/AAC.01578-15]
32 **Benfrid S**, Park KH, Dellarole M, Voss JE, Tamietti C, Pehau-Arnaudet G, Raynal B, Brûlé S, England P, Zhang X, Mikhailova A, Hasan M, Ungeheuer MN, Petres S, Biering SB, Harris E, Sakuntabhai A, Buchy P, Duong V, Dussart P, Coulibaly F, Bontems F, Rey FA, Flamand M. Dengue virus NS1 protein conveys pro-inflammatory signals by docking onto high-density lipoproteins. *EMBO Rep* 2022; **23**: e53600 [PMID: 35607830 DOI: 10.15252/embr.202153600]
33 **Farooq QUA**, Aiman S, Ali Y, Shaukat Z, Ali Y, Khan A, Samad A, Wadood A, Li C. A comprehensive protein interaction map and druggability investigation prioritized dengue virus NS1 protein as promising therapeutic candidate. *PLoS One* 2023; **18**: e0287905 [PMID: 37498862 DOI: 10.1371/journal.pone.0287905]
34 **Migliaccio G**, Tomassini JE, Carroll SS, Tomei L, Altamura S, Bhat B, Bartholomew L, Bosserman MR, Ceccacci A, Colwell LF, Cortese R, De Francesco R, Eldrup AB, Getty KL, Hou XS, LaFemina RL, Ludmerer SW, MacCoss M, McMasters DR, Stahlhut MW, Olsen DB, Hazuda

DJ, Flores OA. Characterization of resistance to non-obligate chain-terminating ribonucleoside analogs that inhibit hepatitis C virus replication in vitro. *J Biol Chem* 2003; **278**: 49164-49170 [PMID: 12966103 DOI: 10.1074/jbc.M305041200]

35 **Olsen DB**, Eldrup AB, Bartholomew L, Bhat B, Bosserman MR, Ceccacci A, Colwell LF, Fay JF, Flores OA, Getty KL, Grobler JA, LaFemina RL, Markel EJ, Migliaccio G, Prhavc M, Stahlhut MW, Tomassini JE, MacCoss M, Hazuda DJ, Carroll SS. A 7-deaza-adenosine analog is a potent and selective inhibitor of hepatitis C virus replication with excellent pharmacokinetic properties. *Antimicrob Agents Chemother* 2004; **48**: 3944-3953 [PMID: 15388457 DOI: 10.1128/AAC.48.10.3944-3953.2004]

36 **Kamiyama N**, Soma R, Hidano S, Watanabe K, Umekita H, Fukuda C, Noguchi K, Gendo Y, Ozaki T, Sonoda A, Sachi N, Runtuwene LR, Miura Y, Matsubara E, Tajima S, Takasaki T, Eshita Y, Kobayashi T. Ribavirin inhibits Zika virus (ZIKV) replication in vitro and suppresses viremia in ZIKV-infected STAT1-deficient mice. *Antiviral Res* 2017; **146**: 1-11 [PMID: 28818572 DOI: 10.1016/j.antiviral.2017.08.007]

37 **Pilkington V**, Pepperrell T, Hill A. A review of the safety of favipiravir - a potential treatment in the COVID-19 pandemic? *J Virus Erad* 2020; **6**: 45-51 [PMID: 32405421 DOI: 10.1016/S2055-6640(20)30016-9]

38 **Enosi Tuipulotu D**, Fumian TM, Netzler NE, Mackenzie JM, White PA. The Adenosine Analogue NITD008 has Potent Antiviral Activity against Human and Animal Caliciviruses. *Viruses* 2019; **11**: 496 [PMID: 31151251 DOI: 10.3390/v11060496]

39 **Nguyen NM**, Tran CN, Phung LK, Duong KT, Huynh Hle A, Farrar J, Nguyen QT, Tran HT, Nguyen CV, Merson L, Hoang LT, Hibberd ML, Aw PP, Wilm A, Nagarajan N, Nguyen DT, Pham MP, Nguyen TT, Javanbakht H, Klumpp K, Hammond J, Petric R, Wolbers M, Nguyen CT, Simmons CP. A randomized, double-blind placebo controlled trial of balapiravir, a polymerase inhibitor, in adult dengue patients. *J Infect Dis* 2013; **207**: 1442-1450 [PMID: 22807519 DOI: 10.1093/infdis/jis470]

40 **Malin JJ**, Suárez I, Priesner V, Fätkenheuer G, Rybniker J. Remdesivir against COVID-19 and Other Viral Diseases. *Clin Microbiol Rev* 2020; **34**: e00162-20 [PMID: 33055231 DOI: 10.1128/CMR.00162-20]

41 **Caly L**, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Res* 2020; **178**: 104787 [PMID: 32251768 DOI: 10.1016/j.antiviral.2020.104787]

42 **Brigham D**, Narkewicz MR. Profile of Sofosbuvir and Velpatasvir Combination in the Treatment of Chronic Hepatitis C in Children and Adolescents: Current Evidence. *Ther Clin Risk Manag* 2024; **20**: 1-7 [PMID: 38230373 DOI: 10.2147/TCRM.S326099]

43 **Kariyawasam R**, Lachman M, Mansuri S, Chakrabarti S, Boggild AK. A dengue vaccine whirlwind update. *Ther Adv Infect Dis* 2023; **10**: 20499361231167274 [PMID: 37114191 DOI: 10.1177/20499361231167274]

44 **Sinclair SM**, Jones JK, Miller RK, Greene MF, Kwo PY, Maddrey WC. The Ribavirin Pregnancy Registry: An Interim Analysis of Potential Teratogenicity at the Mid-Point of Enrollment. *Drug Saf* 2017; **40**: 1205-1218 [PMID: 28689333 DOI: 10.1007/s40264-017-0566-6]

45 **Hung DT**, Ghula S, Aziz JMA, Makram AM, Tawfik GM, Abozaid AA, Pancharatnam RA, Ibrahim AM, Shabouk MB, Turnage M, Nakhare S, Karmally Z, Kouz B, Le TN, Alhijazeen S, Phuong NQ, Ads AM, Abdelaal AH, Nam NH, Iiyama T, Kita K, Hirayama K, Huy NT. The efficacy and adverse effects of favipiravir on patients with COVID-19: A systematic review and meta-analysis of published clinical trials and observational studies. *Int J Infect Dis* 2022; **120**: 217-227 [PMID: 35470021 DOI: 10.1016/j.ijid.2022.04.035]

46 **Yusuf IH**, Charbel Issa P, Ahn SJ. Hydroxychloroquine-induced Retinal Toxicity. *Front Pharmacol* 2023; **14**: 1196783 [PMID: 37324471 DOI: 10.3389/fphar.2023.1196783]

47 **Nelson DR**, Zeuzem S, Andreone P, Ferenci P, Herring R, Jensen DM, Marcellin P, Pockros PJ, Rodríguez-Torres M, Rossaro L, Rustgi VK, Sepe T, Sulkowski M, Thomason IR, Yoshida EM, Chan A, Hill G. Balapiravir plus peginterferon alfa-2a (40KD)/ribavirin in a randomized trial of hepatitis C genotype 1 patients. *Ann Hepatol* 2012; **11**: 15-31 [PMID: 22166557 DOI: 10.1016/S1665-2681(19)31482-6]

48 **Wong CKH**, Au ICH, Cheng WY, Man KKC, Lau KTK, Mak LY, Lui SL, Chung MSH, Xiong X, Lau EHY, Cowling BJ. Remdesivir use and risks of acute kidney injury and acute liver injury among patients hospitalised with COVID-19: a self-controlled case series study. *Aliment Pharmacol Ther* 2022; **56**: 121-130 [PMID: 35318694 DOI: 10.1111/apt.16894]

49 **Shimizu K**, Hirata H, Kabata D, Tokuhira N, Koide M, Ueda A, Tachino J, Shintani A, Uchiyama A, Fujino Y, Ogura H. Ivermectin administration is associated with lower gastrointestinal complications and greater ventilator-free days in ventilated patients with COVID-19: A propensity score analysis. *J Infect Chemother* 2022; **28**: 548-553 [PMID: 35016823 DOI: 10.1016/j.jiac.2021.12.024]

50 **Heischmann S**, Dzieciatkowska M, Hansen K, Leibfritz D, Christians U. The Immunosuppressant Mycophenolic Acid Alters Nucleotide and Lipid Metabolism in an Intestinal Cell Model. *Sci Rep* 2017; **7**: 45088 [PMID: 28327659 DOI: 10.1038/srep45088]

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ORIGINAL ARTICLE

Retrospective Study

Diagnosis of West Nile virus infections: Evaluation of different laboratory methods

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Abstract

BACKGROUND

The diagnosis of West Nile virus (WNV) is challenging due to short-term and low-level viremia, flavivirus cross-reactivity, and long immunoglobulin M (IgM) persistence.

AIM
To evaluate different methods for WNV detection [reverse transcription-polymerase chain reaction (RT-PCR), IgM/IgG antibodies, IgG avidity] in serum, cerebrospinal fluid (CSF), and urine samples of patients with confirmed WNV infection.

METHODS
The study included patients with confirmed WNV neuroinvasive infection (n = 62), asymptomatic WNV seropositive individuals (n = 22), and individuals with false-positive WNV IgM antibodies (n = 30). WNV RNA was detected using RT-PCR. A commercial ELISA was used to detect WNV IgM/IgG antibodies with confirmation of cross-reactive samples using a virus neutralization test (VNT). IgG-positive samples were tested for IgG avidity.

RESULTS
The WNV-RNA detection rates were significantly higher in the urine (54.5%)/serum (46.4%) than in CSF (32.2%). According to the sampling time, the WNV-RNA detection rates in urine collected within 7 days/8-14/≥ 15 days were 29.4/66.6/62.5% (P = 0.042). However, these differences were not observed in the CSF. The median RT-PCR cycle threshold values were significantly lower in urine (32.5, IQR = 28-34) than in CSF (34.5, IQR = 33-36). The frequency of positive WNV IgM and IgG significantly differed according to the sampling time in serum but not in CSF. Positive IgM/IgG antibodies were detected in 84.3/9.3% of serum samples collected within 7 days, 100/71.1% of samples collected 8-14, and 100% samples collected after ≥ 15 days. Recent WNV infection was confirmed by low/borderline avidity index (AI) in 13.6% of asymptomatic individuals. A correlation between ELISA and AI was strong negative for IgM and strong positive for IgG. No significant correlation between ELISA IgG and VNT was found.

CONCLUSION
The frequency of WNV RNA and antibody detection depends on the sampling time and type of clinical samples. IgG avidity could differentiate recent WNV infections from long-persisting IgM antibodies.

Key Words: West Nile virus; Reverse transcription-polymerase chain reaction; Serology; IgG avidity; Cross-reactivity

Core Tip: We analyzed different diagnostic methods in patients with West Nile virus (WNV) neuroinvasive disease and asymptomatic seropositive individuals. The WNV RNA detection rate was significantly higher in the urine/serum than in cerebrospinal fluid (CSF). The RT-PCR cycle threshold (Ct) values were significantly lower in urine than in CSF and serum samples. The frequency of WNV RNA and IgM/IgG antibody detection rates depends on the sampling time and type of clinical samples (CSF or serum). The correlation between ELISA and IgG avidity was negative for IgM and positive for IgG. No correlation was observed between ELISA IgG and virus neutralization test.

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INTRODUCTION

West Nile virus (WNV) is an emerging flavivirus of public health importance. In nature, the WNV transmission cycle includes birds as virus reservoirs and mosquitoes, mainly of the genus *Culex* as vectors. WNV is endemic in many parts of the world (Europe, the United States, the Middle East, Asia, and Africa) causing outbreaks and sporadic infections in both endemic and non-endemic areas[1]. Although the majority of WNV infections (80%) are asymptomatic or present as a non-specific febrile disease (WNV fever), some patients develop neuroinvasive disease (meningitis, encephalitis, myelitis). The mortality rate can reach 10% in severe neuroinvasive forms of the disease[2].

WNV shares many features with some other neuroinvasive arboviruses, especially flaviviruses such as tick-borne encephalitis virus (TBEV), and Usutu virus (USUV). Due to overlapping geographic distributions and clinical symptoms with these infections, the WNV diagnosis should be confirmed using virological methods[3]. The Centers for Disease Control and Prevention/European Center for Disease Control and Prevention (ECDC) laboratory criteria for WNV confirmation include: (1) Direct detection of virus by culture, antigen, or viral RNA; (2) A four-fold rise in antibody titers between acute- and convalescent-phase serum samples; or (3) Virus-specific immunoglobulin M (IgM) confirmed by detection of neutralizing antibodies. The detection of specific IgM antibodies in the cerebrospinal fluid (CSF) and the lack

of IgM to other endemic arboviruses are also criteria for confirmation of WNV neuroinvasive disease (NID)[4,5].

Because the majority of arboviruses, including WNV, have a short period of replication (short-term and low-level viremia), limiting the utility of molecular tests, serology is commonly used for disease confirmation. However, antibody cross-reactivity between viruses within the same serocomplex and persistent antibodies from previous arboviral infections complicate the interpretation of serology results[6].

ELISA and indirect immunofluorescence assay are the most commonly used screening tests for WNV serology. Although next-generation tests have improved performance by selecting the best epitopes and enhancing antigen purity, challenges in serological diagnosis still exist. In samples with cross-reactive antibodies, neutralization tests such as virus neutralization test (VNT) and plaque reduction neutralization test (PRNT), which still represent gold standard serology tests, are necessary for confirmation of the first-line serology[7].

Long IgM persistence is frequently observed in WNV infections. In a study conducted in Greece, WNV IgM antibodies were detectable for more than 3 years in 12% of patients with WNV infection[8]. Moreover, a study from Huston has found WNV IgM persistence (positive or equivocal results) in 42%, 34%, and 23% of WNV-infected patients approximately 1 year, 6 years, and 8 years post-infection, respectively[9]. Long-term persistence of WNV IgM antibodies in the CSF was also observed. In a study among patients with WNV NID from Michigan, IgM antibodies were detectable in the CSF of three patients for 110, 141, and 199 days post-acute phase[10]. In cases with long IgM persistence, IgG avidity determination could be useful for the differentiation of acute *vs* previous WNV infection[11].

Molecular methods such as RT-PCR in blood/serum and CSF samples are limited to the minority of patients who present with ongoing viremia or central nervous system replication[7]. Recent investigations indicated that WNV is excreted in urine longer and at higher levels compared to blood[12].

WNV grows and plaques efficiently on Vero cell culture, usually inducing a cytopathic effect[13]. Virus isolation is time-consuming and laborious[14]. Because WNV is classified as a biosafety level 3 (BSL-3) agent, virus cultivation is restricted to reference laboratories[15].

In Croatia, human WNV infections (WNV NID and WNV fever) have been reported continuously since 2012 in continental counties. In addition, some other flaviviruses are endemic in the same geographic areas, such as TBEV or occur sporadically (USUV)[16].

Because there are still many challenges in flavivirus diagnostics, this study aimed to analyze the characteristics of different methods for the diagnosis of WNV infection.

MATERIALS AND METHODS

Patients

This retrospective study included patients with NID (n = 182) and asymptomatic individuals (n = 352) tested for WNV during the two Croatian outbreaks (2017-2018). Patients with confirmed WNV NID (IgM/IgG positive and/or RT-PCR positive; n = 62), asymptomatic WNV IgM and/or IgG seropositive individuals (n = 22), and individuals with false positive WNV IgM antibodies (n = 30) were analyzed (Figure 1). Serum and CSF samples were collected in all patients, while urine samples were available for 55 patients. The samples were collected in the period from 4-19 days after disease onset (median 9, IQR = 7-13). In addition, paired serum samples taken 2-3 weeks after the first one were collected in 34 patients. Characteristics of patients with WNV NID are presented in Table 1.

Table 1 Characteristics of patients with West Nile virus infection included in the study

Characteristic	Subcategory	*n* (%)[1]
Sex	Male	35 (56.4)
	Female	27 (43.6)
Age in median years (IQR)		68 (58-76)
Clinical diagnosis	Febrile headache	2 (3.2)
	Meningitis	36 (58.1)
	Meningoencephalitis	20 (32.3)
	Meningoencephalomyelitis	2 (3.2)
	Polyradiculoneuritis	2 (3.2)

[1]Unless otherwise indicated. IQR: Interquartile range.

Methods

Serum, CSF, and urine samples were tested for the presence of WNV RNA using real-time RT-PCR as described previously[17]. Initial serological screening (WNV IgM and IgG antibodies) was performed using a commercial ELISA.

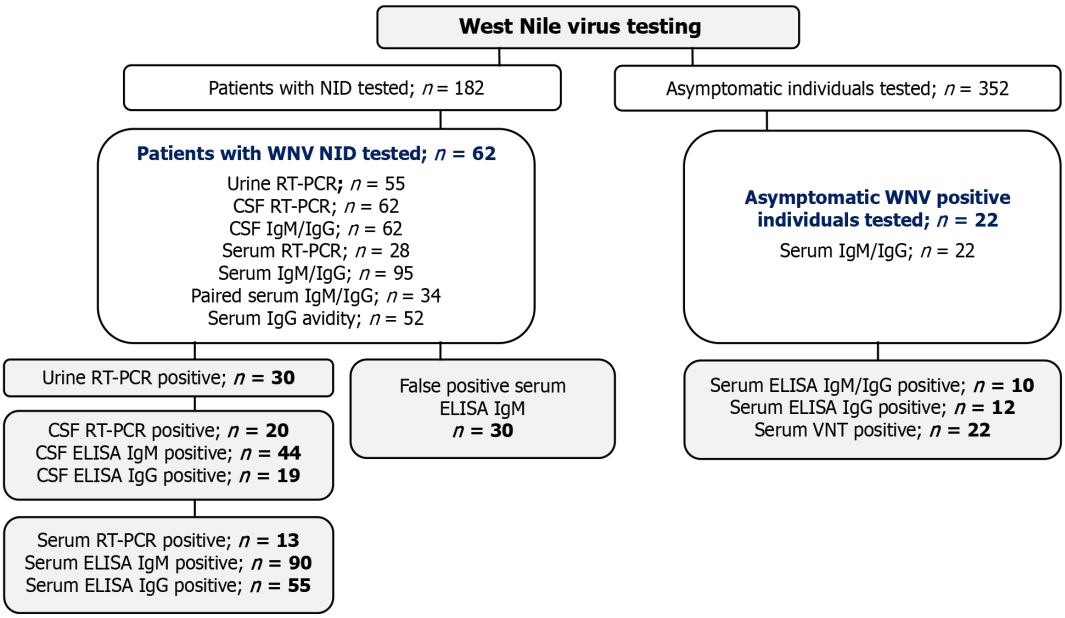

Figure 1 Selection of patients with West Nile virus infection and clinical samples. Samples in gray shadowed boxes were analyzed in the study. CSF: Cerebrospinal fluid; IgG: Immunoglobulin G; IgM: Immunoglobulin M; NID: Neuroinvasive disease; RT-PCR: Reverse transcription-polymerase chain reaction; TBEV: Tick-borne encephalitis virus; USUV: Usutu virus; WNV: West Nile virus.

Samples were also screened for potential cross-reactivity with other flaviviruses endemic in Croatia (TBEV/USUV). WNV cross-reactive samples were confirmed using a VNT[18]. In addition, WNV IgM/IgG positive and IgM negative/IgG positive samples were further tested for IgG avidity to confirm or exclude recent infection[11]. Characteristics of laboratory methods used for the diagnosis of WNV infection are presented in Table 2.

Table 2 Laboratory tests used for the diagnosis of West Nile virus infection

Method	Manufacturer/Protocol	Reference values
RT-PCR	FP: AAG TTG AGT AGA CGG TGC TG; RP: AGA CGG TTC TGA GGG CTT AC; Probe: FAM-CAA CCC CAG GAG GAC TGG-TAMRA	
IgM ELISA	Euroimmun, Lübeck, Germany	Ratio < 0.8 negative; 0.8-1.1 borderline; > 0.1 positive
IgG ELISA	Euroimmun, Lübeck, Germany	RU/mL < 16 negative, 16-22 borderline; > 22 positive
IgG avidity	Euroimmun, Lübeck, Germany	AI < 40% low; 40%-60% borderline; > 60% high
VNT	*In house*	Titer ≥ 10 positive

AI: Avidity index; ELISA: enzyme immunoassay; IgG: Immunoglobulin G; IgM: Immunoglobulin M; RT-PCR: Reverse transcription-polymerase chain reaction; RU: Relative units; VNT: Virus neutralization test.

Statistical analysis

WNV positive detection rates were presented as numbers and percentages with 95% confidence intervals (CI). The differences in the positive WNV RNA and antibody detection rates according to clinical sample and sampling time were compared using a χ^2 test. A Kruskal-Wallis test was used to compare the differences in RT-PCR cycle threshold (Ct) values. The correlation between ELISA IgM/IgG levels and avidity indices (AI) and IgG/VNT titer was calculated using Spearman's rank correlation coefficient. P value < 0.05 was considered statistically significant. For statistical analysis, a Social Science Statistics program was used (https://www.socscistatistics.com/tests/).

RESULTS

WNV RNA detection in serum, CSF, and urine

The WNV-positive detection rate was significantly higher (χ^2 test $P = 0.049$) in the urine (30/55; 54.5%) and serum (13/28; 46.4%) than in CSF samples (20/62; 32.2%). Nucleotide sequencing was successful in 15 (27.3%) urine samples (Figure 2).

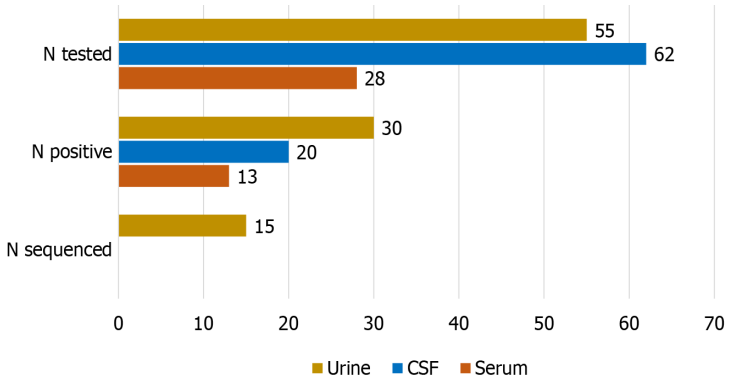

Figure 2 Detection rate of West Nile virus RNA by reverse transcription-polymerase chain reaction. CSF: Cerebrospinal fluid; N: Total sample number.

The RT-PCR positive detection rate depended on the sampling time. In the CSF, the positive detection rate was higher in samples collected within 7 days after disease onset (36.4%) compared to samples collected 8-14 days (31.2%) and ≥ 15 days (25.0%). In contrast, the frequency of WNV RNA positive detection was lower in in urine samples collected within 7 days (29.5%) than in urine samples collected within 8-14 days (66.6%) and ≥ 15 days (62.5%). The observed differences were statistically significant for urine (χ^2 test $P = 0.042$) but not for CSF (χ^2 test $P = 0.828$) (Table 3).

Table 3 Reverse transcription-polymerase chain reaction positive detection rates in cerebrospinal fluid and urine samples, *n* (%)

Days after symptoms onset	WNV RT-PCR CSF		WNV RT-PCR Urine	
	Tested	Positive	Tested	Positive
≤ 7	22 (35.5)	8 (36.4)	17 (30.9)	5 (29.4)
8–14	32 (51.6)	10 (31.2)	30 (54.6)	20 (66.6)
≥ 15	8 (12.9)	2 (25.0)	8 (14.5)	5 (62.5)
Total	62 (100)	20 (32.2)	55 (100)	30 (54.4)

Data are *n* (%). CSF: Cerebrospinal fluid; WNV: West Nile virus; RT-PCR: Reverse transcription-polymerase chain reaction.

The RT-PCR Ct values were significantly lower in urine samples (median 32.5, IQR = 28-34) than in CSF (median 34.5, IQR = 33-36) or serum samples (median 35, IQR = 34-36), (Kruskal-Wallis test $P = 0.047$, Figure 3).

WNV IgM and IgG detection in serum and CSF

WNV antibody detection rates in serum and CSF samples are presented in Figure 4 and Table 4. Using ELISA, IgM antibodies were detected in 90/95 (94.7%) of serum and 44/62 (70.9%) of CSF samples. IgG antibodies were detected in 55 (57.8%) of serum and 19/62 (30.6%) of CSF samples (Figure 4).

The frequency of positive results significantly differed according to sampling time in serum, but not in CSF samples. In serum samples collected within the 7 days after disease onset, the IgM-positive detection rate was 84.3%, compared to 100% in samples collected after more than 8 days (χ^2 test $P = 0.005$). Similarly, the IgG-positive detection rate was lowest in samples collected in the first 7 days (9.3% positive and 6.2% borderline). In samples collected 8-14 days after disease onset, IgG antibodies were positive in 71.1% and borderline in 13.1% of samples. All samples collected ≥ 15 days showed WNV IgG antibodies (χ^2 test $P < 0.001$) (Table 4).

The positive IgM detection rates in the CSF samples collected within 7 days, 8-14 days, and ≥ 15 days were 62.5, 73.9, and 100%, respectively ($P = 0.145$). The frequency of IgG detection was similar in the period ≤ 7 days and 8-14 days (28.1 and 26.1%, respectively), while it was higher in samples collected ≥ 15 days (57.1%; χ^2 test $P = 0.001$) (Table 5).

Samples with false positive WNV IgM antibodies ($n = 30$) were also included in the study. Analyzing the IgM antibody levels, patients with confirmed WNV infection showed a significantly higher IgM (median ratio 3.8, IQR = 3.1-4.3), than

Table 4 Positive detection rate of West Nile virus antibodies in serum samples according to sampling time, *n* (%)

Days after symptoms onset	Tested	WNV IgM antibodies		WNV IgG antibodies	
		Positive	Borderline	Positive	Borderline
≤ 7	32 (33.7)	27 (84.3)	0 (0)	3 (9.3)	2 (6.2)
8 – 14	38 (40.0)	38 (100)	0 (0)	27 (71.1)	5 (13.1)
≥ 15	25 (26.3)	25 (100)	0 (0)	25 (100)	0 (0)
Total	95 (100)	90 (94.7)	0 (0)	55 (57.8)	7 (7.3)

Data are *n* (%). IgG: Immunoglobulin G; IgM: Immunoglobulin M; WNV: West Nile virus.

Table 5 Positive detection rate of West Nile virus antibodies in cerebrospinal fluid samples according to sampling time, *n* (%)

Days after symptoms onset	Tested	WNV IgM antibodies		WNV IgG antibodies	
		Positive	Borderline	Positive	Borderline
≤ 7	32 (51.6)	20 (62.5)	1 (3.1)	9 (28.1)	2 (6.2)
8–14	23 (37.1)	17 (73.9)	0 (0)	6 (26.1)	1 (4.3)
≥ 15	7 (11.3)	7 (100)	0 (0)	4 (57.1)	0 (0)
Total	62 (100)	44 (70.9)	1 (1.6)	19 (30.6)	3 (4.8)

Data are *n* (%). IgG: Immunoglobulin G; IgM: Immunoglobulin M; WNV: West Nile virus.

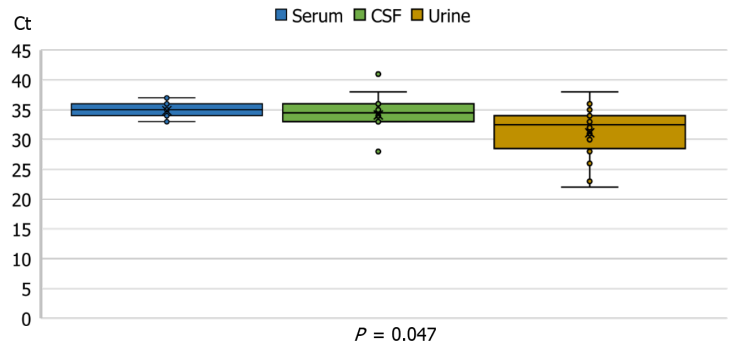

Figure 3 West Nile virus reverse transcription-polymerase chain reaction cycle threshold values in different clinical samples. Plots represent medians with interquartile ranges, inner and outlier points. CSF: Cerebrospinal fluid; Ct: Cycle threshold.

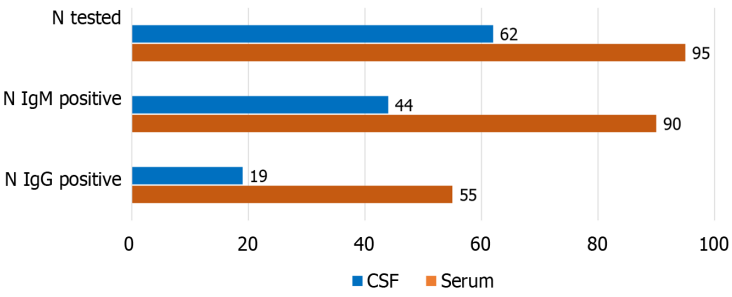

Figure 4 West Nile virus immunoglobulin M and immunoglobulin G detection rates in serum and cerebrospinal fluid. CSF: Cerebrospinal fluid; Ig: Immunoglobulin; N: Total sample number.

patients with false-positive IgM antibodies (median ratio 1.4, IQR = 1.1-1.8) (Kruskall-Wallis test $P < 0.001$) (Figure 5).

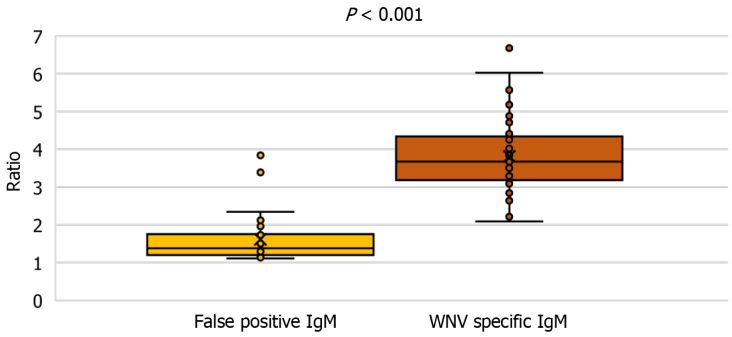

Figure 5 Immunoglobulin M antibody levels in patients with confirmed West Nile virus infection and false positive samples. Plots represent medians with interquartile ranges, inner and outlier points. IgM: Immunoglobulin M; WNV: West Nile virus.

Cross-reactive WNV IgM and IgG antibodies with TBEV/USUV were detected in 4.5% and 38.2% serum samples (Figure 6). The frequency of cross-reactive IgM antibodies was significantly lower than the frequency of cross-reactive IgG antibodies (χ^2 test $P = 0.001$).

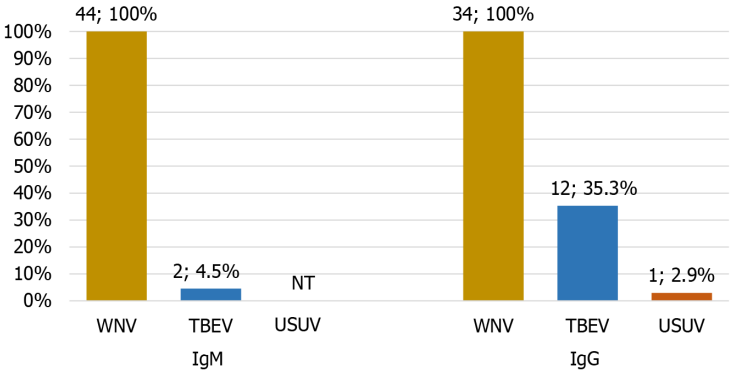

Figure 6 Cross-reactive flavivirus immunoglobulin M immunoglobulin G antibodies detected in patients with West Nile virus infections. IgG: Immunoglobulin G; IgM: Immunoglobulin M; NT: Not tested; TBEV: Tick-borne encephalitis virus; USUV: Usutu virus; WNV: West Nile virus.

WNV IgG avidity determination

IgG avidity was performed in IgM/IgG positive patients with WNV NID ($n = 52$) and asymptomatic WNV seropositive individuals ($n = 22$). In patients with NID, 50 samples showed low AI (median 13%, IQR = 7-21), and two samples showed borderline AI (42 and 40%), respectively.

In asymptomatic WNV seropositive individuals, IgG avidity was performed in IgM-positive/IgG-positive samples ($n = 10$) and IgM-negative/IgG-positive samples ($n = 12$) (Table 6). Three samples (13.6%) showed low/borderline AI indicating acute/recent WNV infection. Using IgG avidity determination, recent WNV infections were confirmed in two IgM-positive patients and one IgM-negative individual.

Table 6 Immunoglobulin G avidity in asymptomatic West Nile virus seropositive individuals

Serology result	IgG avidity index		
	Low	Borderline	High
IgM/IgG positive, $n = 10$	1 (10.0)	1 (10.0)	8 (80.0)
IgM negative/IgG positive, $n = 12$	1 (8.3)	0 (0)	11 (91.7)
Total, $n = 22$	2 (9.1)	1 (4.5)	19 (84.6)

Data are *n* (%). IgG: Immunoglobulin G; IgM: Immunoglobulin M.

A strong negative correlation (Spearman's rho coefficient -0.512, $P < 0.001$) between the ELISA IgM levels (ratio) and AI (%) was observed. Samples with the highest IgM levels were associated with the lowest AI values. For IgG antibodies, a correlation between the IgG levels (RU/mL) and AI (%) was a strong positive (Spearman's rho coefficient 0.802, $P < 0.001$) (Figure 7).

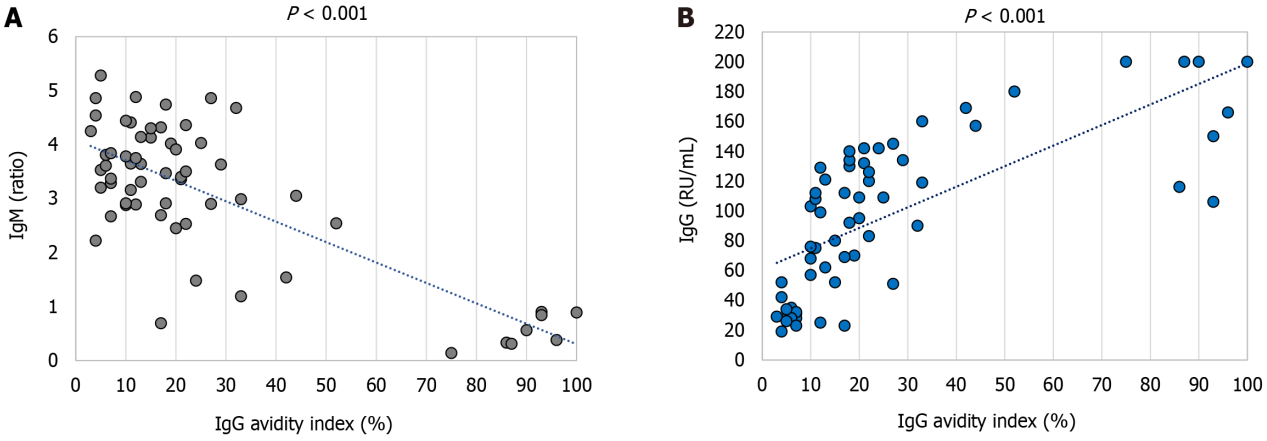

Figure 7 Correlation of immunoglobulin G avidity. A: Immunoglobulin M antibody levels; B: Immunoglobulin G antibody levels. Blue dotted lines represent trendlines. IgG: Immunoglobulin G; IgM: Immunoglobulin M.

A total of 26 IgM and/or IgG-positive samples were confirmed using VNT. No significant correlation between ELISA IgG levels (RU/mL) and VNT titer was found (Spearman's rho coefficient 0.221, $P = 0.265$) (Figure 8).

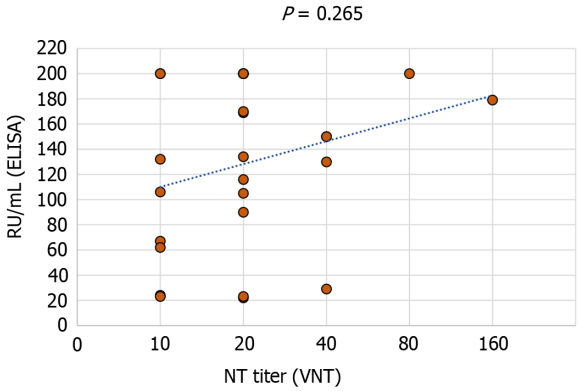

Figure 8 Correlation of immunoglobulin G antibody levels (ELISA) and NT titer (Virus neutralization test). Blue dotted line represents a trendline.

DISCUSSION

The diagnosis of WNV in humans is challenging due to the short arbovirus viremic phase, low-level viremia (humans are dead-end hosts), the cross-reactivity between flaviviruses, and long-term IgM persistence[6,8,11].

In the present study, WNV RNA positive detection rates depend on the sample type (46.4% in serum, 32.2% in CSF, and 54.4% in urine samples). Similarly, in a study from Italy, patients with WNV NID and WNV fever had detectable WNV RNA in urine at a higher rate (43.8%) than WNV RNA in plasma (37.5%) and CSF (7.1%)[19]. Furthermore, in a study conducted in Israel, whole blood samples were most frequently positive using RT-PCR (86.8%), followed by urine samples (58.3%). The positivity of serum and plasma was 26% and 20%, respectively, while CSF was RT-PCR positive only in 16.5% of patients. All samples were collected on average 11 days after symptom onset. These results suggested that in patients with acute WNV fever, WNV RNA is present in whole blood significantly more frequently than any other sample type tested[20].

Although the period in which WNV RNA is detectable in the CSF varies, it can last several weeks in some patients[1]. In our study, the frequency of WNV RNA detection differs according to the sampling time. In the period within 7 days after symptom onset, 36.4% of CSF samples were RT-PCR positive compared to 31.2% 8-14 days and 25.0% ≥ 15 days after symptom onset. In contrast, the frequency of positive WNV RNA detection rate in urine was lowest in samples collected within 7 days (29.4%) compared to 66.6% and 62.5% for samples collected afterward. In a recently published study from Serbia, WNV infection was confirmed by positive WNV RT-PCR in serum and/or CSF samples in 46.3% of patients.

Thirty-one percent more cases were confirmed using urine WNV RNA testing. In contrast to our results, there was no association between the sampling time and WNV RNA urine positivity[12].

Consistent with our findings (the lowest median RT-PCR Ct in urine 32.5, compared to serum 35, and CSF 34.5), in a study from Israel, the WNV viral load in urine was higher than that of whole blood, CSF, serum, and plasma, even though the urine sensitivity was lower than that of whole blood[20].

WNV-specific antibody testing is currently the most widely used approach for the diagnosis of WNV infection. WNV IgM and IgG antibodies are typically detectable by days 4 and 8 after the onset of clinical symptoms[21]. Serological testing of patients included in the present study showed IgM and IgG antibodies in 94.7% and 57.8% of serum samples, respectively. In addition, 70.9% of CSF samples were IgM positive and 30.6% were IgG positive. In the early acute phase of the illness, there is a negative serological window period of detection, as the antibodies have not yet been developed [14]. Our study showed a negative IgM serology in 15.7% of serum and 37.5% of CSF samples collected within 7 days after disease onset. No false negative serum samples were detected after day 8. However, analyzing the CSF serology, 26.1% of CSF samples tested IgM negative in the period 8-14 days, whereas all samples were positive after 14 days. The IgG positivity in serum was 9.3, 71.1, and 100% in samples collected ≤ 7 days, 8-14 days, and ≥ 15 days. In the CSF samples, IgG antibody detection rates were 28.1 and 26.1% in samples collected by day 14 and 57.1% in samples collected after 14 days.

Serological cross-reactivity between flaviviruses is common due to antigenic similarities, especially between the viruses that belong to the same serocomplex. Both species-specific and cross-reactive antibodies are produced during flavivirus infections[22]. People who live in areas where different arboviruses are endemic gradually accumulate cross-reactive antibodies from previous exposures with increasing age[6]. Therefore, it can be challenging to identify the most recent infection in patients with multiple exposures to different flaviviruses[23]. Cross-reacting antibodies are frequently observed in ELISA, while some degree of cross-reactivity was also found in a more specific VNT[24]. Given that WNV and USUV are antigenically closer by genomic phylogeny than TBEV, cross-reactions between WNV and USUV in VNT are usually more common[25]. In our study, ELISA revealed WNV cross-reactivity with TBEV and USUV. IgM cross-reactivity was significantly lower (4.5% of serum samples) than IgG cross-reactivity (38.2% of serum samples). WNV IgG antibodies cross-reacted with TBEV in 35.3% of samples and USUV in 2.9% of samples.

Despite their obvious clinical relevance, IgM antibodies experience false-positive results, which can result in WNV misdiagnosis[26]. Thirty samples with false positive WNV IgM antibodies were also analyzed in the present study. Comparing the levels of false positive IgM antibodies and WNV-specific antibodies, significant differences were observed. WNV-specific antibody levels were significantly higher (median ratio 3.8) than false positive IgM levels (median ratio 1.4).

The diagnostic implications of serum WNV IgM persistence are noteworthy since the presence of IgM antibodies is generally considered evidence of an acute or recent WNV infection. Since the long-term persistence of IgM antibodies is frequently observed in patients with WNV infection[8-10], this should be taken into account when interpreting serology results. IgG avidity was useful to differentiate between recent and previous WNV infection in both patients with NID and WNV fever[11]. In the present study, using avidity determination, 80.0% of tested IgM/IgG-positive asymptomatic individuals were classified as having recent WNV infection, while 20.0% were classified as having previous WNV exposure (long-persisting IgM antibodies). Furthermore, 8.3% of IgM negative/IgG positive individuals had low IgG avidity, suggestive of recent infection.

Our study found a strong negative correlation between IgM levels and IgG avidity, consistent with previous findings in Croatian patients with confirmed WNV infection. The samples with the highest WNV IgM levels had the lowest AI values, indicating that determination of IgG avidity is not required in cases with very high IgM results[11].

Because of its high specificity, neutralization tests are the most reliable serological assays. A correlation between neutralizing flavivirus antibody detection and the presence of specific IgG in blood specimens was observed in some studies[27]. In this study, VNT was used for confirmation of cross-reactive serum samples. However, we found no correlation between WNV-neutralizing antibody titers with binding antibody levels (ELISA). Similarly, some studies have found that the ELISA titers in convalescent WNV patients did not correlate with neutralization nor did neutralization titers increase over time[28].

A limitation of this study that needs to be addressed is the small number of patients tested. Therefore, further studies on a large cohort of WNV patients are needed to confirm our observations.

CONCLUSION

The frequency of WNV RNA and antibody detection depend on the sampling time and type of clinical samples, which should be considered when interpreting the results. Testing should include flaviviruses that circulate in a specific area to exclude cross-reactive antibodies. IgG avidity determination is a useful diagnostic method for differentiating IgM positivity in recent infections from long-persisting WNV IgM antibodies.

FOOTNOTES

Author contributions: Vilibic-Cavlek T and Bogdanic M conceptualized and designed the research; Vilibic-Cavlek T, Bogdanic M and Sabadi D acquired clinical data; Antolasic Lj and Milasincic L and Hruskar Z performed laboratory analysis; Savic V, Barbic Lj, Stevanovic V, Miletic G, Coric I, Mrzljak A, and Listes E performed data collection, and analysis of the results; Vilibic-Cavlek T, Bogdanic

M and Savic V wrote the manuscript; Savini G critically revised the manuscript; Vilibic-Cavlek T and Savic V obtained the funds for this research; All authors have read and approved the final version of the manuscript.

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

Conflict-of-interest statement: All authors reported no conflicts of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at tatjana.vilibic-cavlek@hzjz.hr. Consent for data sharing was not obtained but the presented data are anonymized and the risk of identification is low. No additional data are available.

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REFERENCES

1 **Habarugira G**, Suen WW, Hobson-Peters J, Hall RA, Bielefeldt-Ohmann H. West Nile Virus: An Update on Pathobiology, Epidemiology, Diagnostics, Control and "One Health" Implications. *Pathogens* 2020; **9** [PMID: 32707644 DOI: 10.3390/pathogens9070589]
2 **Roberts JA**, Kim CY, Dean A, Kulas KE, St George K, Hoang HE, Thakur KT. Clinical and Diagnostic Features of West Nile Virus Neuroinvasive Disease in New York City. *Pathogens* 2024; **13** [PMID: 38787234 DOI: 10.3390/pathogens13050382]
3 **Simonin Y**. Usutu, West Nile, and Tick-Borne Encephalitis Viruses. *Viruses* 2022; **14** [PMID: 36298675 DOI: 10.3390/v14102120]
4 **McDonald E**, Mathis S, Martin SW, Erin Staples J, Fischer M, Lindsey NP. Surveillance for West Nile virus disease - United States, 2009-2018. *Am J Transplant* 2021; **21**: 1959-1974 [PMID: 33939278 DOI: 10.1111/ajt.16595]
5 **Riccetti N**, Ferraccioli F, Fasano A, Stilianakis NI. Demographic characteristics associated with West Nile virus neuroinvasive disease - A retrospective study on the wider European area 2006-2021. *PLoS One* 2023; **18**: e0292187 [PMID: 37768957 DOI: 10.1371/journal.pone.0292187]
6 **Kasbergen LMR**, Nieuwenhuijse DF, de Bruin E, Sikkema RS, Koopmans MPG. The increasing complexity of arbovirus serology: An in-depth systematic review on cross-reactivity. *PLoS Negl Trop Dis* 2023; **17**: e0011651 [PMID: 37738270 DOI: 10.1371/journal.pntd.0011651]
7 **Piantadosi A**, Kanjilal S. Diagnostic Approach for Arboviral Infections in the United States. *J Clin Microbiol* 2020; **58** [PMID: 32938736 DOI: 10.1128/JCM.01926-19]
8 **Papa A**, Anastasiadou A, Delianidou M. West Nile virus IgM and IgG antibodies three years post- infection. *Hippokratia* 2015; **19**: 34-36 [PMID: 26435644]
9 **Murray KO**, Garcia MN, Yan C, Gorchakov R. Persistence of detectable immunoglobulin M antibodies up to 8 years after infection with West Nile virus. *Am J Trop Med Hyg* 2013; **89**: 996-1000 [PMID: 24062481 DOI: 10.4269/ajtmh.13-0232]
10 **Kapoor H**, Signs K, Somsel P, Downes FP, Clark PA, Massey JP. Persistence of West Nile Virus (WNV) IgM antibodies in cerebrospinal fluid from patients with CNS disease. *J Clin Virol* 2004; **31**: 289-291 [PMID: 15494271 DOI: 10.1016/j.jcv.2004.05.017]
11 **Vilibic-Cavlek T**, Kristofic B, Savic V, Kolaric B, Barbic L, Tabain I, Peric L, Sabadi D, Miklausic B, Potocnik-Hunjadi T, Zember S, Stevanovic V, Listes E, Savini G. Diagnostic significance of immunoglobulin G avidity in symptomatic and asymptomatic West Nile virus infection. *Rev Soc Bras Med Trop* 2018; **51**: 591-595 [PMID: 30304263 DOI: 10.1590/0037-8682-0482-2017]
12 **Cvjetković IH**, Radovanov J, Kovačević G, Turkulov V, Patić A. Diagnostic value of urine qRT-PCR for the diagnosis of West Nile virus neuroinvasive disease. *Diagn Microbiol Infect Dis* 2023; **107**: 115920 [PMID: 37390574 DOI: 10.1016/j.diagmicrobio.2023.115920]
13 **McAuley AJ**, Beasley DW. Propagation and Titration of West Nile Virus on Vero Cells. *Methods Mol Biol* 2016; **1435**: 19-27 [PMID: 27188547 DOI: 10.1007/978-1-4939-3670-0_3]
14 **Chan KR**, Ismail AA, Thergarajan G, Raju CS, Yam HC, Rishya M, Sekaran SD. Serological cross-reactivity among common flaviviruses. *Front Cell Infect Microbiol* 2022; **12**: 975398 [PMID: 36189346 DOI: 10.3389/fcimb.2022.975398]
15 **Mayo DR**, Beckwith WH 3rd. Inactivation of West Nile virus during serologic testing and transport. *J Clin Microbiol* 2002; **40**: 3044-3046 [PMID: 12149375 DOI: 10.1128/JCM.40.8.3044-3046.2002]
16 **Vilibic-Cavlek T**, Janev-Holcer N, Bogdanic M, Ferenc T, Vujica Ferenc M, Krcmar S, Savic V, Stevanovic V, Ilic M, Barbic L. Current

Status of Vector-Borne Diseases in Croatia: Challenges and Future Prospects. *Life (Basel)* 2023; **13** [PMID: 37763260 DOI: 10.3390/life13091856]

17 **Tang Y**, Anne Hapip C, Liu B, Fang CT. Highly sensitive TaqMan RT-PCR assay for detection and quantification of both lineages of West Nile virus RNA. *J Clin Virol* 2006; **36**: 177-182 [PMID: 16675298 DOI: 10.1016/j.jcv.2006.02.008]

18 **Di Gennaro A**, Lorusso A, Casaccia C, Conte A, Monaco F, Savini G. Serum neutralization assay can efficiently replace plaque reduction neutralization test for detection and quantitation of West Nile virus antibodies in human and animal serum samples. *Clin Vaccine Immunol* 2014; **21**: 1460-1462 [PMID: 25100824 DOI: 10.1128/CVI.00426-14]

19 **Barzon L**, Pacenti M, Franchin E, Pagni S, Martello T, Cattai M, Cusinato R, Palù G. Excretion of West Nile virus in urine during acute infection. *J Infect Dis* 2013; **208**: 1086-1092 [PMID: 23821721 DOI: 10.1093/infdis/jit290]

20 **Lustig Y**, Mannasse B, Koren R, Katz-Likvornik S, Hindiyeh M, Mandelboim M, Dovrat S, Sofer D, Mendelson E. Superiority of West Nile Virus RNA Detection in Whole Blood for Diagnosis of Acute Infection. *J Clin Microbiol* 2016; **54**: 2294-2297 [PMID: 27335150 DOI: 10.1128/JCM.01283-16]

21 **Lustig Y**, Sofer D, Bucris ED, Mendelson E. Surveillance and Diagnosis of West Nile Virus in the Face of Flavivirus Cross-Reactivity. *Front Microbiol* 2018; **9**: 2421 [PMID: 30369916 DOI: 10.3389/fmicb.2018.02421]

22 **Rathore APS**, St John AL. Cross-Reactive Immunity Among Flaviviruses. *Front Immunol* 2020; **11**: 334 [PMID: 32174923 DOI: 10.3389/fimmu.2020.00334]

23 **Mansfield KL**, Horton DL, Johnson N, Li L, Barrett ADT, Smith DJ, Galbraith SE, Solomon T, Fooks AR. Flavivirus-induced antibody cross-reactivity. *J Gen Virol* 2011; **92**: 2821-2829 [PMID: 21900425 DOI: 10.1099/vir.0.031641-0]

24 **Oliveira RA**, de Oliveira-Filho EF, Fernandes AI, Brito CA, Marques ET, Tenório MC, Gil LH. Previous dengue or Zika virus exposure can drive to infection enhancement or neutralisation of other flaviviruses. *Mem Inst Oswaldo Cruz* 2019; **114**: e190098 [PMID: 31411310 DOI: 10.1590/0074-02760190098]

25 **Lorusso A**, Marini V, Di Gennaro A, Ronchi GF, Casaccia C, Carelli G, Passantino G, D'Alterio N, D'Innocenzo V, Savini G, Monaco F, Horton DL. Antigenic relationship among zoonotic flaviviruses from Italy. *Infect Genet Evol* 2019; **68**: 91-97 [PMID: 30517880 DOI: 10.1016/j.meegid.2018.11.023]

26 **Landry ML**. Immunoglobulin M for Acute Infection: True or False? *Clin Vaccine Immunol* 2016; **23**: 540-545 [PMID: 27193039 DOI: 10.1128/CVI.00211-16]

27 **Musso D**, Despres P. Serological Diagnosis of Flavivirus-Associated Human Infections. *Diagnostics (Basel)* 2020; **10** [PMID: 32423058 DOI: 10.3390/diagnostics10050302]

28 **Throsby M**, Goudsmit J, Kruif JD. The Human Antibody Response Against WNV. In: West Nile Encephalitis Virus Infection. Emerging Infectious Diseases of the 21st Century. New York: Springer New York, 2009: 401-416 [DOI: 10.1007/978-0-387-79840-0_18]

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ORIGINAL ARTICLE

Retrospective Study

COVID-19 in pregnancy: Perinatal outcomes and complications

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Abstract

BACKGROUND

The risk of severe coronavirus disease 2019 (COVID-19) in pregnant women is elevated.

AIM

To examine the outcomes of pregnant women with COVID-19 and report perinatal outcomes and complications, while providing a brief review of current literature.

METHODS

The study included pregnant women presenting from April 2020 to February 2022 to the emergency department (ED) of a tertiary hospital. We retrospectively recorded the maternal and perinatal files, including patient epidemiological and clinical characteristics, laboratory values, outcomes, treatment modalities and associations were explored.

RESULTS

Among the 60 pregnant women, 25% required hospitalization, all of whom were symptomatic. Preterm delivery occurred in 30% of cases. Ten percent of neonates required admission to the neonatal intensive care unit, and 5% were classified as

small for their gestational age. All mothers survived COVID-19 and pregnancy, with 6.6% requiring invasive mechanical ventilation. Preterm delivery rates did not differ between hospitalized and non-hospitalized pregnant women; composite unfavorable perinatal outcomes, including stillbirth, small for gestational age, or neonatal intensive care unit (ICU) admission, did not significantly increase in the cases hospitalized for COVID-19 (P = 0.09). The odds of hospitalization increased 2.3-fold for each day of delayed ED presentation [adj. OR (95%CI: 1.46-3.624), P < 0.001]. Comorbidity status was an independent predictor of hospitalization, albeit with marginal significance [adj. OR = 16.13 (95%CI: 1.021-255.146), P = 0.048]. No independent predictors of adverse fetal outcome (composite) were identified, and eventual hospitalization failed to reach statistical significance by a slight margin (P = 0.054).

CONCLUSION

Delayed ED presentation and comorbidities increase hospitalization odds. This study highlights the importance of continuous and specific guidance for managing pregnant COVID-19 patients, including timely and appropriate interventions to minimize maternal and perinatal morbidity and mortality.

Key Words: SARS-CoV-2; COVID-19; Pregnancy; Maternal outcomes; Preterm delivery

Core Tip: Pregnant individuals' risk of contracting severe disease from severe acute respiratory syndrome coronavirus 2 infection is elevated. If hospitalization for coronavirus disease 2019 is indicated for expecting mothers, it is crucial the medical treatment to take place in a facility equipped to monitor both maternal and fetal health. Early detection and management of these cases are paramount for optimal results regarding motherhood and newborn care outcomes.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a multisystem disease that has a variety of effects on pregnant women and their bearing[1]. Pregnant women with COVID-19 have experienced drastic rises in severe maternal illness and mortality, as well as neonatal complications[2,3]. Risk factors for severe maternal morbidity in pregnancy may also increase risk of COVID-19 illness in pregnancy[4].

Women with COVID-19 had higher rates of preterm birth and delivery, intrauterine growth restriction with low birth weight and higher incidences of fetal distress and maternal death than women without COVID-19[1,2,5,6]. Analysis of 1249634 delivery hospitalizations in the US from March 2020 to September 2021, revealed that pregnant women with COVID-19 are more likely to experience stillbirth than those without the virus. (Adjusted RR: 1.90; 95%CI: 1.69-2.15)[7]. Higher magnitude of association was observed during delta variant[7]. As far as complications are concerned, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is also implicated in the occurrence of severe complications, including acute kidney injury and disseminated intravascular coagulation, in pregnant populations. The penultimate complication of acute respiratory distress syndrome (ARDS) is also much more likely to occur in pregnant individuals, requiring use of positive pressure oxygenation or even invasive mechanical ventilation[8]. Women with COVID-19 had a greater risk of admission to intensive care unit (ICU)/high-dependency unit. Among all ICU admissions, women with COVID-19 diagnosis stayed longer than women without COVID-19[2,8].

Given the unique biological characteristics of the mother and fetus, therapeutic interventions during pregnancy necessitate distinct clinical management. Although recommendations for treatment plans are the same for both pregnant and non-pregnant individuals[9], a number of issues need to be considered. Vertical transmission, differences in pharmacokinetics and pharmacodynamics, drug toxicities, and post-natal care are only among the issues that physicians should consider when managing COVID-19 pregnant patients.

We aimed to report our center's experience with COVID-19 pregnant patients, exploring previously reported clinical, therapeutic correlations and outcomes, and set a basis for further discussion.

MATERIALS AND METHODS

We retrospectively recorded the maternal and perinatal outcomes of SARS-CoV-2 positive pregnant women presenting from April 2020 – February 2022 in the emergency obstetric department of a tertiary hospital. Our study was conducted

in accordance with the declaration of Helsinki and principles of good clinical practice and was approved by our institution's review and ethics board (477/24-11/2022).

The study included pregnant women of any gestational age who tested positive for SARS-CoV-2 using reverse transcription polymerase chain reaction and presented to our emergency obstetric department during the study period. Patient demographics, clinical characteristics, pertinent medical history, clinical manifestations, and laboratory values were documented upon presentation. Outcomes and treatment modalities were also recorded, and associations explored.

Preterm delivery is defined as birth occurring before 37 completed weeks of gestation. Gestational age refers to the length of pregnancy calculated from the first day of the last menstrual period. Small for gestational age (SGA) is defined as birth weight below the 10th percentile for the gestational age.

All quantitative variables are described as the median and interquartile range. All qualitative variables are described as absolute frequencies (n) and percentages. χ^2 test and Fischer's exact test were used to evaluate the relationships between categorical variables. The Shapiro-Wilk test was used to assess the normality of observed values. Independent-samples *t*-test was performed for comparison of normally distributed continuous variables across groups. Mann-Whitney *U* test was used for the rest of the continuous variables. Logistic regression was utilized for constructing prediction models for the need for hospitalization.

RESULTS

Sixty pregnant women were included in our cohort. The median age of the sample was 30 years (IQR, 24-35). The median gestational age at the time of presentation was 36 weeks (IQR, 30-38). Almost half of the women were asymptomatic at presentation (45%). Symptomatic patients presented at a median time of 3 (IQR, 2-7) days following symptom onset, mostly complaining of fever (54.5%) and cough (57.6%), dyspnea while at rest (33.3%), while 33.3% experienced premature contractions. One (1.66%) of them was diagnosed with gestational hypertension (but not preeclampsia), 3 (5%) had gestational diabetes, and 3 (5%) were obese, with a body mass index (BMI) greater than 30. All the women were unvaccinated against SARS-CoV-2 during the study period.

Eventually, 15 participants (25%) were admitted to the hospital. Thirteen (21.6%) women received antiviral medication (remdesivir), whereas 25 (41.6%) received corticosteroids. At the time that this cohort study was carried out nirmatrelvir/ritonavir, monoclonal antibodies, tocilizumab, baricitinib had either not received approval or experience was absent in pregnant population, hence not utilized in our center.

With regard to the recorded pregnancy outcomes, 18 (30%) neonates were delivered preterm, and 4 (6.6%) pregnancies were complicated by obstetric implications (hydramnios, preeclampsia) resulting in fetal distress. Two (3.3%) cesarean sections (CS) due to maternal ARDS were performed. In total, two stillbirths (3.3%) were noted, and one pregnancy ended in miscarriage (1.6%). Six (10%) neonates eventually required admission to the neonatal intensive care unit (NICU), whereas three (5%) were classified as SGA. All mothers survived both COVID-19 and pregnancy, despite 4 (6.6%) requiring invasive mechanical ventilation during the course of their hospitalization. The clinical features of all pregnant women and the treatment modalities used, as well as their recorded hospitalization and pregnancy outcomes, are summarized in Table 1.

A subgroup analysis of the clinical characteristics and laboratory values of the women admitted to the hospital for the disease was performed. Adverse perinatal outcomes were collectively evaluated as a single composite endpoint, comprising all possible adverse events related to the fetus or neonate, that is, stillbirth, NICU admission, and SGA. Women in need of hospitalization presented with more severe conditions, as reflected in the significant difference in clinical manifestations including dyspnea, severity indices (World Health Organization Clinical Progression Scale, P/F < 400), and laboratory values including lymphopenia, white blood cells, lactate dehydrogenase, C-reactive protein, and ferritin, while presented later in the course of disease (6 *vs* 0 days since onset in hospitalized and non-hospitalized women, respectively, $P < 0.001$) (Table 2). All obese individuals, defined as those with a BMI > 30 kg/m^2, required hospitalization ($P = 0.03$). The remaining recorded comorbidities did not seem to play a role in the decision to admit, as reflected by the non-significant difference across groups in comorbidity status ($P = 0.25$). Cramer's V for increased BMI was calculated to be 0.397, for a decreased P/F ratio of 0.631, whereas for dyspnea it was 0.821, suggesting a strong correlation of all three parameters with hospital admission.

The gestational age differed significantly between the two groups (30th *vs* 36th median week of presentation for hospitalized and non-hospitalized women, respectively), while the presence of contractions was significantly higher in the non-hospitalized subgroup of pregnant women ($P = 0.026$). None of the asymptomatic individuals were admitted to the hospital. Preterm delivery rates were not different among hospitalization sub-groups. The composite outcome of adverse perinatal events, including stillbirth, SGA, and NICU admission, did not significantly increase in women hospitalized for COVID-19 ($P = 0.09$).

Multiple logistic regression revealed that when accounting for a woman's age, gestational age, and comorbidity status, defined as having at least one reported comorbid condition, days since symptom onset was an independent predictor of hospitalization. For each day of delayed ED presentation, the odds of hospitalization increased 2.3-fold [adj. OR (95%CI: 1.46-3.624), $P < 0.001$]. Comorbidity status proved to be an independent predictor of hospitalization with marginal significance [adj. OR = 16.13 (95%CI: 1.021-255.146), $P = 0.048$]. No independent predictors of adverse fetal outcome (composite) were identified, with eventual hospitalization failing to reach statistical significance only by a slight margin ($P = 0.054$).

Table 1 Patient characteristics (*n* = 60)

Demographics	
Age median (IQR)	30 (24-35)
Gestational week median (IQR)	36 (30-38)
Comorbidities (%)	
Hypertension	1 (1.66)
Diabetes	3 (5)
Hypothyroidism	5 (8.5)
BMI > 30	3 (5)
Hematologic condition	4 (6.8)
Former OB/GYN pathology	3 (5)
Symptomatology	
Days from onset median (IQR)	3 (2-7)
Asymptomatic (%)	27 (45)
Fever (%/symptomatic)	18 (54.5)
Dyspnea (%/symptomatic)	11 (33.3)
Cough (%/symptomatic)	19 (57.6)
Contractions (%/symptomatic)	11 (33.3)
Pregnancy outcomes (%)	
Preterm delivery	18 (30)
Fetal clinical abnormalities	4 (6.6)
Neonatal ICU admission	6 (10)
Miscarriage	1 (1.66)
Still birth	2 (3.33)
SGA	3 (5)
CS due to ARDS	2 (3.33)
Maternal outcomes (%)	
Hospitalization	15 (25)
Survival	60 (100)
IMV	4 (6.6)
Pharmacologic management (%)	
Corticosteroids	25 (41.6)
Remdesivir	13 (21.6)

BMI: Body mass index; OB/GYN: Obstetric/gynecologic; ICU: Intensive care unit; SGA: Small for gestational age; CS: Cesarean section; ARDS: Acute respiratory distress syndrome; IMV: Invasive mechanical ventilation.

DISCUSSION

In our cohort of patients, only a fourth of the unvaccinated pregnant individuals required hospitalization due to disease severity, as reflected in the severity indices. Delay in presentation and the presence of comorbidities seem to predict the need for hospitalization but not composite adverse perinatal events or pregnancy outcomes.

Our findings align with previous authors reporting that the majority of COVID-19 pregnant individuals present and remain asymptomatic through follow-up[5]. When symptoms appear, they do not seem to differ from non-pregnant individuals, although one report noted that pregnant individuals were less likely to present fever, myalgia, dyspnea or cough[1,5,10]. Also, in a subsequent study, fever and cough among pregnant women were reported to be even lower[11]. Delay in presentation was identified as a risk factor for severe disease, hence the need for hospitalization, similar to others[12]. This comes as no surprise since, as the disease progresses, viral toxicity is complicated by the presence of

Table 2 Hospitalized *vs* non-hospitalized pregnant women, *n* (%)

	Hospitalized (*n* = 15)	Not hospitalized (*n* = 45)	*P* value
Demographics			
Age median (IQR)	34 (23-40)	29.67 (26-34)	0.36
Former smokers	2 (13.3)	6 (13.3)	0.584
BMI > 30	3 (20)	0 (0)	0.013
At least one co-morbidity	4 (26.6)	6 (13.3)	0.25
Gestational week median (IQR)	30 (26-36)	36 (34-38)	0.031
Clinical manifestations			
Asymptomatic	0 (0)	27 (37.7)	< 0.001
Days from onset median (IQR)	7 (2-9)	2 (2-3)	0.006
Fever (symptomatic)	10 (66.6)	8 (17.7)	0.2
Dyspnoea (symptomatic)	11 (73.3)	0 (0)	< 0.001
Cough (symptomatic)	11 (73.3)	8 (17.7)	0.09
Contractions (symptomatic)	2 (13.3)	16 (35.5)	0.026
Disease severity			
WHO CPS median (IQR)	5 (5-6)	2 (1-4)	< 0.001
P/F < 400	10 (66.6)	3 (20)	< 0.001
Need for NIV	6 (13.3)	0 (0)	< 0.001
Need for IMV	4 (8.8)	0 (0)	< 0.001
Pharmacological management			
Corticosteroids	13 (86.6)	12 (26.6)	< 0.001
Remdesivir	8 (53.3)	5 (11.1)	0.02
Pregnancy outcomes			
Preterm delivery	5 (33.3)	13 (28.8)	0.745
Composite adverse perinatal	5 (33.3)	6 (13.3)	0.09
Laboratory values median (IQR)			
WBC (K/μL)	6980 (5580-8320)	9110 (7380-11150)	0.01
LYMPH (K/μL)	1.142 (0.785-1.570)	1.300 (1.020-1.880)	0.102
CPK (ng/mL)	69 (20-94)	65.5 (35-164)	0.179
LDH (IU/L)	318 (258-376)	210 (178-283)	0.001
CRP (mg/dL)	5.94 (2.09-9.81)	2.34 (1.02-4.47)	0.028
Ferritin (ng/mL)	149 (101-140)	74 (34.5-95.5)	0.001
D-Dimers (μg/mL)	1.470 (0.670-2.180)	1.492 (1.045-2.215)	0.303

WHO CPS: World Health Organization Clinical Progression Score for COVID-19; BMI: Body mass index; P/F: PaO2/FiO2 ratio; NIV: Non-invasive ventilation; IMV: Invasive mechanical ventilation; WBC: White blood cells; LYMPH: Lymphocyte count; CPK: Creatine phosphokinase; LDH: Lactate dehydrogenase; CRP: C-reactive protein.

systemic inflammatory response[13].

The extent of vertical transmission (in utero, intrapartum, early postnatal period) remains inconclusive and fluctuates between 1.6% and 6.3%, while the overall rate of congenital infection has been reported to be 2.7%[14,15]. Severe COVID-19, ICU admission or death of the mother, and postnatal infection have all been recognized as risk factors for mother-to-child transmission[14]. To this end, specific strains of the SARS-COV-2 may theoretically increase the likelihood of vertical transmission by causing more severe maternal illness. This is proven for the delta, gamma, and alpha strains. On the other hand, omicron strain results in less placental injury and might be associated with a lower risk of vertical transmission[16]. The median gestational week at the time of presentation at our cohort was found to be 36 weeks (IQR,

24-35), meaning that most of the pregnant women presenting to our center were infected in the third semester of their pregnancy (n = 46, 76.7%); thus, no birth defects would be likely at least attributed to SARS-CoV-2 considering almost completion of organogenesis by the time of infection. Nonetheless, results from an international retrospective cohort study that compared obstetric and neonatal outcomes of SARS-CoV-2-positive patients according to gestational age at the time of infection, showed that maternal infection after 20 weeks of gestation increased the risk for a composite of adverse obstetric outcomes, and maternal infection after 26 weeks increased the risk for a composite of adverse neonatal outcomes, in contrast to earlier infection during pregnancy[17]. Global data have shown that the risk of miscarriage or congenital anomalies does not seem to be increased above baseline in COVID-19 pregnant women, with an overall miscarriage rate of 11%[18-24]. Pre-term delivery was observed in almost 30% of our patients, which could be partly attributed to higher stress during the pandemic and alterations in maternity services[25,26]. However, in general, increased pre-term and moderately increased cesarean section rates have been observed among pregnancies with COVID-19[27-29]. In addition, a study noted a decline in vaginal delivery rates which could be due to the perception that opting for cesarean deliveries could potentially reduce the likelihood of COVID-19 transmission[30]. Nonetheless, the limitation of inability to distinguish between spontaneous and iatrogenic preterm birth persists in many studies. As also noted by these authors, it seems that many third-trimester cases are delivered by planned cesarean, pre-emptively driven by the unproven belief that the management of severe maternal respiratory disease would be improved by delivery. Thus, although spontaneous pre-term deliveries do occur, the reason for the observed rise in pre-term and cesarean section rates seems to be multifactorial[31-33]. Similarly, rates of stillbirth, even though they remained low, cannot be detached from potential co-founders, including disruptions to maternal care and maternal supportive services[25,26,34].

The therapeutic management of the pregnant individuals with COVID-19 does not differ significantly compared to the usual management of the non-pregnant COVID-19 patients, according to the current guidelines. However, there are some exceptions regarding the pharmacological parameters of the treatment due to the differences in volume distribution, the potential toxicities for the fetus, the existence of the vertical transmission, and the lactation and the post-natal care in general. In total, only 21.6% of our cohort received remdesivir with favorable results and no adverse perinatal outcomes**.** Experience in therapy has mostly come from the compassionate use program and the Gilead Global Safety Database[35,36]. Compassionate use of remdesivir among 86 pregnant women hospitalized with COVID-19 between March 21, 2020, and June 16, 2020, was correlated with high rates of recovery, while rapid deployment valve (RDV) did not raise any new safety signals[35,37]. Early RDV administration was linked to better clinical outcomes, including lower rates of ICU admission and decreased progression to critical disease, prompt improvement, and recovery by day 7 in pregnant individuals hospitalized with COVID-19[38]. In a similar way, early RDV administration was linked to better pregnancy outcomes, such as significantly lower rates of preterm delivery and COVID-19-related maternal death in pregnant patients admitted to hospital[38,39]. A systematic review and a meta-analysis further supported RDV's promising results in pregnant women with COVID-19 infection, with elevated transaminases as the only notable adverse event, requiring monitoring[40,41].

In our cohort of patients, 41.6% received corticosteroids, the majority of whom were in need of hospitalization. Dexamethasone was associated with a decrease mortality in COVID-19 patients requiring oxygen therapy, thus embedded in international guidelines since the beginning of the pandemic[9,42]. Nonetheless, from the trials that followed, pregnant patients were included only in REMAP-CAP (number of pregnant patients unknown)[43] and RECOVERY (0.06% of participants were pregnant)[42]. RECOVERY trial compared higher dose corticosteroids with standard dose and found that treatment with higher doses leads to increased mortality in COVID-19 patients with hypoxia, who do not require ventilatory support. However, only 3 of the participants were pregnant[44]. The choice of corticosteroid during pregnancy has been based on whether the fetus or the mother was the intended recipient of the medication. In the former scenario, it is preferable for mothers to receive hydrocortisone, methylprednisolone, prednisolone, and prednisone, which are converted by placental enzymes into inactive metabolites and limit fetal exposure to 10%[45-47]. In the latter scenario, when the fetus is being treated, synthetic fluorinated corticosteroids, such dexamethasone, are chosen in order to stimulate fetal lung maturation[45-48]. Associations between corticosteroid administration and adverse pregnancy outcomes, including gestational diabetes, pre-eclampsia, intrauterine growth restriction, congenital malformations, and preterm birth, have been variably reported in the past[46-49]. Also, repeated courses of dexamethasone have been associated with long-term adverse outcomes, such as impaired neurodevelopment [50]. However, evaluating the safety of corticosteroids, and dexamethasone in particular, in this context is still difficult due to a number of confounders, including co-medications and the overrepresentation of high-risk pregnancies in these trials, which inevitably introduce bias[46].

At the time this cohort study was carried out, approval and experience with other agents including tocilizumab, anakinra, baricitinib, nirmatrelvir/ritonavir, tixagevimab/cilgavimab and molnupiravir was scarce or absent; hence we chose not to administer to our patients. Of note, to date, there are recommendations against the use of molnupiravir in pregnant patients due to reported fetal toxicity in animal studies unless there are no other options available and therapy is clearly indicated[9].

None of our patients was vaccinated even though recommendations clearly state that undergoing COVID-19 vaccination–preferably with a non-vector vaccine–should be carried out in all unvaccinated people planning pregnancy or those who are already pregnant[51]. The same applies to booster shots as well as to the updated COVID-19 vaccine [51]. Vaccination early in gestation appears to have higher benefit against maternal risk of COVID-19 related hospitalization, death, and pregnancy complications[52,53]. In a recent study, the effectiveness of mRNA vaccination against SARS-CoV-2 infection after the second dose was 89.5%, a lower risk of stillbirth was observed in the vaccinated cohort compared with the unvaccinated group, and there was no evidence of adverse maternal or neonatal outcomes[54]. Other authors reported that the stillbirth rate among vaccinated women was almost half compared to those who were unvaccinated[55]. In addition, a study noted that the increased pre-term birth risk, which was more pronounced during

the early years of the pandemic, disappeared in regions with high vaccination rates[56]. Completing the COVID-19 immunization series by the mother during pregnancy was also linked to a lower incidence of COVID-19 hospitalization in infants less than six months of age[57]. A recent meta-analysis provided further evidence that COVID-19 vaccination is safe and highly effective in preventing maternal SARS-CoV-2 infection without increasing the risk of adverse maternal and neonatal outcomes[58]. Research conducted in Scotland revealed that an overwhelming majority of SARS-CoV-2 infections, hospital visits and critical care admissions were due to unvaccinated pregnant women. Furthermore, all baby deaths reported were attributed to the same demographic[59]. Even with the predominance of the omicron variant, which is typically associated with less severe maternal and neonatal manifestations, the importance of vaccine administration remains significant as demonstrated by a recent study[55,60].

Study limitations

This study cohort was conducted mostly in the absence of available vaccine against SARS-CoV-2. Even when first vaccine regimens were approved, hesitancy prevailed among pregnant population similar to other vaccines, as also highlighted in our study that no vaccinated pregnant patients were included. Similarly, no oral antivirals were available at the time this study took place[61]. Moreover, our data were produced while non-omicron variants were prevailing. It remains to be seen whether similar manifestations and outcomes would be observed in presence of new variants, even though presence of delta variant was associated with more severe outcomes even among pregnant patients[62]. However, reported data thus far regarding infection with the omicron variant during pregnancy indicate that it results in less severe disease and fewer adverse maternal and neonatal outcomes[55,63].

CONCLUSION

For pregnant individuals, the risk of contracting severe disease from SARS-CoV-2 infection is elevated: It is essential for these patients to consult with a healthcare professional at any sign of symptoms. If hospitalization for COVID-19 is indicated for expecting mothers, medical treatment should take place in a facility equipped to monitor both maternal and fetal health. Early detection and management are paramount for optimal results regarding motherhood and newborn care outcomes.

FOOTNOTES

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REFERENCES

1 **Zambrano LD**, Ellington S, Strid P, Galang RR, Oduyebo T, Tong VT, Woodworth KR, Nahabedian JF 3rd, Azziz-Baumgartner E, Gilboa

SM, Meaney-Delman D; CDC COVID-19 Response Pregnancy and Infant Linked Outcomes Team. Update: Characteristics of Symptomatic Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status - United States, January 22-October 3, 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69**: 1641-1647 [PMID: 33151921 DOI: 10.15585/mmwr.mm6944e3]

2 **Villar J**, Gunier RB, Papageorghiou AT. Further Observations on Pregnancy Complications and COVID-19 Infection-Reply. *JAMA Pediatr* 2021; **175**: 1185-1186 [PMID: 34398219 DOI: 10.1001/jamapediatrics.2021.2606]

3 **Auger N**, Ukah UV, Wei SQ, Healy-Profitós J, Lo E, Dayan N. Impact of Covid-19 on risk of severe maternal morbidity. *Crit Care* 2023; **27**: 344 [PMID: 37670329 DOI: 10.1186/s13054-023-04584-6]

4 **Sentilhes L**, De Marcillac F, Jouffrieau C, Kuhn P, Thuet V, Hansmann Y, Ruch Y, Fafi-Kremer S, Deruelle P. Coronavirus disease 2019 in pregnancy was associated with maternal morbidity and preterm birth. *Am J Obstet Gynecol* 2020; **223**: 914.e1-914.e15 [PMID: 32553908 DOI: 10.1016/j.ajog.2020.06.022]

5 **Allotey J**, Stallings E, Bonet M, Yap M, Chatterjee S, Kew T, Debenham L, Llavall AC, Dixit A, Zhou D, Balaji R, Lee SI, Qiu X, Yuan M, Coomar D, Sheikh J, Lawson H, Ansari K, van Wely M, van Leeuwen E, Kostova E, Kunst H, Khalil A, Tiberi S, Brizuela V, Broutet N, Kara E, Kim CR, Thorson A, Oladapo OT, Mofenson L, Zamora J, Thangaratinam S; for PregCOV-19 Living Systematic Review Consortium. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. *BMJ* 2020; **370**: m3320 [PMID: 32873575 DOI: 10.1136/bmj.m3320]

6 **Aabakke AJM**, Petersen TG, Wøjdemann K, Ibsen MH, Jonsdottir F, Rønneberg E, Andersen CS, Hammer A, Clausen TD, Milbak J, Burmester L, Zethner R, Lindved B, Thorsen-Meyer A, Khalil MR, Henriksen B, Jønsson L, Andersen LLT, Karlsen KK, Pedersen ML, Hedermann G, Vestgaard M, Thisted D, Fallesen AN, Johansson JN, Møller DC, Dubietyte G, Andersson CB, Farlie R, Skaarup Knudsen AK, Hansen L, Hvidman L, Sørensen AN, Rathcke SL, Rubin KH, Petersen LK, Jørgensen JS, Krebs L, Bliddal M. Risk factors for and pregnancy outcomes after SARS-CoV-2 in pregnancy according to disease severity: A nationwide cohort study with validation of the SARS-CoV-2 diagnosis. *Acta Obstet Gynecol Scand* 2023; **102**: 282-293 [PMID: 36695168 DOI: 10.1111/aogs.14512]

7 **DeSisto CL**, Wallace B, Simeone RM, Polen K, Ko JY, Meaney-Delman D, Ellington SR. Risk for Stillbirth Among Women With and Without COVID-19 at Delivery Hospitalization - United States, March 2020-September 2021. *MMWR Morb Mortal Wkly Rep* 2021; **70**: 1640-1645 [PMID: 34818318 DOI: 10.15585/mmwr.mm7047e1]

8 **Metz TD**, Clifton RG, Hughes BL, Sandoval GJ, Grobman WA, Saade GR, Manuck TA, Longo M, Sowles A, Clark K, Simhan HN, Rouse DJ, Mendez-Figueroa H, Gyamfi-Bannerman C, Bailit JL, Costantine MM, Sehdev HM, Tita ATN, Macones GA; National Institute of Child Health and Human Development Maternal-Fetal Medicine Units (MFMU) Network. Association of SARS-CoV-2 Infection With Serious Maternal Morbidity and Mortality From Obstetric Complications. *JAMA* 2022; **327**: 748-759 [PMID: 35129581 DOI: 10.1001/jama.2022.1190]

9 Pregnancy | COVID-19 Treatment Guidelines. Accessed May 3, 2024. Available from: https://www.covid19treatmentguidelines.nih.gov/special-populations/pregnancy/

10 **Khan DSA**, Hamid LR, Ali A, Salam RA, Zuberi N, Lassi ZS, Das JK. Differences in pregnancy and perinatal outcomes among symptomatic versus asymptomatic COVID-19-infected pregnant women: a systematic review and meta-analysis. *BMC Pregnancy Childbirth* 2021; **21**: 801 [PMID: 34852783 DOI: 10.1186/s12884-021-04250-1]

11 **Wang H**, Li N, Sun C, Guo X, Su W, Song Q, Liang Q, Liang M, Ding X, Lowe S, Bentley R, Sun Y. The association between pregnancy and COVID-19: A systematic review and meta-analysis. *Am J Emerg Med* 2022; **56**: 188-195 [PMID: 35413655 DOI: 10.1016/j.ajem.2022.03.060]

12 **Dananché C**, Elias C, Hénaff L, Amour S, Kuczewski E, Gustin MP, Escuret V, Saadatian-Elahi M, Vanhems P. Baseline clinical features of COVID-19 patients, delay of hospital admission and clinical outcome: A complex relationship. *PLoS One* 2022; **17**: e0261428 [PMID: 34995292 DOI: 10.1371/journal.pone.0261428]

13 **Parasher A**. COVID-19: Current understanding of its Pathophysiology, Clinical presentation and Treatment. *Postgrad Med J* 2021; **97**: 312-320 [DOI: 10.1136/postgradmedj-2020-138577]

14 **Allotey J**, Chatterjee S, Kew T, Gaetano A, Stallings E, Fernández-García S, Yap M, Sheikh J, Lawson H, Coomar D, Dixit A, Zhou D, Balaji R, Littmoden M, King Y, Debenham L, Llavall AC, Ansari K, Sandhu G, Banjoko A, Walker K, O'Donoghue K, van Wely M, van Leeuwen E, Kostova E, Kunst H, Khalil A, Brizuela V, Broutet N, Kara E, Kim CR, Thorson A, Oladapo OT, Zamora J, Bonet M, Mofenson L, Thangaratinam S; PregCOV-19 Living Systematic Review Consortium. SARS-CoV-2 positivity in offspring and timing of mother-to-child transmission: living systematic review and meta-analysis. *BMJ* 2022; **376**: e067696 [PMID: 35296519 DOI: 10.1136/bmj-2021-067696]

15 **Peng Z**, Zhang J, Shi Y, Yi M. Research progresses in vertical transmission of SARS-CoV-2 among infants born to mothers with COVID-19. *Future Virol* 2022 [PMID: 35173797 DOI: 10.2217/fvl-2021-0213]

16 **De Luca D**, Vauloup-Fellous C, Benachi A, Vivanti A. Transmission of SARS-CoV-2 from mother to fetus or neonate: What to know and what to do? *Semin Fetal Neonatal Med* 2023; **28**: 101429 [PMID: 36935314 DOI: 10.1016/j.siny.2023.101429]

17 **Badr DA**, Picone O, Bevilacqua E, Carlin A, Meli F, Sibiude J, Mattern J, Fils JF, Mandelbrot L, Lanzone A, De Luca D, Jani JC, Vivanti AJ. Severe Acute Respiratory Syndrome Coronavirus 2 and Pregnancy Outcomes According to Gestational Age at Time of Infection. *Emerg Infect Dis* 2021; **27**: 2535-2543 [PMID: 34352196 DOI: 10.3201/eid2710.211394]

18 **Cosma S**, Carosso AR, Cusato J, Borella F, Carosso M, Bovetti M, Filippini C, D'Avolio A, Ghisetti V, Di Perri G, Benedetto C. Coronavirus disease 2019 and first-trimester spontaneous abortion: a case-control study of 225 pregnant patients. *Am J Obstet Gynecol* 2021; **224**: 391.e1-391.e7 [PMID: 33039396 DOI: 10.1016/j.ajog.2020.10.005]

19 **la Cour Freiesleben N**, Egerup P, Hviid KVR, Severinsen ER, Kolte AM, Westergaard D, Fich Olsen L, Prætorius L, Zedeler A, Christiansen AH, Nielsen JR, Bang D, Berntsen S, Ollé-López J, Ingham A, Bello-Rodríguez J, Storm DM, Ethelberg-Findsen J, Hoffmann ER, Wilken-Jensen C, Jørgensen FS, Westh H, Jørgensen HL, Nielsen HS. SARS-CoV-2 in first trimester pregnancy: a cohort study. *Hum Reprod* 2021; **36**: 40-47 [PMID: 33145598 DOI: 10.1093/humrep/deaa311]

20 **Rotshenker-Olshinka K**, Volodarsky-Perel A, Steiner N, Rubenfeld E, Dahan MH. COVID-19 pandemic effect on early pregnancy: are miscarriage rates altered, in asymptomatic women? *Arch Gynecol Obstet* 2021; **303**: 839-845 [PMID: 33169234 DOI: 10.1007/s00404-020-05848-0]

21 **Hernández-Díaz S**, Smith LH, Wyszynski DF, Rasmussen SA. First trimester COVID-19 and the risk of major congenital malformations-International Registry of Coronavirus Exposure in Pregnancy. *Birth Defects Res* 2022; **114**: 906-914 [PMID: 35929997 DOI: 10.1002/bdr2.2070]

22 **van Baar JAC**, Kostova EB, Allotey J, Thangaratinam S, Zamora JR, Bonet M, Kim CR, Mofenson LM, Kunst H, Khalil A, van Leeuwen E, Keijzer J, Strikwerda M, Clark B, Verschuuren M, Coomarasamy A, Goddijn M, van Wely M; PregCOV-19 Living Systematic Review Consortium. COVID-19 in pregnant women: a systematic review and meta-analysis on the risk and prevalence of pregnancy loss. *Hum Reprod*

Update 2024; **30**: 133-152 [PMID: 38016805 DOI: 10.1093/humupd/dmad030]

23 **Calvert C**, Carruthers J, Denny C, Donaghy J, Hopcroft LEM, Hopkins L, Goulding A, Lindsay L, McLaughlin T, Moore E, Taylor B, Loane M, Dolk H, Morris J, Auyeung B, Bhaskaran K, Gibbons CL, Katikireddi SV, O'Leary M, McAllister D, Shi T, Simpson CR, Robertson C, Sheikh A, Stock SJ, Wood R. A population-based matched cohort study of major congenital anomalies following COVID-19 vaccination and SARS-CoV-2 infection. *Nat Commun* 2023; **14**: 107 [PMID: 36609574 DOI: 10.1038/s41467-022-35771-8]

24 **Reppucci ML**, Kaizer AM, Prendergast C, Acker SN, Mandell EW, Euser AG, Diaz-Miron J. Incidence of congenital complications related to COVID-19 infection during pregnancy. *J Neonatal Perinatal Med* 2023; **16**: 227-234 [PMID: 37092239 DOI: 10.3233/NPM-221122]

25 **Shah PS**, Ye XY, Yang J, Campitelli MA. Preterm birth and stillbirth rates during the COVID-19 pandemic: a population-based cohort study. *CMAJ* 2021; **193**: E1164-E1172 [PMID: 34344771 DOI: 10.1503/cmaj.210081]

26 **Srivastava K**. Covid-19: Why has India had a spike in stillbirths? *BMJ* 2021; **374**: n2133 [PMID: 34479855 DOI: 10.1136/bmj.n2133]

27 **Katz D**, Bateman BT, Kjaer K, Turner DP, Spence NZ, Habib AS, George RB, Toledano RD, Grant G, Madden HE, Butwick AJ, Lynde G, Minehart RD, Beilin Y, Houle TT, Sharpe EE, Kodali B, Bharadwaj S, Farber MK, Palanisamy A, Prabhu M, Gonzales NY, Landau R, Leffert L. The Society for Obstetric Anesthesia and Perinatology Coronavirus Disease 2019 Registry: An Analysis of Outcomes Among Pregnant Women Delivering During the Initial Severe Acute Respiratory Syndrome Coronavirus-2 Outbreak in the United States. *Anesth Analg* 2021; **133**: 462-473 [PMID: 33830956 DOI: 10.1213/ANE.0000000000005592]

28 **Jering KS**, Claggett BL, Cunningham JW, Rosenthal N, Vardeny O, Greene MF, Solomon SD. Clinical Characteristics and Outcomes of Hospitalized Women Giving Birth With and Without COVID-19. *JAMA Intern Med* 2021; **181**: 714-717 [PMID: 33449067 DOI: 10.1001/jamainternmed.2020.9241]

29 **Gharacheh M**, Kalan ME, Khalili N, Ranjbar F. An increase in cesarean section rate during the first wave of COVID-19 pandemic in Iran. *BMC Public Health* 2023; **23**: 936 [PMID: 37226119 DOI: 10.1186/s12889-023-15907-1]

30 **Feldman KM**, Jagannatham S, Hussain FN, Strauss TS, Al-ibraheemi Z, Ashmead G, Brustman L. 12 Observations from an inner city hospital during COVID-19: preterm birth rate and mode of delivery. *Am J Obstet Gynecol* 2021; **224**: S8 [DOI: 10.1016/j.ajog.2020.12.024]

31 **Smith LH**, Dollinger CY, VanderWeele TJ, Wyszynski DF, Hernández-Díaz S. Timing and severity of COVID-19 during pregnancy and risk of preterm birth in the International Registry of Coronavirus Exposure in Pregnancy. *BMC Pregnancy Childbirth* 2022; **22**: 775 [PMID: 36258186 DOI: 10.1186/s12884-022-05101-3]

32 **Mak AHM**, Cicero S, Hui PW. Impact of COVID-19 pandemic on preterm delivery. *J Obstet Gynaecol Res* 2023; **49**: 1539-1544 [PMID: 36988181 DOI: 10.1111/jog.15643]

33 **Piroozi B**, Moradi G, Khoramipoor K, Mahmoodi H, Zandvakili F, Ebrazeh A, Shokri A, Moradpour F. Is the surge in cesarean section rates during the COVID-19 pandemic truly substantiated? *BMC Pregnancy Childbirth* 2024; **24**: 275 [PMID: 38609859 DOI: 10.1186/s12884-024-06492-1]

34 **Schwartz DA**, Mulkey SB, Roberts DJ. SARS-CoV-2 placentitis, stillbirth, and maternal COVID-19 vaccination: clinical-pathologic correlations. *Am J Obstet Gynecol* 2023; **228**: 261-269 [PMID: 36243041 DOI: 10.1016/j.ajog.2022.10.001]

35 **Burwick RM**, Yawetz S, Stephenson KE, Collier AY, Sen P, Blackburn BG, Kojic EM, Hirshberg A, Suarez JF, Sobieszczyk ME, Marks KM, Mazur S, Big C, Manuel O, Morlin G, Rose SJ, Naqvi M, Goldfarb IT, DeZure A, Telep L, Tan SK, Zhao Y, Hahambis T, Hindman J, Chokkalingam AP, Carter C, Das M, Osinusi AO, Brainard DM, Varughese TA, Kovalenko O, Sims MD, Desai S, Swamy G, Sheffield JS, Zash R, Short WR. Compassionate Use of Remdesivir in Pregnant Women With Severe Coronavirus Disease 2019. *Clin Infect Dis* 2021; **73**: e3996-e4004 [PMID: 33031500 DOI: 10.1093/cid/ciaa1466]

36 **Food and Drug Administration**. Center for drug evaluation and research Approval Package for. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/201292Orig1s001.pdf

37 **National Library of Medicine**. PK and Safety of Remdesivir for Treatment of COVID-19 in Pregnant and Non-Pregnant Women in the US. Accessed May 3, 2024. Available from: https://clinicaltrials.gov/study/NCT04582266?tab=results

38 **Eid J**, Abdelwahab M, Colburn N, Day S, Cackovic M, Rood KM, Costantine MM. Early Administration of Remdesivir and Intensive Care Unit Admission in Hospitalized Pregnant Individuals With Coronavirus Disease 2019 (COVID-19). *Obstet Gynecol* 2022; **139**: 619-621 [PMID: 35134032 DOI: 10.1097/AOG.0000000000004734]

39 **Nasrallah S**, Nguyen AQ, Hitchings L, Wang JQ, Hamade S, Maxwell GL, Khoury A, Gomez LM. Pharmacological treatment in pregnant women with moderate symptoms of coronavirus disease 2019 (COVID-19) pneumonia. *J Matern Fetal Neonatal Med* 2022; **35**: 5970-5977 [PMID: 33771091 DOI: 10.1080/14767058.2021.1903426]

40 **Budi DS**, Pratama NR, Wafa IA, Putra M, Wardhana MP, Wungu CDK. Remdesivir for pregnancy: A systematic review of antiviral therapy for COVID-19. *Heliyon* 2022; **8**: e08835 [PMID: 35128114 DOI: 10.1016/j.heliyon.2022.e08835]

41 **Di Gennaro F**, Guido G, Frallonardo L, Segala FV, De Nola R, Damiani GR, De Vita E, Totaro V, Barbagallo M, Nicastri E, Vimercati A, Cicinelli E, Liuzzi G, Veronese N, Saracino A. Efficacy and safety of therapies for COVID-19 in pregnancy: a systematic review and meta-analysis. *BMC Infect Dis* 2023; **23**: 776 [PMID: 37946100 DOI: 10.1186/s12879-023-08747-2]

42 **RECOVERY Collaborative Group**, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A, Elmahi E, Prudon B, Green C, Felton T, Chadwick D, Rege K, Fegan C, Chappell LC, Faust SN, Jaki T, Jeffery K, Montgomery A, Rowan K, Juszczak E, Baillie JK, Haynes R, Landray MJ. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med* 2021; **384**: 693-704 [PMID: 32678530 DOI: 10.1056/NEJMoa2021436]

43 **Angus DC**, Derde L, Al-Beidh F, Annane D, Arabi Y, Beane A, van Bentum-Puijk W, Berry L, Bhimani Z, Bonten M, Bradbury C, Brunkhorst F, Buxton M, Buzgau A, Cheng AC, de Jong M, Detry M, Estcourt L, Fitzgerald M, Goossens H, Green C, Haniffa R, Higgins AM, Horvat C, Hullegie SJ, Kruger P, Lamontagne F, Lawler PR, Linstrum K, Litton E, Lorenzi E, Marshall J, McAuley D, McGlothin A, McGuinness S, McVerry B, Montgomery S, Mouncey P, Murthy S, Nichol A, Parke R, Parker J, Rowan K, Sanil A, Santos M, Saunders C, Seymour C, Turner A, van de Veerdonk F, Venkatesh B, Zarychanski R, Berry S, Lewis RJ, McArthur C, Webb SA, Gordon AC; Writing Committee for the REMAP-CAP Investigators, Al-Beidh F, Angus D, Annane D, Arabi Y, van Bentum-Puijk W, Berry S, Beane A, Bhimani Z, Bonten M, Bradbury C, Brunkhorst F, Buxton M, Cheng A, De Jong M, Derde L, Estcourt L, Goossens H, Gordon A, Green C, Haniffa R, Lamontagne F, Lawler P, Litton E, Marshall J, McArthur C, McAuley D, McGuinness S, McVerry B, Montgomery S, Mouncey P, Murthy S, Nichol A, Parke R, Rowan K, Seymour C, Turner A, van de Veerdonk F, Webb S, Zarychanski R, Campbell L, Forbes A, Gattas D, Heritier S, Higgins L, Kruger P, Peake S, Presneill J, Seppelt I, Trapani T, Young P, Bagshaw S, Daneman N, Ferguson N, Misak C, Santos M, Hullegie S, Pletz M, Rohde G, Rowan K, Alexander B, Basile K, Girard T, Horvat C, Huang D, Linstrum K, Vates J, Beasley R, Fowler R, McGloughlin S, Morpeth S, Paterson D, Venkatesh B, Uyeki T, Baillie K, Duffy E, Fowler R, Hills T, Orr K, Patanwala A, Tong S, Netea M,

Bihari S, Carrier M, Fergusson D, Goligher E, Haidar G, Hunt B, Kumar A, Laffan M, Lawless P, Lother S, McCallum P, Middeldopr S, McQuilten Z, Neal M, Pasi J, Schutgens R, Stanworth S, Turgeon A, Weissman A, Adhikari N, Anstey M, Brant E, de Man A, Lamonagne F, Masse MH, Udy A, Arnold D, Begin P, Charlewood R, Chasse M, Coyne M, Cooper J, Daly J, Gosbell I, Harvala-Simmonds H, Hills T, MacLennan S, Menon D, McDyer J, Pridee N, Roberts D, Shankar-Hari M, Thomas H, Tinmouth A, Triulzi D, Walsh T, Wood E, Calfee C, O'Kane C, Shyamsundar M, Sinha P, Thompson T, Young I, Bihari S, Hodgson C, Laffey J, McAuley D, Orford N, Neto A, Detry M, Fitzgerald M, Lewis R, McGlothlin A, Sanil A, Saunders C, Berry L, Lorenzi E, Miller E, Singh V, Zammit C, van Bentum Puijk W, Bouwman W, Mangindaan Y, Parker L, Peters S, Rietveld I, Raymakers K, Ganpat R, Brillinger N, Markgraf R, Ainscough K, Brickell K, Anjum A, Lane JB, Richards-Belle A, Saull M, Wiley D, Bion J, Connor J, Gates S, Manax V, van der Poll T, Reynolds J, van Beurden M, Effelaar E, Schotsman J, Boyd C, Harland C, Shearer A, Wren J, Clermont G, Garrard W, Kalchthaler K, King A, Ricketts D, Malakoutis S, Marroquin O, Music E, Quinn K, Cate H, Pearson K, Collins J, Hanson J, Williams P, Jackson S, Asghar A, Dyas S, Sutu M, Murphy S, Williamson D, Mguni N, Potter A, Porter D, Goodwin J, Rook C, Harrison S, Williams H, Campbell H, Lomme K, Williamson J, Sheffield J, van't Hoff W, McCracken P, Young M, Board J, Mart E, Knott C, Smith J, Boschert C, Affleck J, Ramanan M, D'Souza R, Pateman K, Shakih A, Cheung W, Kol M, Wong H, Shah A, Wagh A, Simpson J, Duke G, Chan P, Cartner B, Hunter S, Laver R, Shrestha T, Regli A, Pellicano A, McCullough J, Tallott M, Kumar N, Panwar R, Brinkerhoff G, Koppen C, Cazzola F, Brain M, Mineall S, Fischer R, Biradar V, Soar N, White H, Estensen K, Morrison L, Smith J, Cooper M, Health M, Shehabi Y, Al-Bassam W, Hulley A, Whitehead C, Lowrey J, Gresha R, Walsham J, Meyer J, Harward M, Venz E, Williams P, Kurenda C, Smith K, Smith M, Garcia R, Barge D, Byrne D, Byrne K, Driscoll A, Fortune L, Janin P, Yarad E, Hammond N, Bass F, Ashelford A, Waterson S, Wedd S, McNamara R, Buhr H, Coles J, Schweikert S, Wibrow B, Rauniyar R, Myers E, Fysh E, Dawda A, Mevavala B, Litton E, Ferrier J, Nair P, Buscher H, Reynolds C, Santamaria J, Barbazza L, Homes J, Smith R, Murray L, Brailsford J, Forbes L, Maguire T, Mariappa V, Smith J, Simpson S, Maiden M, Bone A, Horton M, Salerno T, Sterba M, Geng W, Depuydt P, De Waele J, De Bus L, Fierens J, Bracke S, Reeve B, Dechert W, Chassé M, Carrier FM, Boumahni D, Benettaib F, Ghamraoui A, Bellemare D, Cloutier È, Francoeur C, Lamontagne F, D'Aragon F, Carbonneau E, Leblond J, Vazquez-Grande G, Marten N, Wilson M, Albert M, Serri K, Cavayas A, Duplaix M, Williams V, Rochwerg B, Karachi T, Oczkowski S, Centofanti J, Millen T, Duan E, Tsang J, Patterson L, English S, Watpool I, Porteous R, Miezitis S, McIntyre L, Brochard L, Burns K, Sandhu G, Khalid I, Binnie A, Powell E, McMillan A, Luk T, Aref N, Andric Z, Cviljevic S, Đimoti R, Zapalac M, Mirković G, Baršić B, Kutleša M, Kotarski V, Vujaklija Brajković A, Babel J, Sever H, Dragija L, Kušan I, Vaara S, Pettilä L, Heinonen J, Kuitunen A, Karlsson S, Vahtera A, Kiiski H, Ristimäki S, Azaiz A, Charron C, Godement M, Geri G, Vieillard-Baron A, Pourcine F, Monchi M, Luis D, Mercier R, Sagnier A, Verrier N, Caplin C, Siami S, Aparicio C, Vautier S, Jeblaoui A, Fartoukh M, Courtin L, Labbe V, Leparco C, Muller G, Nay MA, Kamel T, Benzekri D, Jacquier S, Mercier E, Chartier D, Salmon C, Dequin P, Schneider F, Morel G, L'Hotellier S, Badie J, Berdaguer FD, Malfroy S, Mezher C, Bourgoin C, Megarbane B, Voicu S, Deye N, Malissin I, Sutterlin L, Guitton C, Darreau C, Landais M, Chudeau N, Robert A, Moine P, Heming N, Maxime V, Bossard I, Nicholier TB, Colin G, Zinzoni V, Maquigneau N, Finn A, Kreß G, Hoff U, Friedrich Hinrichs C, Nee J, Pletz M, Hagel S, Ankert J, Kolanos S, Bloos F, Petros S, Pasieka B, Kunz K, Appelt P, Schütze B, Kluge S, Nierhaus A, Jarczak D, Roedl K, Weismann D, Frey A, Klinikum Neukölln V, Reill L, Distler M, Maselli A, Bélteczki J, Magyar I, Fazekas Á, Kovács S, Szőke V, Szigligeti G, Leszkoven J, Collins D, Breen P, Frohlich S, Whelan R, McNicholas B, Scully M, Casey S, Kernan M, Doran P, O'Dywer M, Smyth M, Hayes L, Hoiting O, Peters M, Rengers E, Evers M, Prinssen A, Bosch Ziekenhuis J, Simons K, Rozendaal W, Polderman F, de Jager P, Moviat M, Paling A, Salet A, Rademaker E, Peters AL, de Jonge E, Wigbers J, Guilder E, Butler M, Cowdrey KA, Newby L, Chen Y, Simmonds C, McConnochie R, Ritzema Carter J, Henderson S, Van Der Heyden K, Mehrtens J, Williams T, Kazemi A, Song R, Lai V, Girijadevi D, Everitt R, Russell R, Hacking D, Buehner U, Williams E, Browne T, Grimwade K, Goodson J, Keet O, Callender O, Martynoga R, Trask K, Butler A, Schischka L, Young C, Lesona E, Olatunji S, Robertson Y, José N, Amaro dos Santos Catorze T, de Lima Pereira TNA, Neves Pessoa LM, Castro Ferreira RM, Pereira Sousa Bastos JM, Aysel Florescu S, Stanciu D, Zaharia MF, Kosa AG, Codreanu D, Marabi Y, Al Qasim E, Moneer Hagazy M, Al Swaidan L, Arishi H, Muñoz-Bermúdez R, Marin-Corral J, Salazar Degracia A, Parrilla Gómez F, Mateo López MI, Rodriguez Fernandez J, Cárcel Fernández S, Carmona Flores R, León López R, de la Fuente Martos C, Allan A, Polgarova P, Farahi N, McWilliam S, Hawcutt D, Rad L, O'Malley L, Whitbread J, Kelsall O, Wild L, Thrush J, Wood H, Austin K, Donnelly A, Kelly M, O'Kane S, McClintock D, Warnock M, Johnston P, Gallagher LJ, Mc Goldrick C, Mc Master M, Strzelecka A, Jha R, Kalogirou M, Ellis C, Krishnamurthy V, Deelchand V, Silversides J, McGuigan P, Ward K, O'Neill A, Finn S, Phillips B, Mullan D, Oritz-Ruiz de Gordoa L, Thomas M, Sweet K, Grimmer L, Johnson R, Pinnell J, Robinson M, Gledhill L, Wood T, Morgan M, Cole J, Hill H, Davies M, Antcliffe D, Templeton M, Rojo R, Coghlan P, Smee J, Mackay E, Cort J, Whileman A, Spencer T, Spittle N, Kasipandian V, Patel A, Allibone S, Genetu RM, Ramali M, Ghosh A, Bamford P, London E, Cawley K, Faulkner M, Jeffrey H, Smith T, Brewer C, Gregory J, Limb J, Cowton A, O'Brien J, Nikitas N, Wells C, Lankester L, Pulletz M, Williams P, Birch J, Wiseman S, Horton S, Alegria A, Turki S, Elsefi T, Crisp N, Allen L, McCullagh I, Robinson P, Hays C, Babio-Galan M, Stevenson H, Khare D, Pinder M, Selvamoni S, Gopinath A, Pugh R, Menzies D, Mackay C, Allan E, Davies G, Puxty K, McCue C, Cathcart S, Hickey N, Ireland J, Yusuff H, Isgro G, Brightling C, Bourne M, Craner M, Watters M, Prout R, Davies L, Pegler S, Kyeremeh L, Arbane G, Wilson K, Gomm L, Francia F, Brett S, Sousa Arias S, Elin Hall R, Budd J, Small C, Birch J, Collins E, Henning J, Bonner S, Hugill K, Cirstea E, Wilkinson D, Karlikowski M, Sutherland H, Wilhelmsen E, Woods J, North J, Sundaran D, Hollos L, Coburn S, Walsh J, Turns M, Hopkins P, Smith J, Noble H, Depante MT, Clarey E, Laha S, Verlander M, Williams A, Huckle A, Hall A, Cooke J, Gardiner-Hill C, Maloney C, Qureshi H, Flint N, Nicholson S, Southin S, Nicholson A, Borgatta B, Turner-Bone I, Reddy A, Wilding L, Chamara Warnapura L, Agno Sathianathan R, Golden D, Hart C, Jones J, Bannard-Smith J, Henry J, Birchall K, Pomeroy F, Quayle R, Makowski A, Misztal B, Ahmed I, KyereDiabour T, Naiker K, Stewart R, Mwaura E, Mew L, Wren L, Willams F, Innes R, Doble P, Hutter J, Shovelton C, Plumb B, Szakmany T, Hamlyn V, Hawkins N, Lewis S, Dell A, Gopal S, Ganguly S, Smallwood A, Harris N, Metherell S, Lazaro JM, Newman T, Fletcher S, Nortje J, Fottrell-Gould D, Randell G, Zaman M, Elmahi E, Jones A, Hall K, Mills G, Ryalls K, Bowler H, Sall J, Bourne R, Borrill Z, Duncan T, Lamb T, Shaw J, Fox C, Moreno Cuesta J, Xavier K, Purohit D, Elhassan M, Bakthavatsalam D, Rowland M, Hutton P, Bashyal A, Davidson N, Hird C, Chhablani M, Phalod G, Kirkby A, Archer S, Netherton K, Reschreiter H, Camsooksai J, Patch S, Jenkins S, Pogson D, Rose S, Daly Z, Brimfield L, Claridge H, Parekh D, Bergin C, Bates M, Dasgin J, McGhee C, Sim M, Hay SK, Henderson S, Phull MK, Zaidi A, Pogreban T, Rosaroso LP, Harvey D, Lowe B, Meredith M, Ryan L, Hormis A, Walker R, Collier D, Kimpton S, Oakley S, Rooney K, Rodden N, Hughes E, Thomson N, McGlynn D, Walden A, Jacques N, Coles H, Tilney E, Vowell E, Schuster-Bruce M, Pitts S, Miln R, Purandare L, Vamplew L, Spivey M, Bean S, Burt K, Moore L, Day C, Gibson C, Gordon E, Zitter L, Keenan S, Baker E, Cherian S, Cutler S, Roynon-Reed A, Harrington K, Raithatha A, Bauchmuller K, Ahmad N, Grecu I, Trodd D, Martin J, Wrey Brown C, Arias AM, Craven T, Hope D, Singleton J, Clark S, Rae N, Welters I, Hamilton DO, Williams K, Waugh V, Shaw D, Puthucheary Z, Martin T, Santos F, Uddin R, Somerville A, Tatham KC, Jhanji S, Black E, Dela Rosa A, Howle R, Tully R, Drummond A, Dearden J, Philbin J, Munt S, Vuylsteke A, Chan C, Victor S, Matsa R, Gellamucho M, Creagh-Brown B, Tooley J, Montague L, De Beaux F, Bullman L, Kersiake I, Demetriou C, Mitchard S, Ramos L, White K, Donnison P, Johns M, Casey R, Mattocks L, Salisbury S, Dark P,

Claxton A, McLachlan D, Slevin K, Lee S, Hulme J, Joseph S, Kinney F, Senya HJ, Oborska A, Kayani A, Hadebe B, Orath Prabakaran R, Nichols L, Thomas M, Worner R, Faulkner B, Gendall E, Hayes K, Hamilton-Davies C, Chan C, Mfuko C, Abbass H, Mandadapu V, Leaver S, Forton D, Patel K, Paramasivam E, Powell M, Gould R, Wilby E, Howcroft C, Banach D, Fernández de Pinedo Artaraz Z, Cabreros L, White I, Croft M, Holland N, Pereira R, Zaki A, Johnson D, Jackson M, Garrard H, Juhaz V, Roy A, Rostron A, Woods L, Cornell S, Pillai S, Harford R, Rees T, Ivatt H, Sundara Raman A, Davey M, Lee K, Barber R, Chablani M, Brohi F, Jagannathan V, Clark M, Purvis S, Wetherill B, Dushianthan A, Cusack R, de Courcy-Golder K, Smith S, Jackson S, Attwood B, Parsons P, Page V, Zhao XB, Oza D, Rhodes J, Anderson T, Morris S, Xia Le Tai C, Thomas A, Keen A, Digby S, Cowley N, Wild L, Southern D, Reddy H, Campbell A, Watkins C, Smuts S, Touma O, Barnes N, Alexander P, Felton T, Ferguson S, Sellers K, Bradley-Potts J, Yates D, Birkinshaw I, Kell K, Marshall N, Carr-Knott L, Summers C. Effect of Hydrocortisone on Mortality and Organ Support in Patients With Severe COVID-19: The REMAP-CAP COVID-19 Corticosteroid Domain Randomized Clinical Trial. *JAMA* 2020; **324**: 1317-1329 [PMID: 32876697 DOI: 10.1001/jama.2020.17022]

44 **RECOVERY Collaborative Group**. Higher dose corticosteroids in patients admitted to hospital with COVID-19 who are hypoxic but not requiring ventilatory support (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet* 2023; **401**: 1499-1507 [PMID: 37060915 DOI: 10.1016/S0140-6736(23)00510-X]

45 **Kemp MW**, Newnham JP, Challis JG, Jobe AH, Stock SJ. The clinical use of corticosteroids in pregnancy. *Hum Reprod Update* 2016; **22**: 240-259 [PMID: 26590298 DOI: 10.1093/humupd/dmv047]

46 **Flint J**, Panchal S, Hurrell A, van de Venne M, Gayed M, Schreiber K, Arthanari S, Cunningham J, Flanders L, Moore L, Crossley A, Purushotham N, Desai A, Piper M, Nisar M, Khamashta M, Williams D, Gordon C, Giles I; BSR and BHPR Standards, Guidelines and Audit Working Group. BSR and BHPR guideline on prescribing drugs in pregnancy and breastfeeding-Part I: standard and biologic disease modifying anti-rheumatic drugs and corticosteroids. *Rheumatology (Oxford)* 2016; **55**: 1693-1697 [PMID: 26750124 DOI: 10.1093/rheumatology/kev404]

47 **WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group**, Sterne JAC, Murthy S, Diaz JV, Slutsky AS, Villar J, Angus DC, Annane D, Azevedo LCP, Berwanger O, Cavalcanti AB, Dequin PF, Du B, Emberson J, Fisher D, Giraudeau B, Gordon AC, Granholm A, Green C, Haynes R, Heming N, Higgins JPT, Horby P, Jüni P, Landray MJ, Le Gouge A, Leclerc M, Lim WS, Machado FR, McArthur C, Meziani F, Møller MH, Perner A, Petersen MW, Savovic J, Tomazini B, Veiga VC, Webb S, Marshall JC. Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis. *JAMA* 2020; **324**: 1330-1341 [PMID: 32876694 DOI: 10.1001/jama.2020.17023]

48 **Committee on Obstetric Practice**. Committee Opinion No. 713: Antenatal Corticosteroid Therapy for Fetal Maturation. *Obstet Gynecol* 2017; **130**: e102-e109 [PMID: 28742678 DOI: 10.1097/AOG.0000000000002237]

49 **Bandoli G**, Palmsten K, Forbess Smith CJ, Chambers CD. A Review of Systemic Corticosteroid Use in Pregnancy and the Risk of Select Pregnancy and Birth Outcomes. *Rheum Dis Clin North Am* 2017; **43**: 489-502 [PMID: 28711148 DOI: 10.1016/j.rdc.2017.04.013]

50 **Ninan K**, Liyanage SK, Murphy KE, Asztalos EV, Mcdonald SD. Evaluation of Long-term Outcomes Associated With Preterm Exposure to Antenatal Corticosteroids. *JAMA Pediatr* 2022; **176**: e220483 [DOI: 10.1001/jamapediatrics.2022.0483]

51 **American College of Obstetricians and Gynecologists**. COVID-19 Vaccination Considerations for Obstetric–Gynecologic Care. Accessed May 3, 2024. Available from: https://www.acog.org/clinical/clinical-guidance/practice-advisory/articles/2020/12/covid-19-vaccination-considerations-for-obstetric-gynecologic-care?utm_source=higher-logic&utm_medium=email&utm_content=sept-14&utm_campaign=acog2022-digest

52 **Rottenstreich A**, Zarbiv G, Oiknine-Djian E, Vorontsov O, Zigron R, Kleinstern G, Wolf DG, Porat S. The Effect of Gestational Age at BNT162b2 mRNA Vaccination on Maternal and Neonatal Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antibody Levels. *Clin Infect Dis* 2022; **75**: e603-e610 [PMID: 35171998 DOI: 10.1093/cid/ciac135]

53 **Yang YJ**, Murphy EA, Singh S, Sukhu AC, Wolfe I, Adurty S, Eng D, Yee J, Mohammed I, Zhao Z, Riley LE, Prabhu M. Association of Gestational Age at Coronavirus Disease 2019 (COVID-19) Vaccination, History of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection, and a Vaccine Booster Dose With Maternal and Umbilical Cord Antibody Levels at Delivery. *Obstet Gynecol* 2022; **139**: 373-380 [PMID: 34963127 DOI: 10.1097/AOG.0000000000004693]

54 **Prasad S**, Kalafat E, Blakeway H, Townsend R, O'Brien P, Morris E, Draycott T, Thangaratinam S, Le Doare K, Ladhani S, von Dadelszen P, Magee LA, Heath P, Khalil A. Systematic review and meta-analysis of the effectiveness and perinatal outcomes of COVID-19 vaccination in pregnancy. *Nat Commun* 2022; **13**: 2414 [PMID: 35538060 DOI: 10.1038/s41467-022-30052-w]

55 **Iannaccone A**, Gellhaus A, Reisch B, Dzietko M, Schmidt B, Mavarani L, Kraft K, Andresen K, Kimmig R, Pecks U, Schleußner E. The Importance of Vaccination, Variants and Time Point of SARS-CoV-2 Infection in Pregnancy for Stillbirth and Preterm Birth Risk: An Analysis of the CRONOS Register Study. *J Clin Med* 2024; **13** [PMID: 38541748 DOI: 10.3390/jcm13061522]

56 **Torche F**, Nobles J. Vaccination, immunity, and the changing impact of COVID-19 on infant health. *Proc Natl Acad Sci U S A* 2023; **120**: e2311573120 [PMID: 38011548 DOI: 10.1073/pnas.2311573120]

57 **Halasa NB**, Olson SM, Staat MA, Newhams MM, Price AM, Pannaraj PS, Boom JA, Sahni LC, Chiotos K, Cameron MA, Bline KE, Hobbs CV, Maddux AB, Coates BM, Michelson KN, Heidemann SM, Irby K, Nofziger RA, Mack EH, Smallcomb L, Schwartz SP, Walker TC, Gertz SJ, Schuster JE, Kamidani S, Tarquinio KM, Bhumbra SS, Maamari M, Hume JR, Crandall H, Levy ER, Zinter MS, Bradford TT, Flori HR, Cullimore ML, Kong M, Cvijanovich NZ, Gilboa SM, Polen KN, Campbell AP, Randolph AG, Patel MM; Overcoming Covid-19 Investigators. Maternal Vaccination and Risk of Hospitalization for Covid-19 among Infants. *N Engl J Med* 2022; **387**: 109-119 [PMID: 35731908 DOI: 10.1056/NEJMoa2204399]

58 **Rahmati M**, Yon DK, Lee SW, Butler L, Koyanagi A, Jacob L, Shin JI, Smith L. Effects of COVID-19 vaccination during pregnancy on SARS-CoV-2 infection and maternal and neonatal outcomes: A systematic review and meta-analysis. *Rev Med Virol* 2023; **33**: e2434 [PMID: 36896895 DOI: 10.1002/rmv.2434]

59 **Stock SJ**, Carruthers J, Calvert C, Denny C, Donaghy J, Goulding A, Hopcroft LEM, Hopkins L, McLaughlin T, Pan J, Shi T, Taylor B, Agrawal U, Auyeung B, Katikireddi SV, McCowan C, Murray J, Simpson CR, Robertson C, Vasileiou E, Sheikh A, Wood R. SARS-CoV-2 infection and COVID-19 vaccination rates in pregnant women in Scotland. *Nat Med* 2022; **28**: 504-512 [PMID: 35027756 DOI: 10.1038/s41591-021-01666-2]

60 **Barros FC**, Gunier RB, Rego A, Sentilhes L, Rauch S, Gandino S, Teji JS, Thornton JG, Kachikis AB, Nieto R, Craik R, Cavoretto PI, Winsey A, Roggero P, Rodriguez GB, Savasi V, Kalafat E, Giuliani F, Fabre M, Benski AC, Coronado-Zarco IA, Livio S, Ostrovska A, Maiz N, Castedo Camacho FR, Peterson A, Deruelle P, Giudice C, Casale RA, Salomon LJ, Soto Conti CP, Prefumo F, Mohamed Elbayoumy EZ, Vale M, Hernández V, Chandler K, Risso M, Marler E, Cáceres DM, Crespo GA, Ernawati E, Lipschuetz M, Ariff S, Takahashi K, Vecchiarelli C, Hubka T, Ikenoue S, Tavchioska G, Bako B, Ayede AI, Eskenazi B, Bhutta ZA, Kennedy SH, Papageorghiou AT, Villar J; INTERCOVID-

2022 International Consortium. Maternal vaccination against COVID-19 and neonatal outcomes during Omicron: INTERCOVID-2022 study. *Am J Obstet Gynecol* 2024 [PMID: 38367758 DOI: 10.1016/j.ajog.2024.02.008]

61 **Akinosoglou K**, Schinas G, Rigopoulos EA, Polyzou E, Tzouvelekis A, Adonakis G, Gogos C. COVID-19 Pharmacotherapy in Pregnancy: A Literature Review of Current Therapeutic Choices. *Viruses* 2023; **15** [PMID: 36992497 DOI: 10.3390/v15030787]

62 **Deng J**, Ma Y, Liu Q, Du M, Liu M, Liu J. Association of Infection with Different SARS-CoV-2 Variants during Pregnancy with Maternal and Perinatal Outcomes: A Systematic Review and Meta-Analysis. *Int J Environ Res Public Health* 2022; **19** [PMID: 36498007 DOI: 10.3390/ijerph192315932]

63 **Xiao H**, Chen C, Huang S, Zhang W, Cai S, Hou X, Luo Y, Lin Y. Effects of novel coronavirus Omicron variant infection on pregnancy outcomes: a retrospective cohort study from Guangzhou. *Front Med (Lausanne)* 2023; **10**: 1256080 [PMID: 38170092 DOI: 10.3389/fmed.2023.1256080]

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ORIGINAL ARTICLE

Retrospective Study

Candidemia chronicles: Retrospective analysis of candidemia epidemiology, species distribution, and antifungal susceptibility patterns in Bahrain

Nermin Kamal Saeed, Safiya Almusawi, Mohammed Al-Beltagi

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Abstract

BACKGROUND

Invasive fungal infections, particularly candidemia, pose significant clinical challenges globally. Understanding local epidemiology, species distribution, and antifungal susceptibility patterns is crucial for effective management despite regional variations.

AIM

To investigate the epidemiology, species distribution, antifungal susceptibility patterns, and associated risk factors of candidemia among patients in Bahrain from 2021 to 2023.

METHODS

This retrospective study analyzed demographic data, *Candida* species distribution, antifungal susceptibility profiles, and risk factors among candidemia patients treated at a tertiary care hospital in Bahrain over three years. Data was collected from medical records and analyzed using descriptive statistics.

RESULTS

A total of 430 candidemia cases were identified. The mean age of patients was 65.7 years, with a mortality rate of 85.5%. *Candida albicans* (*C. albicans*) was the most common species, followed by *Candida parapsilosis*, *Candida tropicalis (C. tropicalis)*, and emerging multidrug-resistant *Candida auris* (*C. auris*). Antifungal susceptibility varied across species, with declining susceptibility to azoles observed, particularly among *C. albicans* and *C. tropicalis*. Major risk factors included central venous catheters, broad-spectrum antibiotics, and surgical procedures.

CONCLUSION

This study highlights the substantial burden of candidemia among older adults in Bahrain, characterized by diverse *Candida* species. It also concerns levels of antifungal resistance, notably in *C. auris*. The findings underscore the importance of local epidemiological surveillance and tailored treatment strategies to improve outcomes and mitigate the spread of multidrug-resistant *Candida* species. Future research should focus on molecular resistance mechanisms and optimizing therapeutic approaches to address this growing public health concern.

Key Words: Candidemia; Fungal infections; Antifungal resistance; Epidemiology; Risk factors; Antifungal susceptibility; Bahrain

Core Tip: This study underscores the significant burden of candidemia in Bahrain, highlighting demographic trends, species distribution, antifungal susceptibility patterns, and critical risk factors over three years. The emergence of multidrug-resistant *Candida* species, particularly *Candida auris*, poses challenges to clinical management. High mortality rates underscore the critical need for early detection, prompt initiation of appropriate antifungal therapy guided by susceptibility testing, and rigorous infection control measures. Healthcare providers should prioritize antimicrobial stewardship, minimize invasive procedures when possible, and enhance surveillance efforts to mitigate the rising threat of antifungal resistance and improve patient outcomes in candidemia management.

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INTRODUCTION

Candida is a type of yeast that naturally occurs in small amounts in the human body. It can be found naturally in the gastrointestinal tract, mouth, and genital area. *Candida* typically lives in harmony with other microorganisms and usually does not cause any harm. Nonetheless, specific circumstances, such as a weakened immune system or an imbalance in the body's natural microbiota, can cause *Candida* to overgrow and result in infections. The most typical form of *Candida* infection is candidiasis, which can manifest in several ways, including oral thrush, vaginal yeast infection, cutaneous candidiasis, and invasive candidiasis[1,2]. Human disease caused by *Candida* species is attributed to at least 15 distinct types. However, most invasive diseases, accounting for over 95%, can be traced back to the six most prevalent pathogens. These include *Candida albicans* (*C. albicans*), *Candida auris* (*C. auris*), *Candida glabrata* (*C. glabrata*), *Candida krusei*, *Candida parapsilosis* (*C. parapsilosis*), and *Candida tropicalis* (*C. tropicalis*) in certain regions[3].

Candidemia is a serious bloodstream infection caused by *Candida* species and is the most common form of invasive candidiasis. Its high morbidity and mortality rates pose a significant threat to healthcare facilities worldwide[4]. This infection is particularly dangerous for individuals with weakened immune systems and those hospitalized for an extended period. The early identification and management of this infection is often delayed, leading to severe clinical decline and fatalities that occur before the detection of *Candida* in blood cultures[5]. Understanding the incidence, predisposing factors, and antifungal susceptibility patterns of *Candida* isolates in specific regions is crucial for effectively preventing and managing this infection[6].

The prevalence of candidemia varies among different geographical regions and healthcare facilities. Factors like patient demographics, local prescribing practices, and infection control measures influence this prevalence[7]. Furthermore, understanding the risk factors associated with candidemia is vital for early identification and implementation of preventive measures. Several known risk factors, such as prolonged hospitalization, use of broad-spectrum antibiotics, and presence of central venous catheters (CVCs), have been implicated in candidemia[8]. Meanwhile, addressing the antifungal susceptibility patterns of *Candida* isolates is crucial to ensure proper treatment strategies. Resistance to commonly used antifungal agents, such as fluconazole and echinocandins, has been reported in various regions worldwide[9].

Investigating the antifungal susceptibility profiles of *Candida* isolates in Bahrain will aid in optimizing empirical therapy and formulating guidelines for antifungal use in clinical practice. Addressing the antifungal susceptibility

patterns of *Candida* isolates is crucial to ensuring proper treatment strategies. Resistance to commonly used antifungal agents, such as fluconazole and echinocandins, has been reported in various regions globally. By examining the antifungal susceptibility profiles of *Candida* isolates in Bahrain, we can optimize empirical therapy and establish guidelines for antifungal use in clinical practice[10,11]. Like other parts of the world, the United Kingdom of Bahrain, a small island nation in the Arabian Gulf, has witnessed an increase in candidemia cases over the past decade[12]. However, no available comprehensive studies specifically focused on candidemia in Bahrain exist. There is no available data concerning the infection rate, the type of *Candida* species implicated in candidemia, and their antifungal susceptibility patterns.

Bahrain boasts a well-developed healthcare system that provides comprehensive medical services to its population. The country has a mix of public and private healthcare facilities, with the public sector being the primary provider of health services[13]. The Ministry of Health oversees public healthcare services, ensuring access to primary, secondary, and tertiary care. Bahrain's healthcare system is funded through government subsidies, employer contributions, and out-of-pocket payments[14]. Bahrain has a population of approximately 1.7 million people, with a significant expatriate community. The population growth rate is around 2%, driven by both natural increase and immigration[15]. The population includes a substantial proportion of young adults and working-age individuals, but there is also a growing elderly population due to increasing life expectancy. Bahrain's healthcare system is characterized by its accessibility, comprehensive services, and a blend of public and private healthcare facilities[16]. The government's commitment to providing free healthcare to citizens and the presence of a significant private sector cater to the diverse needs of the population.

This research article aims to bridge this knowledge gap by providing a detailed analysis of candidemia cases in Bahrain between 2021 and 2022. By examining candidemia within a central tertiary care hospital setting in Bahrain, this study seeks to shed light on the local burden of candidemia, providing valuable insights into the epidemiology of this infection, exploring the risk factors specific to the studied cohort, shedding light on the regional determinants contributing to candidemia incidence in the United Kingdom of Bahrain.

MATERIALS AND METHODS

Study design and setting

We retrospectively observed and analyzed the *Candida* profile of the patients admitted to the various departments of Salmanyia Medical Complex, United Kingdom of Bahrain, between January 2021 and December 2023, with positive blood culture of *Candida* species. Data were extracted and reviewed from the inpatients' electronic health medical records from all government hospitals' inpatients. The demographics, clinical data, microbiological profile, and included patient outcomes were extracted and tabulated using the Microsoft Excel database. We ensured that the data collection was systematic and consistent.

Definitions

Candidemia is defined by direct fungal detection in blood cultures. Despite blood culture being the gold standard to diagnose candidemia, it has many limitations, such as low sensitivity (between 21% and 71% in some studies) and the long time needed for the culture to yield results. It also cannot detect deep-seated infections after resolving candidemia. Other tests, such as anti-mannan antibodies, *Candida* mannan antigen, and B-d-glucan, can help to get rapid results but with high false positive results[17,18]. The study involved patients who had tested positive for *Candida* in their blood culture at least once. When a patient had multiple positive cultures, a new episode was only considered if the interval between it and the previous episode was more than two weeks[19]. Isolates were considered duplicates and ignored when identified from the same patient with the same *Candida* species and antifungal profile.

Laboratory technique

Clinical samples were ordered when fungal infection was clinically suspected or in high-risk patients. We incubated the blood culture bottles using BD Bactec (BioMérieux®). We identified the fungal species using Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS, Bruker®) and used broth microdilution method (Bruker®) to identify the antifungal susceptibility following the manufacturer's instructions[20]. We tested fungal susceptibility to 5-fluorocytosine, amphotericin B, anidulafungin, caspofungin, fluconazole, itraconazole, micafungin, posaconazole, and voriconazole[21]. We used the epidemiological cutoff values according to European Committee on Antimicrobial Susceptibility Testing recommendations. Phenotypic detection was done using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Daltonics, Germany). For *C. auris*, Food and Drug Administration Epidemiological cutoff values were used to interpret the minimal inhibitory concentrations[22].

Statistical analysis

For the statistical analysis, we utilized TexaSoft, WINKS Sialodacryoadenitis virus Software 2011 (Sixth Edition, Cedar Hill, Texas, United States) while collating all data on Excel 2017 (Microsoft, Redmond, WA, United States). We calculated the frequencies and percentages for different categorical variables and performed cross-tabulation between each two categorical variables. We conducted a χ^2 test to detect significant relationships between the variables and considered a P value of less than 0.05 statistically significant. A biomedical statistician conducted the statistical review of this study, and all data were anonymized.

Table 1 Demographic data of patients affected with fungemia over the three years of the study, *n* (%)

		2021	2022	2023	Total
Total number		161	143	126	430
Age (years)	mean ± SD	66.9 ± 26.7	68.6 ± 18.8	62.87 ± 19	65.7 ± 18.8
	Median	70	72	68	70
Sex	Male	84 (52.2)	79	62	225
	Female	77 (47.8)	64	64	205
	Male:female ratio	1.1:1	1.23:1	0.96:1	1.1:1
Nationality	Bahraini	131 (81.4)	123 (86)	111 (88)	365 (85)
	Non-Bahraini	30 (18.6)	20 (14)	15 (12)	65 (15)
Prognosis	Live	23 (14.3)	26 (18.2)	13 (10)	62 (14.5)
	Deceased	138 (85.7)	117 (81.8)	113 (90)	368 (85.5)
Candida species	*Candida albicans*	48 (30)	22 (15.4)	33 (26.2)	103 (24)
	Candida tropicalis	30 (18.6)	29 (20.3)	23 (18.2)	82 (19)
	Candida parapsilosis	29 (18)	36 (25.2)	29 (23)	94 (22)
	Candida glabrata	19 (11.8)	7 (5)	10 (8)	36 (8)
	Candida auris	13 (8)	38 (26.5)	27 (23)	78 (18)
	Candida species	6 (3,7)	1 (0.7)	0	7 (1.6)
	Candida dubliniensis	4 (2.5)	1 (0.7)	1 (0.7)	6 (1.3)
	Candida lusitaniae	3 (1.9)	1 (0.7)	2 (1.4)	6 (1.3)
	Candida orthopsilosis	3 (1.9)	3 (2)	1 (0.7)	7 (1.6)
	Candida guilliermondii	2 (1.25)	0	0	2 (0.4)
	Candida metapsilosis	2 (1.25)	3 (2)	1 (0.7)	6 (1.3)
	Candida rugosa	2 (1.25)	0		
	Candida krusei	0	2 (1.4)	1 (0.7)	3 (0.7)
	Candida Cyberlindnera fabianii	1 (0.65)	0	0	1 (0.2)
	Rhodotorula mucilaginosa var mucilaginos	1 (0.65)	0	0	1 (0.2)

Ethical approval

The Secondary Care Research Committee of Salmaniya Medical Complex, Ministry of Health, United Kingdom of Bahrain, has approved the study. However, ethical consideration was unnecessary as it was a non-interventional, retrospective study that did not expose patient data.

RESULTS

This retrospective study investigated the prevalence, risk factors, and antifungal susceptibility patterns of candidemia in patients admitted to Salmaniya Medical Complex, Bahrain, between January 2021 and December 2023. Table 1 displays the demographic data of patients diagnosed with candidemia during the study period. Over this period, 430 patients were identified with candidemia. The data reveals a slight decrease in cases yearly: 161 cases in 2021, 143 in 2022, and 126 in 2023. The mean age of the patients remained relatively stable, around 65.7 years ± 18.8 years, with a median age consistently around 70 years. The male-to-female ratio was nearly equal across the years. The gender distribution was nearly balanced, with a male-to-female ratio of approximately 1.1:1; males constituted 52.3% (225/430) and females 47.7% (205/430) of the cases. Bahraini nationals consistently comprised the majority of the patients. They represented 85% (365/430) of the patients, while non-Bahrainis comprised 15% (65/430). The overall mortality rate among these patients was 85.5% (368/430). The mortality rate among candidemia patients was alarmingly high, averaging 85.5% over the three years. This high mortality rate underscores the severity and impact of candidemia on the affected population.

The distribution of *Candida* species detected from patients with fungemia, also presented in Table 1, Figures 1, and 2, varied across the years. *C. albicans* was the most frequently isolated species, accounting for 24% (103/430) of the cases. *C. parapsilosis* was identified in 22% (94/430) of patients, *C. tropicalis* in 19% (82/430), *C. auris* in 18% (78/430), and *C. glabrata*

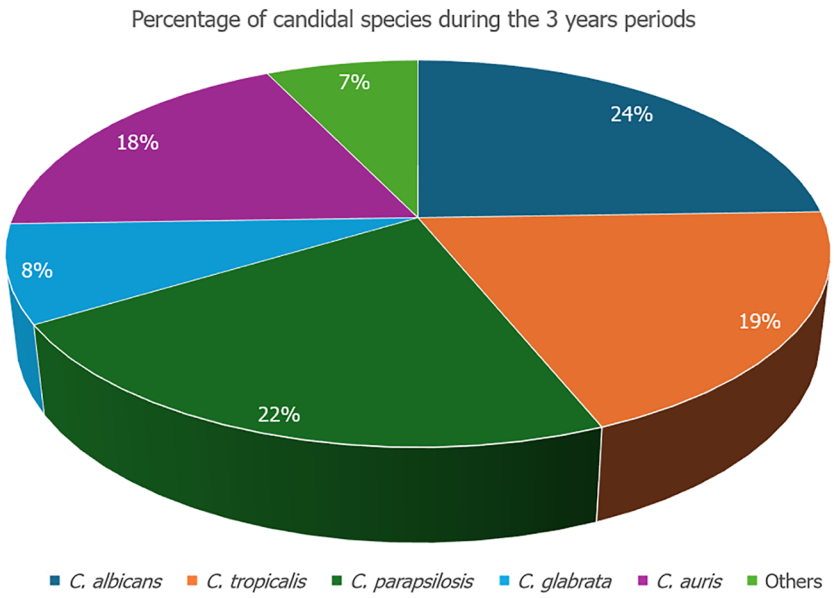

Figure 1 The percentage of candida species during the 3 years periods. *C. albicans*: *Candida albicans*; *C. tropicalis*: *Candida tropicalis*; *C. parapsilosis*: *Candida parapsilosis*; *C. glabrata*: *Candida glabrata*; *C. auris*: *Candida auris*.

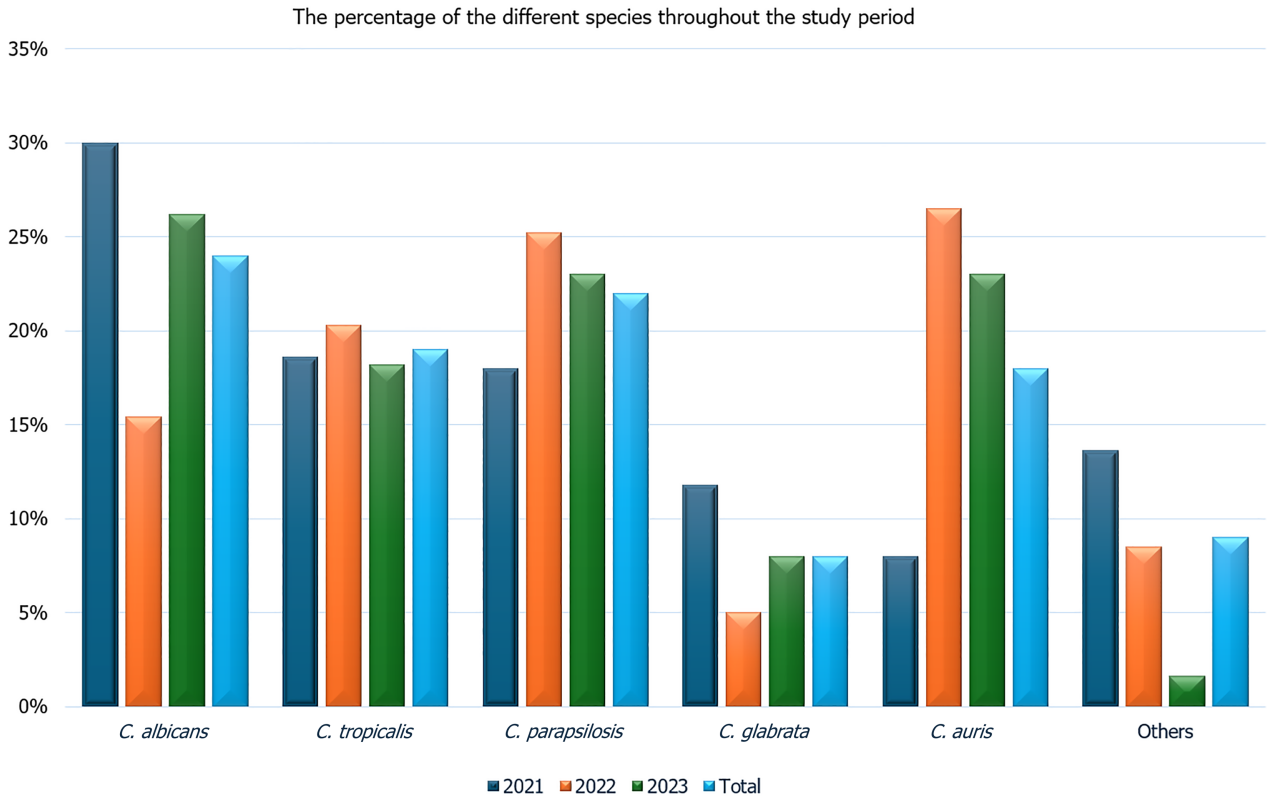

Figure 2 The percentage of the different species throughout the study period. *C. albicans*: *Candida albicans*; *C. tropicalis*: *Candida tropicalis*; *C. parapsilosis*: *Candida parapsilosis*; *C. glabrata*: *Candida glabrata*; *C. auris*: *Candida auris*.

in 8% (36/430). Examining the distribution of *Candida* species over the three years, *C. albicans* was consistently the most common species, though its prevalence fluctuated, being highest in 2021 (30%) and lowest in 2022 (15.4%). *C. parapsilosis* showed an increasing trend, rising from 18% in 2021 to 25.2% in 2022, then slightly decreasing to 23% in 2023. *C. tropicalis* maintained a relatively stable presence, around 18%-20% each year. The incidence of *C. auris* saw a significant rise, from 8% in 2021 to 26.5% in 2022, then slightly decreased to 23% in 2023. *C. glabrata* showed a decreasing trend, from 11.8% in 2021 to 5% in 2022 and 8% in 2023. Other less common *Candida* species, such as *C. dubliniensis*, *C. lusitaniae*, *C. orthopsilosis*, and others, remained relatively rare throughout the study period.

Table 2 summarizes the risk factors associated with invasive fungal infections. Several key risk factors were identified among patients with candidemia. The use of CVCs and other intravascular devices was particularly high, reported in 89.4% of patients in 2021, 92% in 2022, and 90% in 2023. Recent major surgery was noted in 18.5% of patients in 2021, 20% in 2022, and 15% in 2023. Vascular anastomosis was observed in 2%, 5%, and 3% of patients in 2021, 2022, and 2023, respectively. Necrotizing pancreatitis was reported in 0% of patients in 2021, 2% in 2022, and 4% in 2023. *Candida* colonization was present in 93.4% of patients in 2021, 87% in 2022, and 91% in 2023. Broad-spectrum antibiotic exposure was extremely common, observed in 98.7% of patients in 2021, 95% in 2022, and 96% in 2023. Dialysis was required in 17.2% of patients in 2021, 15% in 2022, and 19% in 2023. Parenteral nutrition was administered to 76.8% of patients in 2021, 81% in 2022, and 77% in 2023. Corticosteroid use was noted in 32.4% of patients in 2021, 42% in 2022, and 35% in 2023. Prior antifungal treatment was recorded in 47.7% of patients in 2021, 53% in 2022, and 49% in 2023.

Table 3 provides the antifungal susceptibility profiles for the most common *Candida* species detected among the patients. *C. albicans* demonstrated 100% susceptibility to amphotericin B across all three years. However, susceptibility to fluconazole declined from 87.5% in 2021 to 52.3% in 2023. Voriconazole susceptibility also showed variability, with the highest rate in 2021 (79%) and the lowest in 2022 (63.6%). Susceptibility to posaconazole and itraconazole decreased over the years, with posaconazole dropping from 68.2% in 2021 to 36.4% in 2023, and itraconazole from 75% to 13.6%. Susceptibility to echinocandins (micafungin, anidulafungin, and caspofungin) remained at 100%.

C. tropicalis displayed a high mortality rate, with susceptibility to amphotericin B slightly decreasing from 100% in 2021 to 95.5% overall. Susceptibility to fluconazole was very low, starting at 15.7% in 2021 and dropping to 0% by 2023. Voriconazole, posaconazole, and itraconazole also showed poor susceptibility rates, generally declining over the years. No susceptibility to echinocandins was recorded across the three years. *C. parapsilosis* maintained 100% susceptibility to amphotericin B, with fluconazole susceptibility remaining high but slightly fluctuating, from 82% in 2021 to 76.5% in 2023. Susceptibility to voriconazole, posaconazole, and itraconazole remained relatively stable and high across the years. Echinocandin susceptibility remained consistently at 100%.

C. glabrata showed complete susceptibility to amphotericin B throughout the study period. Fluconazole susceptibility was consistently 0%, and susceptibility to echinocandins varied, with micafungin, anidulafungin, and caspofungin showing better results in 2022 and 2023 compared to 2021. *C. auris* had a 100% susceptibility rate to amphotericin B across all years. Susceptibility to fluconazole was initially 0% in 2021, slightly increasing to 4% in 2022, and then returning to 0% in 2023. Echinocandin susceptibility was high in 2021 and 2022 but showed some resistance in 2023, particularly with caspofungin (4.5%).

While the most common *Candida* species (*C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, and C. auris*) generally remained susceptible to amphotericin B (over 83% susceptible across all species and years) and flucytosine (over 95% susceptible), however, concerning trends in susceptibility were observed for other antifungal agents. For example, susceptibility to fluconazole declined significantly for *C. tropicalis* (15.7% in 2021 to 0% in 2023) and *C. albicans* (87.5% in 2021 to 52.3% in 2023). In addition, while all *Candida* species remained largely susceptible to caspofungin (over 88% susceptible), some isolates of *C. glabrata* showed intermediate susceptibility. Overall, the trend of antifungal susceptibility highlights the ongoing challenge of treating candidemia, with notable resistance patterns emerging in commonly used antifungal agents, particularly fluconazole and voriconazole, for certain *Candida* species. This emphasizes the need for continued monitoring and tailored treatment approaches to manage candidemia effectively.

DISCUSSION

This is the first study to estimate the burden of invasive fungal infection in the United Kingdom of Bahrain without previous epidemiological or clinical studies. The findings from this study highlight several critical aspects of candidemia among patients from 2021 to 2023, underscoring the evolving nature of fungal infections and the associated challenges in clinical management in the United Kingdom. The demographic data reveal that candidemia predominantly affects older adults, with a mean age of 65.7 years and a high mortality rate of 85.5%. The gender distribution was nearly balanced, with a slight male predominance. Bahraini nationals constituted most cases, reflecting the local demographic distribution and healthcare access patterns. Studies from neighboring Gulf countries such as Saudi Arabia and the United Arab Emirates (UAE) have reported similar demographic profiles among candidemia patients, with a predominance of elderly individuals and a slight male preponderance[23,24]. Many studies showed that older age is associated with a high mortality rate and a trend of higher resistance rate to multiple antifungal drugs[25], partially explaining the high mortality rate observed in the current study. Older age is usually associated with high rates of comorbid conditions, aging-related pathophysiological changes, *Candida* l colonization, and polypharmacy[26,27]. The high mortality rates observed in our study (85.5%) align closely with findings from these regions[28-31], indicating a consistent challenge in managing invasive fungal infections despite advances in medical care. Bahrain's high mortality rate from candidemia is influenced by factors such as uneven access to healthcare facilities, variability in care standards due to a diverse healthcare workforce, and suboptimal infection control practices. The patient population, particularly older adults with multiple comorbidities like diabetes and cardiovascular diseases, is highly vulnerable. High usage of CVCs and broad-spectrum antibiotics further increases infection risks. In comparison, high-income countries with robust healthcare infrastructure, advanced infection control, and comprehensive chronic disease management see lower mortality rates. In contrast, low-income and middle-income countries often face higher mortality due to resource constraints and limited healthcare access. To improve outcomes, Bahrain could enhance infection control, implement antimicrobial stewardship, expand healthcare access, invest in healthcare worker training, manage chronic diseases more effectively, and conduct ongoing research and surveillance.

Table 2 Risk factors associated with fungal infections

Risk factors	2021 (*n* = 161)	2022 (*n* = 143)	2023 (*n* = 126)
Recent major surgery	18.5	20	15
Vascular 0 anastomosis	2	5	3
Necrotizing pancreatitis	0	2	4
Central venous catheters and other intravascular devices	89.4	92	90
Candida colonization	93.4	87	91
Exposure to broad spectrum antibiotics	98.7	95	96
Dialysis	17.2	15	19
Parenteral nutrition	76.8	81	77
Corticosteroids	32,4	42	35
Antifungal Rx	47.7	53	49

Species distribution and trends

The distribution of *Candida* species varied over the three years, with *C. albicans* being the most frequently isolated species, followed by *C. parapsilosis*, *C. tropicalis*, and *C. auris*. Notably, *C. auris*, known for its multidrug-resistant properties, represented a significant proportion of the isolates (18%). The emergence of *C. auris* as a major pathogen is particularly concerning due to its ability to cause outbreaks and its high resistance to multiple antifungal agents[32]. *C. auris* has a profound impact on patient outcomes and healthcare practices due to its multidrug-resistant nature, which complicates treatment and leads to higher mortality rates. Its persistence in healthcare environments and resistance to standard antifungal treatments pose significant challenges for outbreak management, necessitating enhanced infection control measures and improved diagnostic capabilities. In Bahrain, the emergence of *C. auris* is influenced by several healthcare practices and environmental factors. High utilization of CVCs and broad-spectrum antibiotics increases the risk of candidemia by providing pathways for *C. auris* to enter the bloodstream and promoting fungal resistance. Inadequate antifungal stewardship further exacerbates the problem. Environmental factors also play a role; the warm and humid climate of Bahrain creates an ideal environment for fungal growth, while insufficient cleaning and disinfection practices in healthcare settings facilitate the persistence and spread of *C. auris*. Additionally, the aging population and rising number of individuals with chronic conditions heighten the risk of invasive fungal infections. Addressing these issues requires enhanced infection control practices, improved environmental hygiene, and effective antifungal stewardship.

The fluctuating prevalence of other *Candida* species can be attributed to factors such as the use of broad-spectrum antifungals and antibiotics, healthcare practices like central venous catheterization, environmental conditions, and regional differences. While *C. albicans* remains a predominant species across the Gulf[23,33], the emergence of *C. auris* as a significant pathogen observed in our study has also been noted in Saudi Arabia and the UAE[34]. This multidrug-resistant species poses a formidable clinical challenge due to its ability to cause outbreaks and its intrinsic resistance to many antifungal agents[35]. Globally, candidemia epidemiology shows considerable variability influenced by regional healthcare practices, antimicrobial stewardship, and patient demographics[36]. Studies from Europe, China, and North America often highlight *C. glabrata* and *C. parapsilosis* as prominent species with varying levels of antifungal resistance[37-40]. As observed in our study, the trend of increasing resistance to azole antifungals, particularly among *C. tropicalis* and *C. glabrata,* mirrors findings from diverse geographic regions[41,42]. This underscores the global concern regarding the emergence of multidrug-resistant *Candida* species and the implications for clinical management.

Risk factors analysis

Several risk factors were consistently associated with candidemia, including using old age, CVCs, exposure to broad-spectrum antibiotics, and parenteral nutrition. These factors are well-known predisposing conditions for invasive fungal infections, as they disrupt normal host defenses and create environments conducive to fungal growth[8,43]. Identifying and understanding risk factors associated with candidemia are crucial for effective management and prevention strategies[44]. Our study identifies several significant risk factors consistent with previous literature and highlights regional variations that warrant attention.

Our study's high prevalence of CVCs and other intravascular devices among candidemia patients (89.4% in 2021, 92% in 2022, and 90% in 2023) underscores their role as major risk factors. These devices provide a conduit for *Candida* species to enter the bloodstream, leading to invasive infections[45]. Similar findings have been reported globally and in other Gulf region studies, emphasizing the importance of strict adherence to infection control measures and catheter care protocols to mitigate these risks[46,47]. Another significant risk factor observed in our study is the widespread use of broad-spectrum antibiotics, reported in 98.7% of patients in 2021, 95% in 2022, and 96% in 2023. Antibiotic therapy disrupts the normal flora, creating an environment conducive to *Candida* overgrowth and subsequent infection[48]. This association has been well-established in the literature and underscores the critical need for antimicrobial stewardship programs to optimize antibiotic use and reduce the incidence of secondary fungal infections[49].

Our findings indicate variable prevalence rates for recent major surgery (18.5% to 20%) and vascular anastomosis (2% to 5%) across the study years. These surgical procedures can compromise host defenses and predispose patients to candidemia, consistent with findings from other studies globally[50,51]. High rates of *Candida* colonization (93.4% to 91%) and dialysis (17.2% to 19%) further highlight their role as predisposing factors. Colonization serves as a reservoir for subsequent bloodstream infections[52], while dialysis procedures increase the risk of catheter-related infections and systemic fungal spread[53]. Our cohort's use of parenteral nutrition (76.8% to 81%) and corticosteroids (32.4% to 42%) underscores their immunosuppressive effects, which can impair host defenses against *Candida* species. These findings align with previous studies highlighting their association with increased candidemia risk[54,55].

Our findings align with previous studies from the Gulf region and globally, confirming the universal nature of these risk factors in predisposing patients to candidemia. Studies from Saudi Arabia and the UAE report similar risk factor profiles, emphasizing the consistent challenges posed by invasive fungal infections across the region[56]. Studies globally have also identified CVCs, broad-spectrum antibiotics, and surgical interventions as predominant risk factors for candidemia[24,57,58]. The prevalence of specific risk factors across regions varies, influenced by local healthcare practices, patient demographics, and antimicrobial use policies[59].

Understanding the epidemiology and risk factors associated with candidemia is essential for guiding clinical practice and implementing targeted prevention strategies[60]. It is the healthcare professionals who play a crucial role in implementing these strategies. Multifaceted approaches, including infection control measures, antimicrobial stewardship, and early detection strategies, are critical in reducing the incidence and improving outcomes of candidemia. Future research should focus on tailored interventions to mitigate specific risk factors identified in different healthcare settings, ultimately enhancing patient care and safety[61].

Antifungal susceptibility trends

Antifungal susceptibility patterns among *Candida* species are crucial for guiding empirical therapy and optimizing treatment outcomes in candidemia[62]. Our study provides valuable insights into the susceptibility profiles of commonly isolated *Candida* species over three years, highlighting trends and implications for clinical practice. Our findings indicate variable susceptibility profiles among *Candida* species to different antifungal agents. *C. albicans*, the predominant species in our study, demonstrated consistent susceptibility to amphotericin B and 5-fluorocytosine across the years, with varying susceptibility to azoles and echinocandins. While susceptibility to echinocandins remained relatively stable, *C. albicans* showed declining susceptibility to fluconazole and voriconazole over the years, with fluconazole susceptibility dropping from 87.5% in 2021 to 52.3% in 2023. This trend indicates a growing resistance to azole antifungals, commonly used in clinical practice. However, echinocandin susceptibility (micafungin, anidulafungin, and caspofungin) remained at 100%, suggesting that these agents are still effective for treating *C. albicans* infections.

Comparatively, studies from other regions also report similar resistance patterns. Research from Europe and North America indicates increasing resistance to azoles, particularly fluconazole, among *C. albicans* isolates[63,64]. This global trend underscores the need for continuous monitoring and the potential necessity of adjusting empirical treatment protocols to incorporate agents with sustained efficacy, such as echinocandins or rifapentine[65]. Region-specific studies, such as those from the Gulf region, further corroborate our findings. For instance, recent reports highlight a growing prevalence of azole-resistant *C. albicans* strains, echoing our observed decline in susceptibility[66-68]. These comparative insights emphasize the universal nature of antifungal resistance trends and the importance of local epidemiological data in guiding treatment decisions.

C. tropicalis species exhibited high susceptibility to amphotericin B but decreased susceptibility to fluconazole (starting at 15.7% in 2021 and reaching 0% by 2023). This species also showed poor susceptibility to other azoles, highlighting the necessity for alternative antifungal strategies. Echinocandin susceptibility remained variable but generally lower than that of other agents. The consistent susceptibility to amphotericin B offers a viable treatment option for infections caused by *C. tropicalis*. The increasing resistance of *C. tropicalis* to antifungal agents, particularly azoles like fluconazole, is a significant concern. Overuse and misuse of antifungal medications, environmental factors, hospital settings, genetic mutations, and the global spread of resistant strains all contribute to this issue[69,70]. Comparative studies from other regions reflect similar resistance patterns. For example, research from Asia and Europe has also documented declining fluconazole susceptibility among *C. tropicalis* isolates. A study in China reported fluconazole resistance rates rising from 16.7% in 2010 to 87.5%–100% after 2014[71], paralleling our findings of complete resistance by 2023. Similarly, European surveillance data highlight increasing resistance to azoles among *C. tropicalis*, corroborating our observations[24]. Arastehfar *et al*[72] showed an increase in the prevalence of fluconazole-non-susceptible *C. tropicalis* blood isolates in Turkey reaching. In the Gulf region, studies have different patterns in azole resistance among *C. tropicalis* isolates. Reports from Saudi Arabia showed 95% susceptibility to azole antifungals[31], while studies from Kuwait have shown a susceptibility rate of up to 100%, indicating the effects of different factors on antifungal susceptibility[27]. These findings across different geographical locations emphasize the global nature of this resistance issue. Echinocandin susceptibility among *C. tropicalis* remains a topic of concern, as variability in efficacy has been noted in several studies[73]. For instance, Swedish research indicates fluctuating susceptibility rates, aligning with the variable echinocandin susceptibility observed in our study[74]. This variability necessitates careful consideration of local susceptibility data when choosing echinocandin therapy.

In the current study, *C. parapsilosis* maintained high susceptibility to fluconazole and other azoles and echinocandins, which were observed consistently across the study years, making it more manageable than other species. This highlights a favorable susceptibility profile that aligns with previous reports. The high susceptibility of *C. parapsilosis* to fluconazole, other azoles, and echinocandins observed in our study can be attributed to several factors[75]. One primary reason is the inherent genetic makeup of *C. parapsilosis*, which appears less prone to developing resistance mutations than other *Candida* species, such as *C. glabrata* and *C. auris*[76]. Additionally, *C. parapsilosis* is less commonly exposed to selective

Table 3 Antifungal susceptibility for the most common fungal strains detected from patients with fungaemia

Candida species	Year	Number	Age (years)	Male:female	Death	Amphotericin B	5-fluorocytosine	Fluconazole	Voriconazole	Posaconazole	Itraconazole	Micafungin	Anidulafungin	Caspofungin
Candida albicans	2021	48	65.1 ± 18	0.7	89.5	100	N/A	87.5	79	68.2	75	13	21	100
	2022	24	71.6 ± 18.7	1.2:1	77.3	100	N/A	63.6	63.6	45.5	45.5	54.5	36.4	100
	2023	33	66.5 ± 15.6	1.5:1	80	100	N/A	52.3	71.4	66.6	36.4	13.6	13.6	100
	Total	103	66 ± 18.8	1:1	83	100	N/A	71	74.5	62.2	51	21.4	21	100
Candida tropicalis	2021	31	66.6± 14.7	1.9:1	89.6	100	N/A	15.7	10.5	5.5	10.5	N/A	0	0
	2022	29	65.6 ± 22	0.93	72	82	N/A	9	9	0	0	N/A	0	0
	2023	23	67.4 ± 19.4	1.1:1	92	100	N/A	0	0	0	0	0	0	0
	Total	83	67± 18.3	1.2:1	83	95.5	N/A	8.8	6.6	2.2	4.6	0	0	0
Candida parapsilosis	2021	27	61.2 ± 22.2	1.3:1	92	100	N/A	82	100	91	100	100	100	100
	2022	36	70.4 ± 14.9	1.1:1	87	100	N/A	68	95	95	95	100	100	100
	2023	29	61.7 ± 20.6	0.93:1	85.7	100	N/A	76.5	94	94	88.2	100	100	100
	Total	93	64.9 ± 196	1.1:1	88.5	100	N/A	75.7	95.6	93.7	93.2	100	100	100
Candida glabrata	2021	18	64.2 ± 13.7	2.2:1	94.7	100	N/A	0	N/A	N/A	N/A	100	54	54
	2022	7	70.6 ± 11.7	2.5:1	66.6	100	N/A	0	N/A	N/A	N/A	100	100	100
	2023	10	64.3	1:4	100	100	N/A	0	N/A	N/A	N/A	100	100	100
	Total	35	65.4 ± 15.4	1.25:1	88.8	100	N/A	0	N/A	N/A	N/A	100	64	64
Candida auris	2021	13	67.5 ± 21	1.2:1	100	100	N/A	0	N/A	N/A	N/A	87.5	87.5	87.5
	2022	38	72.4 ± 15.5	1.5:1	85.3	100	N/A	4	N/A	N/A	N/A	100	100	0
	2023	27	58.3 ± 18.9	0.93:1	100	100	N/A	0	N/A	N/A	N/A	100	100	4.5
	Total	78	66.6 ± 18.8	1.2:1	87.5	100	N/A	2	N/A	N/A	N/A	98	98	13.2

pressure from antifungal treatments in clinical settings, reducing the likelihood of resistance development[77]. Moreover, *C. parapsilosis* is known to have lower intrinsic resistance mechanisms, such as reduced efflux pump activity and less frequent mutations in key genes like *ERG11* and *FKS1*, which are commonly associated with azole and echinocandin resistance, respectively[78]. The lower usage of antifungal agents against *C. parapsilosis* infections, possibly due to its lower virulence and pathogenicity than other *Candida* species, also contributes to its sustained susceptibility profile[79]. However, the emergence of resistance in even a small proportion of isolates necessitates ongoing surveillance.

C. glabrata, known for its intrinsic resistance, showed complete resistance to fluconazole with a consistent 0% susceptibility rate and variable susceptibility to echinocandins across the study period. Recent evidence indicates that most

invasive candidiasis caused by *C. glabrata* is associated with biofilm growth. *C. glabrata* biofilms exhibit antifungal resistance due to their compact, dense structure of yeast cells nested in an extracellular matrix rich in proteins and carbohydrates, particularly β-1,3 glucan. Many genes are linked to biofilm formation in *C. glabrata*, such as EPA6, which encodes adhesin controlled by multiple factors, including the CgYak1p kinase, chromatin remodeling Swi/Snf complex components, subtelomeric silencing, and the transcription factor CgCst6[80-82]. This resistance pattern underscores the challenges in treating infections caused by this species. The variable resistance to Echinocandin emphasizes the need for susceptibility testing to guide therapy for *C. glabrata* infections[83].

Since it was identified in 2009 by a Japanese scientist, *C. auris* has been reported worldwide[84]. Emerging as a global concern due to multidrug resistance, *C. auris* in our study exhibited high resistance to fluconazole but variable susceptibility to echinocandins (particularly caspofungin, which showed some resistance in 2023) and amphotericin B. Our findings confirm the worldwide concern about this emerging multidrug-resistant fungus that is rapidly spreading throughout the world as being reported from more than 30 countries with the shift from multi-drug resistant to pan-drug resistant[85]. Lockhart *et al*[86] reported a simultaneous rise of multidrug-resistant *C. auris* on three continents, using whole-genome sequencing, suggesting closely simultaneous and new independent emergence of various clonal populations on three continents. Therefore, infection with *C. auris* is expected to be associated with high mortality rates due to its resistance to multiple classes of antifungal drugs[87]. The observed resistance trends underscore the need for vigilance and rapid detection methods to ensure appropriate and effective treatment when managing infections caused by this elusive pathogen.

Rising antifungal resistance rates pose significant challenges in managing invasive fungal infections. Increased resistance to common antifungals, such as fluconazole, reduces the effectiveness of standard treatments, leading to more severe and prolonged infections. This situation often necessitates the use of stronger, more toxic, and costly antifungal agents, which can increase patient morbidity and healthcare expenses. To combat this issue, early and accurate diagnostics are crucial for selecting appropriate therapies based on resistance profiles. Antifungal stewardship programs play a vital role by optimizing antifungal use, curbing unnecessary prescriptions, and monitoring resistance patterns. These programs should also focus on adjusting treatment protocols as resistance trends evolve. Enhanced infection control practices, including stringent hygiene and isolation measures, are necessary to prevent the spread of resistant strains. Additionally, educating healthcare professionals about resistance trends and investing in research for new antifungal agents are essential. Collaborative efforts among healthcare institutions, public health agencies, and research organizations are key to effective surveillance and resistance management.

Clinical implications

The high mortality rate associated with candidemia, coupled with the increasing resistance to commonly used antifungals, underscores the importance of susceptibility testing and individualized treatment approaches in managing candidemia[88]. Rapid identification of *Candida* species and their susceptibility profiles is essential to guide timely and appropriate antifungal therapy[89]. Additionally, preventive measures, such as reducing broad-spectrum antibiotic use and minimizing the duration of central venous catheter use, are crucial in mitigating the risk of candidemia[90]. Clinicians should consider local epidemiological data and susceptibility profiles when selecting empirical therapy, especially in critically ill patients at high risk for invasive fungal infections[91]. Future research should focus on molecular mechanisms of resistance, novel antifungal agents, and strategies to optimize antifungal therapy efficacy while minimizing the development of resistance. Collaborative efforts are essential to combatting the rising threat of multidrug-resistant *Candida* species and improving patient outcomes in candidemia management. To mitigate the risks associated with candidemia, healthcare providers should implement several practical strategies. First, they should adhere to strict infection control practices, such as proper hand hygiene, sterilization of medical equipment, and isolation protocols for infected patients, to prevent the spread of infection[92]. Second, the judicious use of antibiotics should be prioritized to avoid unnecessary use of broad-spectrum antibiotics, which can disrupt normal flora and promote fungal growth. Third, healthcare providers should monitor and promptly remove CVCs when they are no longer necessary to reduce the risk of catheter-related infections[93]. Fourth, early diagnostic methods, such as molecular assays and rapid fungal cultures, should be employed to quickly identify the causative pathogens and their resistance profiles, allowing for targeted therapy. Fifth, antifungal stewardship programs should be established to optimize the use of antifungal agents, ensuring appropriate prescribing practices and reducing the development of resistance[94]. Finally, continuous education and training programs for healthcare professionals on the latest guidelines and best practices in infection control and antifungal management are essential to keep them updated and enhance patient care[95].

Limitations

This study has several limitations that warrant consideration. Firstly, it was conducted at a single center, which may restrict the generalizability of findings to other healthcare settings within Bahrain or regions with different healthcare practices and patient demographics. Variability in local healthcare practices, infrastructure, and patient populations means that the findings might not be applicable to different settings or larger geographic areas, potentially affecting the broader applicability of the conclusions drawn from the study. Secondly, retrospective data collection can introduce several biases that may affect the validity of study findings. For instance, incomplete or inconsistent medical records can lead to missing data on key variables, such as patient demographics or clinical outcomes, skewing the results. Recall bias may occur if data are dependent on historical records that are not uniformly detailed. Moreover, the sample size for certain *Candida* species and specific years might be relatively small, affecting the precision of the observed prevalence estimates and resistance patterns. Variability in antifungal susceptibility testing methods across the study period could also influence the accuracy and comparability of susceptibility data reported. Furthermore, differences in species identification methods or techniques over the study period might impact the reported distribution of *Candida* species and their

resistance profiles. The reliance on documented risk factors from medical records may not capture all relevant clinical variables or potential confounders influencing candidemia risk. Additionally, the study population included diverse patient groups with varying underlying conditions and comorbidities, introducing heterogeneity that could affect the interpretation of results and their generalizability. While the study spanned three years, longer-term trends and potential fluctuations in candidemia incidence, species distribution, and resistance patterns over a broader timeframe were not fully explored. Moreover, specific aspects of clinical management (*e.g.*, treatment protocols and adherence to guidelines) were not extensively addressed, which could impact outcomes and observed resistance patterns. Lastly, the findings may not fully reflect broader regional or global epidemiological trends in candidemia due to local healthcare practices, antimicrobial use policies, and unique demographic characteristics specific to Bahrain. Addressing these limitations in future research can enhance the robustness and applicability of studies on candidemia in Bahrain and similar settings, providing more comprehensive insights into invasive fungal infections and guiding effective clinical management strategies.

Recommendations

Enhanced surveillance and ongoing epidemiological studies are crucial to monitor candidemia trends, species distribution, and antifungal resistance patterns. This proactive approach will enable early detection of multidrug-resistant strains like *C. auris* and inform timely public health responses. Implementing robust antifungal stewardship programs is essential to optimize antifungal use, promote judicious prescribing of azoles, and prioritize effective agents based on local susceptibility data. Strengthening infection prevention and control measures, particularly focusing on reducing central venous catheter-related infections, is paramount. This includes strict adherence to catheter care protocols, vigilant monitoring, and timely removal of unnecessary catheters. Continuous education and training for healthcare providers on candidemia recognition, diagnosis, and management are critical, emphasizing early initiation of appropriate antifungal therapy tailored to local resistance profiles and patient-specific risks. Supporting research into molecular mechanisms of antifungal resistance and fostering the development of novel antifungal agents and diagnostics will enhance treatment options. Multidisciplinary collaboration among clinical microbiologists, infectious disease specialists, pharmacists, and infection control teams is essential for comprehensive candidemia management strategies. Lastly, increasing public awareness about candidemia risk factors and infection prevention practices will empower patients and caregivers to advocate for safer healthcare practices. Implementing these recommendations will be pivotal in addressing candidemia challenges in Bahrain, improving patient outcomes, and reducing the impact of invasive fungal infections in healthcare settings.

CONCLUSION

In conclusion, this study provides valuable insights into the epidemiology, species distribution, antifungal susceptibility patterns, and associated risk factors of candidemia among patients in Bahrain from 2021 to 2023. The findings underscore the significant burden of invasive fungal infections, particularly among older adults, with a notable mortality rate and a diverse spectrum of *Candida* species. The emergence of multidrug-resistant species, including *C. auris*, presents ongoing challenges in clinical management and highlights the importance of surveillance and timely intervention strategies. Antifungal susceptibility trends reveal varying degrees of resistance among different *Candida* species, emphasizing the need for tailored treatment approaches guided by local susceptibility data. Common risk factors such as CVCs, broad-spectrum antibiotics, and surgical interventions underscore the importance of infection control measures and antimicrobial stewardship to mitigate candidemia risk. Despite its limitations, including single-center retrospective design and potential biases inherent to observational studies, this research contributes to understanding candidemia in Bahrain's healthcare landscape. Future studies should focus on longitudinal surveillance, molecular mechanisms of resistance, and optimizing therapeutic strategies to improve patient outcomes and mitigate the rising threat of multidrug-resistant *Candida* species. Overall, this study provides a foundation for targeted interventions and policy development aimed at reducing the incidence and improving the management of candidemia in Bahrain and similar healthcare settings globally.

FOOTNOTES

Author contributions: Saeed NK, Almusawi S, and Al-Biltagi M contributed substantially to this retrospective study's design and implementation; Saeed NK and Almusawi S were primarily responsible for data collection, ensuring accuracy and completeness; Saeed NK took the lead in drafting the initial manuscript; Almusawi S contributed significantly to the writing and organization of the content; Al-Biltagi M provided critical revisions, offering expert insights and guidance on the manuscript's scientific and clinical content; all authors reviewed and approved the final version of the manuscript.

Institutional review board statement: The Secondary Care Research Committee of Salmaniya Medical Complex, Ministry of Health, United Kingdom of Bahrain, has approved the study. However, ethical consideration was unnecessary as it was a non-interventional, retrospective study that did not expose patient data.

Informed consent statement: No informed consent was needed as the study was a retrospective analysis of the laboratory data without personnel information.

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Data sharing statement: The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request. All data have been anonymized to ensure confidentiality and are shared in accordance with relevant ethical guidelines and institutional policies.

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REFERENCES

1 **Basmaciyan L**, Bon F, Paradis T, Lapaquette P, Dalle F. "Candida Albicans Interactions With The Host: Crossing The Intestinal Epithelial Barrier". *Tissue Barriers* 2019; **7**: 1612661 [PMID: 31189436 DOI: 10.1080/21688370.2019.1612661]
2 **Talapko J**, Juzbašić M, Matijević T, Pustijanac E, Bekić S, Kotris I, Škrlec I. Candida albicans-The Virulence Factors and Clinical Manifestations of Infection. *J Fungi (Basel)* 2021; **7**: 79 [PMID: 33499276 DOI: 10.3390/jof7020079]
3 **McCarty TP**, White CM, Pappas PG. Candidemia and Invasive Candidiasis. *Infect Dis Clin North Am* 2021; **35**: 389-413 [PMID: 34016283 DOI: 10.1016/j.idc.2021.03.007]
4 **Pfaller MA**, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* 2007; **20**: 133-163 [PMID: 17223626 DOI: 10.1128/CMR.00029-06]
5 **Epelbaum O**, Chasan R. Candidemia in the Intensive Care Unit. *Clin Chest Med* 2017; **38**: 493-509 [PMID: 28797491 DOI: 10.1016/j.ccm.2017.04.010]
6 **Logan C**, Martin-Loeches I, Bicanic T. Invasive candidiasis in critical care: challenges and future directions. *Intensive Care Med* 2020; **46**: 2001-2014 [PMID: 32990778 DOI: 10.1007/s00134-020-06240-x]
7 **Mamali V**, Siopi M, Charpantidis S, Samonis G, Tsakris A, Vrioni G, On Behalf Of The Candi-Candi Network. Increasing Incidence and Shifting Epidemiology of Candidemia in Greece: Results from the First Nationwide 10-Year Survey. *J Fungi (Basel)* 2022; **8**: 116 [PMID: 35205870 DOI: 10.3390/jof8020116]
8 **Poissy J**, Damonti L, Bignon A, Khanna N, Von Kietzell M, Boggian K, Neofytos D, Vuotto F, Coiteux V, Artru F, Zimmerli S, Pagani JL, Calandra T, Sendid B, Poulain D, van Delden C, Lamoth F, Marchetti O, Bochud PY; FUNGINOS; Allfun French Study Groups. Risk factors for candidemia: a prospective matched case-control study. *Crit Care* 2020; **24**: 109 [PMID: 32188500 DOI: 10.1186/s13054-020-2766-1]
9 **Badiee P**, Alborzi A. Susceptibility of clinical Candida species isolates to antifungal agents by E-test, Southern Iran: A five year study. *Iran J Microbiol* 2011; **3**: 183-188 [PMID: 22530086]
10 **S U**, Sumana MN. Retrospective analysis on distribution and antifungal susceptibility profile of Candida in clinical samples: a study from Southern India. *Front Public Health* 2023; **11**: 1160841 [PMID: 37261242 DOI: 10.3389/fpubh.2023.1160841]
11 **Alkhars N**, Gaca A, Zeng Y, Al-Jallad N, Rustchenko E, Wu TT, Eliav E, Xiao J. Antifungal Susceptibility of Oral Candida Isolates from Mother-Infant Dyads to Nystatin, Fluconazole, and Caspofungin. *J Fungi (Basel)* 2023; **9**: 580 [PMID: 37233291 DOI: 10.3390/jof9050580]
12 **Kim EJ**, Lee E, Kwak YG, Yoo HM, Choi JY, Kim SR, Shin MJ, Yoo SY, Cho NH, Choi YH. Trends in the Epidemiology of Candidemia in Intensive Care Units From 2006 to 2017: Results From the Korean National Healthcare-Associated Infections Surveillance System. *Front Med (Lausanne)* 2020; **7**: 606976 [PMID: 33392229 DOI: 10.3389/fmed.2020.606976]
13 **Katoue MG**, Cerda AA, García LY, Jakovljevic M. Healthcare system development in the Middle East and North Africa region: Challenges, endeavors and prospective opportunities. *Front Public Health* 2022; **10**: 1045739 [PMID: 36620278 DOI: 10.3389/fpubh.2022.1045739]
14 **Musaiger AO**, Awadhalla MS, Al-Mannai M, AlSawad M, Asokan GV. Dietary habits and sedentary behaviors among health science university students in Bahrain. *Int J Adolesc Med Health* 2017; **29** [PMID: 26251982 DOI: 10.1515/ijamh-2015-0038]
15 **Al-Snan NR**, Messaoudi S, R Babu S, Bakhiet M. Population genetic data of the 21 autosomal STRs included in GlobalFiler kit of a population sample from the Kingdom of Bahrain. *PLoS One* 2019; **14**: e0220620 [PMID: 31415577 DOI: 10.1371/journal.pone.0220620]
16 **Wirayuda AAB**, Al-Mahrezi A, Chan MF. Factors Impacting Life Expectancy in Bahrain: Evidence from 1971 to 2020 Data. *Int J Health Serv* 2022; 207314221129052 [PMID: 36214193 DOI: 10.1177/00207314221129052]
17 **Theel ES**, Doern CD. β-D-glucan testing is important for diagnosis of invasive fungal infections. *J Clin Microbiol* 2013; **51**: 3478-3483 [PMID: 23850953 DOI: 10.1128/JCM.01737-13]
18 **Duettmann W**, Koidl C, Krause R, Lackner G, Woelfler A, Hoenigl M. Specificity of mannan antigen and anti-mannan antibody screening in patients with haematological malignancies at risk for fungal infection. *Mycoses* 2016; **59**: 374-378 [PMID: 26916753 DOI: 10.1111/myc.12482]
19 **Pappas PG**, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis* 2016; **62**: e1-50 [PMID: 26679628 DOI: 10.1093/cid/civ933]

20 **Lin HH**, Tseng KH, Tien N, Lin YT, Yu J, Hsueh PR, Cho DY. Evaluation of the Rapid Sepsityper protocol and specific MBT-Sepsityper module for the identification of bacteremia and fungemia using Bruker Biotyper MALDI-TOF MS. *J Microbiol Immunol Infect* 2022; **55**: 1330-1333 [PMID: 35981943 DOI: 10.1016/j.jmii.2022.07.005]

21 **Arendrup MC**, Cuenca-Estrella M, Lass-Flörl C, Hope W; EUCAST-AFST. EUCAST technical note on the EUCAST definitive document EDef 7.2: method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts EDef 7.2 (EUCAST-AFST). *Clin Microbiol Infect* 2012; **18**: E246-E247 [PMID: 22563750 DOI: 10.1111/j.1469-0691.2012.03880.x]

22 **Frías-De-León MG**, Hernández-Castro R, Vite-Garín T, Arenas R, Bonifaz A, Castañón-Olivares L, Acosta-Altamirano G, Martínez-Herrera E. Antifungal Resistance in Candida auris: Molecular Determinants. *Antibiotics (Basel)* 2020; **9**: 568 [PMID: 32887362 DOI: 10.3390/antibiotics9090568]

23 **Alkhalifa W**, Alhawaj H, Alamri A, Alturki F, Alshahrani M, Alnimr A. Clinical and Microbiological Characteristics of Candidemia Cases in Saudi Arabia. *Infect Drug Resist* 2023; **16**: 4489-4503 [PMID: 37457797 DOI: 10.2147/IDR.S411865]

24 **Al Dhaheri F**, Thomsen J, Everett D, Denning DW. Mapping the Burden of Fungal Diseases in the United Arab Emirates. *J Fungi (Basel)* 2024; **10**: 353 [PMID: 38786708 DOI: 10.3390/jof10050353]

25 **Barchiesi F**, Orsetti E, Mazzanti S, Trave F, Salvi A, Nitti C, Manso E. Candidemia in the elderly: What does it change? *PLoS One* 2017; **12**: e0176576 [PMID: 28493896 DOI: 10.1371/journal.pone.0176576]

26 **Guimarães T**, Nucci M, Mendonça JS, Martinez R, Brito LR, Silva N, Moretti ML, Salomão R, Colombo AL. Epidemiology and predictors of a poor outcome in elderly patients with candidemia. *Int J Infect Dis* 2012; **16**: e442-e447 [PMID: 22486857 DOI: 10.1016/j.ijid.2012.02.005]

27 **Wang H**, Liu N, Yin M, Han H, Yue J, Zhang F, Shan T, Guo H, Wu D. The epidemiology, antifungal use and risk factors of death in elderly patients with candidemia: a multicentre retrospective study. *BMC Infect Dis* 2014; **14**: 609 [PMID: 25420435 DOI: 10.1186/s12879-014-0609-x]

28 **Alobaid K**, Ahmad S, Asadzadeh M, Mokaddas E, Al-Sweih N, Albenwan K, Alfouzan W, Al-Obaid I, Jeragh A, Al-Roomi E, Khan Z, Joseph L, Varghese S. Epidemiology of Candidemia in Kuwait: A Nationwide, Population-Based Study. *J Fungi (Basel)* 2021; **7**: 673 [PMID: 34436212 DOI: 10.3390/jof7080673]

29 **Uwaydah AH**, Abdelmaksoud SI, Atta A, AlQasimi A. Candidemia A Review Over 5 Years at A 300 Beds Military Hospital (Serving Military Personnel and Their Families) In the United Arab Emirates. *J Nur Healthcare* 2016; **1**: 1-4 [DOI: 10.33140/JNH/01/02/00007]

30 **Ellis M**, Hedstrom U, Jumaa P, Bener A. Epidemiology, presentation, management and outcome of candidemia in a tertiary care teaching hospital in the United Arab Emirates, 1995-2001. *Med Mycol* 2003; **41**: 521-528 [PMID: 14725327 DOI: 10.1080/13693780310001645337]

31 **Alhatmi H**, Almansour S, Abanamy R, Akbar A, Abalkhail M, Alharbi A, Alsaedy A, Mahmoud E, Alalwan B, AlJohani S, Aldibasi OS, Bosaeed M, Alothman A. Clinical Characteristics and Outcome of Candidemia: Experience from a Tertiary Referral Center in Saudi Arabia. *Saudi J Med Med Sci* 2022; **10**: 125-130 [PMID: 35602394 DOI: 10.4103/sjmms.sjmms_625_21]

32 **Ahmad S**, Alfouzan W. Candida auris: Epidemiology, Diagnosis, Pathogenesis, Antifungal Susceptibility, and Infection Control Measures to Combat the Spread of Infections in Healthcare Facilities. *Microorganisms* 2021; **9**: 807 [PMID: 33920482 DOI: 10.3390/microorganisms9040807]

33 **Khan Z**, Ahmad S, Al-Sweih N, Mokaddas E, Al-Banwan K, Alfouzan W, Al-Obaid I, Al-Obaid K, Asadzadeh M, Jeragh A, Joseph L, Varghese S, Vayalil S, Al-Musallam O. Changing trends in epidemiology and antifungal susceptibility patterns of six bloodstream Candida species isolates over a 12-year period in Kuwait. *PLoS One* 2019; **14**: e0216250 [PMID: 31042770 DOI: 10.1371/journal.pone.0216250]

34 **Alshahrani FS**, Elgujja AA, Alsubaie S, Ezreqat SA, Albarraq AM, Barry M, Binkhamis K, Alabdan L. Description of Candida auris Occurrence in a Tertiary Health Institution in Riyadh, Saudi Arabia. *Healthcare (Basel)* 2023; **11**: 3150 [PMID: 38132040 DOI: 10.3390/healthcare11243150]

35 **Thomsen J**, Abdulrazzaq NM, Oulhaj A, Nyasulu PS, Alatoom A, Denning DW, Al Dhaheri F; UAE AMR Surveillance Consortium, Menezes GA, Moubareck CA, Senok A, Everett DB. Emergence of highly resistant Candida auris in the United Arab Emirates: a retrospective analysis of evolving national trends. *Front Public Health* 2023; **11**: 1244358 [PMID: 38292390 DOI: 10.3389/fpubh.2023.1244358]

36 **Chakrabarti A**, Sood P. On the emergence, spread and resistance of Candida auris: host, pathogen and environmental tipping points. *J Med Microbiol* 2021; **70**: 001318 [PMID: 33599604 DOI: 10.1099/jmm.0.001318]

37 **Parslow BY**, Thornton CR. Continuing Shifts in Epidemiology and Antifungal Susceptibility Highlight the Need for Improved Disease Management of Invasive Candidiasis. *Microorganisms* 2022; **10**: 1208 [PMID: 35744725 DOI: 10.3390/microorganisms10061208]

38 **Branco J**, Miranda IM, Rodrigues AG. Candida parapsilosis Virulence and Antifungal Resistance Mechanisms: A Comprehensive Review of Key Determinants. *J Fungi (Basel)* 2023; **9**: 80 [PMID: 36675901 DOI: 10.3390/jof9010080]

39 **Miranda-Cadena K**, Marcos-Arias C, Mateo E, Aguirre JM, Quindós G, Eraso E. Prevalence and antifungal susceptibility profiles of Candida glabrata, Candida parapsilosis and their close-related species in oral candidiasis. *Arch Oral Biol* 2018; **95**: 100-107 [PMID: 30096698 DOI: 10.1016/j.archoralbio.2018.07.017]

40 **Pfaller MA**, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. Twenty Years of the SENTRY Antifungal Surveillance Program: Results for Candida Species From 1997-2016. *Open Forum Infect Dis* 2019; **6**: S79-S94 [PMID: 30895218 DOI: 10.1093/ofid/ofy358]

41 **Song Y**, Chen X, Yan Y, Wan Z, Liu W, Li R. Prevalence and Antifungal Susceptibility of Pathogenic Yeasts in China: A 10-Year Retrospective Study in a Teaching Hospital. *Front Microbiol* 2020; **11**: 1401 [PMID: 32719663 DOI: 10.3389/fmicb.2020.01401]

42 **Pfaller MA**, Messer SA, Boyken L, Tendolkar S, Hollis RJ, Diekema DJ. Geographic variation in the susceptibilities of invasive isolates of Candida glabrata to seven systemically active antifungal agents: a global assessment from the ARTEMIS Antifungal Surveillance Program conducted in 2001 and 2002. *J Clin Microbiol* 2004; **42**: 3142-3146 [PMID: 15243073 DOI: 10.1128/JCM.42.7.3142-3146.2004]

43 **Dixit D**, Jen P, Maxwell TD, Smoke S, McCracken JA, Cardinale-King M, Haribhakti A, Patel P, Cani E, Choi SC, Jagpal S, Varughese T, Tatem LL, Bhowmick T. Risk Factors and Clinical Outcomes of Candidemia Associated With Severe COVID-19. *Crit Care Explor* 2022; **4**: e0762 [PMID: 36119397 DOI: 10.1097/CCE.0000000000000762]

44 **da Silva CM**, de Carvalho AMR, Macêdo DPC, Jucá MB, Amorim RJM, Neves RP. Candidemia in Brazilian neonatal intensive care units: risk factors, epidemiology, and antifungal resistance. *Braz J Microbiol* 2023; **54**: 817-825 [PMID: 36892755 DOI: 10.1007/s42770-023-00943-1]

45 **Yamin DH**, Husin A, Harun A. Risk Factors of Candida parapsilosis Catheter-Related Bloodstream Infection. *Front Public Health* 2021; **9**: 631865 [PMID: 34458217 DOI: 10.3389/fpubh.2021.631865]

46 **Amabile E**, Totaro M, Cappelli LV, Minotti C, Micozzi A. A Case of Central Venous Catheter-Related Candida parapsilosis Fungemia Evolved to Disseminated Infection in a Neutropenic Patient with Blast Crisis of Chronic Myeloid Leukemia. *Mediterr J Hematol Infect Dis* 2024; **16**: e2024013 [PMID: 38223476 DOI: 10.4084/MJHID.2024.013]

47 **Aghili SR**, Shokohi T, Boroumand MA, Hashemi Fesharaki S, Salmanian B. Intravenous Catheter-Associated Candidemia due to Candida membranaefaciens: The First Iranian Case. *J Tehran Heart Cent* 2015; **10**: 101-105 [PMID: 26110010]

48 **Li H**, Miao MX, Jia CL, Cao YB, Yan TH, Jiang YY, Yang F. Interactions between Candida albicans and the resident microbiota. *Front Microbiol* 2022; **13**: 930495 [PMID: 36204612 DOI: 10.3389/fmicb.2022.930495]

49 **Reed EE**, West JE, Keating EA, Pancholi P, Balada-Llasat JM, Mangino JE, Bauer KA, Goff DA. Improving the management of candidemia through antimicrobial stewardship interventions. *Diagn Microbiol Infect Dis* 2014; **78**: 157-161 [PMID: 24316015 DOI: 10.1016/j.diagmicrobio.2013.11.012]

50 **Kilic AU**, Basaga SM, Cevahir F, Cakir O, Doganay M, Alp E. Risk prediction for candidemia in surgical intensive care unit patients. *North Clin Istanb* 2020; **7**: 348-353 [PMID: 33043259 DOI: 10.14744/nci.2020.27136]

51 **Berdal JE**, Haagensen R, Ranheim T, Bjørnholt JV. Nosocomial candidemia; risk factors and prognosis revisited; 11 years experience from a Norwegian secondary hospital. *PLoS One* 2014; **9**: e103916 [PMID: 25079361 DOI: 10.1371/journal.pone.0103916]

52 **Nascimento T**, Inácio J, Guerreiro D, Patrício P, Proença L, Toscano C, Diaz P, Barroso H. Insights into Candida Colonization in Intensive Care Unit Patients: A Prospective Multicenter Study. *J Fungi (Basel)* 2024; **10**: 378 [PMID: 38921364 DOI: 10.3390/jof10060378]

53 **Pyrgos V**, Ratanavanich K, Donegan N, Veis J, Walsh TJ, Shoham S. Candida bloodstream infections in hemodialysis recipients. *Med Mycol* 2009; **47**: 463-467 [PMID: 18798046 DOI: 10.1080/13693780802369332]

54 **Quesada C**, Aceituno J, Suárez R, Mazariegos C. Fungemia Related to Parenteral Nutrition. *Curr Trop Med Rep* 2017; **4**: 172-177 [DOI: 10.1007/s40475-017-0120-8]

55 **Li Z**, Denning DW. The Impact of Corticosteroids on the Outcome of Fungal Disease: a Systematic Review and Meta-analysis. *Curr Fungal Infect Rep* 2023; **17**: 54-70 [PMID: 36852004 DOI: 10.1007/s12281-023-00456-2]

56 **AlMaghrabi RS**, Al-Musawi T, Albaksami O, Subhi AL, Fakih RE, Stone NR. Challenges in the Management of Invasive Fungal Infections in the Middle East: Expert Opinion to Optimize Management Using a Multidisciplinary Approach. *Cureus* 2023; **15**: e44356 [PMID: 37779746 DOI: 10.7759/cureus.44356]

57 **Tukenmez Tigen E**, Bilgin H, Perk Gurun H, Dogru A, Ozben B, Cerikcioglu N, Korten V. Risk factors, characteristics, and outcomes of candidemia in an adult intensive care unit in Turkey. *Am J Infect Control* 2017; **45**: e61-e63 [PMID: 28359611 DOI: 10.1016/j.ajic.2017.02.022]

58 **Rajni E**, Chaudhary P, Garg VK, Sharma R, Malik M. A complete clinico-epidemiological and microbiological profile of candidemia cases in a tertiary-care hospital in Western India. *Antimicrob Steward Healthc Epidemiol* 2022; **2**: e37 [PMID: 36310808 DOI: 10.1017/ash.2021.235]

59 **Xiao Z**, Wang Q, Zhu F, An Y. Epidemiology, species distribution, antifungal susceptibility and mortality risk factors of candidemia among critically ill patients: a retrospective study from 2011 to 2017 in a teaching hospital in China. *Antimicrob Resist Infect Control* 2019; **8**: 89 [PMID: 31161036 DOI: 10.1186/s13756-019-0534-2]

60 **Hou J**, Deng J, Liu Y, Zhang W, Wu S, Liao Q, Ma Y, Kang M. Epidemiology, Clinical Characteristics, Risk Factors, and Outcomes of Candidemia in a Large Tertiary Teaching Hospital in Western China: A Retrospective 5-Year Study from 2016 to 2020. *Antibiotics (Basel)* 2022; **11**: 788 [PMID: 35740194 DOI: 10.3390/antibiotics11060788]

61 **Liu SH**, Mitchell H, Nasser Al-Rawahi G. Epidemiology and associated risk factors for candidemia in a Canadian tertiary paediatric hospital: An 11-year review. *J Assoc Med Microbiol Infect Dis Can* 2023; **8**: 29-39 [PMID: 37008577 DOI: 10.3138/jammi-2022-0021]

62 **Shah DN**, Yau R, Weston J, Lasco TM, Salazar M, Palmer HR, Garey KW. Evaluation of antifungal therapy in patients with candidaemia based on susceptibility testing results: implications for antimicrobial stewardship programmes. *J Antimicrob Chemother* 2011; **66**: 2146-2151 [PMID: 21700622 DOI: 10.1093/jac/dkr244]

63 **Fothergill AW**, Sutton DA, McCarthy DI, Wiederhold NP. Impact of new antifungal breakpoints on antifungal resistance in Candida species. *J Clin Microbiol* 2014; **52**: 994-997 [PMID: 24403302 DOI: 10.1128/JCM.03044-13]

64 **Flowers SA**, Colón B, Whaley SG, Schuler MA, Rogers PD. Contribution of clinically derived mutations in ERG11 to azole resistance in Candida albicans. *Antimicrob Agents Chemother* 2015; **59**: 450-460 [PMID: 25385095 DOI: 10.1128/AAC.03470-14]

65 **Wang Y**, He Y, Cai T, Lei Z, Lei W, Cao Y, Wu J. A mechanism study on the synergistic effects of rifapentine and fluconazole against fluconazole-resistant Candida albicans in vitro. *Heliyon* 2024; **10**: e27346 [PMID: 38515731 DOI: 10.1016/j.heliyon.2024.e27346]

66 **Zarrinfar H**, Kord Z, Fata A. High incidence of azole resistance among Candida albicans and C. glabrata isolates in Northeastern Iran. *Curr Med Mycol* 2021; **7**: 18-21 [PMID: 35528623 DOI: 10.18502/cmm.7.3.7801]

67 **Dhasarathan P**, AlSalhi MS, Devanesan S, Subbiah J, Ranjitsingh AJA, Binsalah M, Alfuraydi AA. Drug resistance in Candida albicans isolates and related changes in the structural domain of Mdr1 protein. *J Infect Public Health* 2021; **14**: 1848-1853 [PMID: 34794907 DOI: 10.1016/j.jiph.2021.11.002]

68 **Al Halteet S**, Abdel-Hadi A, Hassan M, Awad M. Prevalence and Antifungal Susceptibility Profile of Clinically Relevant Candida Species in Postmenopausal Women with Diabetes. *Biomed Res Int* 2020; **2020**: 7042490 [PMID: 33294451 DOI: 10.1155/2020/7042490]

69 **Forastiero A**, Mesa-Arango AC, Alastruey-Izquierdo A, Alcazar-Fuoli L, Bernal-Martinez L, Pelaez T, Lopez JF, Grimalt JO, Gomez-Lopez A, Cuesta I, Zaragoza O, Mellado E. Candida tropicalis antifungal cross-resistance is related to different azole target (Erg11p) modifications. *Antimicrob Agents Chemother* 2013; **57**: 4769-4781 [PMID: 23877676 DOI: 10.1128/AAC.00477-13]

70 **Lima R**, Ribeiro FC, Colombo AL, de Almeida JN Jr. The emerging threat antifungal-resistant Candida tropicalis in humans, animals, and environment. *Front Fungal Biol* 2022; **3**: 957021 [PMID: 37746212 DOI: 10.3389/ffunb.2022.957021]

71 **Fan X**, Tsui CKM, Chen X, Wang P, Liu ZJ, Yang CX. High prevalence of fluconazole resistant Candida tropicalis among candiduria samples in China: An ignored matter of concern. *Front Microbiol* 2023; **14**: 1125241 [PMID: 36937265 DOI: 10.3389/fmicb.2023.1125241]

72 **Arastehfar A**, Hilmioğlu-Polat S, Daneshnia F, Hafez A, Salehi M, Polat F, Yaşar M, Arslan N, Hoşbul T, Ünal N, Metin DY, Gürcan Ş, Birinci A, Koç AN, Pan W, Ilkit M, Perlin DS, Lass-Flörl C. Recent Increase in the Prevalence of Fluconazole-Non-susceptible Candida tropicalis Blood Isolates in Turkey: Clinical Implication of Azole-Non-susceptible and Fluconazole Tolerant Phenotypes and Genotyping. *Front Microbiol* 2020; **11**: 587278 [PMID: 33123116 DOI: 10.3389/fmicb.2020.587278]

73 **Szekely J**, Rakchang W, Rattanaphan P, Kositpantawong N. Fluconazole and echinocandin resistance of Candida species in invasive candidiasis at a university hospital during pre-COVID-19 and the COVID-19 outbreak. *Epidemiol Infect* 2023; **151**: e146 [PMID: 37622338 DOI: 10.1017/S0950268823001346]

74 **Axner-Elings M**, Botero-Kleiven S, Jensen RH, Arendrup MC. Echinocandin susceptibility testing of Candida isolates collected during a 1-year period in Sweden. *J Clin Microbiol* 2011; **49**: 2516-2521 [PMID: 21543574 DOI: 10.1128/JCM.00201-11]

75 **Daneshnia F**, de Almeida Júnior JN, Arastehfar A, Lombardi L, Shor E, Moreno L, Verena Mendes A, Goreth Barberino M, Thomaz

Yamamoto D, Butler G, Perlin DS, Colombo AL. Determinants of fluconazole resistance and echinocandin tolerance in C. parapsilosis isolates causing a large clonal candidemia outbreak among COVID-19 patients in a Brazilian ICU. *Emerg Microbes Infect* 2022; **11**: 2264-2274 [PMID: 36066554 DOI: 10.1080/22221751.2022.2117093]

76 **Franconi I**, Rizzato C, Poma N, Tavanti A, Lupetti A. Candida parapsilosis sensu stricto Antifungal Resistance Mechanisms and Associated Epidemiology. *J Fungi (Basel)* 2023; **9**: 798 [PMID: 37623569 DOI: 10.3390/jof9080798]

77 **Ning Y**, Xiao M, Perlin DS, Zhao Y, Lu M, Li Y, Luo Z, Dai R, Li S, Xu J, Liu L, He H, Liu Y, Li F, Guo Y, Chen Z, Xu Y, Sun T, Zhang L. Decreased echinocandin susceptibility in Candida parapsilosis causing candidemia and emergence of a pan-echinocandin resistant case in China. *Emerg Microbes Infect* 2023; **12**: 2153086 [PMID: 36440795 DOI: 10.1080/22221751.2022.2153086]

78 **Murphy SE**, Bicanic T. Drug Resistance and Novel Therapeutic Approaches in Invasive Candidiasis. *Front Cell Infect Microbiol* 2021; **11**: 759408 [PMID: 34970504 DOI: 10.3389/fcimb.2021.759408]

79 **Trofa D**, Gácser A, Nosanchuk JD. Candida parapsilosis, an emerging fungal pathogen. *Clin Microbiol Rev* 2008; **21**: 606-625 [PMID: 18854483 DOI: 10.1128/CMR.00013-08]

80 **Hassan Y**, Chew SY, Than LTL. Candida glabrata: Pathogenicity and Resistance Mechanisms for Adaptation and Survival. *J Fungi (Basel)* 2021; **7**: 667 [PMID: 34436206 DOI: 10.3390/jof7080667]

81 **Sardi JCO**, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJS. Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *J Med Microbiol* 2013; **62**: 10-24 [PMID: 23180477 DOI: 10.1099/jmm.0.045054-0]

82 **Silva S**, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiol Rev* 2012; **36**: 288-305 [PMID: 21569057 DOI: 10.1111/j.1574-6976.2011.00278.x]

83 **Alexander BD**, Johnson MD, Pfeiffer CD, Jiménez-Ortigosa C, Catania J, Booker R, Castanheira M, Messer SA, Perlin DS, Pfaller MA. Increasing echinocandin resistance in Candida glabrata: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. *Clin Infect Dis* 2013; **56**: 1724-1732 [PMID: 23487382 DOI: 10.1093/cid/cit136]

84 **Kumar H**, Mukherjee D, Banerjee S, Upadhyay P, Sharma V, Akilimali A. Candida Auris: An Emerging Multidrug-Resistant Fungal Pathogen in the United States and the Urgent Call for Action. *Microbiol Insights* 2023; **16**: 11786361231200836 [PMID: 37745089 DOI: 10.1177/11786361231200836]

85 **Ademe M**, Girma F. Candida auris: From Multidrug Resistance to Pan-Resistant Strains. *Infect Drug Resist* 2020; **13**: 1287-1294 [PMID: 32440165 DOI: 10.2147/IDR.S249864]

86 **Lockhart SR**, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP, Colombo AL, Calvo B, Cuomo CA, Desjardins CA, Berkow EL, Castanheira M, Magobo RE, Jabeen K, Asghar RJ, Meis JF, Jackson B, Chiller T, Litvintseva AP. Simultaneous Emergence of Multidrug-Resistant Candida auris on 3 Continents Confirmed by Whole-Genome Sequencing and Epidemiological Analyses. *Clin Infect Dis* 2017; **64**: 134-140 [PMID: 27988485 DOI: 10.1093/cid/ciw691]

87 **Alvarez-Moreno CA**, Morales-López S, Rodriguez GJ, Rodriguez JY, Robert E, Picot C, Ceballos-Garzon A, Parra-Giraldo CM, Le Pape P. The Mortality Attributable to Candidemia in C. auris Is Higher than That in Other Candida Species: Myth or Reality? *J Fungi (Basel)* 2023; **9**: 430 [PMID: 37108885 DOI: 10.3390/jof9040430]

88 **Wang E**, Farmakiotis D, Yang D, McCue DA, Kantarjian HM, Kontoyiannis DP, Mathisen MS. The ever-evolving landscape of candidaemia in patients with acute leukaemia: non-susceptibility to caspofungin and multidrug resistance are associated with increased mortality. *J Antimicrob Chemother* 2015; **70**: 2362-2368 [PMID: 25855759 DOI: 10.1093/jac/dkv087]

89 **Alves J**, Alonso-Tarrés C, Rello J. How to Identify Invasive Candidemia in ICU-A Narrative Review. *Antibiotics (Basel)* 2022; **11**: 1804 [PMID: 36551461 DOI: 10.3390/antibiotics11121804]

90 **Cobrado L**, Silva-Dias A, Azevedo MM, Rodrigues A. Anti-Candida activity of antimicrobial impregnated central venous catheters. *Antimicrob Resist Infect Control* 2017; **6**: 110 [PMID: 29142743 DOI: 10.1186/s13756-017-0269-x]

91 **Hoenigl M**, Enoch DA, Wichmann D, Wyncoll D, Cortegiani A. Exploring European Consensus About the Remaining Treatment Challenges and Subsequent Opportunities to Improve the Management of Invasive Fungal Infection (IFI) in the Intensive Care Unit. *Mycopathologia* 2024; **189**: 41 [PMID: 38704761 DOI: 10.1007/s11046-024-00852-3]

92 **Ahmad S**, Asadzadeh M. Strategies to Prevent Transmission of Candida auris in Healthcare Settings. *Curr Fungal Infect Rep* 2023; **17**: 36-48 [PMID: 36718372 DOI: 10.1007/s12281-023-00451-7]

93 **Chan XHS**, O'Connor CJ, Martyn E, Clegg AJ, Choy BJK, Soares AL, Shulman R, Stone NRH, De S, Bitmead J, Hail L, Brealey D, Arulkumaran N, Singer M, Wilson APR. Reducing broad-spectrum antibiotic use in intensive care unit between first and second waves of COVID-19 did not adversely affect mortality. *J Hosp Infect* 2022; **124**: 37-46 [PMID: 35339638 DOI: 10.1016/j.jhin.2022.03.007]

94 **Chakrabarti A**, Mohamed N, Capparella MR, Townsend A, Sung AH, Yura R, Muñoz P. The Role of Diagnostics-Driven Antifungal Stewardship in the Management of Invasive Fungal Infections: A Systematic Literature Review. *Open Forum Infect Dis* 2022; **9**: ofac234 [PMID: 35873300 DOI: 10.1093/ofid/ofac234]

95 **Barratt R**, Gilbert GL. Education and training in infection prevention and control: Exploring support for national standards. *Infect Dis Health* 2021; **26**: 139-144 [PMID: 33461891 DOI: 10.1016/j.idh.2020.12.002]

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ORIGINAL ARTICLE

Observational Study

Acceptance of COVID-19 vaccine and its related determinants in Nigeria: An online survey

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Abstract

BACKGROUND

Vaccine hesitancy is a major challenge in the fight against the coronavirus disease 2019 (COVID-19) pandemic. Identifying the sociodemographic factors associated with vaccine acceptance among Nigerians is crucial for improving vaccine uptake.

AIM

To assess the acceptance rate of COVID-19 vaccine and its related determinants among Nigerians.

METHODS

An online cross-sectional survey (observational study) was conducted between February 2021 and May 2021, using a questionnaire hosted on SurveyMonkey. The invitation to take part in the poll was sent out to participants through social networking platforms. A logistic regression was used to determine which sociodemographic factors were associated with vaccine acceptance constructs.

RESULTS

A total of 1800 persons responded to the survey, a larger proportion of whom were males (53.9%) and within the age group of 21-30 years (29.4%) and earned an average income of less than $500 per month (43.3%). Only 0.56% of participants had a high perceived risk of COVID-19 infection, while only 1.11% had a perceived risk of dying from COVID-19. The perception rate of the COVID-19 vaccine among participants was 51.1%, while the acceptance rate was 63.9%. There was no significant association between the COVID-19 vaccine acceptance rate and related determinants assessed, particularly age ($\chi^2 = 3.049$, $P = 0.550$), sex ($\chi^2 = 0.102$, $P = 0.749$), average income ($\chi^2 = 3.802$, $P = 0.875$), and religion ($\chi^2 = 2.819$, $P = 0.420$). Participants with chronic conditions demonstrated a higher acceptance rate compared to the general population.

CONCLUSION

Despite the positive perception observed and substantial vaccine acceptance rate among the study participants, more public health interventions are still needed to enhance vaccine acceptability in Nigeria.

Key Words: Acceptance; COVID-19; Determinants; Hesitancy; Nigerians; Online survey; Vaccine

Core Tip: This study assessed coronavirus disease 2019 vaccine acceptance among Nigerians through an online survey with 1800 respondents. Despite a substantial acceptance rate of 63.9%, sociodemographic factors (age, sex, income, and religion) did not significantly influence vaccine uptake. Positive perceptions of vaccination were common, yet financial barriers affected acceptance rates. Key findings suggest enhancing public health education, economic support, and trust-building measures to improve vaccine uptake. Notably, individuals with chronic conditions were more inclined to accept the vaccine, underscoring the need for targeted interventions to achieve broader immunization coverage and herd immunity in Nigeria.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19), which first surfaced in China in December 2019 and has since spread to practically every nation on the planet, has led to numerous fatalities and financial difficulties throughout the globe[1,2]. The pandemic exacerbated issues in developing nations due to fewer medical facilities, higher poverty rates, and limited access to immunizations. Inequitable vaccine distribution may lead to increased hospitalizations, deaths, and the emergence of new disease strains in these nations[3,4]. A variety of methods, including physical isolation, mobility limitations, and vaccination campaigns, have been employed to tackle the COVID-19 pandemic[5]. Vaccines are critical for avoiding and managing infectious disease epidemics, ultimately saving millions of lives[6]. However, the broader acceptance of vaccines by the public is crucial to halting the epidemic. Despite the availability of vaccination services, various factors contribute to some people's distrust of vaccines and reluctance to utilize them[7,8].

Vaccination is one of the most cost-effective methods for preventing and controlling infectious diseases. However, certain individuals and segments of the public are opposed to vaccination[9]. The volume of knowledge on why individuals reject and accept vaccines is growing. Governments worldwide have recommended preventive measures such as wearing masks, maintaining social distance, frequent hand washing, and lockdowns to limit the spread of COVID-19 and minimize fatalities[10]. However, factors such as religious bias, politics, and language barriers have been identified as obstacles to vaccine roll-out and acceptance[11-13]. According to the World Health Organization, vaccine skepticism is one of the most serious threats to global health[14]. The current COVID-19 immunization has a poor acceptance rate due to budgetary constraints and a history of COVID-19 illness. Presenting simple, comprehensible information about vaccines and strongly persuading individuals to receive them is the most effective strategy to combat vac-cine aversion[15, 16].

Patients are more likely to get vaccinated and are less hesitant if medical workers have a positive attitude toward vaccines[17]. The level of knowledge and attitude regarding immunization that healthcare professionals have will impact their likelihood of using it and promoting it to their patients. Since healthcare workers are on the front lines of the current epidemic, governments have prioritized immunizing them first. However, there have been increasing cases of vaccine refusal[18,19]. Policymakers, academics, and health officials need to understand how well the COVID-19 vaccine is accepted by healthcare workers and what influences their acceptance or rejection to develop effective strategies to reduce vaccination hesitancy. While more than half of the world's population (59.5%) has received the COVID-19 vaccine, significant disparities in immunization rates persist across nations[20,21]. The Horn of Africa has the world's lowest immunization rate, at less than 20%. Delays in vaccination may be due to perceived dangers, religious beliefs, and inadequate information and awareness[22].

MATERIALS AND METHODS

Rationale of the study

Understanding the factors influencing vaccine acceptance is essential not only for managing the COVID-19 pandemic but also for addressing future infectious diseases that require vaccination. Insights gained from studying COVID-19 vaccine acceptance can inform strategies to enhance vaccine uptake for other infectious diseases, thereby improving global health outcomes.

Hypotheses

Hypothesis 1: Sociodemographic factors such as age, sex, income, and religion are significant predictors of COVID-19 vaccine acceptance among Nigerians.

Hypothesis 2: Individuals with chronic conditions in Nigeria are more likely to accept the COVID-19 vaccine compared to those without chronic conditions.

Study design

This observational study collected data on COVID-19 vaccine acceptance and related sociodemographic factors, without manipulating any variables or providing interventions.

Setting/period: The nation-wide online web-based survey was conducted from February 1, 2021 to May 31, 2021.

Cross-sectional: The data was collected at a single point in time, providing a snapshot of the population's attitudes and behaviors during the survey period.

Participants

Target population: The Nigerian populace.

Inclusion/exclusion criteria: Consenting respondents with access to the internet participated in the online survey.

Survey instrument: The questionnaire was hosted on SurveyMonkey, designed to gather information on vaccine acceptance, perceived risk, and sociodemographic factors. The survey was structured into several sections. The initial section provided an introduction with information about previous studies and included a page on informed consent. The subsequent sections, accessible only to those who agreed to participate, collected demographic information, participants'

attitudes toward vaccinations and social distancing, and the impact of the COVID-19 pandemic on their work lives. Questions were inspired by previous studies[15,20,23]. Each participant completed the survey after providing informed consent, which took approximately 10-15 min.

Mode of distribution: The invitation to participate was disseminated *via* social networking platforms, including Facebook, Twitter, and WhatsApp, targeting a potentially broad audience within the Nigerian population.

Validity of the instrument: To ensure the validity of the instrument, both face and content validity were assessed. Experts in public health and survey methodology reviewed the questionnaire to ensure it was appropriate and comprehensive for the study objectives. Demographic information was collected to identify potential sampling biases, including differences between participants and non-participants. This helped address the potential bias that might arise from the selective participation of certain demographic groups.

Variables

Independent variables: Sociodemographic factors such as age, sex, income, religion, and the presence of chronic conditions.

Dependent variable: COVID-19 vaccine acceptance, measured as the primary outcome.

Other variables: Perceived risk of infection and death from COVID-19.

Data sources and measurement

The data for this study were sourced from an online cross-sectional survey conducted between February 2021 and May 2021. The survey was hosted on SurveyMonkey, and participants were recruited through social networking platforms, which facilitated broad access across the Nigerian population.

Variables of interest and measurement

COVID-19 vaccine acceptance: Vaccine acceptance was assessed using specific questions within the survey designed to capture participant willingness to receive the COVID-19 vaccine. This was measured as a categorical variable, with participants indicating whether they were willing to accept the vaccine or not.

Sociodemographic factors: These variables (age, sex, income, and religion) were self-reported by participants through the survey. Age was captured in predefined categories (*e.g.*, 21-30 years), sex was reported as male or female, income was categorized based on monthly earnings (*e.g.*, less than $500 per month), and religion was also categorized (*e.g.*, Christianity, Islam).

Chronic conditions: Participants were asked if they had any chronic health conditions, with the presence of such conditions being noted as a binary variable (yes/no).

Perceived risk of COVID-19 infection and death: Perceived risk was assessed through survey questions where participants rated their perceived likelihood of contracting COVID-19 and their perceived risk of dying from it. These perceptions were measured on a percentage scale (*e.g.*, 0%, 1%-10%, 11%-20%, 21%-30%, *etc.*).

Comparability of assessment methods: All participants in the study completed the same standardized survey, ensuring that the methods of data collection and measurement were consistent across the entire sample. As the study did not involve multiple groups subjected to different assessment methods, the comparability of assessment methods was inherently maintained. This uniform approach minimized the risk of measurement bias and ensured that the data collected were comparable across all respondents.

Bias

It is important to acknowledge potential bias related to the mode of survey distribution. The survey was disseminated *via* social media platforms, which means that individuals without access to these platforms were not represented in the sample. This limitation may affect the generalizability of the findings. Due to the nature of social media distribution, the total number of individuals who received the survey invitation could not be determined. Therefore, the exact response rate could not be calculated. This represents a limitation of the study, as it prevents us from assessing the representativeness of the sample accurately.

Data completeness and usability

After data collection, responses were reviewed for completeness and usability. This involved checking for any missing or incomplete responses that could compromise the data quality. Only fully completed questionnaires were included in the analysis to maintain the integrity of the data. By considering these limitations and addressing potential biases, this study aimed to provide a comprehensive understanding of the acceptance of the COVID-19 vaccine and its related determinants among Nigerians.

Sample size

A total of 1800 participants completed the survey. This sample size was not predetermined prior to the survey (because

the research team aimed to achieve a sample size that would be sufficiently large to provide the necessary statistical power for detecting potential associations between sociodemographic factors and COVID-19 vaccine acceptance). Rather, it was determined by the number of respondents who actually engaged with the survey during the collection period from February 2021 to May 2021. This final number of participants was influenced by factors such as the reach of the survey distribution channels and participant engagement.

Quantitative variables

In the study, quantitative variables, such as age, income, and perceived risk of COVID-19 infection and death, were initially collected in their continuous or original forms. However, for the purpose of analysis, these variables were grouped into categorical ranges to facilitate logistic regression and χ^2 tests.

Groupings and rationale

Age: Age was categorized into predefined groups, such as 21-30 years, 31-40 years, *etc.* This grouping allowed for easier interpretation of the data and comparison across different demographic groups. It also helped in managing potential variability and simplified the analysis, making it more straightforward to identify trends or associations with vaccine acceptance.

Income: Income was categorized into ranges (*e.g.*, less than $500 per month, $500-$1000 per month, *etc.*) to reflect different socioeconomic strata within the population. This categorization helped in assessing whether income levels influenced vaccine acceptance and made it easier to interpret the effects of economic factors on participants' decisions.

Perceived risk of COVID-19 infection and death: Perceived risk was categorized into percentage ranges (*e.g.*, 0%, 1%-10%, 11%-20%, *etc.*). This grouping method allowed the study to assess how different levels of perceived risk correlated with vaccine acceptance. It also helped in simplifying the analysis by reducing the complexity of dealing with continuous data, making it easier to identify patterns and draw conclusions.

Statistical analysis

Quantitative data were analyzed using Statistical Package for the Social Sciences, version 25 (IBM Corp., Armonk, NY, United States), in alignment with the study's objectives. The analysis focused on respondents' answers to closed-ended questions with a limited set of response options, where quantitative techniques were appropriate.

Statistical methods and control for confounding: Descriptive statistics, including frequency distributions, percentages, mean scores, and standard deviations, were calculated and presented in tabular form. χ^2 tests were employed for bivariate analyses to explore the associations between sociodemographic factors and vaccine acceptance. To control for potential confounding variables, logistic regression analysis was conducted, allowing for the adjustment of multiple sociodemographic factors simultaneously. The level of statistical significance was set at $P = 0.05$.

Subgroup and interaction analysis: Subgroup analyses were performed to examine interactions between key sociodemographic variables and vaccine acceptance. For example, separate logistic regression models were run for different age groups, income levels, and participants with chronic conditions to identify whether these subgroups exhibited different patterns of vaccine acceptance. Interaction terms were also tested within the regression models to assess whether the effect of one variable on vaccine acceptance was modified by another variable (*e.g.*, the interaction between age and income).

Handling of missing data: There was no missing data in this study. All respondents completed the survey in full, ensuring a complete dataset for analysis. As a result, no specific methods for handling missing data were required. The integrity of the dataset was maintained throughout the analysis process.

Analytical methods: To ensure that the findings were as representative as possible, the following analytical methods were employed: (1) Descriptive statistics: Frequency distributions, percentages, mean scores, and standard deviations were computed to describe the characteristics of the sample and the distribution of responses; (2) χ^2 tests: They were used for bivariate analyses to explore the associations between sociodemographic variables (*e.g.*, age, sex, and income) and vaccine acceptance. χ^2 tests helped identify significant relationships within the sample; (3) Logistic regression analysis: This method was employed to assess the relationship between sociodemographic factors and vaccine acceptance while adjusting for potential confounders. Logistic regression allowed for the evaluation of the influence of multiple variables simultaneously; and (4) Weighting considerations: Although the study used a convenience sampling method, the analysis considered potential biases by comparing the sample demographics with known population characteristics where possible. This comparison helped in interpreting the findings and understanding their generalizability.

Interpretation and limitations

The cross-sectional design of the study provided a snapshot of vaccine acceptance at a single point in time. Due to the non-random sampling method, the results were interpreted with caution regarding their generalizability to the broader Nigerian population. The findings reflected the attitudes and perceptions of the sample group and may not fully capture the diversity of the entire population.

Sensitivity analyses

In this study, sensitivity analyses were conducted to ensure the robustness and reliability of the findings and ensure that the results were not unduly affected by methodological choices or specific assumptions. The following sensitivity analyses were performed.

Alternative cutoff points for vaccine acceptance: To test the stability of the vaccine acceptance measure, different cutoff points for categorizing participants as having poor or good acceptance rate were examined. This analysis aimed to determine whether the conclusions regarding vaccine acceptance remained consistent across different classification schemes. It helped verify that the results were not unduly influenced by the choice of cutoff points.

Subgroup analyses: Additional analyses were performed within specific subgroups, such as different age ranges, income levels, and chronic condition statuses. These subgroup analyses were conducted to check whether the main findings held true within different segments of the population and to identify any potential variations in vaccine acceptance based on these factors.

RESULTS

Participants

A total of 1800 Nigerians participated in the survey.

Reporting numbers at each stage of the study: (1) Potentially eligible participants: The survey was distributed through social networking platforms. The exact number of individuals who received the survey invitation could not be determined, as the survey was disseminated broadly without precise tracking of potential reach; (2) Examined for eligibility: All individuals who clicked on the survey link were considered as having accessed the survey. There was no formal examination of eligibility beyond the initial access, as the survey was open to any respondent who chose to pa-rticipate; (3) Confirmed eligible: Since the study was a cross-sectional online survey, eligibility criteria were not formally applied beyond the general inclusion of all consenting respondents. The study assumed that those who participated were representative of the target population; and (4) Included in the study: A total of 1800 respondents participated in the survey and provided complete responses. These individuals were included in the analysis. As this was a cross-sectional study, no follow-up was required or conducted. All 1800 respondents who completed the survey were included in the data analysis.

Reasons for non-participation at each stage: The survey did not track individual responses beyond submission. However, potential reasons for non-participation could include: (1) Non-engagement: Individuals may not have engaged with the survey invitation or chosen to participate within the study period; (2) Incomplete responses: Participants who started the survey but did not complete it were excluded from submission; and (3) Technical issues: Some individuals may have experienced technical problems accessing or completing the survey.

The sociodemographic characteristics of the study participants are presented in Table 1. A larger proportion of the respondents were male (53.9%) and within the age group of 21-30 years (29.4%), earned an average income of less than $500 per month (43.3%), and lived in urban areas (90.6%). Table 2 shows the existence of underlying conditions among the study participants. A small percentage reported underlying conditions, including diabetes (8.3%), heart disease (2.2%), pulmonary disease (1.1%), with none indicating hypertension (0%). Table 3 presents the impacts of the COVID-19 pandemic on the work life of the study participants. According to the survey, 48.9% of respondents were employed, with most perceiving the COVID-19 pandemic as having negative effects on their careers. Nearly one-third of the respondents were receiving less pay for their work.

The perception of vaccination and social distancing measures among the study participants is presented in Table 4. The majority of respondents had a positive perception of vaccination and social distancing measures. Most participants strongly agreed that vaccines are important for their health (43.9%), being vaccinated is important for the health of others in my community (47.8%), getting vaccines is a good way to protect oneself from disease (41.1%), and social distancing can protect oneself (53.9%), children (57.2%), and parents (53.9%) from COVID-19. A vast majority (over 95%) of participants with underlying conditions strongly agreed on the importance, effectiveness, and trustworthiness of vaccines and the recommendations from healthcare providers. All of them (100%) strongly agreed that vaccination is crucial for community health and that social distancing protects against COVID-19. Social distancing is widely recognized as beneficial for personal and familial protection against COVID-19. However, there is some uncertainty regarding the risks of new vaccines, with mixed responses (Table 5).

The association between the sociodemographic characteristics and perception of vaccination as well as social distancing measures among the study participants is presented in Table 6. There was no significant association between the observed COVID-19 vaccine acceptance rate (63.9%) and related determinants including age ($\chi^2 = 3.049$, $P = 0.550$), sex ($\chi^2 = 0.102$, $P = 0.749$), location ($\chi^2 = 0.005$, $P = 0.941$), average income ($\chi^2 = 3.802$, $P = 0.875$), and religion ($\chi^2 = 2.819$, $P = 0.420$). Nine hundred twenty participants (51.1%) had good perception, while 880 (48.9%) had poor perception of vaccination and social distancing measures (Figure 1).

The perceived risk of COVID-19 infection among participants was as follows: 0% (600), 1%-10% (450), 11%-20% (180), 21%-30% (140), 31%-40% (110), and higher percentages. Only ten people considered themselves to have 81%-90% risk of getting infected (Figure 2). On the other hand, the perceived risk of dying from COVID-19 if infected was as follows: 0% (740), 1%-10% (470), 11%-20% (160), 21%-30% (120), 31%-40% (30), *etc*. Twenty people considered themselves to have an

Table 1 Sociodemographic characteristics of the study participants

Variable	Categories	Frequency
Age group in yr	≤ 20	310 (17.2)
	21-30	530 (29.4)
	31-40	460 (25.6)
	41-50	380 (21.1)
	> 51	120 (6.7)
Sex	Female	830 (46.1)
	Male	970 (53.9)
Location	Rural	170 (9.4)
	Urban	1630 (90.6)
Average income	Less than $500 per month	780 (43.3)
	$1000-$1999 per month	190 (10.6)
	$2000-$2999 per month	240 (13.3)
	$3000-$4999 per month	110 (6.1)
	$5000-$7999 per month	60 (3.3)
	$500-$999 per month	280 (15.6)
	$8000-$9999 per month	40 (2.2)
	$10000-$12999 per month	30 (1.7)
	$13000 or more per month	70 (3.9)
Religion	Catholic	280 (15.6)
	Christian/Protestant/Methodist/Lutheran/Baptist	1130 (62.8)
	Muslim	380 (21.1)
	Other	10 (0.6)

Data are presented as *n* (%).

Table 2 Existence of underlying conditions among the study participants

Underlying conditions	Frequency (%)
Have diabetes	150 (8.3)
Have heart disease	40 (2.2)
Have pulmonary disease	20 (1.1)
Have hypertension	0 (0.0)

Data are presented as *n* (%).

81%-90% risk of dying if infected (Figure 3). The occurrence of COVID-19 and the severity among the study participants is presented in Table 7. Ten of them (5.6%) indicated that they have been diagnosed with COVID-19 by a doctor, of which only 2.2% had very serious cases, while 3.3% of the cases were not very serious.

Table 8 shows the COVID-19 vaccine acceptance rates among the study participants. A vaccine with 95% efficacy if the government was offering it as a free and optional vaccine would be accepted by 1380 participants (76.7%). Only 70.0% of the study participants indicated that they would buy and get vaccinated if the vaccine was 95% effective, had a 5% chance of a side effect like a fever or local pain, and was sold for US$50, while 1150 (63.9%) said they would buy and get vaccinated if the vaccine cost was US$100 (Figure 4). All the participants with underlying conditions (100%) indicated acceptance of the vaccine when it was 95% effective and offered for free. Acceptance rates remained high (85.7%-95.2%) even with reduced effectiveness and potential higher side effect risks. They showed 100% acceptance for vaccine prices up to US$100. However, acceptance slightly decreased to 95.2% at a price of US$200. The vaccine had full acceptance at

Table 3 Impacts of the coronavirus disease 2019 pandemic on work and study life of the participants

Variable	Categories	Frequency
Are you currently	Employed for wages	880 (48.9)
	Homemaker	10 (0.6)
	Out of work for 1 year or more	30 (1.7)
	Out of work for less than 1 year	80 (4.4)
	Self-employed	150 (8.3)
	Student	650 (36.1)
How much has your work changed as a result of the COVID-19 pandemic	I was let go from my job	80 (4.4)
	I work fewer hours	340 (18.9)
	I work more hours	250 (13.9)
	No change. I work the same amount	530 (29.4)
	Not applicable (not working)	600 (33.3)
How much has your salary changed as a result of the COVID-19 pandemic	I am getting paid less	660 (36.7)
	I am getting paid more	40 (2.2)
	No change. I am getting paid the same	1100 (61.1)
In the past week, how often have you gone to work or school outside of the home	0 day	260 (14.4)
	1 day	30 (1.7)
	2 days	150 (8.3)
	3 days	160 (8.9)
	4 days	180 (10.0)
	5 days	510 (28.3)
	6 days	180 (10.0)
	7 days	330 (18.3)
Did you wear a mask at work/school	No	20 (1.1)
	Not applicable (not going out a whole week)	150 (8.3)
	Yes, during my whole time at work/school	1020 (56.7)
	Yes, for part of the time at work/school	610 (33.9)
In the past week, how often have you gone to a grocery store or other food vendor	0 day	170 (9.4)
	1 day	510 (28.3)
	2 days	520 (28.9)
	3 days	360 (20.0)
	4 days	40 (2.2)
	5 days	90 (5.0)
	6 days	50 (2.8)
	7 days	60 (3.3)
Did you wear a mask at the grocery store or other food vendor	No	130 (7.2)
	Not applicable (not going out to grocery store or other food vendor whole week)	80 (4.4)
	Yes, during my whole time at the store	1280 (71.1)
	Yes, for part of the time at the store	310 (17.2)

Data are presented as *n* (%). COVID-19: Coronavirus disease 2019.

Table 4 Perception of vaccination and social distancing measures among the study participants

Variable	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
Vaccines are important for my health	790 (43.9)	780 (43.3)	170 (9.4)	20 (1.1)	40 (2.2)
Vaccines are effective	650 (36.1)	870 (48.3)	240 (13.3)	10 (0.6)	30 (1.7)
Being vaccinated is important for the health of others in my community	860 (47.8)	740 (41.1)	140 (7.8)	50 (2.8)	10 (0.6)
All routine vaccines recommended by the healthcare workers are beneficial	580 (32.2)	850 (47.2)	300 (16.7)	60 (3.3)	10 (0.6)
New vaccines carry more risks than older vaccines	330 (18.3)	490 (27.2)	670 (37.2)	220 (12.2)	90 (5.0)
The information I receive about vaccines from the government is reliable and trustworthy	130 (7.2)	610 (33.9)	770 (42.8)	220 (12.2)	70 (3.9)
Getting vaccines is a good way to protect me from disease	740 (41.1)	820 (45.6)	150 (8.3)	50 (2.8)	40 (2.2)
Generally, I follow vaccine recommendations from my doctor or health care provider	650 (36.1)	950 (52.8)	140 (7.8)	50 (2.8)	10 (0.6)
Social distancing can protect yourself from COVID-19	970 (53.9)	730 (40.6)	70 (3.9)	20 (1.1)	10 (0.6)
Social distancing can protect your child or children from COVID-19	1030 (57.2)	650 (36.1)	100 (5.7)	20 (1.1)	0 (0.0)
Social distancing can protect your parents from COVID-19	1030 (57.2)	670 (37.2)	80 (4.4)	20 (1.1)	0 (0.0)

Data are presented as *n* (%). COVID-19: Coronavirus disease 2019.

Table 5 Perception of vaccination and social distancing measures among those with underlying conditions

Variable	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
Vaccines are important for my health	200 (95.2)	10 (4.76)	0 (0.0)	0 (0.0)	0 (0.0)
Vaccines are effective	200 (95.2)	10 (4.76)	0 (0.0)	0 (0.0)	0 (0.0)
Being vaccinated is important for the health of others in my community	210 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
All routine vaccines recommended by the healthcare workers are beneficial	200 (95.2)	10 (4.76)	0 (0.0)	0 (0.0)	0 (0.0)
New vaccines carry more risks than older vaccines	0 (0.0)	20 (9.52)	80 (38.1)	100 (47.6)	10 (4.76)
The information I receive about vaccines from the government is reliable and trustworthy	200 (95.2)	10 (4.76)	0 (0.0)	0 (0.0)	0 (0.0)
Getting vaccines is a good way to protect me from disease	200 (95.2)	10 (4.76)	0 (0.0)	0 (0.0)	0 (0.0)
Generally, I follow vaccine recommendations from my doctor or health care provider	200 (95.2)	10 (4.76)	0 (0.0)	0 (0.0)	0 (0.0)
Social distancing can protect yourself from COVID-19	210 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Social distancing can protect your child or children from COVID-19	200 (95.2)	5 (2.38)	5 (2.38)	0 (0.0)	0 (0.0)
Social distancing can protect your parents from COVID-19	200 (95.2)	5 (2.38)	5 (2.38)	0 (0.0)	0 (0.0)

Data are presented as *n* (%). COVID-19: Coronavirus disease 2019.

lower price points (US$5, US$12.5, US$25, US$50, and US$100). Overall, the study indicated strong willingness to accept the COVID-19 vaccine among participants with underlying conditions, influenced by the effectiveness, side effects, and cost of the vaccine (Table 9).

The association between the sociodemographic characteristics and COVID-19 vaccine acceptance rates among the study participants is presented in Table 10. No significant associations ($P > 0.05$) were found between sociodemographic characteristics and COVID-19 vaccine acceptance rates. The overall mean score for vaccine acceptance among participants showed significant differences ($P < 0.05$) between those willing to accept the vaccine (8.44 ± 1.14) and those not willing

Table 6 Association between sociodemographic and perception of vaccination and social distancing measures among the study participants

Variable	Categories	Poor perception	Good perception	Total	χ^2	*P* value	Odds ratio (95%CI)
Age group (yr)	≤ 20	220 (12.2)	90 (5.0)	310 (17.2)	8.449	0.076	
	21-30	260 (14.4)	270 (15.0)	530 (29.4)			1.25 (0.94-1.65)
	31-40	200 (11.1)	260 (14.4)	460 (25.6)			
	41-50	160 (8.9)	220 (12.2)	380 (21.1)			
	> 51	40 (2.2)	80 (4.4)	120 (6.7)			
Sex	Female	450 (25.0)	380 (21.1)	830 (46.1)	1.75	0.186	
	Male	430 (23.9)	540 (30.0)	970 (53.9)			0.74 (0.38-1.44)
Location	Rural	110 (6.1)	60 (3.3)	170 (9.4)	1.88	0.17	
	Urban	770 (42.8)	860 (47.8)	1630 (90.6)			0.50 (0.16-1.51)
Average income	Less than $500 per month	410 (22.8)	370 (20.6)	780 (43.3)			1.17 (0.14-9.43)
	$500-$999 per month	110 (6.1)	170 (9.4)	280 (15.6)			0.94 (0.17-5.27)
	$1000-$1999 per month	80 (4.4)	110 (6.1)	190 (10.6)	7.15	0.521	
	$2000-$2999 per month	110 (6.1)	130 (7.2)	240 (13.3)			0.38 (0.07-2.21)
	$3000-$4999 per month	40 (2.2)	70 (3.9)	110 (6.1)			1.19 (0.46-3.10)
	$5000-$7999 per month	30 (1.7)	30 (1.7)	60 (3.3)			1.33 (0.34-5.13)
	$8000-$9999 per month	20 (1.1)	20 (1.1)	40 (2.2)			1.59 (0.64-3.96)
	$10000-$12999 per month	30 (1.7)	0 (0)	30 (1.7)			1.26 (0.44-3.65)
	$13000 or more per month	50 (2.8)	20 (1.1)	70 (3.9)			0.00 (0.00-0.00)
Religion	Catholic	150 (8.3)	130 (7.2)	280 (15.6)	2.819	0.42	
	Christian/Protestant/Methodist/Lutheran/Baptist	580 (32.2)	550 (30.6)	1130 (62.8)			0.00 (0.00-0.00)
	Muslim	150 (8.3)	230 (12.8)	38 (21.1)			0.00 (0.00-0.00)
	Other	0 (0.0)	10 (0.6)	1 (0.6)			0.00 (0.00-0.00)

Data are presented as *n* (%). CI: Confidence interval.

(4.18 ± 1.66) (Table 11).

Unadjusted and confounder-adjusted estimates

Unadjusted estimates: The study found no significant associations between sociodemographic characteristics (age, sex, location, average income, and religion) and COVID-19 vaccine acceptance rates. For instance, the χ^2 test results showed non-significant *P* values for these variables (age: $\chi^2 = 3.049$, $P = 0.550$; sex: $\chi^2 = 0.102$, $P = 0.749$; location: $\chi^2 = 0.005$, $P = 0.941$; income: $\chi^2 = 3.802$, $P = 0.875$; religion: $\chi^2 = 2.819$, $P = 0.420$).

Confounder-adjusted estimates: Logistic regression analysis was conducted to assess the association between sociodemographic factors and vaccine acceptance while adjusting for potential confounders. However, as no significant associations were found in the unadjusted analyses, the adjusted estimates were not substantially different.

Category boundaries for continuous variables

Perceived risk of COVID-19 infection: 0% (600 respondents), 1%-10% (450 respondents), 11%-20% (180 respondents), 21%-30% (140 respondents), 31%-40% (110 respondents), and higher percentages, including 81%-90% (10 respondents).

Perceived risk of dying from COVID-19: 0% (740 respondents), 1%-10% (470 respondents), 11%-20% (160 respondents), 21%-30% (120 respondents), 31%-40% (30 respondents), and higher percentages, including 81%-90% (20 respondents).

Translation of relative risk into absolute risk: The study did not specifically translate relative risk into absolute risk. However, the provided percentages of perceived risk can be used to infer the perceived likelihood of infection and death among participants. For instance, if 48.9% perceived their risk of infection to be between 1%-10%, it suggests that nearly half of the participants consider their risk relatively low.

Table 7 Occurrence of the coronavirus disease 2019 and the severity among the study participants

Variable	Categories	Frequency
Diagnosed with COVID-19 by a doctor	No	1700 (94.4)
	Yes	100 (5.6)
How serious was the course of illness when you were infected with COVID-19	Not applicable	1700 (94.4)
	Not very serious	60 (3.3)
	Somewhat serious	40 (2.2)
Do you know people in your immediate social environment (close friends or family members) who are or have been infected with COVID-19	Do not know	420 (23.3)
	No	670 (37.2)
	Yes	710 (39.4)
How serious was the course of illness when your friend or family member was infected with the COVID-19? If you know multiple people who have had COVID-19, think about the most recent one	Not applicable	1090 (60.6)
	Not very serious	260 (14.4)
	Somewhat serious	300 (16.7)
	Very serious	150 (8.3)
Have you seen or read about individuals infected with the COVID-19 on social media or TV	No	30 (1.7)
	Yes	1770 (98.3)
How serious was the course of illness for the most recent COVID-19 case you have seen or read about in social media or on the TV	No idea	30 (1.7)
	Not very serious	260 (14.4)
	Somewhat serious	620 (34.4)
	Very serious	890 (49.4)
Have you ever heard that your local health facilities stop the vaccination service for kids due to COVID-19 pandemic	No	1550 (86.1)
	Yes	250 (13.9)

Data are presented as *n* (%). COVID-19: Coronavirus disease 2019.

Other analyses performed: (1) Subgroup and interaction analyses: Subgroup analyses were performed to explore variations in vaccine acceptance based on underlying conditions. Participants with underlying conditions showed high acceptance rates, with 100% acceptance for a vaccine with 95% efficacy if offered free. Sensitivity to price and effectiveness was also examined, revealing high acceptance even with variations in vaccine cost and potential side effects; and (2) Sensitivity analyses: Sensitivity analyses involved testing different cutoff points for categorizing vaccine acceptance and examining the effect of various model specifications on the findings. The study tested alternative thresholds for vaccine acceptance and adjusted for different sociodemographic factors in the regression models. Additionally, analyses of subgroups, such as those with underlying conditions, were conducted to assess the stability of the main findings across different groups.

DISCUSSION

The results of the current study, which examined COVID-19 vaccine acceptance and its determinants among 1800 respondents in Nigeria, revealed several key findings and trends that can be compared with previous studies conducted in Nigeria and beyond.

Summary of key results

The study revealed a vaccine acceptance rate of 63.9% among the Nigerian participants. This indicates a majority willingness to receive the COVID-19 vaccine under the conditions described in the survey. A significant portion of respondents demonstrated a positive perception of both the COVID-19 vaccine and social distancing measures. Specifically, 43.9% of participants strongly agreed on the importance of vaccines for personal health, and 47.8% recognized their importance for community health. In addition, 53.9% of participants believed that social distancing could protect oneself and family from COVID-19. Participants with chronic conditions showed even higher vaccine acceptance, with 100% indicating acceptance for a vaccine with 95% efficacy offered free of charge. This highlights an enhanced willingness among those who may perceive a higher personal risk or benefit from vaccination.

Table 8 Coronavirus disease 2019 vaccine acceptance rates among the study participants

Variable	Categories	Frequency
Imagine that a new COVID-19 vaccine has just been developed. It has received the same testing as the adult influenza vaccine. The government is offering it as a FREE and optional vaccine. Would you accept a COVID-19 vaccine if the vaccine was 95% effective	No	420 (23.3)
	Yes	1380 (76.7)
The vaccine is 50% effective, with a 5% chance of a side effect like a fever (50% effective means that there is a 50% reduction in disease among those vaccinated compared to those unvaccinated)	No	860 (47.8)
	Yes	940 (52.2)
The vaccine is 95% effective, with a 20% chance of a side effect like a fever (95% effective means that there is a 95% reduction in disease among those vaccinated compared to those unvaccinated)	No	550 (30.6)
	Yes	1250 (69.4)
The vaccine is 75% effective, with a 5% chance of a side effect like a fever (75% effective means that there is a 75% reduction in disease among those vaccinated compared to those unvaccinated)	No	400 (22.2)
	Yes	1400 (77.8)
The vaccine is 75% effective, with a 20% chance of a side effect like a fever (75% effective means that there is a 75% reduction in disease among those vaccinated compared to those unvaccinated)	No	990 (55.0)
	Yes	810 (45.0)
For a COVID-19 vaccine that is 95% effective and a 5% chance of a side effect like fever or local pain? If the price was US$50 for complete vaccination. Would you buy the vaccine and get vaccinated	No	540 (30.0)
	Yes	1260 (70.0)
If the COVID-19 vaccine price was US$100. Would you buy the vaccine and get vaccinated	No	650 (36.1)
	Yes	1150 (63.9)
If the COVID-19 vaccine price was US$200. Would you still buy the vaccine and get vaccinated	No	570 (31.7)
	Yes	1230 (68.3)
If the COVID-19 vaccine price is reduced to US$25. Would you buy the vaccine and get vaccinated	No	550 (30.6)
	Yes	1250 (69.4)
If the COVID-19 vaccine price was US$12.5. Would you buy the vaccine and get vaccinated	No	1210 (67.2)
	Yes	590 (32.8)
If the COVID-19 vaccine price was US$5 only. Would you buy the vaccine and get vaccinated	No	630 (35.0)
	Yes	1170 (65.0)

Data are presented as *n* (%). COVID-19: Coronavirus disease 2019.

No significant association was found between age and vaccine acceptance, with odds ratios (OR) ranging from 0.94 to 1.65 across different age groups. Male participants showed a lower OR of vaccine acceptance (OR = 0.74, 95%CI: 0.38-1.44), though this result was not statistically significant. Urban residents had a lower adjusted OR (OR = 0.50, 95%CI: 0.16-1.51) for vaccine acceptance compared to rural residents, but this result was also not statistically significant. The OR varied across different income brackets, with no consistent trend and confidence intervals suggesting limited precision. No significant differences were found in vaccine acceptance across different religious groups, with OR indicating no significant associations. Participants' perceived risk of COVID-19 infection and death was low, with a majority estimating their risk in the lower percentage range (0%-10%). This lower perceived risk may impact their motivation for vaccine uptake. High acceptance was noted when the vaccine was offered free with 95% efficacy. Acceptance rates remained high even when the vaccine cost increased, though there was a slight decline as the price rose to US$200.

The study indicated an overall COVID-19 vaccine acceptance rate of 63.9%, which aligns with similar trends observed in related research, though variations exist across different demographic and professional groups.

Sociodemographic characteristics and vaccine acceptance

The relationship between sociodemographic factors and COVID-19 vaccine acceptance in Nigeria has yielded mixed results across studies. The current study found no significant association between factors like age, sex, income, and religion and vaccine acceptance, aligning with findings by Iwuafor *et al*[24], who also reported no significant predictors of vaccine refusal among healthcare professionals in Cross River State. This suggests that vaccine hesitancy may be more influenced by individual perceptions and external factors such as misinformation than by demographic variables. However, other studies reported significant associations between sociodemographic factors and vaccine acceptance. Olu-Abiodun *et al*[25] found that vaccine acceptance rates varied widely (20.0%-58.2%) across demographics, with concerns over vaccine safety, conspiracy theories, and adverse effects playing key roles.

Eze *et al*[26] identified sex, religion, ethnicity, and geographical location as significant predictors, noting that male sex, Christianity, and Northern residence were positively associated with acceptance. Al-Mustapha *et al*[27] also found age and income to be significant predictors, with older individuals and those with higher incomes more likely to accept the

Table 9 Coronavirus disease 2019 vaccine acceptance rates among those with underlying conditions

Variable	Categories	Frequency
Imagine that a new COVID-19 vaccine has just been developed. It has received the same testing as the adult influenza vaccine. The government is offering it as a free and optional vaccine. Would you accept a COVID-19 vaccine if the vaccine was 95% effective?	No	0 (0.0)
	Yes	210 (100.0)
The vaccine was 50% effective, with a 5% chance of a side effect like a fever? (50% effective means that there is a 50% reduction in disease among those vaccinated compared to those unvaccinated)	No	10 (4.8)
	Yes	200 (95.2)
The vaccine was 95% effective, with a 20% chance of a side effect like a fever? (95% effective means that there is a 95% reduction in disease among those vaccinated compared to those unvaccinated)	No	30 (14.3)
	Yes	180 (85.7)
The vaccine was 75% effective, with a 5% chance of a side effect like a fever? (75% effective means that there is a 75% reduction in disease among those vaccinated compared to those unvaccinated)	No	25 (11.9)
	Yes	185 (88.09)
The vaccine was 75% effective, with a 20% chance of a side effect like a fever? (75% effective means that there is a 75% reduction in disease among those vaccinated compared to those unvaccinated)	No	30 (14.3)
	Yes	180 (85.7)
For a COVID-19 vaccine that was 95% effective and a 5% chance of a side effect like a fever or local pain? If the price is US$50 for complete vaccination. Would you buy the vaccine and get vaccinated?	No	0 (0.0)
	Yes	210 (100)
If the COVID-19 vaccine price was US$100. Would you buy the vaccine and get vaccinated?	No	0 (0.0)
	Yes	210 (100.0)
If the COVID-19 vaccine price was US$200. Would you buy the vaccine and get vaccinated?	No	10 (4.8)
	Yes	200 (95.2)
If the COVID-19 vaccine price was reduced to US$25. Would you buy the vaccine and get vaccinated?	No	0 (0.0)
	Yes	210 (100.0)
If the COVID-19 vaccine price was US$12.5. Would you buy the vaccine and get vaccinated?	No	0 (0.0)
	Yes	210 (100.0)
If the COVID-19 vaccine price was US$5 only. Would you buy the vaccine and get vaccinated?	No	0 (0.0)
	Yes	210 (100.0)

Data are presented as *n* (%). COVID-19: Coronavirus disease 2019.

vaccine. Alice *et al*[28] reported that increasing age, male sex, and trust in government and public health authorities were associated with higher vaccine acceptance. Njoga *et al*[29] emphasized the role of geopolitical zones, education, and occupation in vaccine hesitancy, particularly among healthcare workers, academics, and students.

While our study focused on age, sex, location, average income, and religion of the study participants, we recognize the importance of education and socioeconomic status as determinants of vaccine acceptance. Previous studies[30-34] have shown that higher education levels and socioeconomic status correlate with greater vaccine acceptance due to better health literacy and perceived risk. These contrasting findings suggest that the influence of sociodemographic factors on vaccine acceptance may vary by context, and targeted interventions considering these variables may be necessary to address vaccine hesitancy effectively in Nigeria.

Perception

Our study revealed that a majority of respondents have a positive perception of vaccination and social distancing measures. Specifically, 43.9% strongly agreed on the importance of vaccines for health, and 47.8% believed vaccination is crucial for community health. These perceptions are comparable to the findings by Tijani *et al*[35], who reported a significant association between educational level and monthly income with vaccine uptake, highlighting the importance of awareness and financial stability in fostering positive perceptions towards vaccination.

Impact of COVID-19 on work life

The current study indicated that nearly half of the respondents (48.9%) felt that the COVID-19 pandemic negatively impacted their careers, with many reporting reduced pay. This economic impact likely influenced their perceptions and acceptance of vaccination, similar to findings by Zakari *et al*[36], where economic factors and skepticism about vaccine efficacy were major reasons for hesitancy among university community members.

Table 10 Association between sociodemographic and the coronavirus disease 2019 vaccine acceptance rates among the study participants

Variable	Categories	Poor	Good	Total	χ^2	*P* value	Odds ratio (95%CI)
Age group (yr)	≤ 20	70 (3.9)	240 (13.3)	310 (17.2)	3.049	0.55	
	21-30	200 (11.1)	330 (18.3)	530 (29.4)			0.76 (0.57-1.02)
	31-40	180 (10.0)	280 (15.6)	460 (25.6)			
	41-50	150 (8.3)	230 (12.8)	380 (21.1)			
	> 51	50 (2.8)	70 (3.9)	120 (6.7)			
Sex	Female	310 (17.2)	520 (28.9)	830 (46.1)	0.102	0.749	
	Male	340 (18.9)	630 (35.0)	970 (53.9)			0.83 (0.42-1.62)
Location	Rural	60 (3.3)	110 (6.1)	170 (9.4)	0.005	0.941	
	Urban	590 (32.8)	1040 (57.8)	1630 (90.6)			0.90 (0.29-2.78)
Average income	Less than $500 per month	310 (17.2)	470 (26.1)	780 (43.3)			2.74 (0.25-30.32)
	$500-$999 per month	110 (6.1)	170 (9.4)	280 (15.6)			4.14 (0.44-38.90)
	$1000-$1999 per month	40 (2.2)	150 (8.3)	190 (10.6)	3.802	0.875	
	$2000-$2999 per month	90 (5.0)	150 (8.3)	240 (13.3)			0.81 (0.16-4.05)
	$3000-$4999 per month	40 (2.2)	70 (3.9)	110 (6.1)			1.25 (0.47-3.29)
	$5000-$7999 per month	10 (0.6)	50 (2.8)	60 (3.3)			1.33 (0.34-5.22)
	$8000-$9999 per month	10 (0.6)	30 (1.7)	40 (2.2)			1.08 (0.43-2.68)
	$10000-$12999 per month	10 (0.6)	20 (1.1)	30 (1.7)			2.76 (0.81-9.42)
	$13000 or more per month	30 (1.7)	40 (2.2)	70 (3.9)			1.14 (0.09-13.78)
Religion	Catholic	100 (5.6)	180 (10.0)	280 (15.6)	1.804	0.614	
	Christian/Protestant/Methodist/Lutheran/Baptist	440 (24.4)	690 (38.3)	1130 (62.8)			0.00 (0.00-0.00)
	Muslim	110 (6.1)	270 (15.0)	380 (21.1)			0.00 (0.00-0.00)
	Other	0 (0.0)	10 (0.6)	10 (0.6)			0.00 (0.00-0.00)

Data are presented as *n* (%). CI: Confidence interval.

Table 11 Overall mean score of the coronavirus disease 2019 vaccine acceptance rates among the study participants

Variable	Poor	Good	*P* value
Overall score on perception of vaccination and social distancing measures among the study participants	8.49 ± 2.72	9.04 ± 1.88	0.112
Overall score on the coronavirus disease 2019 vaccine acceptance rates among the study participants.	4.18 ± 1.66	8.44 ± 1.14	0.000

Acceptance rates and determinants

A notable finding from the current study was the high acceptance rate (76.7%) for a vaccine with 95% efficacy if provided for free, which dropped to 63.9% if the vaccine costs $100. This trend of price sensitivity aligns with Padonou *et al*[37], who also observed that financial constraints and economic conditions significantly affected vaccine acceptance rates in Benin. In comparison to the findings from Omale *et al*[38], where health workers' acceptance was primarily driven by perceptions of vaccine importance and safety, the respondents in the current study similarly indicated good perception (51.1%) *vs* poor perception (48.9%) of vaccination measures. This further emphasizes that improving perceptions through education and communication is crucial for enhancing vaccine uptake.

More than two-thirds of respondents in this study expressed willingness to be vaccinated for various reasons, while 64% indicated readiness to get vaccinated for a range of reasons. In terms of willingness to be vaccinated, the result of this current study is higher than those from Italy (53.7%), Russia (54.9%), Poland (56.3%), the United States (56.9%), Greece (57.7%), and France (58%)[39-41]. On the other side, countries such as Ecuador (97.0%), Malaysia (94.3%), Indonesia (93.3%), and China (91.3%) had the highest rates of vaccine acceptability, whilst Jordan (28.4%) and Kuwait (23.6%) had the lowest[40]. Since these factors were shown to be connected with the willingness to become vaccinated, the variation in vaccination rates might be attributed to the respondent's level of education, employment, or social standing.

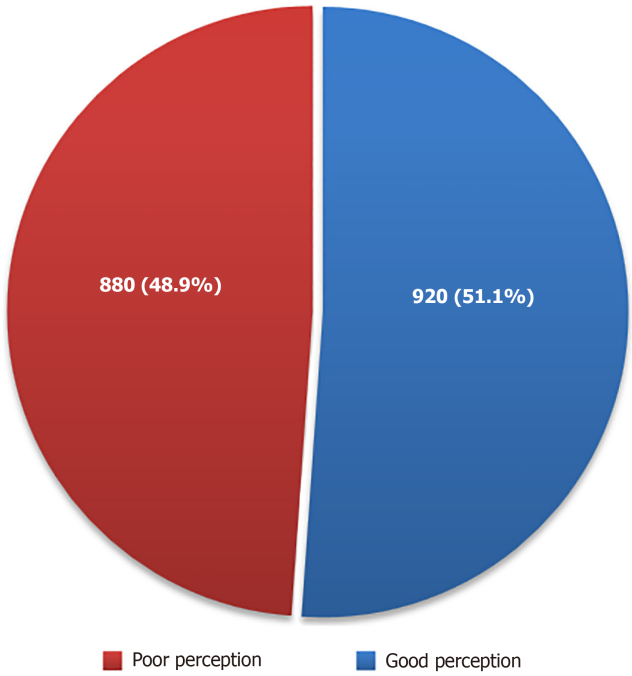

Figure 1 Perception on vaccination and social distancing measures among the study participants.

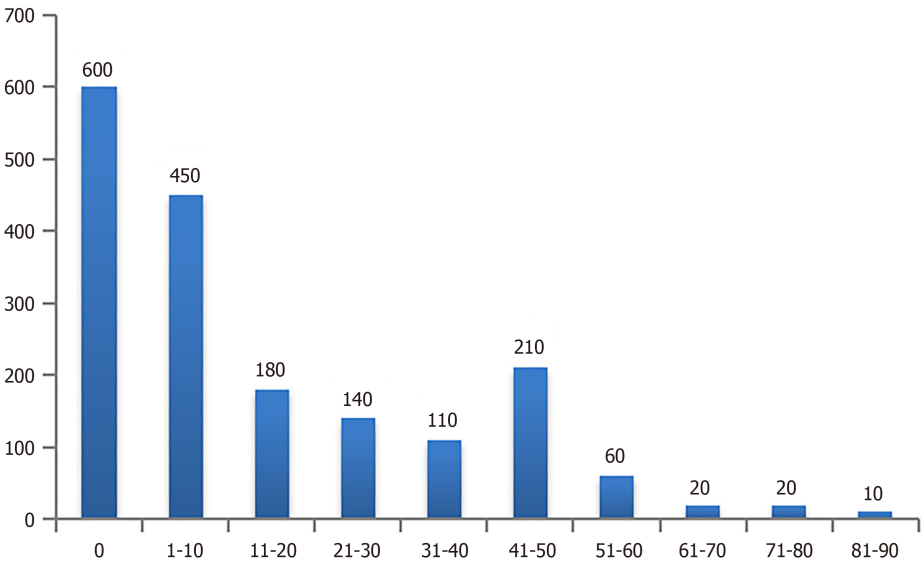

Figure 2 Perceived risk of getting infected with the coronavirus disease 2019 in the next month.

In addition, it has been reported that non-governmental groups make significant improvements to the vaccination programs in countries that have a low or moderate income. People living in remote areas and communities that are difficult to access now have easier access to vaccinations. It is possible that many people would pick the non-governmental organization sector to supervise vaccine distribution since they do not put much faith in official organizations. This might be the result of factors such as delay caused by government bureaucracy (delays the administration of vaccinations), nepotism (selects vaccine recipients rather than those who are most susceptible), and corruption (influences the cost of vaccines and how widely they are distributed)[38].

The COVID-19 vaccination has been deemed safe and effective by 60% and 60.1% of individuals, as determined by different systematic reviews and meta-analyses[40,41]. According to the findings of an in-depth study as well as a meta-analysis, 81.65% of the general population was eager to be vaccinated against COVID-19. However, this number was far lower than those who actually received the vaccine[42]. This might provide an explanation for how the COVID-19 epidemic is spreading throughout the world and impacting a variety of countries. It is possible that the gap might be explained by the fact that those who participated in the poll came from a wide array of social and cultural backgrounds. It is also conceivable that some of them do not have access to reliable social media channels where people are sharing authentic information regarding the COVID-19 vaccination. There is a possibility that the differences might be due to di-

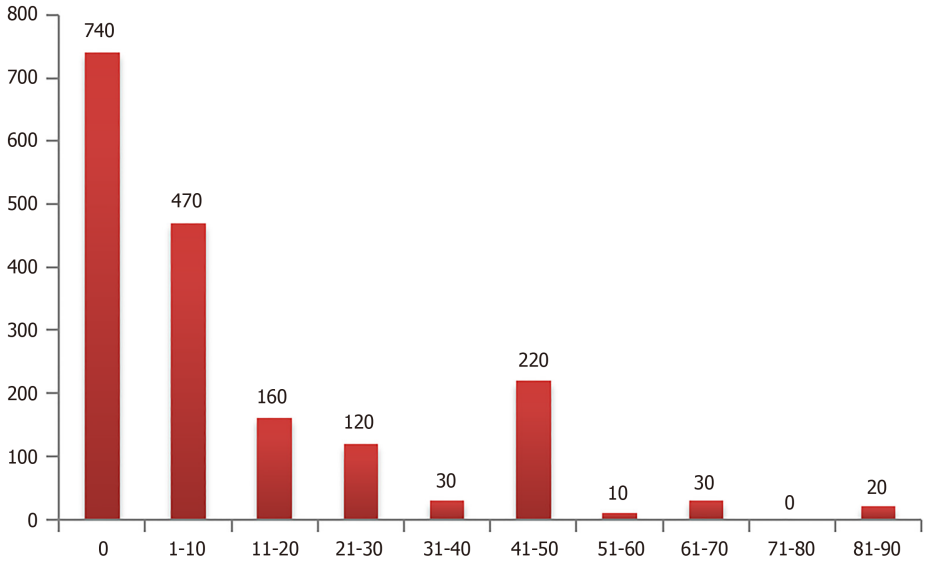

Figure 3 Perceived risk of dying from the coronavirus disease 2019 if infected.

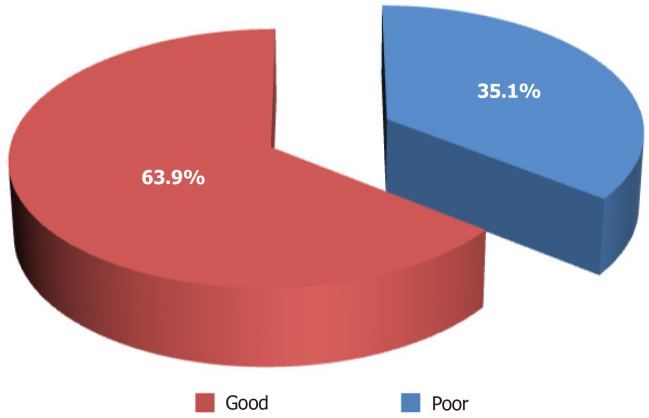

Figure 4 Overall coronavirus disease 2019 vaccine acceptance rate among the study participants.

vergent points of view on the relevance and importance of the vaccine.

Furthermore, our study observed that a higher percentage of male respondents supported vaccination compared to female respondents. This was in line with the results of a prior study that showed males were much more willing to acquire vaccinations. This might be due to the fact that roughly two-thirds of the males who participated in this study were literate. As a result, they were probably aware of the potentially harmful effects of COVID-19, which increased the likelihood that they would acquire the vaccine. When the vaccine became available, most people elected to get it because they were terrified of getting COVID-19. This fear may have prompted them to make this decision. The lack of understanding on the safety of the vaccine and the absence of clinical trials were the two factors that were brought up as the most common vaccination roadblocks or reasons for skepticism. This was in line with the findings of the majority of the studies that were carried out to establish why some individuals did not acquire the COVID 19 vaccination[43].

Perceived risks

The study assessed perceived risks of infection and death from COVID-19, revealing that a significant number of respondents felt they had minimal risk, with 740 participants considering their risk of dying from COVID-19 as 0%. This low perceived risk could be a contributing factor to vaccine hesitancy, as seen in Olawa *et al*[44], where mistrust in the government and vaccine benefits led to lower acceptance rates.

Perceived sickness risks have been connected to health-related activity[45]. However, the majority of research on risk perceptions focused primarily on the possibility of being ill[46]. The majority of the respondents in this study had a higher perception of not being infected with COVID-19 as most indicated a lower percentage risk level of getting infected with COVID-19 and risk of hospitalization and dying from COVID-19 if infected. Findings, especially in Sub-Saharan nations, have shown that COVID-19 infected people have no or mild symptoms. This implies that the likelihood of infection may not give much insight into how individuals perceive risk and how this influences their health practices, which may be due to herd immunity of the respondents[47].

The degree of association between these risk measures was relatively weak, and they cannot be used as proxies. Although the majority of participants in this study had good knowledge regarding vaccination, some also exhibited comparatively lower percentages of COVID-19 acceptability, and this might be due to low level of exposure and the perceived risk of dying among the respondents. Moreover, respondents in the age group of 51 years and above demonstrated the lowest level of acceptability, which corroborated with the submission of Fojnica *et al*[48].

If these people had been better informed about the vaccines and the degree to which the government was unprepared for them, it is possible that they would have been more worried about the accessibility and safety of the immunizations. On the contrary, they had a tendency to become worse. On the other hand, herd immunity against the COVID-19 outbreak may be feasible if the government makes measures that are open and transparent and implements a range of credible diplomatic actions in order to secure enough immunizations for widespread inoculation[49,50].

Vaccine hesitancy

Compared to the study by Mustapha *et al*[51], which found a vaccine acceptance rate of 40.0% among students, the acceptance rate of the current study was higher. However, both studies highlight the need for targeted education and public health campaigns to address misconceptions and improve vaccine uptake. The present study also suggested the necessity of making vaccines more affordable and accessible, which aligns with recommendations from Kolawole *et al*[52] and Mahmood *et al*[53], who emphasized the role of public health professionals and trust in vaccines.

History of underlying conditions and openness to receiving the COVID-19 vaccine

Our findings indicate a significant relationship between a history of chronic illness and openness to receiving the COVID-19 vaccine. People who had a previous diagnosis of a chronic illness had a higher likelihood of responding fa-vorably to the COVID-19 vaccination than those who did not have such a diagnosis. Most of the participants with un-derlying conditions strongly agree on the importance, effectiveness, and protective benefits of vaccines, indicating a high level of acceptance and trust. This trust in vaccines and the reliability of information from healthcare workers and the government is crucial for achieving high vaccination coverage and community immunity. Public health campaigns should leverage this trust to disseminate important messages and encourage compliance with vaccination schedules.

However, some uncertainty remains regarding the risks associated with new vaccines, highlighting the need for clear, evidence-based information to prevent hesitancy. The implications of high COVID-19 vaccine acceptance rates among individuals with underlying conditions are significant for public health, policy, and society. High acceptance, especially for a free vaccine that is 95% effective, underscores the willingness to get vaccinated, which is vital for achieving herd immunity and controlling the spread of COVID-19. Even with some side effects, acceptance remains high, though slightly lower with increased side effect likelihood. This emphasizes the importance of transparent risk-benefit communication. Vaccines with higher efficacy rates are more likely to be accepted, suggesting that public health efforts should focus on distributing and promoting the most effective vaccines.

Affordability is a key factor, with 100% acceptance for vaccines priced up to $100 and a slight decrease at $200. Policymakers should ensure vaccines are affordable or free, especially for vulnerable populations with underlying conditions. Subsidies, insurance coverage, and financial assistance programs can help maintain high vaccination rates. Ensuring equitable access to vaccines is essential for those more susceptible to severe outcomes from COVID-19. This is consistent with previous studies[54-58]. Both an Australian study and a report by the World Health Organization support these findings[59-61]. This research highlights the need to establish a mechanism that would enable recipients of the COVID-19 vaccination who have a history of chronic diseases to acquire the vaccine. This necessity is emphasized by our findings.

Studies on vaccine history, also known as past vaccination behavior, are accurate predictors of future vaccination behavior[62,63]. A previous survey found that between 2010 and 2015, only 3.4% to 44.1% of healthcare workers in the Kingdom of Saudi Arabia received the influenza vaccine[13]. In addition, earlier studies that used a cross-sectional approach indicated that if people in Russia and Indonesia were not given sufficient knowledge regarding the efficacy and safety of vaccines, they would not support them. This was the case in both countries[62]. In addition, there is a major shortage of COVID-19 vaccines in Nigeria since the country does not get them on a consistent basis or in adequate quantities. In addition, the lack of contracts with alternative healthcare providers abroad has made it difficult to provide immunizations at the appropriate period[63-65]. The opinions of the majority of people about vaccinations in general have been significantly changed, due to concerns surrounding the safety of vaccines and doubts regarding vaccine supply.

Limitations of the study

The study on COVID-19 vaccine acceptance among Nigerians, while providing valuable insights, had several limitations that could affect the interpretation of the results.

Sampling bias (non-random sampling): The study used an online survey distributed through social networking platforms. This method of recruitment can introduce selection bias, as it may not be representative of the entire population. Individuals with higher internet access and engagement with social media are more likely to participate, potentially skewing the sample towards a younger, more urban, and more internet-savvy demographic. This sampling bias could lead to an overrepresentation of younger and more educated individuals who might have higher vaccine acceptance compared to those with less internet access or lower education levels. The extent of this bias is unclear, but it could be substantial given that a significant proportion of participants were in the 21-30 age group and lived in urban areas.

Response bias: The study relied on self-reported data, which can introduce response bias. Participants might provide socially desirable answers or overstate their acceptance of the vaccine to align with perceived social norms. This could

lead to an overestimation of vaccine acceptance and a more positive perception of vaccination and social distancing measures. The impact of response bias is difficult to quantify but could be considerable, especially in the context of sensitive topics like vaccine acceptance.

Response rate calculation: Due to the nature of social media distribution, it was impossible to determine the total number of individuals who received the survey invitation. Without this data, accurately calculating the response rate is unfeasible, which limits our ability to assess the representativeness and potential response bias of the sample. The inability to calculate the response rate significantly affects the assessment of sample representativeness and potential biases. It poses a high risk of both overestimating and underestimating the true attitudes and acceptance rates within the general population. The bias could lean towards overrepresenting more engaged and possibly more positive respondents, while underrepresenting less engaged groups who might have different views on the vaccine and social distancing measures.

Measurement issues: The study used percentage-based categories for perceived risk rather than more granular or standardized scales. This method may lack precision and could lead to misclassification of participants' perceptions. This imprecision could obscure the true distribution of perceived risk and affect the accuracy of the associations with vaccine acceptance. The magnitude of this imprecision could vary but may limit the ability to detect more silent differences in perceived risk.

Cross-sectional design: As a cross-sectional study, it captured data at a single point in time, making it challenging to infer causality or changes in vaccine acceptance over time. The snapshot nature of the data may not reflect shifts in attitudes or external factors affecting vaccine acceptance that occurred after the study period. This limitation is inherent to cross-sectional studies and affects all results similarly, though the exact impact on conclusions about causality is significant.

Sample size: While the sample size of 1800 participants is relatively large, it was not calculated based on formal power analysis. This means the study may have limited power to detect smaller associations or differences. Without an adequate power analysis, the study might not detect significant associations that exist, or it might find associations that are not robust. The lack of power analysis could affect the reliability of the study's conclusions, particularly for subgroup analyses where sample sizes are smaller.

Confounding variables: Despite adjusting for some sociodemographic factors, other potential confounders such as health literacy, exposure to misinformation, and prior experiences with vaccines were not assessed. The study did not consider education and socioeconomic status, which are crucial determinants of vaccine acceptance. Previous research indicated that individuals with higher education levels and better socioeconomic positions were more likely to understand the benefits of vaccination and perceive higher risks of COVID-19, influencing their acceptance rates. Unmeasured confounders could skew the results in either direction, making it challenging to isolate the true determinants of vaccine acceptance. The magnitude of this bias is difficult to estimate, but could be significant, particularly if these unmeasured factors are strongly related to both sociodemographic characteristics and vaccine acceptance.

Overall interpretation: The study provided valuable insights into the level of COVID-19 vaccine acceptance and the general perception of vaccination and social distancing among Nigerians. However, the limitations regarding sampling methods, response bias, and measurement precision necessitate a cautious interpretation of the results. The relatively high acceptance rate and positive perceptions suggest a generally supportive attitude towards COVID-19 vaccination among the surveyed group, particularly among those with underlying health conditions. The non-random sampling method and potential response biases imply that the findings may not fully represent the broader Nigerian population. The results should be interpreted with caution, acknowledging that the actual acceptance rates and perceptions might differ in the general population.

Generalizability: The findings may not be generalizable to all Nigerians or other populations due to the specific nature of the sample (*e.g.*, higher internet usage and urban focus). This limits the ability to apply the findings broadly and could result in different vaccine acceptance patterns in other demographic groups. The extent of this generalizability issue is substantial, given the specific sociodemographic characteristics of the sample.

Recommendations for future studies

Based on the above limitations, we hereby make the following recommendations: (1) Future studies should employ a multimodal distribution strategy, including face-to-face interviews, telephone surveys, and distribution through traditional media channels (*e.g.*, radio, television) to reach a broader and more diverse population, including those without internet access; (2) It is crucial to incorporate education levels and detailed socioeconomic status indicators in future surveys. These factors significantly influence vaccine acceptance and would provide a clearer understanding of the determinants of vaccine uptake; (3) Conduct longitudinal studies to track changes in vaccine acceptance over time. This approach would help identify trends, evaluate the long-term effectiveness of public health interventions, and observe shifts in public perception as the pandemic evolves; (4) To mitigate social desirability bias, consider using anonymous surveys and validated instruments designed to reduce the impact of self-reporting biases. Additionally, incorporating qualitative methods (*e.g.*, focus groups and in-depth interviews) can provide deeper insights into the reasons behind vaccine acceptance or hesitancy; (5) Based on the study findings, public health campaigns should be tailored to address the specific concerns and barriers identified in the survey. These campaigns should emphasize the safety, efficacy, and importance of COVID-19 vaccines, particularly targeting groups with lower acceptance rates; (6) Governments and

policymakers should consider providing vaccines for free or at subsidized rates to alleviate financial barriers. Economic support measures, such as compensation for potential side effects or paid time off for vaccination, could also enhance acceptance; (7) Engage community leaders, healthcare providers, and influencers to build trust and disseminate accurate information about COVID-19 vaccines. This grassroots approach can help counteract misinformation and skepticism, particularly in communities with historically low trust in governmental or medical institutions; and (8) Increase the number and accessibility of vaccination centers, especially in rural and underserved areas. Mobile clinics and community-based vaccination drives can help reach populations with limited access to healthcare facilities.

By addressing these limitations and implementing the recommended strategies, future research and public health efforts can more effectively enhance vaccine acceptance and uptake, contributing to better control of the COVID-19 pandemic in Nigeria.

CONCLUSION

The findings of this study provided valuable insights into the determinants of COVID-19 vaccine acceptance in Nigeria, highlighting the significant role of economic factors, perceptions of vaccine efficacy and safety, and the impact of the pandemic on individuals' lives. Despite the observed positive perception and a substantial acceptance rate of 63.9% among the study participants, the analysis revealed that sociodemographic factors such as age, sex, income, and religion did not significantly influence vaccine uptake. However, individuals with a history of chronic conditions demonstrated a higher likelihood of accepting the vaccine, underscoring the importance of targeted interventions. To enhance vaccine acceptability and achieve herd immunity, it is imperative to implement comprehensive public health strategies. These should include economic support to alleviate financial barriers, extensive educational campaigns to improve understanding of vaccine efficacy and safety, and trust-building measures to counteract misinformation and skepticism towards vaccines and governmental initiatives. Ministries of health, legislators, health planners, and other stakeholders must intensify efforts to disseminate accurate and reliable information regarding COVID-19 vaccines. By focusing on effective public health education and tailored interventions for specific demographic groups, it is possible to improve overall vaccine uptake and better manage the COVID-19 pandemic in Nigeria.

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FOOTNOTES

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Informed consent statement: All study participants provided informed consent by ticking the 'informed consent' box in the Google Form prior to study enrollment; without this consent, they were unable to take the survey.

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REFERENCES

1 **Okai GA**, Abekah-Nkrumah G. The level and determinants of COVID-19 vaccine acceptance in Ghana. *PLoS One* 2022; **17**: e0270768 [PMID: 35802742 DOI: 10.1371/journal.pone.0270768]
2 **Dhama K**, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, Singh KP, Chaicumpa W, Bonilla-Aldana DK, Rodriguez-Morales AJ. Coronavirus Disease 2019-COVID-19. *Clin Microbiol Rev* 2020; **33**: e00028- e00020 [PMID: 32580969 DOI: 10.1128/CMR.00028-20]
3 **Ehrenberg JP**, Utzinger J, Fontes G, da Rocha EMM, Ehrenberg N, Zhou XN, Steinmann P. Efforts to mitigate the economic impact of the COVID-19 pandemic: potential entry points for neglected tropical diseases. *Infect Dis Poverty* 2021; **10**: 2 [PMID: 33397510 DOI: 10.1186/s40249-020-00790-4]
4 **Burki T**. Global COVID-19 vaccine inequity. *Lancet Infect Dis* 2021; **21**: 922-923 [PMID: 34174236 DOI: 10.1016/S1473-3099(21)00344-3]
5 **Ahmed MAM**, Colebunders R, Gele AA, Farah AA, Osman S, Guled IA, Abdullahi AAM, Hussein AM, Ali AM, Siewe Fodjo JN. COVID-19 Vaccine Acceptability and Adherence to Preventive Measures in Somalia: Results of an Online Survey. *Vaccines (Basel)* 2021; **9**: 543 [PMID: 34064159 DOI: 10.3390/vaccines9060543]
6 **Kwok KO**, Li KK, Wei WI, Tang A, Wong SYS, Lee SS. Editor's Choice: Influenza vaccine uptake, COVID-19 vaccination intention and vaccine hesitancy among nurses: A survey. *Int J Nurs Stud* 2021; **114**: 103854 [PMID: 33326864 DOI: 10.1016/j.ijnurstu.2020.103854]
7 **Ripabelli G**, Tamburro M, Buccieri N, Adesso C, Caggiano V, Cannizzaro F, Di Palma MA, Mantuano G, Montemitro VG, Natale A, Rodio L, Sammarco ML. Active Surveillance of Adverse Events in Healthcare Workers Recipients After Vaccination with COVID-19 BNT162b2 Vaccine (Pfizer-BioNTech, Comirnaty): A Cross-Sectional Study. *J Community Health* 2022; **47**: 211-225 [PMID: 34628568 DOI: 10.1007/s10900-021-01039-3]
8 **Sallam M**. COVID-19 Vaccine Hesitancy Worldwide: A Concise Systematic Review of Vaccine Acceptance Rates. *Vaccines (Basel)* 2021; **9**: 160 [PMID: 33669441 DOI: 10.3390/vaccines9020160]
9 **Paterson P**, Meurice F, Stanberry LR, Glismann S, Rosenthal SL, Larson HJ. Vaccine hesitancy and healthcare providers. *Vaccine* 2016; **34**: 6700-6706 [PMID: 27810314 DOI: 10.1016/j.vaccine.2016.10.042]
10 **Manning ML**, Gerolamo AM, Marino MA, Hanson-Zalot ME, Pogorzelska-Maziarz M. COVID-19 vaccination readiness among nurse faculty and student nurses. *Nurs Outlook* 2021; **69**: 565-573 [PMID: 33610324 DOI: 10.1016/j.outlook.2021.01.019]
11 **Askarian M**, Fu LY, Taghrir MH, Borazjani R, Shayan Z, Taherifard E, Taherifard E, Akbarialiabad H, Longtin Y, Askarian A, Kavousi S. COVID-19 Vaccination Acceptance in Iran, a Nationwide Survey on Factors Associated with the Willingness toward Getting Vaccinated. *Int J Prev Med* 2022; **13**: 130 [PMID: 36452471 DOI: 10.4103/ijpvm.ijpvm_261_21]
12 **Pelčić G**, Karačić S, Mikirtichan GL, Kubar OI, Leavitt FJ, Cheng-Tek Tai M, Morishita N, Vuletić S, Tomašević L. Religious exception for vaccination or religious excuses for avoiding vaccination. *Croat Med J* 2016; **57**: 516-521 [PMID: 27815943 DOI: 10.3325/cmj.2016.57.516]
13 **McFadden SM**, Demeke J, Dada D, Wilton L, Wang M, Vlahov D, Nelson LE. Confidence and Hesitancy During the Early Roll-out of COVID-19 Vaccines Among Black, Hispanic, and Undocumented Immigrant Communities: a Review. *J Urban Health* 2022; **99**: 3-14 [PMID: 34940933 DOI: 10.1007/s11524-021-00588-1]
14 **Thangaraju P**, Venkatesan S. WHO Ten threats to global health in 2019: Antimicrobial resistance. *CUMJ* 2019; **44**: 1150-1151 [DOI: 10.17826/cumj.514157]
15 **Wagner AL**, Rajamoorthy Y, Taib NM. Impact of economic disruptions and disease experiences on COVID-19 vaccination uptake in Asia: A study in Malaysia. *Narra J* 2021; **1**: e42 [PMID: 38449462 DOI: 10.52225/narraj.v1i2.42]
16 **Hassan W**, Kazmi SK, Tahir MJ, Ullah I, Royan HA, Fahriani M, Nainu F, Rosa SGV. Global acceptance and hesitancy of COVID-19 vaccination: A narrative review. *Narra J* 2021; **1**: e57 [PMID: 38450215 DOI: 10.52225/narra.v1i3.57]
17 **Asma S**, Akan H, Uysal Y, Poçan AG, Sucaklı MH, Yengil E, Gereklioğlu Ç, Korur A, Başhan İ, Erdogan AF, Özşahin AK, Kut A. Factors effecting influenza vaccination uptake among health care workers: a multi-center cross-sectional study. *BMC Infect Dis* 2016; **16**: 192 [PMID: 27142774 DOI: 10.1186/s12879-016-1528-9]
18 **Chew NWS**, Lee GKH, Tan BYQ, Jing M, Goh Y, Ngiam NJH, Yeo LLL, Ahmad A, Ahmed Khan F, Napolean Shanmugam G, Sharma AK, Komalkumar RN, Meenakshi PV, Shah K, Patel B, Chan BPL, Sunny S, Chandra B, Ong JJY, Paliwal PR, Wong LYH, Sagayanathan R, Chen JT, Ying Ng AY, Teoh HL, Tsivgoulis G, Ho CS, Ho RC, Sharma VK. A multinational, multicentre study on the psychological outcomes and associated physical symptoms amongst healthcare workers during COVID-19 outbreak. *Brain Behav Immun* 2020; **88**: 559-565 [PMID: 32330593 DOI: 10.1016/j.bbi.2020.04.049]
19 **Sun S**, Lin D, Operario D. Interest in COVID-19 vaccine trials participation among young adults in China: Willingness, reasons for hesitancy, and demographic and psychosocial determinants. *Prev Med Rep* 2021; **22**: 101350 [PMID: 33816087 DOI: 10.1016/j.pmedr.2021.101350]
20 **Lazarus JV**, Ratzan SC, Palayew A, Gostin LO, Larson HJ, Rabin K, Kimball S, El-Mohandes A. A global survey of potential acceptance of a COVID-19 vaccine. *Nat Med* 2021; **27**: 225-228 [PMID: 33082575 DOI: 10.1038/s41591-020-1124-9]
21 **Karafillakis E**, Larson HJ; ADVANCE consortium. The benefit of the doubt or doubts over benefits? A systematic literature review of perceived risks of vaccines in European populations. *Vaccine* 2017; **35**: 4840-4850 [PMID: 28760616 DOI: 10.1016/j.vaccine.2017.07.061]
22 **Harapan H**, Anwar S, Yufika A, Sharun K, Gachabayov M, Fahriani M, Husnah M, Raad R, Abdalla RY, Adam RY, Khiri NM, Ismaeil MI, Ismail AY, Kacem W, Dahman NB, Teyeb Z, Aloui K, Hafsi M, Ferjani M, Deeb DA, Emad D, Abbas KS, Monib FA, Sami FS, Subramaniam R, Panchawagh S, Anandu S, Haque MA, Ferreto LE, Briones MF, Morales RB, Díaz SA, Aburto JT, Rojas JE, Balogun EO, Enitan SS, Yomi AR, Durosinmi A, Ezigbo ED, Adejumo EN, Babadi E, Kakemam E, Malik NI, Ullah I, Rosiello DF, Emran TB, Wendt GW, Arab-Zozani M,

Wagner AL, Mudatsir M. Vaccine hesitancy among communities in ten countries in Asia, Africa, and South America during the COVID-19 pandemic. *Pathog Glob Health* 2022; **116**: 236-243 [PMID: 34928187 DOI: 10.1080/20477724.2021.2011580]

23 **Boche B**, Kebede O, Damessa M, Gudeta T, Wakjira D. Health Professionals' COVID-19 Vaccine Acceptance and Associated Factors in Tertiary Hospitals of South-West Ethiopia: A Multi-Center Cross- Sectional Study. *Inquiry* 2022; **59**: 469580221083181 [PMID: 35285341 DOI: 10.1177/00469580221083181]

24 **Iwuafor A**, Ogban G, Ita O, Offiong A, Owai P, Udoh U, Elem D. Determinants of COVID-19 vaccine acceptance amongst doctors practising in Cross River State, Nigeria. *Af J Clin Exp Micro* 2023; **24**: 147-157 [DOI: 10.4314/ajcem.v24i2.4]

25 **Olu-Abiodun O**, Abiodun O, Okafor N. COVID-19 vaccination in Nigeria: A rapid review of vaccine acceptance rate and the associated factors. *PLoS One* 2022; **17**: e0267691 [PMID: 35544545 DOI: 10.1371/journal.pone.0267691]

26 **Eze UA**, Ndoh KI, Ibisola BA, Onwuliri CD, Osiyemi A, Ude N, Chime AA, Ogbor EO, Alao AO, Abdullahi A. Determinants for Acceptance of COVID-19 Vaccine in Nigeria. *Cureus* 2021; **13**: e19801 [PMID: 34963828 DOI: 10.7759/cureus.19801]

27 **Al-Mustapha AI**, Okechukwu O, Olayinka A, Muhammed OR, Oyewo M, Owoicho SA, Abubakar AT, Olabisi A, Jibril A, Ereh S, Fakayode OE, Ogundijo OA, Elelu N, Adetunji VO. A national survey of COVID-19 vaccine acceptance in Nigeria. *Vaccine* 2022; **40**: 4726-4731 [PMID: 35764433 DOI: 10.1016/j.vaccine.2022.06.050]

28 **Alice TE**, Okonofua M, Adeke A, Obi A. Willingness to Accept a COVID-19 Vaccine in Nigeria: A Population-based Cross-sectional Study. *CAJPH* 2021; **7**: 53 [DOI: 10.11648/j.cajph.20210702.12]

29 **Njoga EO**, Mshelbwala PP, Abah KO, Awoyomi OJ, Wangdi K, Pewan SB, Oyeleye FA, Galadima HB, Alhassan SA, Okoli CE, Kwaja EZ, Onwumere-Idolor OS, Atadiose EO, Awoyomi PO, Ibrahim MA, Lawan KM, Zailani SA, Salihu MD, Rupprecht CE. COVID-19 Vaccine Hesitancy and Determinants of Acceptance among Healthcare Workers, Academics and Tertiary Students in Nigeria. *Vaccines (Basel)* 2022; **10**: 626 [PMID: 35455375 DOI: 10.3390/vaccines10040626]

30 **Nindrea RD**, Usman E, Katar Y, Sari NP. Acceptance of COVID-19 vaccination and correlated variables among global populations: A systematic review and meta-analysis. *Clin Epidemiol Glob Health* 2021; **12**: 100899 [PMID: 34746514 DOI: 10.1016/j.cegh.2021.100899]

31 **Reed-Thryselius S**, Fuss L, Rausch D. The Relationships Between Socioeconomic Status, COVID-19 Risk Perceptions, and the Adoption of Protective Measures in a Mid-Western City in the United States. *J Community Health* 2022; **47**: 464-474 [PMID: 35129800 DOI: 10.1007/s10900-022-01070-y]

32 **Fenta ET**, Tiruneh MG, Delie AM, Kidie AA, Ayal BG, Limenh LW, Astatkie BG, Workie NK, Yigzaw ZA, Bogale EK, Anagaw TF. Health literacy and COVID-19 vaccine acceptance worldwide: A systematic review. *SAGE Open Med* 2023; **11**: 20503121231197869 [PMID: 37823070 DOI: 10.1177/20503121231197869]

33 **Kirbiš A**. The Impact of Socioeconomic Status, Perceived Threat and Healthism on Vaccine Hesitancy. *Sustainability* 2023; **15**: 6107 [DOI: 10.3390/su15076107]

34 **Montagni I**, Ouazzani-Touhami K, Mebarki A, Texier N, Schück S, Tzourio C; CONFINS group. Acceptance of a Covid-19 vaccine is associated with ability to detect fake news and health literacy. *J Public Health (Oxf)* 2021; **43**: 695-702 [PMID: 33693905 DOI: 10.1093/pubmed/fdab028]

35 **Tijani B**, Filani T, Oluyide O, Odis A, Ezike E, Adewemimo A, Benjamin A, Joseph I, Sagar M, Akinreni T. COVID-19 Vaccine Uptake and its Determinants: Findings From A Web-Based Survey in Nigeria. *EJMED* 2023; **5**: 48-52 [DOI: 10.24018/ejmed.2023.5.4.1795]

36 **Zakari S**, Ogbu CO, Zakari H, Chioma ES, Ijimbili SB, Idoko JE, Emmanuel AO, Nnenna AD, Agwara BC, Adokiye ES, Idowu AO, Edegbene OA, Anejo-okopi J. Acceptance and hesitancy of COVID-19 vaccine among university community members of Otukpo, Nigeria: a cross-sectional study. *Discov glob soc* 2023; **1**: 5 [DOI: 10.1007/s44282-023-00013-9]

37 **Padonou SGR**, Kakaï Glèlè C, Accrombessi M, Adegbite BR, Dangbenon E, Bah H, Akogbeto E, Bah Chabi AI, Kaucley L, Sourakatou S, Dossou A, Batonon A, Bissouma-Ledjou T, Hounkpatin B. Assessment of COVID-19 Vaccine Acceptance and Its Associated Factors during the Crisis: A Community-Based Cross-Sectional Study in Benin. *Vaccines (Basel)* 2023; **11**: 1104 [PMID: 37376493 DOI: 10.3390/vaccines11061104]

38 **Omale UI**, Oka OU, Amuzie CI, Uduma VU, Adeke AS, Ikegwuonu CO, Nkwo GE, Nwali UIA, Iyare O, Ewah RL, Nnachi OO, Ukpabi OO, Okeke IM. COVID-19 vaccination acceptance (uptake, hesitancy, intention to receive and timeliness of the intention to receive) and the determinants among health workers in Ebonyi state, Nigeria: an analytical cross-sectional study. *BMJ Open* 2023; **13**: e068668 [PMID: 37438061 DOI: 10.1136/bmjopen-2022-068668]

39 **Cordina M**, Lauri MA, Lauri J. Attitudes towards COVID-19 vaccination, vaccine hesitancy and intention to take the vaccine. *Pharm Pract (Granada)* 2021; **19**: 2317 [PMID: 33828623 DOI: 10.18549/PharmPract.2021.1.2317]

40 **Kourlaba G**, Kourkouni E, Maistreli S, Tsopela CG, Molocha NM, Triantafyllou C, Koniordou M, Kopsidas I, Chorianopoulou E, Maroudi-Manta S, Filippou D, Zaoutis TE. Willingness of Greek general population to get a COVID-19 vaccine. *Glob Health Res Policy* 2021; **6**: 3 [PMID: 33509291 DOI: 10.1186/s41256-021-00188-1]

41 **Isiguzo GC**, Stefanovics E, Unamba NN, Mbam TT, Anyaehie UG, Chukwu CC, Anyaehie UB, Osy-Eneze C, Ibezim EO, Okoro UG, Njoku PO, Adimekwe AI, Ibediro K, Stefanovics G, Iheanacho T. Perceptions of the COVID-19 Vaccine and Willingness to Receive Vaccination among Health Workers in Nigeria: A Cross-sectional Study. *Niger J Clin Pract* 2024; **27**: 102-108 [PMID: 38317042 DOI: 10.4103/njcp.njcp_537_23]

42 **Kamal AM**, Sarkar T, Khan MM, Roy SK, Khan SH, Hasan SMM, Hossain MS, Dell CA, Seale H, Islam MS. Factors Affecting Willingness to Receive COVID-19 Vaccine Among Adults: A Cross-sectional Study in Bangladesh. *JHM* 2023; **25**: 414-423 [DOI: 10.1177/09735984211050691]

43 **Robinson E**, Jones A, Lesser I, Daly M. International estimates of intended uptake and refusal of COVID-19 vaccines: A rapid systematic review and meta-analysis of large nationally representative samples. *Vaccine* 2021; **39**: 2024-2034 [PMID: 33722411 DOI: 10.1016/j.vaccine.2021.02.005]

44 **Olawa B**, Lawal A, Odoh I, Azikiwe J, Olawole A, Odusina E, Ayodele I, Ajayi O. Mistrust in government and COVID-19 vaccination acceptance in Nigeria: investigating the indirect roles of attitudes towards vaccination. *J Egypt Public Health Assoc* 2023; **98**: 1 [PMID: 36745270 DOI: 10.1186/s42506-023-00129-5]

45 **Nehal KR**, Steendam LM, Campos Ponce M, van der Hoeven M, Smit GSA. Worldwide Vaccination Willingness for COVID-19: A Systematic Review and Meta-Analysis. *Vaccines (Basel)* 2021; **9**: 1071 [PMID: 34696179 DOI: 10.3390/vaccines9101071]

46 **Wang Q**, Yang L, Jin H, Lin L. Vaccination against COVID-19: A systematic review and meta-analysis of acceptability and its predictors. *Prev Med* 2021; **150**: 106694 [PMID: 34171345 DOI: 10.1016/j.ypmed.2021.106694]

47 **Gachabayov M**, Sharun K, Felsenreich DM, Nainu F, Anwar S, Yufika A, Ophinni Y, Yamada C, Fahriani M, Husnah M, Raad R, Khiri NM,

Abdalla RY, Adam RY, Ismaeil MI, Ismail AY, Kacem W, Teyeb Z, Aloui K, Hafsi M, Ferjani M, Dahman NBH, Deeb DA, Emad D, Abbas KS, Monib FA, Sami FS, Ramanarayanan S, Panchawagh S, Anandu S, Haque MA, Ferreto LE, Briones MF, Morales RB, Lazcano-Díaz S, Aburto JT, Rojas JE, Balogun EO, Kusuma HI, Yeni CM, Utami NA, Enitan SS, Yomi AR, Durosinmi A, Adejumo EN, Ezigbo ED, Babadi E, Kakemam E, Ullah I, Malik NI, Rosiello F, Emran TB, Imelda E, Wendt GW, Arab-Zozani M, Dhama K, Mudatsir M, Harapan H. Perceived risk of infection and death from COVID-19 among community members of low- and middle-income countries: A cross-sectional study. *F1000Res* 2022; **11**: 345 [PMID: 36128553 DOI: 10.12688/f1000research.109575.2]

48 **Fojnica A**, Osmanovic A, Đuzic N, Fejzic A, Mekic E, Gromilic Z, Muhovic I, Kurtovic-Kozaric A. COVID-19 vaccine acceptance and rejection in an adult population in Bosnia and Herzegovina. *PLoS One* 2022; **17**: e0264754 [PMID: 35226708 DOI: 10.1371/journal.pone.0264754]

49 **Rhodes A**, Hoq M, Measey MA, Danchin M. Intention to vaccinate against COVID-19 in Australia. *Lancet Infect Dis* 2021; **21**: e110 [PMID: 32941786 DOI: 10.1016/S1473-3099(20)30724-6]

50 **Yıldırım M**, Güler A. Positivity explains how COVID-19 perceived risk increases death distress and reduces happiness. *Pers Individ Dif* 2021; **168**: 110347 [PMID: 32843780 DOI: 10.1016/j.paid.2020.110347]

51 **Mustapha M**, Lawal BK, Sha'aban A, Jatau AI, Wada AS, Bala AA, Mustapha S, Haruna A, Musa A, Ahmad MH, Iliyasu S, Muhammad S, Mohammed FZ, Ahmed AD, Zainal H. Factors associated with acceptance of COVID-19 vaccine among University health sciences students in Northwest Nigeria. *PLoS One* 2021; **16**: e0260672 [PMID: 34843594 DOI: 10.1371/journal.pone.0260672]

52 **Kolawole OM**, Aun II, Ogah JI, Folahan FF, Kolawole CF. Determinants of COVID-19 vaccine uptake in Kwara State, Nigeria: The role of public health management professionals in driving behavioral change. *GHES* 2024; **2**: 2462 [DOI: 10.36922/ghes.2462]

53 **Mahmood MY**, Ashiru A, Shettima FB, Abdullahi AA, Ngulde AM, Abbas ZU, Jibrin IM, Musami UB, Wakawa IA. Personality Traits as Key Determinants of COVID-19 Vaccine Uptake among Healthcare Workers in Nigeria. *NJM* 2023; **32**: 293-301 [DOI: 10.4103/njm.njm_40_23]

54 **Elsehrawy MG**. Acceptance to take COVID-19 vaccine and its relation to COVID-19 infection. *Ital J Med* 2024; **18** [DOI: 10.4081/itjm.2024.1725]

55 **Gunawardhana N**, Baecher K, Boutwell A, Pekwarake S, Kifem M, Ngong MG, Fondzeyuf A, Halle-Ekane G, Mbah R, Tih P, Dionne-Odom J, Tebit DM. COVID-19 vaccine acceptance and perceived risk among pregnant and non-pregnant adults in Cameroon, Africa. *PLoS One* 2022; **17**: e0274541 [PMID: 36099295 DOI: 10.1371/journal.pone.0274541]

56 **Kreps S**, Prasad S, Brownstein JS, Hswen Y, Garibaldi BT, Zhang B, Kriner DL. Factors Associated With US Adults' Likelihood of Accepting COVID-19 Vaccination. *JAMA Netw Open* 2020; **3**: e2025594 [PMID: 33079199 DOI: 10.1001/jamanetworkopen.2020.25594]

57 **Masoud M**, Bassyouni RH, Abdel-wahed WY, Al Hawamdeh MI, Nassar FM, Arishi N, Ziad A, Elsidig LA, Hamed NS. Acceptance of COVID-19 vaccination and associated factors in Middle East countries: a multinational study. *AJM* 2024; **60**: 1-10 [DOI: 10.1080/20905068.2023.2292915]

58 **Tran VD**, Pak TV, Gribkova EI, Galkina GA, Loskutova EE, Dorofeeva VV, Dewey RS, Nguyen KT, Pham DT. Determinants of COVID-19 vaccine acceptance in a high infection-rate country: a cross-sectional study in Russia. *Pharm Pract (Granada)* 2021; **19**: 2276 [PMID: 33828622 DOI: 10.18549/PharmPract.2021.1.2276]

59 **Gallè F**, Sabella EA, Roma P, De Giglio O, Caggiano G, Tafuri S, Da Molin G, Ferracuti S, Montagna MT, Liguori G, Orsi GB, Napoli C. Knowledge and Acceptance of COVID-19 Vaccination among Undergraduate Students from Central and Southern Italy. *Vaccines (Basel)* 2021; **9**: 638 [PMID: 34200835 DOI: 10.3390/vaccines9060638]

60 **Ferrer R**, Klein WM. Risk perceptions and health behavior. *Curr Opin Psychol* 2015; **5**: 85-89 [PMID: 26258160 DOI: 10.1016/j.copsyc.2015.03.012]

61 **Boonsaeng T**, Carpio CE, Guerrero P, Sarasty O, Borja I, Hudson D, Macharia A, Shibia M. Perceived Risks of Infection, Hospitalization, and Death From COVID-19 at the Equator: Ecuador and Kenya. *Disaster Med Public Health Prep* 2021; **17**: e34 [PMID: 34392868 DOI: 10.1017/dmp.2021.268]

62 **Bartsch SM**, O'Shea KJ, Ferguson MC, Bottazzi ME, Wedlock PT, Strych U, McKinnell JA, Siegmund SS, Cox SN, Hotez PJ, Lee BY. Vaccine Efficacy Needed for a COVID-19 Coronavirus Vaccine to Prevent or Stop an Epidemic as the Sole Intervention. *Am J Prev Med* 2020; **59**: 493-503 [PMID: 32778354 DOI: 10.1016/j.amepre.2020.06.011]

63 **Hossain MA**, Hossain KMA, Sakel M, Kabir MF, Saunders K, Faruqui R, Hossain MS, Uddin Z, Kader M, Walton LM, Haque MO, Shafin R, Chakrovorty SK, Jahid IK. Knowledge, Attitudes, Behavioural Practises, and Psychological Impact Relating to COVID-19 Among People Living With Spinal Cord Injury During In-Patient Rehabilitation in Bangladesh. *Front Neurol* 2021; **12**: 739354 [PMID: 35197912 DOI: 10.3389/fneur.2021.739354]

64 **Olaoye I**, Ekong A, Samuel A, Kelaiditi E, Myrissa K, Jacdonmi T, Gboyega F. Public beliefs and willingness to accept COVID-19 vaccines among adults in South-Western Nigeria: A cross-sectional study. *AIMS Public Health* 2023; **10**: 1-15 [PMID: 37063349 DOI: 10.3934/publichealth.2023001]

65 **Abate BB**, Tilahun BD, Yayeh BM. Global COVID-19 vaccine acceptance level and its determinants: an umbrella review. *BMC Public Health* 2024; **24**: 5 [PMID: 38166750 DOI: 10.1186/s12889-023-17497-4]

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SYSTEMATIC REVIEWS

Review of Albanian studies suggests the need for further efforts to counteract significant hepatitis B virus prevalence

Jerina Jaho, Fatjona Kamberi, Enkeleint A Mechili, Agreta Bicaj, Paola Carnì, Leonardo Baiocchi

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Abstract

BACKGROUND

Hepatitis B virus (HBV) is categorized as one of the smallest enveloped DNA viruses and is the prototypical virus of the Hepatoviridae family. It is usually transmitted through body fluids such as blood, semen, and vaginal secretions. The majority (more than 95%) of immunocompetent adults infected with HBV spontaneously clear the infection. In the context of the high prevalence of HBV infection in Albania, the research gap is characterized by the lack of studies aimed at advancing the current understanding and improving the prevailing situation. The main objective of this study was to address the low rate of HBV diagnosis and the lack of a comprehensive national program to facilitate widespread diagnosis.

AIM

To analyze the prevalence of HBV infection in Albania and elucidate the persistently high prevalence despite efforts and measures implemented.

METHODS

Using a systematic literature review, we collected existing research on the epidemiology of HBV in Albania from PubMed, Cochrane Library, Google Scholar, and Albanian Medical Journals, focusing on studies published after the 1980s and conducted solely in the Albanian population.

RESULTS

The findings reveal a dynamic shift in HBV prevalence in Albania over several decades. Initially high, the prevalence gradually declined following the implementation of screening and vaccination programs. However, the prevalence rates have remained notably high, exceeding 8% in recent years. Contributing factors include vertical transmission, inadequate healthcare infrastructure, and challenges in screening and diagnosis. Studies among Albanian refugees in neighboring countries also reported high prevalence rates, emphasizing the need for transnational interventions. Despite advancements in screening, vaccination, and healthcare infrastructure, Albania continues to face a substantial burden of HBV infection.

CONCLUSION

The persistence of high prevalence underscores the complexity of the issue, requiring ongoing efforts to ensure a comprehensive understanding and effective mitigation. Addressing gaps in vaccination coverage, improving access to screening and diagnosis, and enhancing public awareness are crucial steps toward reducing HBV prevalence in Albania.

Key Words: Albania; Hepatitis B virus; Epidemiology; Vaccination; Systematic review

Core Tip: This study aimed to comprehensively analyze the prevalence of hepatitis B virus infection in Albania and elucidate the persistently high prevalence despite implemented efforts and measures.

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INTRODUCTION

Hepatitis B virus (HBV) is classified as one of the smallest enveloped DNA viruses and serves as a prototype within the Hepadnaviridae family.

It is often transmitted through body fluids such as blood, semen, and vaginal secretions. The majority (more than 95%) of immunocompetent adults infected with HBV can clear the infection spontaneously[1,2].

HBV infection is highly variable in both presentation and severity. Some people clear the infection spontaneously, while others endure a lifetime of chronic complications, including hepatitis, cirrhosis, and cancer[3].

Serological markers of HBV infection include hepatitis B surface antigen (HBsAg), indicating active infection; hepatitis B e antigen (HBeAg), indicating active viral replication; anti-HBe, reflecting loss of HBeAg synthesis due to immunologic containment or viral gene mutations; anti-HBc, indicating past or current infection; and anti-HBs, which serve as a marker for recovery from acute infection or vaccination-induced immunity[4,5].

The epidemiology of hepatitis B exhibits significant geographical variation, which is a distinctive aspect of its prevalence patterns. At the same time, it is one of the most pervasive infectious diseases on a global scale[6]. According to estimates from the World Health Organization (WHO) 2019[7], the global burden of chronic hepatitis B was substantial, encompassing approximately 296 million people. This prevalence was accompanied by an annual incidence of 1.5 million new infections.

The epidemiology of hepatitis B can be described in terms of the prevalence of HBsAg in a population, broadly classified into high- (> 8% HBsAg prevalence), intermediate- (2%–7%), and low-prevalence areas (< 2%)[8].

There is a compelling association between the route of transmission, the genotype of HBV, and the epidemiological distribution of HBV infection in various countries[9].

The implementation of a mass HBV immunization program was recommended by the WHO since 1991, and has dramatically decreased the prevalence of HBV infection in many countries[10].

The primary objective of this study was to discern trends in the prevalence of HBV in Albania and to elucidate the persistently high prevalence, despite concerted efforts and implemented measures.

The research gap within the context of the high prevalence of HBV in Albania is characterized by a lack of studies aimed at advancing current understanding and improving the prevailing situation. This deficiency is notably accentuated by the absence of a comprehensive national program, limited diagnostic initiatives, and an insufficient scale of diagnostic interventions. Addressing these gaps is imperative to inform evidence-based strategies for effective prevention and control of HBV in Albania.

The primary objective of this study was to address the infrequent diagnostic rates of HBV and the lack of comprehensive national programs facilitating widespread diagnosis. Furthermore, the study aimed to analyze factors that contribute to the persistent high incidence of HBV, despite sporadic diagnoses. This investigation will specifically explore key elements such as immunization, advances in hospital hygiene, improvements in diagnostic tools, increased access-

ibility to diagnostic services in public and private entities, and the availability of cost-effective vaccines. The overarching goal was to gain insight into the multifaceted dynamics that influence the incidence of HBV, ultimately forming strategies to improve diagnostic rates and mitigate the prevailing high prevalence.

MATERIALS AND METHODS

This study employed a systematic literature review methodology to comprehensively analyze existing research on the epidemiology of HBV in Albania. The primary objective was to identify the prevalent trends and determine the causes that contribute to sustained high prevalence despite the implemented efforts. The research was conducted across PubMed, Cochrane Library, Google Scholar, and Albanian Medical Journals, with keywords used in English or Albanian language, depending on the database (Albanian language keywords exclusively for articles published in Albanian). The inclusion criteria encompass full papers and articles published after 1980s, focusing on studies/systematic literature reviews conducted solely in the Albanian population and specifically addressing the epidemiology of HBV in Albania.

The following keywords and terms were used in the PubMed Database: (1) Hepatitis B; (2) HBV prevalence; and (3) Albania. The search string utilized was as follows: (("hepatitis B" [All fields] OR "HBV" [all fields] OR ("hepatitis" [all fields] AND "B" [all fields])) AND ("prevalence" [all fields] OR "Albania" [all fields])). Results of the research are reported in Figure 1.

RESULTS

Prevalence of HBV in Albania

In Albania, viral hepatitis B has been and continues to be a serious public health problem.

The endemicity of hepatitis B is described by the prevalence of HBsAg in the general population in a given geographic area. The WHO has classified the prevalence of hepatitis B according to HBsAg expression as high- (> 8%), medium- (2-7%), and low-prevalence (< 2%) countries (< 2%)[8].

Official statistics show that, despite small fluctuations, the incidence of viral hepatitis in the period 1964-1990 was quite high, ranging from 200-400 cases *per* 100000 inhabitants[11]. Studies based on the electrophoresis method have found a HBsAg positivity rate of ~10% in the healthy population in Albania[11]. The implementation of the HBsAg screening program in Albania started around 1975, and since that time the incidence and prevalence of HBV have changed significantly toward improving the situation.

For years, the condition appeared worrying, since the incidence figures were higher in children and adolescents. In a study on the epidemiological situation of HBV (1985), it was emphasized that viral hepatitis in children has increased and specifically in 1977 it was found in 36%, in 1979 in 41%, and in 1981 in 47% of children with acute viral hepatitis admitted to Tirana Pediatric Hospital[12].

The study of the prevalence of hepatitis B in Albania was investigated in different contingents, including immigrants settled in Italy and Greece after the mass exodus of 1991 as a result of the major political events that swept the country.

In a study conducted in 1980, among other things, the presence of the Australia antigen was studied in different contingents of pregnant women. The percentage of carryover according to quarters I, II, and III was 4.4%, 4.3%, and 5%, respectively. This study aimed to study risk factors for HBV transmission and vertical mother-to-child transmission[13].

In 1993, a study was conducted on the epidemiology of viral hepatitis in Albania. The sample consisted of 545 patients from the general population in the period January to August 1993. Vertical transmission was also studied in 193 pregnant women. It was found that 18.3% of the patients who received (n = 100) the examination were positive for HBsAg[11].

Daleko *et al*[14] analyzed the marked prevalence of viral hepatitis in Albanian immigrants originating mainly from the south of Albania. In this study, 1025 refugees located mainly in the prefecture of Ioannina were evaluated. The prevalence of HBsAg was found to be 22.2%.

Similarly, a study on the prevalence of various markers of hepatitis was conducted in pregnant women who had migrated to Greece (1996)[15]. The study sample included 500 participants, of whom 67 pregnant women (13.4%) tested HBsAg+.

Greece and Italy are the two countries with the highest number of Albanians who emigrated after 1991. Studies similar to those conducted in Greece have also been conducted on Albanian immigrants in Italy. In a study conducted by Chironna *et al*[16], the seroprevalence of hepatitis B, C, and D was analyzed in 670 Albanian immigrants in southern Italy in 1997; 13.6% of them were positive for HBsAg, while the prevalence of anti-HBs was 47.6%.

The HEPAGA project, a Greek-Albanian research collaboration, aimed to detect hepatitis B in the young population of Albania. For this reason, 410 young people aged 14-20 years who lived in Albania from September 2001 to February 2002 were examined. In this study conducted by Katsanos *et al*[17], 49 participants who constituted 11.89% of the sample were positive for HBsAg.

Health workers are considered a risk group for parenteral infectious diseases. The aim of the study conducted by Kondili *et al*[18] was to assess the prevalence of HBV and hepatitis C virus (HCV) in Albanian health workers. The study noted that the prevalence of HBsAg in 480 participants was 8.1% while the prevalence of anti-HBc was 70%. In this study, the highest prevalence was observed in the age group of 20-30 years old (11.4%). This study concluded not only the high rate of HBV infection in healthcare workers but also the low vaccination coverage.

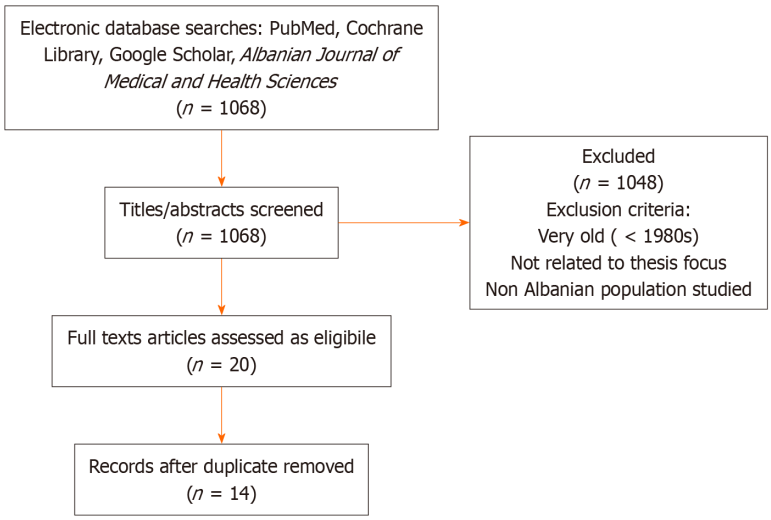

Figure 1 PRISMA flow diagram. The PRISMA flow diagram outlines the selection process for studies included in the systematic review on hepatitis B virus prevalence in Albania. The diagram details each step, from the initial identification of studies through database searches and other sources, to the final inclusion of 14 studies that met the inclusion criteria.

Elefsiniotis *et al*[19] evaluated the prevalence of serological markers in 1333 pregnant women living in Greece but of different nationalities; 30.6% of them had Albanian nationality. Among pregnant women of Albanian nationality, the prevalence of HBsAg was estimated to be 11%, while the prevalence of anti-HBc was 52%. This study highlighted that HBV infection is endemic in pregnant Albanian women.

One of the most important studies conducted on the epidemiology of HBV in Albania is the one conducted by Resuli *et al*[20] who noted that the prevalence of HBsAg and anti-HBs was 9.5% and 28%, respectively, demonstrating that despite the almost twofold decrease in the prevalence of HBsAg in the general population, Albania continues to remain a highly endemic country for HBV.

Milionis[21] conducted a study in young Albanian immigrants living in Greece to study the serological prevalence of HBV and HCV; 504 subjects aged 10-23 participated in the study. In this study, 11.7% of the patients were found to be positive for HBsAg.

Durro and Qyra[22] evaluated in a retrospective study the epidemiological trends of hepatitis B in 79274 blood donors during the period 1999-2009. The prevalence of HBsAg in the examined blood donors was 7.9%. According to the status of the blood donor, the prevalence of HBsAg was 10.5% in commercial blood donors, 8.1% in voluntary blood donors, and 8.6% in blood donors from family members. The prevalence of anti-HBc was 59.1%.

Limited studies have been conducted on the epidemiology of hepatitis B within the Albanian population; however, the available literature provides sufficient information on the prevalence of this virus. In particular, a significant portion of these studies have been conducted in neighboring countries, particularly Italy and Greece, which experienced increased immigration from Albania post-1990. A temporal analysis of these studies reveals a consistent decline in the prevalence of HBsAg. Nevertheless, it is imperative to underscore the scarcity of in-depth investigations conducted after 2009. Despite evident progress, Albania persists with a notable prevalence of hepatitis B exceeding 8%. The most relevant papers regarding HBV epidemiology in Albania are reported in Table 1.

Epidemiological trends

The prevalence of hepatitis B in Albania has exhibited dynamic shifts characterized by a sustained reduction in reported cases (Figure 2). This phenomenon can be attributed to the implementation of various policies and the variable influence of risk factors. Beyond the overarching decline in prevalence and incidence rates in successive years, there are discernible variations in various epidemiological aspects.

Zehender *et al*[23] demonstrated that genotype D2 made its entry into the Albanian population during the latter part of the 1960s. Research findings indicated a continual increase in the effective number of infections until the mid-1990s, at which point a plateau was reached.

This is reflected in a study conducted in children hospitalized for acute viral hepatitis at the Pediatric Hospital in Tirana. A notable increase in cases was observed, specifically in the year 1977, accounting for 36% of cases, in 1979 reaching 42%, and in 1981 escalating to 47% of cases[12].

The incidence of hepatitis B in Albania has been documented in a limited number of studies. Most of these studies have assessed the prevalence in various groups or in the general healthy population. The incidence of viral hepatitis during the years 1964-1990 was notably high, ranging between 200-400 cases *per* 100000 inhabitants. Meanwhile, data from the WHO indicate that the annual incidence of viral hepatitis (all forms combined) in Western European countries ranged from 10 to 50 cases *per* 100000 inhabitants.

These studies indicate that until the 1990s, the incidence and prevalence of HBsAg in Albania were notably high and experienced an upward trend during a specific time period[11].

Table 1 Key studies on hepatitis B virus epidemiology in Albanian population

Ref.	Study region	Year	Age	Number of cases	Prevalence of HBsAg
Angoni *et al*[11]	Albania	1993	Adult	545	18.3%
Dalekos *et al*[14]	Refugees (Greece)	1995	All ages	1025	22.2%
Malamitsi-Puchner *et al*[15]	Albanian refugees	1996	Pregnant women	500	13.4%
Chironna *et al*[16]	Albanian refugees (Italy)	2000	All ages	670	13.6%
Papaevangelou *et al*[25]	Albanian (Greece)	2006	Pregnant women	409	9.8%
Kondili *et al*[18]	Albania	2007	Health care workers	480	8.1%
Katsanos *et al*[17]	Albania	2009	All ages	410	11.89%
Elefsiniotis *et al*[19]	Albanian refugees	2009	Pregnant women	408	11%
Resuli *et al*[20]	Albania	2009	All ages	3880	9.5%
Durro and Qyra[22]	Albania	2009	Blood donors	79274	7.9%
Milionis[21]	Albanian (Greece)	2010	10-23	504	11.7%

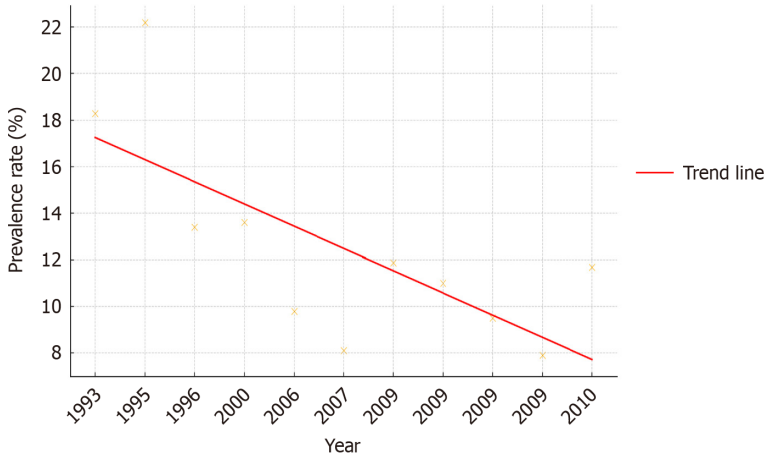

Figure 2 Hepatitis B virus prevalence rates over time. The scatter plot and trend line illustrate the changes in hepatitis B virus prevalence rates in Albania over the years. Data from key studies, summarized in Table 1, show a clear declining trend in hepatitis B virus prevalence from 1993 to 2010.

In the aftermath of Albania's transition in the early 1990s, substantial emigration occurred, impacting not only Albania but also neighboring Italy and Greece. R. Angoni's 1993 study of 545 subjects revealed a notable 18.3% prevalence of HBsAg, indicating significant rates of liver infection. Additionally, 49% of subjects tested positive for anti-HBs, suggesting prior exposure or immunization[11].

Dalekos *et al*[14] conducted a study among Albanian refugees in Greece, estimating a prevalence of hepatitis B of 22.5% among 1025 subjects. Similarly, Roussos *et al*[24] found a comparable prevalence of 22.4% among a smaller sample of 76 Albanian subjects. Subsequent to national vaccination campaigns initiated in 1994, there was a notable decline in prevalence rates reported by studies among Albanian refugees in Greece and Italy, with figures dropping to 13.6%, 13.4%, and 9.8%, respectively[15,16,25].

Resuli *et al*[20] highlighted a significant decrease in HBV prevalence by 50%, estimating a prevalence of only 9.5% among 3880 subjects. Furthermore, the lowest prevalence was observed in a study of Albanian blood donors in 2009, where only 7.9% tested positive, marking a decrease from the prevalence of 9.1% reported in 1999[22]. Furthermore, Kondili *et al*[18] reported a prevalence of 8.1% among 480 Albanian healthcare workers[18].

Comparable studies conducted between 2009 and 2010 demonstrate a persistent prevalence of HBV ranging from 11% to 11.89%. Although indicating a decrease in previous prevalence rates, these findings still underscore a notably high prevalence of HBV within the examined population over this short period[17,19,21]. The analysis of trend and its statistic (analysis of covariance) are reported in Figure 2 and Table 2, respectively.

Factors influencing the high prevalence of HBV in Albania

The persistently high prevalence of HBV in Albania is the result of multifactorial influences, reflecting the ongoing challenges within the healthcare system and societal contexts. While past issues such as the lack of single-use medical devices in hospitals have been addressed to some extent, contemporary factors continue to contribute to the prevalence of

Table 2 Analysis of covariance results for hepatitis B virus prevalence rates[1]

Source	Type III sum of squares	df	Mean square	*F*	*P* value
Corrected model	127.702	2	63.851	7.735	0.013
Intercept	1757.171	1	1757.171	212.864	
Population	5.079	1	5.079	0.615	0.455
Year 2000	103.685	1	103.685	12.560	0.008
Error	66.039	8	8.255		
Total	1914.742	11			
Corrected total	193.741	10			

[1]The analysis of covariance provides strong statistical evidence indicating a significant reduction in hepatitis B virus prevalence rates after the year 2000. The year 2000 variable is a significant predictor ($P = 0.008$), demonstrating a notable difference in prevalence rates before and after this period. The model explains approximately 65.9% of the variance in hepatitis B virus prevalence rates ($R^2 = 0.659$), with an adjusted R^2 of 0.574, indicating a good fit. The population variable ($P = 0.455$) is not significant, showing that sample size variations do not influence hepatitis B virus rates. The intercept is highly significant ($P < 0.001$), suggesting a substantial baseline prevalence rate, which was impacted by interventions and changes over time.

HBV. Among these factors is the persistence of vertical transmission, where infected mothers can still pass the virus to their newborns during childbirth. Furthermore, challenges in access to healthcare services, including screening and vaccination programs, and lack of awareness of HBV transmission and prevention among the general population further exacerbate the prevalence of the virus in Albania.

Troja[13] found a direct correlation between the prevalence of HBV in pregnant women and the number of injections received[13]. This highlights the role of inadequate use of single-use medical devices in hospitals as a contributing factor to HBV transmission[13,15].

Several studies conducted on Albanian refugees in Italy or Greece have emphasized the significant impact of low socioeconomic status and the poor hygienic conditions experienced by its members on the high prevalence of HBV[14,15,17].

Vertical transmission, resulting from factors such as inadequate screening of pregnant women and the use of non-disposable medical equipment, played a significant role in the high prevalence of HBV[11,13]. Hospital practices in Albania also contributed due to poor medical and nursing standards[15]. Resuli *et al*[20] noted that the reduction in the prevalence of HBsAg after infant vaccination mainly stemmed from the prevention of perinatal HBV transmission, highlighting the role of vertical transmission in high prevalence.

The lack of a proper screening for blood and its products also contributed to this endemic prevalence. However, in a study conducted among Albanian blood donors, the prevalence of HBsAg decreased from 9.1% in 1999 to 7.9% in 2009, suggesting an improvement in the transfusion safety of blood and its products[22].

One of the most significant factors contributing to the high prevalence of HBV in Albania was the low coverage of HBV immunization. Numerous studies carried out among the Albanian population, both within Albania and in neighboring countries such as Italy and Greece, have highlighted the low vaccination rates. Despite efforts in prenatal screening and vaccination programs, gaps in coverage and inadequate administration of vaccine doses persist[16,24]. Chironna *et al*[16] found that the prevalence of HBV among children up to 10 years of age was 8.1%, indicating high vertical and horizontal transmission rates and underscoring the inadequacy of immunization efforts.

Despite a high percentage of women who underwent prenatal screening for HBsAg, certain factors, such as delivery in public hospitals and maternal illiteracy, were associated with some women not being tested[25]. Furthermore, Albania's potential higher endemicity of HBV infection compared to neighboring countries may contribute to the elevated prevalence observed among its population[17].

Furthermore, the absence of a nationwide surveillance campaign for HBV infections and the underestimation of vaccination programs exacerbated the situation. In a study, inefficiencies in the monitoring program were highlighted, indicating the need for improved data quality. To enhance the effectiveness of the system, a web-based reporting system, enhanced laboratory tests, and staff training are necessary measures to increase quality, efficiency, and overall usefulness [26].

Measures to counteract high prevalence of HBV in Albania

The measures taken to combat HBV infection in Albania have been multifaceted, addressing various aspects of healthcare infrastructure, prevention, and awareness. Implementing more sensitive laboratory techniques for HBV marker detection, such as ELISA, has advanced diagnostic capabilities[11]. Moreover, vaccination programs targeting newborns, initiated in May 1995, have been integrated into the National Immunization Programs, with infants receiving immunizations at birth, and after 1 and 5 mo[14,20]. Improvements in socioeconomic status and hospital hygiene have contributed to reducing HBV transmission rates[15].

Comprehensive vaccination programs have been crucial, emphasizing nationwide efforts to ensure universal vaccination coverage, particularly among newborns[16]. Furthermore, the adoption of single-use medical devices and improved hospital sanitation practices has helped mitigate the risks of HBV transmission. Establishing viral hepatitis

surveillance systems has allowed monitoring of HBV prevalence over time and tracking the impact of vaccination programs[16].

Prenatal screening has been improved with universal screening of pregnant women, although challenges remain in ensuring coverage among all demographics[25]. Access to screening and testing for HBV infection, especially among high-risk populations, has been prioritized to identify and manage cases effectively[17]. Strengthening healthcare infrastructure, including access to screening, testing, and vaccination services, has been essential in improving HBV management[19].

Targeted vaccination programs have focused on high-risk groups, including healthcare workers, hemodialysis patients, and patients with thalassemia, to curb transmission[20]. Furthermore, the reinforcement of general preventive measures such as safe injection practices, proper sterilization of medical and dental equipment, and blood product screening has contributed to reducing the risks of HBV transmission[22].

Health education initiatives have played a crucial role in raising awareness of HBV transmission routes, preventive measures, and the importance of vaccination, leading to behavior changes and increased coverage of immunization[21, 22]. However, challenges persist, including the need for continuous improvement in monitoring programs, data quality improvement, and staff training to ensure the effectiveness of HBV management strategies[26].

DISCUSSION

The study of HBV prevalence trends in Albania reveals a complex epidemiological landscape influenced by various public health interventions, socioeconomic factors, and migration patterns. Despite efforts to control HBV through vaccination programs and improved healthcare infrastructure, Albania remains a high-endemicity region with a prevalence exceeding 8%. This contrasts with neighboring countries like Greece and Italy, where HBV prevalence has significantly declined due to successful public health measures.

Greece, now classified as a low-endemicity country with a corrected prevalence of 1.88%, still shows substantial variability in HBV prevalence across different regions and among specific populations, such as immigrants. Notably, studies have indicated that Albanian immigrants in Greece exhibit higher HBV prevalence rates than the general Greek population, highlighting the ongoing impact of Albania's high endemicity on neighboring countries[27].

Similarly, Italy has experienced a dramatic decrease in HBV incidence following the implementation of a national universal vaccination program in 1991 and public health campaigns initiated in the 1980s. The incidence of acute hepatitis B in Italy has dropped from 98 cases per 100000 inhabitants in the 1960s to just 0.21 cases per 100000 inhabitants by 2020 [28].

These reductions in HBV prevalence in Greece and Italy underscore the effectiveness of sustained public health efforts, particularly vaccination, in controlling HBV transmission. However, the persistent high prevalence in Albania, as well as among Albanian immigrants in Greece and Italy, suggests that the public health infrastructure in Albania requires further strengthening. Additionally, transnational public health strategies are needed to address the unique challenges posed by migration and to reduce the burden of HBV in the region. Overall, the comparison of HBV prevalence trends between Albania and its neighboring countries highlights the critical importance of robust vaccination programs, effective public health campaigns, and improved healthcare access in reducing HBV prevalence and achieving better public health outcomes.

CONCLUSION

During the period 1964-1990, the incidence of viral hepatitis in Albania exhibited a considerable elevation, fluctuating within the range of 200-400 cases *per* 100000 inhabitants. In recent years a noticeable dearth of comprehensive studies has been observed in the resident population of Albania on the incidence and prevalence of HBV. A notable deficiency in the diagnostic infrastructure for HBV is evident, with the majority of identified cases arising predominantly from voluntary individuals participating in blood donation or routine health examinations. The absence of a national surveillance system dedicated to monitoring HBV infection in Albania constitutes a significant impediment to accurate assessment and targeted management of this public health problem. The efficacy of anti-HBV vaccines has been potentially compromised over certain periods, contributing to the enduring high prevalence of HBV presently. A considerable proportion of people harboring HBV infections remain undiagnosed, exerting a substantial influence on the high prevalence of HBV that prevails in the Albanian population. There is a pressing imperative for in-depth investigations into the incidence and prevalence dynamics of HBV in Albania, underscoring the need for comprehensive and systematic research efforts. Despite the notable strides made through vaccination campaigns, the persistence of a substantial prevalence of HBV in Albania underscores the complexity of the issue, warranting continued efforts for a comprehensive understanding and effective mitigation.

FOOTNOTES

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REFERENCES

1 **Tripathi N**, Mousa OY. Hepatitis B. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2024 [PMID: 32310405]
2 **Global Hepatitis B Vaccination**. Why CDC is Working to Prevent Hepatitis B Globally. Available from: https://www.cdc.gov/global-hepatitis-b-vaccination/why/index.html
3 **Committee on a National Strategy for the Elimination of Hepatitis B and C**; Board on Population Health and Public Health Practice; Health and Medicine Division; National Academies of Sciences, Engineering, and Medicine; Buckley GJ, Strom BL, editors. Eliminating the Public Health Problem of Hepatitis B and C in the United States: Phase One Report. Washington (DC): National Academies Press (US), 2016 [PMID: 27336113 DOI: 10.17226/23407]
4 **Dienstag JL**. Hepatitis B virus infection. *N Engl J Med* 2008; **359**: 1486-1500 [PMID: 18832247 DOI: 10.1056/NEJMra0801644]
5 **Lok AS**, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; **45**: 507-539 [PMID: 17256718 DOI: 10.1002/hep.21513]
6 **Kwon SY**, Lee CH. Epidemiology and prevention of hepatitis B virus infection. *Korean J Hepatol* 2011; **17**: 87-95 [PMID: 21757978 DOI: 10.3350/kjhep.2011.17.2.87]
7 **World Health Organization**. Hepatitis B. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b#:~:text=For%20World%20Hepatitis%20Day%202023,the%202030%20hepatitis%20elimination%20target.
8 **Lavanchy D**. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 2004; **11**: 97-107 [PMID: 14996343 DOI: 10.1046/j.1365-2893.2003.00487.x]
9 **Lin CL**, Kao JH. Hepatitis B virus genotypes and variants. *Cold Spring Harb Perspect Med* 2015; **5**: a021436 [PMID: 25934462 DOI: 10.1101/cshperspect.a021436]
10 **Hou J**, Liu Z, Gu F. Epidemiology and Prevention of Hepatitis B Virus Infection. *Int J Med Sci* 2005; **2**: 50-57 [PMID: 15968340 DOI: 10.7150/ijms.2.50]
11 **Angoni R**. Data on the prevalence of viral hepatitis A, B, C, D, and E infections in Albania. *Buletini i Shkencave Mjekesore* 1998; 70-3
12 **Troja P**, Ndrenika Gj, Taka M, Pepa T. Acute A, B and non-A non-B virus hepatitis in children. *Buletini i Shkencave Mjekesore* 1985; 81-5
13 **Troja P**. Antigen Australia in pregnant women, those who have just given birth, and in newborn children. *Buletini i Shkencave Mjekesore* 1980; 115-20
14 **Dalekos GN**, Zervou E, Karabini F, Tsianos EV. Prevalence of viral markers among refugees from southern Albania: increased incidence of infection with hepatitis A, B and D viruses. *Eur J Gastroenterol Hepatol* 1995; **7**: 553-558 [PMID: 7552639]
15 **Malamitsi-Puchner A**, Papacharitonos S, Sotos D, Tzala L, Psichogiou M, Hatzakis A, Evangelopoulou A, Michalas S. Prevalence study of different hepatitis markers among pregnant Albanian refugees in Greece. *Eur J Epidemiol* 1996; **12**: 297-301 [PMID: 8884198 DOI: 10.1007/BF00145420]
16 **Chironna M**, Germinario C, Lopalco PL, Quarto M, Barbuti S. HBV, HCV and HDV infections in Albanian refugees in Southern Italy (Apulia region). *Epidemiol Infect* 2000; **125**: 163-167 [PMID: 11057972 DOI: 10.1017/s0950268899004215]
17 **Katsanos KH**, Christodoulou DK, Zervou E, Babameto A, Kraja B, Hyphantis H, Karetsos V, Tsonis G, Basho J, Resuli BF, Tsianos EV. Hepatitis B remains a major health priority in Western Balkans: results of a 4-year prospective Greek-Albanian collaborative study. *Eur J Intern Med* 2009; **20**: 698-702 [PMID: 19818290 DOI: 10.1016/j.ejim.2009.07.016]
18 **Kondili LA**, Ulqinaku D, Hajdini M, Basho M, Chionne P, Madonna E, Taliani G, Candido A, Dentico P, Bino S, Rapicetta M. Hepatitis B virus infection in health care workers in Albania: a country still highly endemic for HBV infection. *Infection* 2007; **35**: 94-97 [PMID: 17401713 DOI: 10.1007/s15010-007-6076-1]
19 **Elefsiniotis IS**, Vezali E, Brokalaki H, Tsoumakas K. Hepatitis B markers and vaccination-induced protection rate among Albanian pregnant women in Greece. *World J Gastroenterol* 2009; **15**: 5498-5499 [PMID: 19916183 DOI: 10.3748/wjg.15.5498]
20 **Resuli B**, Prifti S, Kraja B, Nurka T, Basho M, Sadiku E. Epidemiology of hepatitis B virus infection in Albania. *World J Gastroenterol* 2009; **15**: 849-852 [PMID: 19230046 DOI: 10.3748/wjg.15.849]
21 **Milionis C**. Serological markers of Hepatitis B and C among juvenile immigrants from Albania settled in Greece. *Eur J Gen Pract* 2010; **16**: 236-240 [PMID: 20954813 DOI: 10.3109/13814788.2010.525631]
22 **Durro V**, Qyra S. Trends in prevalence of hepatitis B virus infection among Albanian blood donors, 1999-2009. *Virol J* 2011; **8**: 96 [PMID: 21375724 DOI: 10.1186/1743-422X-8-96]

23 **Zehender G**, Ebranati E, Gabanelli E, Shkjezi R, Lai A, Sorrentino C, Lo Presti A, Basho M, Bruno R, Tanzi E, Bino S, Ciccozzi M, Galli M. Spatial and temporal dynamics of hepatitis B virus D genotype in Europe and the Mediterranean Basin. *PLoS One* 2012; **7**: e37198 [PMID: 22662136 DOI: 10.1371/journal.pone.0037198]

24 **Roussos A**, Goritsas C, Pappas T, Spanaki M, Papadaki P, Ferti A. Prevalence of hepatitis B and C markers among refugees in Athens. *World J Gastroenterol* 2003; **9**: 993-995 [PMID: 12717844 DOI: 10.3748/wjg.v9.i5.993]

25 **Papaevangelou V**, Hadjichristodoulou C, Cassimos D, Theodoridou M. Adherence to the screening program for HBV infection in pregnant women delivering in Greece. *BMC Infect Dis* 2006; **6**: 84 [PMID: 16681862 DOI: 10.1186/1471-2334-6-84]

26 **Kureta E**, Basho M, Roshi E, Bino S, Simaku A, Burazeri1 G. Evaluation of the surveillance system for hepatitis B and C in Albania during 2013-2014. *Albanian Med J* 2014; 57-67

27 **Rigopoulou EI**, Gatselis NK, Galanis K, Lygoura V, Gabeta S, Zachou K, Dalekos GN. The changing epidemiology of hepatitis B in Greece. *Ann Gastroenterol* 2021; **34**: 431-437 [PMID: 33948070 DOI: 10.20524/aog.2021.0614]

28 **Sagnelli C**, Sica A, Creta M, Calogero A, Ciccozzi M, Sagnelli E. Epidemiological and clinical aspects of hepatitis B virus infection in Italy over the last 50 years. *World J Gastroenterol* 2022; **28**: 3081-3091 [PMID: 36051347 DOI: 10.3748/wjg.v28.i26.3081]

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SYSTEMATIC REVIEWS

Possible viral agents to consider in the differential diagnosis of blepharoconjunctivitis

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Abstract

BACKGROUND

Blepharoconjunctivitis poses a diagnostic challenge due to its diverse etiology, including viral infections. Blepharoconjunctivits can be acute or chronic, self-limiting, or needing medical therapy.

AIM

To review possible viral agents crucial for accurate differential diagnosis in cases of blepharoconjunctivitis.

METHODS
The PubMed database was searched for records relating to viral blepharoconjunctivitis. The search string generated was "("virally"[All Fields] OR "virals"[All Fields] OR "virology"[MeSH Terms] OR "virology"[All Fields] OR "viral"[All Fields]) AND "Blepharoconjunctivitis"[All Fields]".

RESULTS
A total of 24 publications were generated from the search string. Reference lists from each relevant article were also searched for more information and included in this review. Viral etiologies such as adenovirus, herpes simplex virus (HSV), varicella-zoster virus (VZV), and Epstein-Barr virus (EBV) are frequently implicated. Adenoviral infections manifest with follicular conjunctivitis and preauricular lymphadenopathy, often presenting as epidemic keratoconjunctivitis. HSV and VZV infections can result in herpetic keratitis and may exhibit characteristic dendritic corneal ulcers. EBV, although less common, can cause unilateral or bilateral follicular conjunctivitis, particularly in immunocompromised individuals. Other potential viral agents, such as enteroviruses and molluscum contagiosum virus, should also be considered, especially in pediatric cases.

CONCLUSION
Prompt recognition of these viral etiologies is essential for appropriate management and prevention of complications. Thus, a thorough understanding of the clinical presentation, epidemiology, and diagnostic modalities is crucial for accurate identification and management of viral blepharoconjunctivitis.

Key Words: Viral; Blepharoconjunctivitis; Herpes simplex virus; Varicella-Zoster; Epstein-Barr

Core Tip: Viral blepharoconjunctivitis, an inflammation of the conjunctiva and eyelids caused by viral infections, represents a significant challenge in ophthalmic practice due to its highly contagious nature and potential for widespread outbreaks. The virus not only directly damages the conjunctival epithelial cells but also induces a robust inflammatory response. Understanding the molecular mechanisms underlying these interactions remains a focus of ongoing research, with implications for developing targeted therapies. The self-limiting nature of the condition, with symptoms generally resolving within two to three weeks, poses a diagnostic challenge, as it often necessitates distinguishing it from other types of conjunctivitis to prevent unnecessary use of antibiotics and to implement appropriate infection control measures.

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INTRODUCTION

Overview of blepharoconjunctivitis

Blepharoconjunctivitis is a complex entity that depicts, inflammation of the eyelid and the conjunctiva at once, due to various causes, especially of infectious origin. So in layman's terms, it is a combination of blepharitis and conjunctivitis [1]. The signs and symptoms are similar to those seen in blepharitis, it is worth noting that, chronic blepharitis can trigger conjunctivitis, because of the anatomical juxtaposition between the eyelid structure and the translucent conjunctiva[2]. Signs and Symptoms include pain, conjunctiva injection, eyelid edema and concretion, tearing, ocular irritation, *etc.* There are different mechanisms or patterns of classification, depending on the cause, course, anatomical location affected, *etc.* The most common classification pattern used is the American Academy of Ophthalmology, which divides it into anterior and posterior, depending on what anatomical location is affected by the condition[1]. Management is usually poised towards identifying the cause and treatment to relieve symptoms and recurrence. The diagnosis of the various types of blepharoconjunctivitis is important not only because it directs therapy, but also because it gives both the physician and patient an idea about the prognosis[3,4].

Clinical presentation of blepharoconjunctivitis

Blepharoconjunctivitis is an inflammatory response of the eyelids and the conjunctival tissue to physical, chemical, neoplastic, viral or microbial assault[1]. It is usually characterized by the presence of certain clinical features which can also be associated with other ocular surface disorders. Consequently, the common clinical features of blepharoconjunctivitis are considered to be non-specific. Clinically, the etiology mostly defines the type and nature of the presenting characteristics of blepharoconjunctivitis. The nature of clinical presentation may range from severely ulcerated and disrupted eyelid margins to pouting of the meibomian orifices and mild conjunctival injection. Whereas, the former

presentation is mostly associated with serious cases such as neoplasm, a viral or virulent microbial invasion, trauma[5-8] *etc.*, the latter mostly suggests a non-sight and non-life-threatening etiology. Moreover, the chronicity of the disease can determine the type and nature of the clinical characteristics at the initial presentation. This is because clinically, most blepharoconjunctivitis can be secondary to a poorly managed blepharitis or any long-standing disease of the eyelids[9].

Definition and classification

The term blepharoconjunctivitis refers to the inflammation of the eyelids and the semi-transparent mucous membrane-like conjunctival covering the anterior surface of the globe and the tarsal plate[1]. The conjunctival membrane is continuous with the stratified squamous epithelium of the eyelid at the mucocutaneous junction of the eyelid margin. Consequently, the close apposition between both tissues makes it easier for a long-standing eyelid inflammation to spread to the conjunctival. The word "Blepharoconjunctivis" derives its origin from the Greek words, "Blepharon" meaning eyelid, and "itis" meaning inflammation[10-12] and the Latin word "conjunctival" meaning to connect. Generally, blepharoconjunctivitis can be categorized based on the cause, chronicity, anatomical position of involvement, standardized photo grading scales, presenting clinical features, and associated signs[1,13]. A correct and adequate classification is necessary to establish the severity, and chronicity as well as formulate the most effective treatment regimen.

Symptoms and signs

The patient's presenting complaints may include epiphora, foreign body sensation, ocular itching, ocular discomfort, burning sensation, and grittiness[14,15]. Symptoms such as a ropy and mucopurulent ocular discharge may be reported in cases of allergies, viral invasion, and a chronic bacterial infestation of the lids respectively. Furthermore, some patients may report ocular redness along with other symptoms[15]. The presenting clinical signs can be influenced by the etiology and associated conditions. Severe itching, phylectenular, follicles, papillae, ropy or a mucopurulent discharge and conjunctival injection, may suggest an allergy, a viral or bacterial infestation[16]. Recurrent chalazia, pouting of the meibomian orifice, and telangiectasia of the eyelid and tarsal plate may be suggestive of meibomian gland dysfunction and posterior blepharitis[17,18]. Whereas, eyelash mating, crusting, scaling, and an erythematous eyelid may demonstrate anterior blepharitis[19,20]. Furthermore, severe conjunctival cicatrization, symblepharon, ocular surface desiccation, trichiasis corneal opacity may suggest a serious underlying systemic disease such as Steven Johnson syndrome, pemphigoid, trachoma etcetera. Blepharoconjunctivitis can also be associated with certain skin conditions such as rosacea[21,22]. This is usually associated with facial papule, erythema, telangiectasia *etc.* The ulceration of the eyelid margin as well as the disruption of its normal anatomical layout is usually associated with neoplastic lesions involving the eyelid, an aggressive viral infiltration, or virulent microbial infestations[5-8].

Challenges in diagnosis

One challenge to differentials is the sudden emergence of a new virus or a new strain of an existing virus. It is important to accurately put the monkeypox virus at the back of our mind for cases presenting with acute blepharoconjunctivitis, due to its re-emergence, as this will help proffer early solutions, for better outcomes[23]. Treatment for other conditions may also present with blepharoconjunctivitis as a side effect, for example, Kornhauser *et al*[24] found out during the treatment of a case of glioblastoma multiform with Temozolomide, that blepharoconjunctivitis was a side effect of the treatment regimen. Dupilumab, the monoclonal antibody used in treating eczema and asthma, has also been implicated in triggering blepharoconjunctivitis, especially in patients with atopic dermatitis[25,26].

Blepharoconjunctivitis, inflammation of the eyelids and conjunctiva, can be caused by various infectious agents, including viruses. Of all these conditions, viral blepharoconjunctivitis stands out because it is extremely contagious and has the potential to affect public health greatly. Viral infections, including those caused by herpes simplex virus (HSV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), and other less frequent viruses, significantly contribute to the overall impact of this disorder.

Epidemiological studies emphasize the significance of distinguishing viral blepharoconjunctivitis from alternative sources of conjunctival inflammation. HSV type 1 keratitis impacts almost 1.5 million individuals worldwide, with around 40000 new cases each year. This condition can result in serious complications like corneal ulcers and blindness [27]. This emphasizes the crucial requirement for precise diagnosis and efficient management techniques in order to avoid results that could potentially harm vision. Furthermore, it is important to comprehend viral causes in eye diseases, as VZV-related herpes zoster impacts 10%-20% of individuals who were previously infected, especially those who are 50 years old or older[28,29].

Epstein-Barr virus (EBV), a virus linked to many eye conditions such as uveitis, keratitis, and conjunctivitis, has a notable impact on a substantial number of people globally[30,31]. Notable occurrences of EBV-related sequelae, such as nasopharyngeal cancer and systemic lupus erythematosus, have been recorded with frequency in countries like China and Saudi Arabia[32,33]. These data emphasize the significance of considering EBV when diagnosing ocular inflammation.

The various causes of viral blepharoconjunctivitis, including uncommon pathogens like molluscum contagiosum, enteroviruses, and monkeypox virus, make diagnosis more difficult and emphasize the importance of comprehensive clinical assessment and specific diagnostic techniques. Enteroviral infections can result in acute hemorrhagic conjunctivitis and other disorders affecting the surface of the eye. Additionally, monkeypox has recently been identified as a cause of viral blepharoconjunctivitis[34,35].

This study specifically examines viral-related blepharoconjunctivitis, aiming to clarify the different viral agents implicated, their clinical presentations, diagnostic difficulties, and approaches to treatment. By including epidemiological research into our discussion, we aim to improve our understanding of the significance of precise diagnosis and proper

treatment in reducing the problems linked to viral blepharoconjunctivitis. Common viral agents implicated in the differentials are discussed while other less common pathogens are also mentioned. A literature review on the management of these conditions is also attempted.

MATERIALS AND METHODS

A search of the online PubMed database was conducted using the keywords "viral" and "blepharoconjunctivitis". The search algorithm generated a search string thus; "("virally"[All Fields] OR "virals"[All Fields] OR "virology"[MeSH Terms] OR "virology"[All Fields] OR "viral"[All Fields]) AND "Blepharoconjunctivitis"[All Fields]". The results were scrutinized by two authors for relevance. A PRISMA diagram[4] is shown Figure 1, depicting the search strategy. This study included peer-reviewed research publications that met specific criteria, such as being randomized controlled trials, cohort studies, case-control studies, cross-sectional studies, or case reports. This study included individuals of all age groups who were diagnosed with viral blepharoconjunctivitis or other ocular diseases caused by viral infections. Articles that particularly discuss the study of the occurrence, characteristics, diagnostic techniques, and strategies for managing viral blepharoconjunctivitis were included. We only included research published in English to guarantee the correctness and uniformity of the data extraction.

Reviews, editorials, comments, and publications that were not based on original research were excluded from consideration. Studies that only examined non-viral causes of blepharoconjunctivitis or did not give detailed information about viral causes were also excluded. Articles that lacked significant data on the epidemiology, clinical symptoms, or management of viral blepharoconjunctivitis were not included. Studies published in languages other than English were eliminated because of the possibility of translation problems and variances in terminologies.

RESULTS

This search returned 24 records. 5 studies were excluded due to being out of scope. The records retrieved ranged from 1973 to 2023. Each valid record was further scrutinized for related literature among their references. A further 68 articles were therefore generated.

DISCUSSION

Viral etiologies of blepharoconjunctivitis

Adenovirus: Epidemiology: Adenoviruses constitute the most common cause of highly contagious conjunctivitis in the world. Viral conjunctivitis affects many people yearly with a percentage of the population exhibiting corneal infiltrates [36]. In fact, in a study, epidemiological analysis of retrospective data which also included 231 conjunctival cases, 205 were diagnosed with viral origin (46.3 % male and 53.7 female), thus highlighting the higher prevalence of viral conjunctivitis in comparison to those of bacterial origin[37]. Another study aimed at evaluating the Human adenoviruses and their serotypes in keratoconjunctivitis patients who attended outpatient clinics at Mansoura Ophthalmic Centre was done. Results therefrom, showed human adenoviruses (HAdV) was detected in 38% of samples, and among the serotypes, HAdV D-8 was the most dominant[38]. Viral conjunctivitis spreads in areas where patients have close contacts such as ophthalmic units of Hospitals, schools, nursing homes, and workplaces[39].

Clinical features: HAdV have been known to cause mild respiratory, gastrointestinal, urogenital, and ocular disease [40]. Adenoviral conjunctivitis which presents mainly as acute follicular conjunctivitis with severe symptoms such as epidemic keratoconjunctivitis (EKC) is caused by human adenovirus[36]. Serotypes of Adenoviruses that cause EKC include Adenovirus 8, 19, and 37[41]. Adenovirus type 3 has been implicated in pharyngoconjunctival fever. Furthermore, a wide range of serotypes have also been presumed to cause follicular conjunctivitis[36]. It is a contagious disease that could be associated with community-acquired or nosocomial infections[42]. Adenoviruses have been shown to result in hemorrhagic enteritis in chickens[43]. They have also been implicated as one of the causes of keratitis[44]. In typical cases, patients complain of unilateral redness, tearing, irritation, and photophobia[36]. Care must be taken to differentiate such complaints from diseases like glaucoma[39,45].

Diagnostic methods: Adenoviruses are classically by an increased titer value of antibodies as the patient progresses from an acute to a chronic stage. Polymerase chain reaction based testing[46].

HSV: The Herpesviridae family, comprising Alphaherpesvirinae, Betaherpesvirinae, and Gammaherpesvirinae subfamilies, further delineates into two major genera within Alphaherpesvirinae: Simplexvirus and Varicellovirus[47]. This viral family exhibits the potential for transmission from the genitals to the eye, leading to severe ocular complications such as blepharoconjunctivitis especially among adults[48]. Primary herpes simplex blepharoconjunctivitis also typically affects children and adolescents, with some individuals delaying medical attention until they recognize persistent watering as a lasting consequence of the infection[49].

Blepharoconjunctivitis, a prevalent ocular infection among young individuals, also manifests bilaterally in Iberian hares infected with the myxoma virus and is recognized in avian pox cases[50-52]. Moreover, it can arise as a complication associated with unhygienic use of ocular prosthetics or water pollution[53,54]. Additionally, bacterial

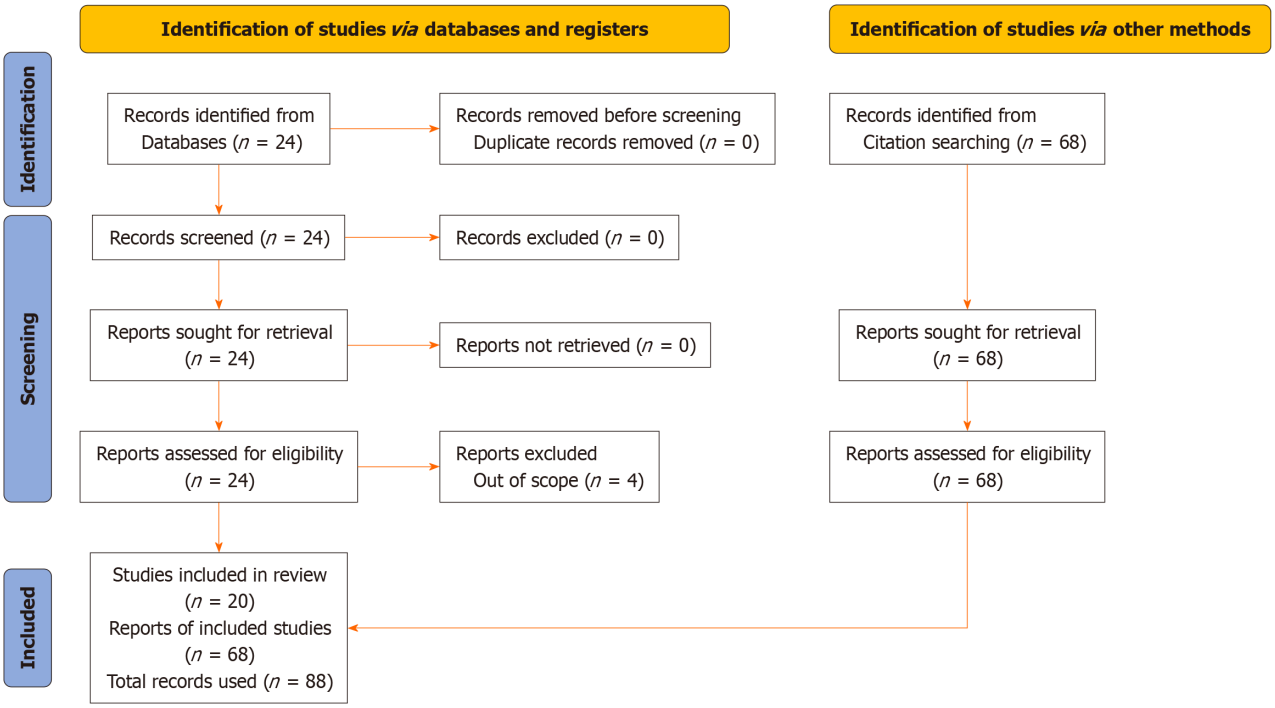

Figure 1 PRISMA diagram depicting the search strategy.

blepharokeratoconjunctivitis can lead to acute bilateral corneal opacity in children, albeit rarely[55]. In humans, herpetic stromal keratitis and blepharoconjunctivitis, partly attributed to immunopathological responses to HSV-1, involve neutrophil infiltration and cytokines such as interleukin (IL)-6, IL-10, IL-12, and interferon-gamma[56]. Tumor-induced blepharoconjunctivitis is observed in immunodeficient patients, like those with Autosomal-recessive hyper-IgE syndrome [57].

Epidemiology: HSV type 1 keratitis affects 1.5 million people globally, with 40000 new cases annually leading to severe eye conditions such as corneal ulcers, which can potentially cause blindness[22]. Although HSV infection is more common in adults, there are reports of it occurring in children through vaginal delivery, leading to ocular diseases[58].

Clinical features: HSV infection of the cornea and ocular adnexa in children can present with several clinical manifestations, including blepharoconjunctivitis, interstitial keratitis, epithelial keratitis, disciform keratitis, neurotrophic keratitis, and stromal keratitis[59]. Herpes simplex virus infection can lead to several ocular diseases, including blepharoconjunctivitis, epithelial keratitis, stromal keratitis, endothelial keratitis, and iritis[60]. The manifestation of these ocular diseases is accompanied by a variety of clinical features. For example, a case of blepharoconjunctivitis was reported in an elderly patient with complete acquired ankyloblepharon, showing clinical signs of preseptal cellulitis[61]. Also, patients who have previously experienced HSV epithelial keratitis are likely to experience increased likelihood of recurrent epithelial keratitis while undergoing acute treatment for HSV stromal keratouveitis[62]. A report by Malik *et al*[63] indicated that congenital HSV-2 infection can lead to bilateral macular hyperpigmented scars. While most HSV ocular diseases are unilateral, in patients with atopy or compromised immune systems, bilateral herpetic keratoconjunctivitis can occur, leading to a more prolonged course with frequent recurrences and severe complications[64].

Diagnostic methods: HSV 1 infection can be definitively diagnosed through polymerase chain reaction testing of blood and cerebrospinal fluid samples[65]. Oral acyclovir is regarded as a treatment option for HSV infection, even in children, once the diagnosis is confirmed[66]. Clinicians should recognize that smallpox vaccination can cause harmful ocular effects, such as preseptal cellulitis and blepharoconjunctivitis, in both vaccine recipients and their contacts to ensure rapid diagnosis and appropriate treatment[67].

VZV: The VZV is one of the several herpes viruses that are infective to humans. It is the causative organism for shingles(herpes zoster) and chicken pox[68]. While chickenpox is generally a self-limiting illness, complications such as bacterial super infections, pneumonia, and central nervous system involvement can occur, particularly in immunocompromised individuals[69,70].

Epidemiology: Scampoli *et al*[28] reported that 10%-20% of previously infected individuals were likely to develop VZV-linked herpes zoster with its attendant sequelae later in life, especially above 50 years. Individual risk herpes zoster has been reported at 25%, with a two-fold increase in individuals older than 85 years of age[29].

Clinical features: Following primary infection, VZV establishes latency in sensory ganglia. Reactivation of the latent virus can lead to herpes zoster, commonly known as shingles. Herpes zoster presents as a painful vesicular rash localized to a dermatome innervated by the affected ganglion[71,72]. Post-herpetic neuralgia, a chronic pain syndrome, is a common complication of herpes zoster, particularly in older adults.

Diagnostic methods: Diagnosis of VZV-linked systemic and ocular diseases is by a combination of clinical/physical examinations and serological assays. Patients usually present with a rash that begins as flat lesions and progressed to

raised lesions accompanied by a rash. The rashes start from the central body mass and progress to the extremities. A polymerase chain reaction (PCR) analysis of the fluid collected from the pustules confirms the presence of VZV[73].

EBV: EBV, a member of the herpes virus family, is one of the most prevalent viruses in humans[30]. Known primarily for causing infectious mononucleosis (mono), EBV is also linked to various cancers and autoimmune diseases[74,75]. Additionally, EBV is associated with ocular manifestations such as blepharoconjunctivitis, as shown by sero-epidemiologic data and confirmed cases where conjunctival disease appeared as an initial symptom of EBV infection[76].

EBV, also referred to as human herpes virus 4, is a double-stranded DNA virus[77]. It was identified in 1964 by Michael Epstein, Yvonne Barr, and Bert Achong through the study of Burkitt lymphoma cell cultures[78]. This virus primarily targets B lymphocytes, a type of white blood cell integral to the immune system[79]. EBV is notable for its capacity to establish a lifelong latent infection within the host, reactivating intermittently without causing symptoms[80].

The EBV genome is about 172 kilobase pairs in length and encodes approximately 85 proteins[81]. Key among these proteins are the EBV nuclear antigens and latent membrane proteins, which are essential for the virus's persistence in the host and the transformation of infected cells[82].

Epidemiology: The epidemiology of EBV exhibits diverse trends and impacts across various populations. In China, studies reveal a high prevalence of EBV among children, with viral loads rising in the presence of co-infections with bacteria or other viruses, contributing to immune disorders like systemic lupus erythematosus and infectious mononucleosis[32]. In Saudi Arabia, EBV is notably associated with nasopharyngeal carcinoma (NPC), showing a high prevalence of EBV infection in patients and a predominance of genotype I, highlighting the need for further research to understand the burden of EBV-associated NPC[33]. In Russia, EBV infection represents a global challenge, with variations in immunological reactivity due to different pathogens, underscoring the necessity for improved epidemiological surveillance and preventive measures[83]. In Brazil, a study links EBV infection with autoimmune inflammatory rheumatic diseases, particularly noting a higher prevalence of active EBV infection in systemic lupus erythematosus patients, especially those undergoing corticosteroid therapy[84].

Clinical features: Ocular manifestations associated with EBV infection are diverse and can affect various structures within the eye. Uveitis stands out as one of the primary presentations, marked by inflammation of the uvea, which encompasses the iris, ciliary body, and choroid[85]. This inflammation typically presents with symptoms like eye redness, pain, and sensitivity to light, and blurred vision, with anterior uveitis being more prevalent in EBV-related cases[31]. Although less common, posterior uveitis affecting the retina and choroid can also occur[86], and EBV-associated uveitis may manifest unilaterally or bilaterally and may recur[87].

Beyond uveitis, EBV infection has been linked to various other ocular complications, including keratitis, conjunctivitis, and optic neuritis[88,89]. Keratitis, involving inflammation of the cornea, can range from mild epithelial defects to severe necrotizing forms[88]. Conjunctivitis, characterized by conjunctival redness and discharge, may coincide with systemic EBV symptoms like fever and malaise[89]. Optic neuritis, affecting the optic nerve, can cause vision impairment and eye pain exacerbated by movement.

Less common but still significant ocular conditions associated with EBV infection include retinal vasculitis, retinitis, and optic neuropathy[90-92]. Retinal vasculitis involves inflammation of retinal blood vessels, potentially leading to vascular occlusion and ischemic retinopathy[90]. Retinitis, characterized by features like retinal hemorrhages and exudates, can also occur[91]. Optic neuropathy related to EBV infection may present with optic disc swelling or optic atrophy, resulting in visual field defects and decreased acuity[92].

Given the range and severity of EBV-associated ocular manifestations, clinicians must maintain a high level of suspicion for EBV in patients presenting with ocular inflammation, especially alongside systemic symptoms suggestive of viral infection. Early recognition and proper management of these EBV-related eye conditions are crucial for preventing vision-threatening complications and preserving visual function.

Diagnostic methods: The diagnosis of ocular conditions associated with EBV often involves a combination of clinical evaluation, serological testing for EBV-specific antibodies, PCR assays for viral DNA, and ocular imaging techniques such as fundoscopy, optical coherence tomography, and fluorescein angiography[93-98]. Treatment strategies for EBV-associated eye diseases typically focus on managing inflammation and controlling viral replication. Topical or systemic corticosteroids may be prescribed to alleviate ocular inflammation, while antiviral agents such as acyclovir or valacyclovir may be used to suppress viral replication[99,100].

Other potential viral agents: Although most cases of viral conjunctivitis have been known to be associated with adenovirus[101] however, some of the most contagious forms of blepharoconjunctivitis are linked to certain enterovirus stereotypes[34].

Enterovirus: Enterovirus is a genus consisting of several other species and subspecies of viruses such as coxsackieviruses, enteroviruses, rhinoviruses, polioviruses and echovirus. It belongs to the viral family Picornaviridae and is renowned for being amongst the most Rampant pathogens on earth[102]. Blepharoconjunctivitis secondary to certain strains of enterovirus is characterized by varying degrees of subconjunctival hemorrhage, conjunctival hyperemia, tarsal conjunctival follicles, profuse tearing, and lid edema. Certain patients may report itching before the onset of conjunctivitis [103]. Acute hemorrhagic conjunctivitis is a highly contagious ocular surface disease that is caused by enterovirus 70 and coxsackievirus A24[104]. Although the condition is relatively benign and resolves within five to seven days it may associated with significant ocular pain if a superficial punctate keratopathy develops. Other less common viral etiology of blepharoconjunctivitis include molluscum contagiosum virus, measles, mumps, and severe acute respiratory syndrome coronavirus 2 virus.

Molluscum contagiosum: this is a DNA virus that belongs to the poxviridae family. It causes dermatosis mostly in the pediatric demographics, sexually active and immunocompromised individuals[105]. Molluscum contagiosum is a round

pinkish umbilicated skin papule that can be found on epithelial surfaces including the skin of the face and eyelids[106]. Furthermore, transmission is usually by contact, fomites, and autoinoculation[107], and It is known for its affinity for epithelial tissue, this is known as "tropism"[108]. Molluscum contagiosum dermatosis is usually self-remitting however, treatment may be required for genital lesions and immunocompromised individuals[104]. Blepharoconjunctivitis may arise secondary to longstanding ocular molluscum contagiosum, which is more common in HIV and pediatric patients with atopic dermatitis[109]. Commonly associated signs include follicular conjunctivitis, mucopurulent discharge, subepithelial epithelial infiltrates, and corneal pannus[110].

Measles and mumps: These are both single-stranded RNA viruses with enveloped virions and equipped with the ability to replicate in a host's cytoplasm. Both cause very contagious diseases in humans although, measles and mumps are preventable with the MMR vaccine[111] both can lead to encephalitis and other potentially life-threatening complications[112-114]. Whereas, measles is generally characterized by upper respiratory symptoms (coryza), cough, and conjunctivitis, Mumps on the other hand is characterized by general symptoms such as pyrexia, tiredness, cephalalgia, malaise, and anorexia which is followed by swelling and inflammation of the parotid[115]. Mump and measles blepharoconjunctivitis are mostly characterized by conjunctival follicles, superficial vessel engorgement, conjunctival hyperemia, pseudomembranes, and photophobia[116].

Monkey pox virus: Scandale *et al*[35] implicated the monkeypox virus as a causative agent of viral blepharoconjunctivitis; reporting a unilateral ocular manifestation, with associated epidermal findings. The examination pointed to a diagnosis of Viral blepharoconjunctivitis in that eye, secondary to a monkeypox infection[23]. This can progress to necrotizing keratoconjunctivitis which would result in significant orbital morbidity[117]. While the monkeypox virus may be self-limiting in its mild forms, antiviral medication is needed in its more serious presentations[118].

Myxoma virus: This is a virus from the genus Leporipoxvirus[119]. It is commonly carried by its murine host and transmitted by the mosquito insect which is endemic to Sun-Saharan Africa[120,121]. Farsang *et al*[122] reported on myxomatosis presenting with conjunctivitis as one of its symptoms in a study out of central Europe.

Table 1 is has been included to show and summarize a differential diagnoses between common causes of viral blepharoconjunctivitis.

Diagnostic modalities for viral blepharoconjunctivitis

Distinguishing between viral infection-related blepharoconjunctivitis and blepharoconjunctivitis caused by other factors requires a meticulous evaluation of clinical symptoms, patient history, and diagnostic tests. The clinical presentation is fundamental. Regarding blepharoconjunctivitis associated with viral infection, the initiation and manifestations are important. Viral blepharoconjunctivitis typically manifests with an abrupt onset. Typical symptoms consist of redness in the conjunctiva, discharge of watery fluid, and a sensation of itching. Different viral strains may exhibit unique characteristics. HSV is a viral infection with typical unilateral manifestation characterized by blister-like sores on the eyelids or conjunctiva. There is a possibility of having dendritic ulcers on the cornea.

VZV is a pathogen, and the condition typically manifests as a painful vesicular rash that is limited to a specific area of the skin, known as a dermatome. It is sometimes accompanied by inflammation of the conjunctiva, which is the thin membrane that covers the front surface of the eye. Adenovirus infection frequently leads to a sudden and highly infectious inflammation of the conjunctiva, characterized by a copious and watery discharge. Linked to preauricular lymphadenopathy. Enteroviruses can lead to a condition called acute hemorrhagic conjunctivitis, which is characterized by redness and swelling of the conjunctiva and eyelids.

The generalized symptoms with different agents can vary. Viral infections might be accompanied by systemic symptoms such as fever, malaise, or a prodromal rash. Causes not related to viruses can give different symptoms. Allergic blepharoconjunctivitis frequently occurs on both sides, accompanied by irritation, tearing, and redness of the conjunctiva. May have a previous record of exposure to allergens and frequently exhibit a viscous discharge. Bacterial blepharoconjunctivitis typically exhibits a purulent discharge that is particularly intense in the morning. It can occur on one side or both sides and may be accompanied by the formation of crusts on the eyelids. Chemical irritants commonly manifest as a sensation of burning and redness after coming into contact with irritants or chemicals. The discharge may become less purulent and frequently improves after the removal of the irritant.

The medical background should be considered in the differential diagnosis. For viral infection, it is fundamental to search for recent contact with persons who have exhibited comparable symptoms, a past occurrence of cold sores (caused by the herpes simplex virus), or shingles (caused by the varicella-zoster virus). Past use of contact lenses can be significant, especially when considering adenoviral infections. For allergies, the clinician should request information regarding recent contact with recognized allergies, such as pollen, dust, or animal dander. Allergic blepharoconjunctivitis commonly occurs during certain seasons or is associated with specific triggers. With bacterial infections, it is fundamental to consider recent occurrences of upper respiratory infections or proximity to individuals with bacterial conjunctivitis. Chemical exposure needs to be evaluated for recent exposure to chemicals or irritants, such as chlorine in swimming pools or domestic cleaning products.

Diagnostic tests can be useful in determining etiology. Viral conjunctivitis can be diagnosed with PCR testing. This assay is highly sensitive to identifying specific viral DNA or RNA, namely for HSV, VZV, and adenovirus. PCR is a diagnostic technique that can verify the existence of the virus and differentiate between various strains. Viral cultures can be used in difficult cases. This refers to the process of growing and isolating viruses in a laboratory setting to study their characteristics and behavior. This method can be utilized to separate and detect the virus from conjunctival swabs. This is very valuable for verifying adenoviral infections. Immunoassays can identify viral antigens or antibodies. For instance, the utilization of direct fluorescent antibody testing can accurately detect adenoviral infections. Gram stain and culture can be used to diagnose bacterial infections. The Gram stain of the discharge and culture can be used to identify bacterial pathogens.

Table 1 Distinguishing features of blepharoconjunctivitis caused by different viruses, including herpes simplex virus, varicella-zoster virus, adenovirus, and enterovirus

Feature	Herpes simplex virus	Varicella-zoster virus	Adenovirus	Enterovirus
Common symptoms	Unilateral conjunctival redness, itching, vesicular lesions on eyelids, dendritic ulcers on cornea	Painful vesicular rash localized to a dermatome, conjunctivitis	Acute conjunctivitis with profuse, watery discharge, preauricular lymphadenopathy	Conjunctival hyperemia, lid swelling, profuse tearing, possible subconjunctival hemorrhage
Discharge type	Clear or watery; may have epithelial defects	Clear or serous; often accompanied by skin lesions	Watery; often profuse and accompanied by preauricular lymphadenopathy	Watery or serous; can be associated with hemorrhage
Onset	Sudden, often following reactivation of latent infection	Sudden, often follows a history of shingles or chickenpox	Acute onset, highly contagious	Acute onset, highly contagious
Preauricular lymphadenopathy	Rare	Rare	Common	Rare
Corneal involvement	Frequent; dendritic ulcers visible on fluorescein staining	Possible; less common but can have corneal involvement	Rare	Rare
Associated systemic symptoms	Fever, malaise, possibly cold sores	Painful rash in a dermatome, fever	Often accompanied by upper respiratory symptoms	May have systemic symptoms like fever, malaise, or rash
Diagnostic tests	PCR for HSV DNA, viral culture, direct fluorescent antibody	PCR for VZV DNA, viral culture	PCR for adenoviral DNA, viral culture	PCR for enteroviral RNA, viral culture
Immunoassays	Detect HSV-specific antigens or antibodies	Detect VZV-specific antigens or antibodies	Detect adenoviral antigens or antibodies	Detect enteroviral antigens or antibodies
Corneal examination	Dendritic ulcers, punctate epithelial keratopathy	Vesicular rash on eyelids and possible corneal involvement	Typically, no corneal involvement	Rarely involves the cornea
Epidemiology	Common, especially in individuals with a history of cold sores or HSV infections	Less common, typically in individuals with recent shingles or chickenpox	Highly contagious; common in children and adults	Less common, often associated with outbreaks
Management	Antiviral medications (*e.g.*, acyclovir), topical or systemic steroids for inflammation	Antiviral medications, supportive care for rash and pain	Supportive care, antihistamines, sometimes antiviral treatment	Supportive care, analgesics for discomfort

PCR: Polymerase chain reaction; HSV: Herpes simplex virus; VZV: Varicella-zoster virus.

Allergy testing can be useful for causes not related to viruses. To accurately identify certain allergies, skin prick testing or serological assays may be required. Schirmer's test can be used to diagnose underlying dry eye diseases. The procedure assesses tear production and aids in the diagnosis of dry eye syndrome or meibomian gland.

Medical assessment is imperative to making a diagnosis. The conjunctival appearance must be considered. Viral conjunctivitis commonly manifests as widespread redness of the conjunctiva. Bacterial infections can exhibit localized hyperemia, which is an increased blood flow in a specific area, along with the presence of purulent discharge. The ocular surface evaluation is fundamental. Dendritic ulcers are a clear sign of HSV infection. Additional viral infections may exhibit a broader and more widespread corneal involvement, whereas allergic or bacterial conjunctivitis often does not directly impact the cornea. Preauricular lymphadenopathy manifests as the enlargement of lymph nodes located in front of the ear. Prevalent in viral conjunctivitis, especially adenoviral infections. This discovery can aid in distinguishing between allergic or bacterial origins.

Clinical examination: Prompt diagnosis of viral blepharoconjunctivitis is essential in other to control the spread of the disease. Subjective symptoms such as eyelid swelling, serofibrinous discharges, ciliary injection, subconjunctival hemorrhage, punctate epithelial keratopathy, and occasional preauricular lymph node inflammation may aid in the diagnosis[123].

Laboratory tests: Careful examination of the eyelid margins and the type of discharge emanating from the eye and the lymph nodes are useful clinical tests that can help the diagnosis of viral blepharoconjunctivitis. The immunochromatography test kit was introduced to diagnose adenoviral infections in 1996[124]. It works by detecting the presence of the presence of hexon proteins which are the outer parts of adenoviruses[125]. A unique viral detection tool that uses tears instead of conjunctival scrapings has also been found useful in the diagnosis of adenoviruses[126,127].

Viral culture: Molecular analyses are the most common method for diagnosing the presence of viruses *in vivo* or *in vitro*, as the time taken to isolate a virus might be long, demands a lot of technical know-how, is expensive, and also requires a special form of setup.

For effective management of viral blepharoconjunctivitis, culture assessment is very important, as determination of the viral agent involved is of paramount importance. With the knowledge that the viral load is a factor in the remission of symptoms and prognosis[127], effective laboratory culture becomes very important, the virus of concern must be determined with high-level accuracy, and the best-responding medication taught in the management of the condition [64]. Although the most common viral agent isolated from the ocular surface over the years, is the herpes simplex virus 1 [128], that does not mean a randomized treatment plan should not be discontinued if resolution seems not to occur. Viruses can be cultured in two or more forms, of which we have the tube culture and the shell vial method.

PCR: Metagenomic deep sequencing, and cenegermin nerve growth factor[129] offer promise as diagnostic, and therapeutic options, respectively. Polymerase chain reaction offers a wide range of advantages, including but not limited to, high sensitivity and specificity, it can also be used to assess a wide range of rotavirus, which is a common cause of diarrhea in children[130,131]. Real-time PCR helps in obtaining insights into viral workups, including those affecting the respiratory tracts in children[132]. Polymerase chain reaction comes highly recommended in cases of recurrent or non-differentiating ocular inflammation. The PCR test is a rapid test for identification of different strains of the adenovirus [133-135].

Imaging studies: Confocal microscopy is a very important non-invasive method of imaging, even in cases where there is already existing keratopathy[136]. Adenovirus infections usually are capable of affecting deeper tissues spreading down to the orbit, hence MRI is a strong tool in the clinical workup of ocular infection, with high-level suspicion for adenovirus [137,138]. Immunochromatography comes in more handy and useful than the enzymatic assay, in terms of specificity and sensitivity, in identifying adenoviral-related conjunctivitis[139]. In almost all cases of viral infection electron microscopy, due to its rapid nature and relatively inexpensiveness, it is an important imaging tool in viral infections[140].

Management strategies for viral blepharoconjunctivitis

Management of viral blepharoconjunctivitis starts with an appropriate diagnosis of the actual causative agent, followed by an appropriate culture plan, if management or treatment must be holistic, and with better prognostic outcome. Although if management is prompt better outcomes are sure, but some cases can end up causing debilitating ocular defects with long-time visual disability and discomfort. A remarkable effect has been found from the use of zalcitabine, sanidine, interferon beta, and anti osteopontin, in the treatment of adenoviral-related conjunctivitis[141], there seems to be no experimental study to show the significant therapeutic effect gotten from the combination of topical corticosteroid and topical cidofovir in treating adenoviral related eye infection[142]. Some cases of ocular tumor especially ocular sebaceous carcinoma can present with signs of blepharoconjunctivitis too, so appropriate diagnosis of a condition is very key in preventing more devastating sequelae[143]. Managing blepharoconjunctivitis irrespective of the cause is poised at limiting surface inflammation, limiting dysfunction involving the meibomian gland and protecting the eyelid integrity [144].

General supportive measures

Management is usually supportive or conservative, especially in uncomplicated cases[24]. The use of a warm compass to relieve symptoms and appropriate ocular hygiene has also been documented. Also, there is an important need to as a matter of importance avoid possible triggers, especially environmental triggers[1]. For cases of blepharoconjunctivitis occurring side by side with other conditions, are managed with proper management of the triggering condition, Termozolamide has been found to have ocular side effects in treating a patient that presents with blepharoconjunctivitis and also multiform gliomas[145] eyelid hygiene enhances better treatment outcome in cases of blepharoconjunctivitis [146].

Complications and their management

Blepharoconjunctivitis can lead to a range of complications if not properly managed. One major complication is chronic discomfort and irritation[146], which can significantly impact a patient's quality of life similar to other diseases[147,148]. Persistent inflammation can in turn cause changes in the structure of the eyelids, such as thickening, scarring, or the development of irregularities in the eyelid margin[149,150]. These structural changes can disrupt the normal function of the eyelids, potentially leading to issues like trichiasis, where eyelashes grow inward and rub against the cornea, causing further irritation and risk of corneal abrasions or ulcers, and eventual blindness[146]

Another significant complication of blepharoconjunctivitis is the increased risk of secondary infections[151]. The chronic inflammation and disruption of the normal eyelid and conjunctival architecture can compromise the natural barriers that protect the eye from pathogens. This can lead to recurrent bacterial or viral infections, which may exacerbate the inflammation and further damage the ocular surface. Additionally, chronic inflammation can result in meibomian gland dysfunction, leading to dry eye syndrome. This condition not only causes discomfort but also increases the risk of further complications like corneal erosion, which can severely impair vision if not treated appropriately. Thus, the management of blepharoconjunctivitis requires a comprehensive approach to mitigate these potential complications and preserve ocular health.

Prevention and control measures

Hygiene practices: As with all infective processes, proper hygiene is the first point of call in preventive considerations. A primary step is maintaining good eyelid hygiene[152]. This involves regular cleaning of the eyelids to remove debris, oils, and bacteria that can contribute to inflammation[152]. Using a clean, damp cloth or a commercially available eyelid scrub, gently wipe along the lash line to keep the area clean[153]. It's recommended to perform this cleaning routine at least once a day, especially if the patient has a predisposition to blepharitis or has experienced recurrent episodes of

conjunctivitis[154].

Another essential step is to practice good hand hygiene, as this helps to prevent the transfer of infectious agents to the eyes[114]. Hands should be washed thoroughly with soap and water before touching the face or eyes[155]. Additionally, individuals should avoid rubbing their eyes, as this can introduce bacteria or viruses and exacerbate irritation[155]. For contact lens users, it is vital to follow proper lens care guidelines, including washing hands before handling lenses, using appropriate cleaning solutions, and replacing lenses as recommended[156,157]. Avoid sharing personal items like towels, cosmetics, and eye drops to minimize the risk of cross-contamination. Implementing these hygiene practices can significantly reduce the likelihood of developing blepharoconjunctivitis and help maintain overall eye health.

Vaccines: Vaccinations are a useful management for viral blepharoconjunctivitis as they directly attack the causal agents. Trifluridine has been suggested as a possible prophylactic choice for adenoviruses and the monkeypox virus[158]. Trifluridine combined with brincidofovir and tecovirimat performed better than single-therapy tecovirimat in resistant monkeypox strains[159]. Multiple vaccines including NIOCH-14, Cidofovir, CMX-001, and ST-246 are in the final stages of development and show some promise in blepharoconjunctivitis prevention[160]. Al-Dwairi *et al*[161] reported that periocular vaccination produced better protection against herpes simplex keratitis than against systemic vaccination. Naidu *et al*[162] however suggested that intramuscular administration of the HSV-VC1 vaccine resulted in complete protection against the McKrae strain of the HSV virus. On the other hand, systemic vaccines have also been known to re-activate dormant herpes simplex virus in ocular tissue[163].

CONCLUSION

In conclusion, viral blepharoconjunctivitis presents a significant clinical challenge due to its highly contagious nature and potential for widespread transmission. This paper has shed light on the complexities surrounding this ocular condition by examining its etiology, clinical manifestations, diagnosis, and management strategies.

FOOTNOTES

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REFERENCES

1 **Fazal MI**, Patel BC. Blepharoconjunctivitis. 2023 Jul 31. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [PMID: 32644328]
2 **Chodosh J**, Pineda R II, Bunya VY, Sundar G, Yen MT, MD, Burkat CN, Kaufman AR. 2024. Monkeypox. Accessed June 7, 2024. Available from: https://eyewiki.org/Monkeypox
3 **McCulley JP**. Blepharoconjunctivitis. *Int Ophthalmol Clin* 1984; **24**: 65-77 [PMID: 6233233 DOI: 10.1097/00004397-198424020-00009]
4 **Zeppieri M**, Gagliano C, Spadea L, Salati C, Chukwuyem EC, Enaholo ES, D'Esposito F, Musa M. From Eye Care to Hair Growth: Bimatoprost. *Pharmaceuticals (Basel)* 2024; **17** [PMID: 38794131 DOI: 10.3390/ph17050561]
5 **Vitiello L**, Lixi F, Coco G, Giannaccare G. Ocular Surface Side Effects of Novel Anticancer Drugs. *Cancers (Basel)* 2024; **16** [PMID:

38254833 DOI: 10.3390/cancers16020344]
6 **Pereira SM**, Lima RV, Muniz MCR, Araújo MBF, de Moraes Ferreira Júnior L, de Queiroz Sales Martins JT, Luz CFC, Cid DAC, da Rocha Lucena D. Congenital herpes simplex with ophthalmic and multisystem features: a case report. *BMC Pediatr* 2023; **23**: 611 [PMID: 38044450 DOI: 10.1186/s12887-023-04423-1]
7 **Nghiem AZ**, Ameen M, Koutroumanos N. Canalicular obstruction associated with dupilumab. *Int Ophthalmol* 2023; **43**: 4791-4795 [PMID: 37843763 DOI: 10.1007/s10792-023-02880-2]
8 **Xu Z**, Oyeniran EO, Xu X, Baumrin EL. Pseudomonal blepharoconjunctivitis causing neutropenic sepsis after allogeneic hematopoietic cell transplantation. *Transpl Infect Dis* 2022; **24**: e13718 [PMID: 34435717 DOI: 10.1111/tid.13718]
9 **Bothun CE**, Decanini A, Bothun ED. Tinea blepharitis and follicular conjunctivitis in a child. *J AAPOS* 2021; **25**: 253-254 [PMID: 34166819 DOI: 10.1016/j.jaapos.2021.04.004]
10 **Subramanian N**. Blepharoplasty. *Indian J Plast Surg* 2008; **41**: S88-S92 [PMID: 20174547 DOI: 10.1055/s-0039-1700479]
11 **Kinash RG**, Fulton NJ. Essential blepharospasm: nursing update. *J Neurosci Nurs* 1990; **22**: 215-219 [PMID: 2144555 DOI: 10.1097/01376517-199008000-00005]
12 **Mehrotra N**, Singh S. Periodontitis. 2023 May 1. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [PMID: 31082170]
13 **Thygeson P**. The etiology and treatment of blepharitis; a study in military personnel. *Mil Surg* 1946; **98**: 191-203 [PMID: 21017790 DOI: 10.1093/milmed/98.3.191]
14 **Floor T**, Henry YP, Kraal-Biezen E. [Corneal perforation due to 'lost' contact lenses]. *Ned Tijdschr Geneeskd* 2019; **163** [PMID: 31647622]
15 **Idu FK**, Efosa AD, Mutali M Jr. Ocular Side Effects of Eyelash Extension Use Among Female Students of the University of Benin, Benin City, Edo State, Nigeria. *Cureus* 2024; **16**: e53047 [PMID: 38410308 DOI: 10.7759/cureus.53047]
16 **Sabhahit SV**, Babu M, V D. Ocular effects of eye cosmetic formulations. *Cutan Ocul Toxicol* 2024; **43**: 154-160 [PMID: 38806172 DOI: 10.1080/15569527.2024.2360735]
17 **Le T**, Can B, Orge F. Herpes Simplex Conjunctivitis and Recurrent Chalazia in a Patient DOCK8 Deficiency. *Ocul Immunol Inflamm* 2022; **30**: 1988-1991 [PMID: 34255603 DOI: 10.1080/09273948.2021.1919309]
18 **Yeu E**, Garg S, Ayres BD, Beckman K, Chamberlain W, Lee B, Raju L, Rao N, Rocha KM, Schallhorn J, Zavodni Z, Mah FS, Farid M; from the ASCRS Cornea Clinical Committee. Current state and future perspectives in the diagnosis of eyelid margin disease: clinical review. *J Cataract Refract Surg* 2024; **50**: 868-875 [PMID: 38758201 DOI: 10.1097/j.jcrs.0000000000001483]
19 **Ahuja AS**, Farford BA, Forouhi M, Abdin R, Salinas M. The Ocular Manifestations of COVID-19 Through Conjunctivitis. *Cureus* 2020; **12**: e12218 [PMID: 33489624 DOI: 10.7759/cureus.12218]
20 **Salvetat ML**, Musa M, Pellegrini F, Salati C, Spadea L, Zeppieri M. Considerations of COVID-19 in Ophthalmology. *Microorganisms* 2023; **11** [PMID: 37764064 DOI: 10.3390/microorganisms11092220]
21 **Musa MJ**, Okoye SG, Enaholo E, Ogwumu KE, Iyami NG. Stevens-Johnson syndrome in an 18-year-old Nigerian female: A case report. *Jrnl Nig Opto Assoc* 2023; **25**: 32-41 [DOI: 10.4314/jnoa.v25i1.6]
22 **Patel NV**, Gupta N, Shetty R. Preferred practice patterns and review on rosacea. *Indian J Ophthalmol* 2023; **71**: 1382-1390 [PMID: 37026270 DOI: 10.4103/IJO.IJO_2983_22]
23 **Bloom J**, Parise M, Saeed O, Holicki C, Mihok B. Monkeypox Presenting with Blepharoconjunctivitis. *Case Rep Ophthalmol* 2023; **14**: 647-653 [PMID: 38023611 DOI: 10.1159/000533914]
24 **Kornhauser T**, Pemberton JD. Temozolomide-associated blepharoconjunctivitis: a case report. *BMC Ophthalmol* 2024; **24**: 162 [PMID: 38609860 DOI: 10.1186/s12886-024-03417-6]
25 **Ferreira S**, Torres T. Conjunctivitis in patients with atopic dermatitis treated with dupilumab. *Drugs Context* 2020; **9** [PMID: 32426016 DOI: 10.7573/dic.2020-2-3]
26 **Liberman P**, Shifera AS, Berkenstock M. Dupilumab-Associated Conjunctivitis in Patients With Atopic Dermatitis. *Cornea* 2020; **39**: 784-786 [PMID: 31985517 DOI: 10.1097/ICO.0000000000002262]
27 **Zannella C**, Chianese A, De Bernardo M, Folliero V, Petrillo F, De Filippis A, Boccia G, Franci G, Rosa N, Galdiero M. Ophthalmic Solutions with a Broad Antiviral Action: Evaluation of Their Potential against Ocular Herpetic Infections. *Microorganisms* 2022; **10** [PMID: 36144330 DOI: 10.3390/microorganisms10091728]
28 **Scampoli P**, Di Martino G, Cedrone F, Odio C, Di Giovanni P, Romano F, Staniscia T. The Burden of Herpes Zoster on Hospital Admissions: A Retrospective Analysis in the Years of 2015-2021 from the Abruzzo Region, Italy. *Vaccines (Basel)* 2024; **12** [PMID: 38793713 DOI: 10.3390/vaccines12050462]
29 **Pinchinat S**, Cebrián-Cuenca AM, Bricout H, Johnson RW. Similar herpes zoster incidence across Europe: results from a systematic literature review. *BMC Infect Dis* 2013; **13**: 170 [PMID: 23574765 DOI: 10.1186/1471-2334-13-170]
30 **Huang W**, Bai L, Tang H. Epstein-Barr virus infection: the micro and macro worlds. *Virol J* 2023; **20**: 220 [PMID: 37784180 DOI: 10.1186/s12985-023-02187-9]
31 **Paroli MP**, Restivo L, Ottaviani E, Nardella C, Abicca I, Spadea L, Paroli M. Clinical Features of Infectious Uveitis in Children Referred to a Hospital-Based Eye Clinic in Italy. *Medicina (Kaunas)* 2022; **58** [PMID: 36422212 DOI: 10.3390/medicina58111673]
32 **Ye Z**, Chen L, Zhong H, Cao L, Fu P, Xu J. Epidemiology and clinical characteristics of Epstein-Barr virus infection among children in Shanghai, China, 2017-2022. *Front Cell Infect Microbiol* 2023; **13**: 1139068 [PMID: 37026057 DOI: 10.3389/fcimb.2023.1139068]
33 **Al-Anazi AE**, Alanazi BS, Alshanbari HM, Masuadi E, Hamed ME, Dandachi I, Alkathiri A, Hanif A, Nour I, Fatani H, Alsaran H, AlKhareeb F, Al Zahrani A, Alsharm AA, Eifan S, Alosaimi B. Increased Prevalence of EBV Infection in Nasopharyngeal Carcinoma Patients: A Six-Year Cross-Sectional Study. *Cancers (Basel)* 2023; **15** [PMID: 36765601 DOI: 10.3390/cancers15030643]
34 **Lévêque N**, Huguet P, Norder H, Chomel JJ. [Enteroviruses responsible for acute hemorrhagic conjunctivitis]. *Med Mal Infect* 2010; **40**: 212-218 [PMID: 19836177 DOI: 10.1016/j.medmal.2009.09.006]
35 **Scandale P**, Raccagni AR, Nozza S. Unilateral Blepharoconjunctivitis due to Monkeypox Virus Infection. *Ophthalmology* 2022; **129**: 1274 [PMID: 36041955 DOI: 10.1016/j.ophtha.2022.08.013]
36 **Omari AA**, Mian SI. Adenoviral keratitis: a review of the epidemiology, pathophysiology, clinical features, diagnosis, and management. *Curr Opin Ophthalmol* 2018; **29**: 365-372 [PMID: 29708932 DOI: 10.1097/ICU.0000000000000485]
37 **Balasopoulou A**, Kokkinos P, Pagoulatos D, Plotas P, Makri OE, Georgakopoulos CD, Vantarakis A. A molecular epidemiological analysis of adenoviruses from excess conjunctivitis cases. *BMC Ophthalmol* 2017; **17**: 51 [PMID: 28438142 DOI: 10.1186/s12886-017-0447-x]

38 **Badawi AE**, Kasem MA, Moemen D, El Sayed Zaki M. Molecular, Epidemiological and Clinical Assessment of Adenoviral Keratoconjunctivitis in Egypt: Institutional Study. *Ocul Immunol Inflamm* 2023; **31**: 1640-1646 [PMID: 35816022 DOI: 10.1080/09273948.2022.2092004]

39 **Salvetat ML**, Zeppieri M, Tosoni C, Parisi L, Brusini P. Non-conventional perimetric methods in the detection of early glaucomatous functional damage. *Eye (Lond)* 2010; **24**: 835-842 [PMID: 19696803 DOI: 10.1038/eye.2009.216]

40 **Gonçalves MA**, de Vries AA. Adenovirus: from foe to friend. *Rev Med Virol* 2006; **16**: 167-186 [PMID: 16710837 DOI: 10.1002/rmv.494]

41 **Shafiei K**, Makvandi M, Teimoori A, Samarbafzadeh A, Khataminia G, Jalilian S, Neisi N, Makvandi K, Haj MS. Frequency of adenovirus serotype 8 in patients with Keratoconjunctivitis, in Ahvaz, Iran. *Iran J Microbiol* 2019; **11**: 129-136 [PMID: 31341567 DOI: 10.18502/ijm.v11i2.1074]

42 **Gupta D 4th**, Daigavane S. A Clinical Case of Viral Keratitis. *Cureus* 2022; **14**: e30311 [PMID: 36407263 DOI: 10.7759/cureus.30311]

43 **Aoki K**, Kaneko H, Kitaichi N, Ohguchi T, Tagawa Y, Ohno S. Clinical features of adenoviral conjunctivitis at the early stage of infection. *Jpn J Ophthalmol* 2011; **55**: 11-15 [PMID: 21331686 DOI: 10.1007/s10384-010-0894-x]

44 **Shivaprasad HL**. Adenovirus group II-like infection in chukar partridges (Alectoris chukar). *Avian Dis* 2008; **52**: 353-356 [PMID: 18646470 DOI: 10.1637/8032-062007-Case.1]

45 **Della Mea G**, Bacchetti S, Zeppieri M, Brusini P, Cutuli D, Gigli GL. Nerve fibre layer analysis with GDx with a variable corneal compensator in patients with multiple sclerosis. *Ophthalmologica* 2007; **221**: 186-189 [PMID: 17440281 DOI: 10.1159/000099299]

46 **Doerfler W**. Adenoviruses. In: Baron S, editor. Medical Microbiology. 4th ed. Galveston (TX): University of Texas Medical Branch at Galvesto, 1996

47 **Khehra N**, Padda IS, Swift CJ. Polymerase Chain Reaction (PCR). 2023 Mar 6. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [PMID: 36943981]

48 **Whitley RJ**. Herpesviruses. In: Baron S, editor. Medical Microbiology. 4th ed. Galveston (TX), 1996

49 **Oh JO**, Kimura SJ, Ostler HB. Acute ocular infection by type 2 herpes simplex virus in adults. *Arch Ophthalmol* 1975; **93**: 1127-1129 [PMID: 172050 DOI: 10.1001/archopht.1975.01010020845003]

50 **Kareem Rhumaid A**, Alak Mahdi Al-Buhilal J, Al-Rubaey NKF, Yassen Al-Zamily K. Prevalence and Antibiotic Susceptibility of Pathogenic Bacteria Associated with Ocular Infections in Adult Patients. *Arch Razi Inst* 2022; **77**: 1917-1924 [PMID: 37123163 DOI: 10.22092/ARI.2022.359510.2437]

51 **Agulló-Ros I**, Jiménez-Martín D, Camacho-Sillero L, Gortázar C, Capucci L, Cano-Terriza D, Zorrilla I, Gómez-Guillamón F, García-Bocanegra I, Risalde MA. Pathological changes and viral antigen distribution in tissues of Iberian hare (Lepus granatensis) naturally infected with the emerging recombinant myxoma virus (ha-MYXV). *Vet Rec* 2023; **192**: e2182 [PMID: 36129410 DOI: 10.1002/vetr.2182]

52 **Ávila-Reyes VA**, Díaz-Morales V, Chávez-Maya F, García-Espinosa G, Sánchez-Godoy FD. Outbreak of Systemic Avian Pox in Canaries (Serinus canaria domestica) Associated with the B1 Subgroup of Avian Pox Viruses. *Avian Dis* 2019; **63**: 525-530 [PMID: 31967439 DOI: 10.1637/12038-011819-Case.1]

53 **Rokohl AC**, Mor JM, Trester M, Koch KR, Heindl LM. [Rehabilitation of Anophthalmic Patients with Prosthetic Eyes in Germany Today - Supply Possibilities, Daily Use, Complications and Psychological Aspects]. *Klin Monbl Augenheilkd* 2019; **236**: 54-62 [PMID: 30567009 DOI: 10.1055/a-0764-4974]

54 **Iftimovici R**, Iordan L, Chelaru M. [Viral pollution studies of water environments]. *Virologie* 1989; **40**: 119-123 [PMID: 2781725]

55 **Wang TJ**, Hsiao CH, Hu FR, Wang IJ, Hou YC. Acute bilateral diffuse corneal opacity in a child. *Cornea* 2007; **26**: 375-378 [PMID: 17413971 DOI: 10.1097/ICO.0b013e31802eaf7a]

56 **Stumpf TH**, Case R, Shimeld C, Easty DL, Hill TJ. Primary herpes simplex virus type 1 infection of the eye triggers similar immune responses in the cornea and the skin of the eyelids. *J Gen Virol* 2002; **83**: 1579-1590 [PMID: 12075076 DOI: 10.1099/0022-1317-83-7-1579]

57 **Papan C**, Hagl B, Heinz V, Albert MH, Ehrt O, Sawalle-Belohradsky J, Neumann J, Ries M, Bufler P, Wollenberg A, Renner ED. Beneficial IFN-α treatment of tumorous herpes simplex blepharoconjunctivitis in dedicator of cytokinesis 8 deficiency. *J Allergy Clin Immunol* 2014; **133**: 1456-1458 [PMID: 24698314 DOI: 10.1016/j.jaci.2014.02.008]

58 **Matos RJC**, Pires JMS, Cortesão D. Management of Neonatal Herpes Simplex Infection: A Rare Case of Blepharoconjunctivitis and Concurrent Epithelial and Stromal Keratitis. *Ocul Immunol Inflamm* 2018; **26**: 625-627 [PMID: 27849421 DOI: 10.1080/09273948.2016.1242017]

59 **Carter SB**, Cohen EJ. Development of Herpes Simplex Virus Infectious Epithelial Keratitis During Oral Acyclovir Therapy and Response to Topical Antivirals. *Cornea* 2016; **35**: 692-695 [PMID: 26989961 DOI: 10.1097/ICO.0000000000000806]

60 **Liu S**, Pavan-Langston D, Colby KA. Pediatric herpes simplex of the anterior segment: characteristics, treatment, and outcomes. *Ophthalmology* 2012; **119**: 2003-2008 [PMID: 22796308 DOI: 10.1016/j.ophtha.2012.05.008]

61 **Campanella PC**, Rosenwasser GO, Sassani JW, Goldberg SH. Herpes simplex blepharoconjunctivitis presenting as complete acquired ankyloblepharon. *Cornea* 1997; **16**: 360-361 [PMID: 9143812 DOI: 10.1097/00003226-199705000-00018]

62 **Wilhelmus KR**, Dawson CR, Barron BA, Bacchetti P, Gee L, Jones DB, Kaufman HE, Sugar J, Hyndiuk RA, Laibson PR, Stulting RD, Asbell PA. Risk factors for herpes simplex virus epithelial keratitis recurring during treatment of stromal keratitis or iridocyclitis. Herpetic Eye Disease Study Group. *Br J Ophthalmol* 1996; **80**: 969-972 [PMID: 8976723 DOI: 10.1136/bjo.80.11.969]

63 **Malik AN**, Hildebrand GD, Sekhri R, Russell-Eggitt IM. Bilateral macular scars following intrauterine herpes simplex virus type 2 infection. *J AAPOS* 2008; **12**: 305-306 [PMID: 18440255 DOI: 10.1016/j.jaapos.2008.01.002]

64 **Souza PM**, Holland EJ, Huang AJ. Bilateral herpetic keratoconjunctivitis. *Ophthalmology* 2003; **110**: 493-496 [PMID: 12623810 DOI: 10.1016/S0161-6420(02)01772-4]

65 **Imamoglu EY**, Gunay M, Cilek EA, Karatekin G. Bilateral blepharoconjunctivitis as the presenting sign of disseminated herpes simplex 1 infection in a preterm neonate. *Ocul Immunol Inflamm* 2014; **22**: 326-329 [PMID: 24784884 DOI: 10.3109/09273948.2013.807346]

66 **Hong J**, Deng SX, Sun X, Xu J. Oral acyclovir for herpes simplex blepharoconjunctivitis in children. *Ophthalmology* 2013; **120**: e28 [PMID: 23732061 DOI: 10.1016/j.ophtha.2013.01.036]

67 **Hu G**, Wang MJ, Miller MJ, Holland GN, Bruckner DA, Civen R, Bornstein LA, Mascola L, Lovett MA, Mondino BJ, Pegues DA. Ocular vaccinia following exposure to a smallpox vaccinee. *Am J Ophthalmol* 2004; **137**: 554-556 [PMID: 15013881 DOI: 10.1016/j.ajo.2003.09.013]

68 **Adishvili L**, Bodokia N, Tsikarishvili S, Tskitishvili A. Adult Varicella Complicated by Deep Venous Thrombosis and Pulmonary Embolism: A Case Report and a Literature Review. *Cureus* 2024; **16**: e59213 [PMID: 38807843 DOI: 10.7759/cureus.59213]

69 **Saksena R**, Thomas BJ, Das R, Nagpal S, Suri PR, Wadhwa RK, Choudhary A, Gaind R, Gupta E. Varicella zoster virus outbreak in a long-

term care unit of a tertiary care hospital in northern India. *Epidemiol Infect* 2024; **152**: e81 [PMID: 38736415 DOI: 10.1017/S0950268824000712]

70 **Biswas J**, Nagpal A, Chopra S, Karna S. Resolution of chicken pox neuroretinitis with oral acyclovir: a case report. *Ocul Immunol Inflamm* 2003; **11**: 315-318 [PMID: 14704904 DOI: 10.1076/ocii.11.4.315.18267]

71 **Takahashi S**, Okabayashi K, Soejima I, Oniki A, Ishihara S, Tomimitsu H. Delayed Superior Orbital Fissure Syndrome Arising More than One Month after Herpes Zoster Ophthalmicus and Meningitis. *Intern Med* 2024 [PMID: 38839332 DOI: 10.2169/internalmedicine.3652-24]

72 **Musa M**, Enaholo E, Aluyi-Osa G, Atuanya GN, Spadea L, Salati C, Zeppieri M. Herpes simplex keratitis: A brief clinical overview. *World J Virol* 2024; **13**: 89934 [PMID: 38616855 DOI: 10.5501/wjv.v13.i1.89934]

73 **Chau VQ**, Hinkle JW, Wu CY, Pakravan P, Volante V, Sengillo JD, Staropoli PC, Miller D, Yannuzzi NA, Albini TA. Outcomes of infectious panuveitis associated with simultaneous multi-positive ocular fluid polymerase chain reaction. *Retina* 2024; **44**: 909-915 [PMID: 38271688 DOI: 10.1097/IAE.0000000000004037]

74 **Soldan SS**, Messick TE, Lieberman PM. Therapeutic approaches to Epstein-Barr virus cancers. *Curr Opin Virol* 2022; **56**: 101260 [PMID: 36174496 DOI: 10.1016/j.coviro.2022.101260]

75 **Dunmire SK**, Hogquist KA, Balfour HH. Infectious Mononucleosis. *Curr Top Microbiol Immunol* 2015; **390**: 211-240 [PMID: 26424648 DOI: 10.1007/978-3-319-22822-8_9]

76 **Murata T**. Epstein-Barr virus: the molecular virology and the associated diseases. *Fujita Med J* 2023; **9**: 65-72 [PMID: 37234394 DOI: 10.20407/fmj.2022-018]

77 **Zhang N**, Zuo Y, Jiang L, Peng Y, Huang X, Zuo L. Epstein-Barr Virus and Neurological Diseases. *Front Mol Biosci* 2021; **8**: 816098 [PMID: 35083281 DOI: 10.3389/fmolb.2021.816098]

78 **O'Gallagher M**, Bunce C, Hingorani M, Larkin F, Tuft S, Dahlmann-Noor A. Topical treatments for blepharokeratoconjunctivitis in children. *Cochrane Database Syst Rev* 2017; **2**: CD011965 [PMID: 28170093 DOI: 10.1002/14651858.CD011965.pub2]

79 **Lieberman PM**. Chromatin Structure of Epstein-Barr Virus Latent Episomes. *Curr Top Microbiol Immunol* 2015; **390**: 71-102 [PMID: 26424644 DOI: 10.1007/978-3-319-22822-8_5]

80 **Hatton OL**, Harris-Arnold A, Schaffert S, Krams SM, Martinez OM. The interplay between Epstein-Barr virus and B lymphocytes: implications for infection, immunity, and disease. *Immunol Res* 2014; **58**: 268-276 [PMID: 24619311 DOI: 10.1007/s12026-014-8496-1]

81 **Smatti MK**, Al-Sadeq DW, Ali NH, Pintus G, Abou-Saleh H, Nasrallah GK. Epstein-Barr Virus Epidemiology, Serology, and Genetic Variability of LMP-1 Oncogene Among Healthy Population: An Update. *Front Oncol* 2018; **8**: 211 [PMID: 29951372 DOI: 10.3389/fonc.2018.00211]

82 **Cancian L**, Bosshard R, Lucchesi W, Karstegl CE, Farrell PJ. C-terminal region of EBNA-2 determines the superior transforming ability of type 1 Epstein-Barr virus by enhanced gene regulation of LMP-1 and CXCR7. *PLoS Pathog* 2011; **7**: e1002164 [PMID: 21857817 DOI: 10.1371/journal.ppat.1002164]

83 **Solomay TV**, Semenenko TA. [Epstein-Barr viral infection is a global epidemiological problem]. *Vopr Virusol* 2022; **67**: 265-273 [PMID: 36097708 DOI: 10.36233/0507-4088-122]

84 **França SAS**, Viana JBGO, Góes HCA, Fonseca RRS, Laurentino RV, Costa IB, Oliveira-Filho AB, Machado LFA. Epidemiology of the Epstein-Barr Virus in Autoimmune Inflammatory Rheumatic Diseases in Northern Brazil. *Viruses* 2022; **14** [PMID: 35458425 DOI: 10.3390/v14040694]

85 **Hsia NY**, Bair H, Lin CY, Lin CJ, Lai CT, Chang CM, Lin JM, Tsai YY. Epstein-Barr Virus Uveitis Confirmed *via* Aqueous Humor Polymerase Chain Reaction and Metagenomics-A Case Report. *Medicina (Kaunas)* 2024; **60** [PMID: 38256358 DOI: 10.3390/medicina60010097]

86 **Alba-Linero C**, Rocha-de-Lossada C, Rachwani-Anil R, Sainz-de-la-Maza M, Sena-Corrales G, Romano V, Rodríguez-Calvo-de-Mora M. Anterior segment involvement in Epstein-Barr virus: a review. *Acta Ophthalmol* 2022; **100**: e1052-e1060 [PMID: 34766457 DOI: 10.1111/aos.15061]

87 **Lee JH**, Agarwal A, Mahendradas P, Lee CS, Gupta V, Pavesio CE, Agrawal R. Viral posterior uveitis. *Surv Ophthalmol* 2017; **62**: 404-445 [PMID: 28012878 DOI: 10.1016/j.survophthal.2016.12.008]

88 **Tang RT**, Gavito-Higuera J, Prospero Ponce CM. A Case of Epstein-Barr Virus Encephalitis and Orbital-Face Inflammation. *Cureus* 2024; **16**: e56888 [PMID: 38659504 DOI: 10.7759/cureus.56888]

89 **Mushiga Y**, Komoto T, Nagai N, Ozawa Y. Effects of intraocular treatments for Epstein-Barr virus (EBV) retinitis: A case report. *Medicine (Baltimore)* 2021; **100**: e28101 [PMID: 35049237 DOI: 10.1097/MD.0000000000028101]

90 **Victor AA**, Sukmana N. RETINAL VASCULITIS ASSOCIATED WITH EPSTEIN-BARR VIRUS INFECTION, A CASE REPORT. *Retin Cases Brief Rep* 2018; **12**: 314-317 [PMID: 28030457 DOI: 10.1097/ICB.0000000000000508]

91 **Hsia YC**, Chin-Hong PV, Levin MH. Epstein-Barr Virus Neuroretinitis in a Lung Transplant Patient. *J Neuroophthalmol* 2017; **37**: 43-47 [PMID: 27525478 DOI: 10.1097/WNO.0000000000000433]

92 **Peponis VG**, Chatziralli IP, Parikakis EA, Chaira N, Katzakis MC, Mitropoulos PG. Bilateral Multifocal Chorioretinitis and Optic Neuritis due to Epstein-Barr Virus: A Case Report. *Case Rep Ophthalmol* 2012; **3**: 327-332 [PMID: 23139677 DOI: 10.1159/000343704]

93 **Keorochana N**. A case report of Epstein-Barr virus-associated retinal vasculitis: successful treatment using only acyclovir therapy. *Int Med Case Rep J* 2016; **9**: 213-218 [PMID: 27524923 DOI: 10.2147/IMCRJ.S107089]

94 **Weller JM**, Bergua A, Mardin CY. Retinopathy in a patient with acute Epstein-Barr virus infection: follow-up analysis using spectral domain optical coherence tomography. *Retin Cases Brief Rep* 2015; **9**: 72-77 [PMID: 25383851 DOI: 10.1097/ICB.0000000000000087]

95 **Savastano MC**, Rispoli M, Di Antonio L, Mastropasqua L, Lumbroso B. Observed positive correlation between Epstein-Barr virus infection and focal choroidal excavation. *Int Ophthalmol* 2014; **34**: 927-932 [PMID: 24193503 DOI: 10.1007/s10792-013-9874-8]

96 **Liu Z**, Ji MF, Huang QH, Fang F, Liu Q, Jia WH, Guo X, Xie SH, Chen F, Liu Y, Mo HY, Liu WL, Yu YL, Cheng WM, Yang YY, Wu BH, Wei KR, Ling W, Lin X, Lin EH, Ye W, Hong MH, Zeng YX, Cao SM. Two Epstein-Barr virus-related serologic antibody tests in nasopharyngeal carcinoma screening: results from the initial phase of a cluster randomized controlled trial in Southern China. *Am J Epidemiol* 2013; **177**: 242-250 [PMID: 23255783 DOI: 10.1093/aje/kws404]

97 **De Paschale M**, Clerici P. Serological diagnosis of Epstein-Barr virus infection: Problems and solutions. *World J Virol* 2012; **1**: 31-43 [PMID: 24175209 DOI: 10.5501/wjv.v1.i1.31]

98 **She RC**, Stevenson J, Phansalkar AR, Hillyard DR, Litwin CM, Petti CA. Limitations of polymerase chain reaction testing for diagnosing acute Epstein-Barr virus infections. *Diagn Microbiol Infect Dis* 2007; **58**: 333-335 [PMID: 17376631 DOI: 10.1016/j.diagmicrobio.2007.01.014]

99 **Sato T**, Kitamura R, Kaburaki T, Takeuchi M. Retinitis associated with double infection of Epstein-Barr virus and varicella-zoster virus: A case report. *Medicine (Baltimore)* 2018; **97**: e11663 [PMID: 30075555 DOI: 10.1097/MD.0000000000011663]

100 **Walling DM**, Flaitz CM, Nichols CM. Epstein-Barr virus replication in oral hairy leukoplakia: response, persistence, and resistance to treatment with valacyclovir. *J Infect Dis* 2003; **188**: 883-890 [PMID: 12964120 DOI: 10.1086/378072]

101 **Durand ML**, Barshak MB, Sobrin L. Eye Infections. *N Engl J Med* 2023; **389**: 2363-2375 [PMID: 38118024 DOI: 10.1056/NEJMra2216081]

102 **Sinclair W**, Omar M. Enterovirus. 2023 Jul 31. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [PMID: 32966001]

103 **Langford MP**, Anders EA, Burch MA. Acute hemorrhagic conjunctivitis: anti-coxsackievirus A24 variant secretory immunoglobulin A in acute and convalescent tear. *Clin Ophthalmol* 2015; **9**: 1665-1673 [PMID: 26392747 DOI: 10.2147/OPTH.S85358]

104 **Wright PW**, Strauss GH, Langford MP. Acute hemorrhagic conjunctivitis. *Am Fam Physician* 1992; **45**: 173-178 [PMID: 1309404]

105 **Meza-Romero R**, Navarrete-Dechent C, Downey C. Molluscum contagiosum: an update and review of new perspectives in etiology, diagnosis, and treatment. *Clin Cosmet Investig Dermatol* 2019; **12**: 373-381 [PMID: 31239742 DOI: 10.2147/CCID.S187224]

106 **Peterson AR**, Nash E, Anderson BJ. Infectious Disease in Contact Sports. *Sports Health* 2019; **11**: 47-58 [PMID: 30106670 DOI: 10.1177/1941738118789954]

107 **Leung AKC**, Barankin B, Hon KLE. Molluscum Contagiosum: An Update. *Recent Pat Inflamm Allergy Drug Discov* 2017; **11**: 22-31 [PMID: 28521677 DOI: 10.2174/1872213X11666170518114456]

108 **Senkevich TG**, Koonin EV, Bugert JJ, Darai G, Moss B. The genome of molluscum contagiosum virus: analysis and comparison with other poxviruses. *Virology* 1997; **233**: 19-42 [PMID: 9201214 DOI: 10.1006/viro.1997.8607]

109 **Singh M**, Acharya M, Gandhi A, Prakash U. Molluscum-related keratoconjunctivitis. *Indian J Ophthalmol* 2019; **67**: 1176 [PMID: 31238446 DOI: 10.4103/ijo.IJO_1808_18]

110 **Ringeisen AL**, Raven ML, Barney NP. Bulbar Conjunctival Molluscum Contagiosum. *Ophthalmology* 2016; **123**: 294 [PMID: 26802706 DOI: 10.1016/j.ophtha.2015.11.022]

111 **Moss WJ**. Measles. *Lancet* 2017; **390**: 2490-2502 [PMID: 28673424 DOI: 10.1016/S0140-6736(17)31463-0]

112 **Naim HY**. Measles virus. *Hum Vaccin Immunother* 2015; **11**: 21-26 [PMID: 25483511 DOI: 10.4161/hv.34298]

113 **White SJ**, Boldt KL, Holditch SJ, Poland GA, Jacobson RM. Measles, mumps, and rubella. *Clin Obstet Gynecol* 2012; **55**: 550-559 [PMID: 22510638 DOI: 10.1097/GRF.0b013e31824df256]

114 **Hviid A**, Rubin S, Mühlemann K. Mumps. *Lancet* 2008; **371**: 932-944 [PMID: 18342688 DOI: 10.1016/S0140-6736(08)60419-5]

115 **Muto T**, Imaizumi S, Kamoi K. Viral Conjunctivitis. *Viruses* 2023; **15** [PMID: 36992385 DOI: 10.3390/v15030676]

116 **Solano D**, Fu L, Czyz CN. Viral Conjunctivitis. 2023 Aug 28. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [PMID: 29262100]

117 **Vasquez-Perez A**, Magan T, Volpe G, Osborne SF, McFaul K, Vahdani K. Necrotizing Blepharoconjunctivitis and Keratitis in Human Monkeypox. *JAMA Ophthalmol* 2023; **141**: 285-288 [PMID: 36757718 DOI: 10.1001/jamaophthalmol.2022.6253]

118 **Rayati Damavandi A**, Semnani F, Hassanpour K. A Review of Monkeypox Ocular Manifestations and Complications: Insights for the 2022 Outbreak. *Ophthalmol Ther* 2023; **12**: 55-69 [PMID: 36512187 DOI: 10.1007/s40123-022-00626-4]

119 **Cardoso B**, García-Bocanegra I, Queirós J, Fernández-López J, Alves PC, Acevedo P. Effect of Myxoma Virus Species Jump on Iberian Hare Populations. *Emerg Infect Dis* 2024; **30**: 1293-1296 [PMID: 38781982 DOI: 10.3201/eid3006.231280]

120 **Brugman VA**, Hernández-Triana LM, Prosser SW, Weland C, Westcott DG, Fooks AR, Johnson N. Molecular species identification, host preference and detection of myxoma virus in the Anopheles maculipennis complex (Diptera: Culicidae) in southern England, UK. *Parasit Vectors* 2015; **8**: 421 [PMID: 26271277 DOI: 10.1186/s13071-015-1034-8]

121 **Rossini E**, Bazzucchi M, Trocchi V, Merzoni F, Bertasio C, Knauf S, Lavazza A, Cavadini P. Identification and Characterisation of a Myxoma Virus Detected in the Italian Hare (Lepus corsicanus). *Viruses* 2024; **16** [PMID: 38543802 DOI: 10.3390/v16030437]

122 **Farsang A**, Makranszki L, Dobos-Kovács M, Virág G, Fábián K, Barna T, Kulcsár G, Kucsera L, Vetési F. Occurrence of atypical myxomatosis in Central Europe: clinical and virological examinations. *Acta Vet Hung* 2003; **51**: 493-501 [PMID: 14680061 DOI: 10.1556/AVet.51.2003.4.7]

123 **Kowanz DH**, Rokohl AC, Heindl LM. [Viral Conjunctivitis: Findings, Therapy, and Prophylaxis]. *Klin Monbl Augenheilkd* 2023; **240**: 1317-1331 [PMID: 37586401 DOI: 10.1055/a-2129-1255]

124 **Ather F**, Zia MA, Habib M, Shah MS. Development of an ELISA for the detection of fowl adenovirus serotype -4 utilizing fiber protein. *Biologicals* 2024; **85**: 101752 [PMID: 38401400 DOI: 10.1016/j.biologicals.2024.101752]

125 **Paulini I**, Siqueira-Silva J, Thomaz L, Rocha L, Harsi C, Bellei N, Granato C. Development of a prototype immunochromatographic test for rapid diagnosis of respiratory adenovirus infection. *Braz J Infect Dis* 2017; **21**: 500-506 [PMID: 28623675 DOI: 10.1016/j.bjid.2017.03.023]

126 **Migita H**, Ueno T, Tsukahara-Kawamura T, Saeki Y, Hanaoka N, Fujimoto T, Uchio E. Evaluation of adenovirus amplified detection of immunochromatographic test using tears including conjunctival exudate in patients with adenoviral keratoconjunctivitis. *Graefes Arch Clin Exp Ophthalmol* 2019; **257**: 815-820 [PMID: 30826875 DOI: 10.1007/s00417-019-04281-7]

127 **Isik P**, Harbiyeli II, Ozturk G, Erdem E, Yagmur M, Yarkin F. The Relationship between Clinical Findings and Viral Load in Adenoviral Keratoconjunctivitis. *Jpn J Infect Dis* 2022; **75**: 592-596 [PMID: 35908877 DOI: 10.7883/yoken.JJID.2022.210]

128 **Mays JB**, Mariem MN, Alabadi HI. Seroprevalence of herpes simplex virus type 1 (Herpesviridae: Simplexvirus: Human alphaherpesvirus 1) in smokers. *Vopr Virusol* 2024; **69**: 187-192 [PMID: 38843024 DOI: 10.36233/0507-4088-220]

129 **Adams BS**, Patel AR. Cenegermin. 2024 Mar 19. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [PMID: 34424642]

130 **Mousavi-Nasab SD**, Sabahi F, Kaghazian H, Paryan M, Mirab Samiee S, Ghaderi M, Zali F, Makvandi M. A Real-Time RT-PCR Assay for Genotyping of Rotavirus. *Iran Biomed J* 2020; **24**: 399-404 [PMID: 32660931 DOI: 10.29252/ibj.24.6.394]

131 **El-Sayed Zaki M**, Abd-El Fatah GA. Rapid detection of oculopathogenic adenovirus in conjunctivitis. *Curr Microbiol* 2008; **56**: 105-109 [PMID: 17985184 DOI: 10.1007/s00284-007-9054-z]

132 **Behera HS**, Srigyan D. Evaluation of Polymerase Chain Reaction over Routine Microbial Diagnosis for the Diagnosis of Fungal Keratitis. *Optom Vis Sci* 2021; **98**: 280-284 [PMID: 33633022 DOI: 10.1097/OPX.0000000000001652]

133 **Laaks D**, Smit DP, Harvey J. Polymerase chain reaction to search for Herpes viruses in uveitic and healthy eyes: a South African perspective. *Afr Health Sci* 2015; **15**: 748-754 [PMID: 26957961 DOI: 10.4314/ahs.v15i3.7]

134 **Gilbert LL**, Dakhama A, Bone BM, Thomas EE, Hegele RG. Diagnosis of viral respiratory tract infections in children by using a reverse transcription-PCR panel. *J Clin Microbiol* 1996; **34**: 140-143 [PMID: 8748290 DOI: 10.1128/jcm.34.1.140-143.1996]

135 **Chang CH**, Sheu MM, Lin KH, Chen CW. Hemorrhagic viral keratoconjunctivitis in Taiwan caused by adenovirus types 19 and 37: applicability of polymerase chain reaction-restriction fragment length polymorphism in detecting adenovirus genotypes. *Cornea* 2001; **20**: 295-300 [PMID: 11322419 DOI: 10.1097/00003226-200104000-00011]

136 **Horton JC**, Miller S. Magnetic Resonance Imaging in Epidemic Adenoviral Keratoconjunctivitis. *JAMA Ophthalmol* 2015; **133**: 960-961 [PMID: 26022084 DOI: 10.1001/jamaophthalmol.2015.1457]

137 **Sharma S**. Diagnosis of infectious diseases of the eye. *Eye (Lond)* 2012; **26**: 177-184 [PMID: 22094299 DOI: 10.1038/eye.2011.275]

138 **Uchio E**, Aoki K, Saitoh W, Itoh N, Ohno S. Rapid diagnosis of adenoviral conjunctivitis on conjunctival swabs by 10-minute immunochromatography. *Ophthalmology* 1997; **104**: 1294-1299 [PMID: 9261316 DOI: 10.1016/s0161-6420(97)30145-6]

139 **Van Rij G**, Klepper L, Peperkamp E, Schaap GJ. Immune electron microscopy and a cultural test in the diagnosis of adenovirus ocular infection. *Br J Ophthalmol* 1982; **66**: 317-319 [PMID: 6280747 DOI: 10.1136/bjo.66.5.317]

140 **Boerner CF**, Lee FK, Wickliffe CL, Nahmias AJ, Cavanagh HD, Straus SE. Electron microscopy for the diagnosis of ocular viral infections. *Ophthalmology* 1981; **88**: 1377-1381 [PMID: 6275326 DOI: 10.1016/s0161-6420(81)34882-9]

141 **Nakagawara K**, Hayashi H, Kawaji K, Sasano M, Kodama EN. Application of human lymphoid cells for the evaluation of antivirals against human adenovirus type 19: Zalcitabine has superior activity compared to cidofovir. *Antivir Chem Chemother* 2020; **28**: 2040206620921319 [PMID: 32345035 DOI: 10.1177/2040206620921319]

142 **Muqit MM**, Foot B, Walters SJ, Mudhar HS, Roberts F, Rennie IG. Observational prospective cohort study of patients with newly-diagnosed ocular sebaceous carcinoma. *Br J Ophthalmol* 2013; **97**: 47-51 [PMID: 23117971 DOI: 10.1136/bjophthalmol-2012-302443]

143 **Uchio E**. [New medical treatment for viral conjunctivitis]. *Nippon Ganka Gakkai Zasshi* 2005; **109**: 962-84; discussion 985 [PMID: 16408491]

144 **Romanowski EG**, Araullo-Cruz T, Gordon YJ. Topical corticosteroids reverse the antiviral effect of topical cidofovir in the Ad5-inoculated New Zealand rabbit ocular model. *Invest Ophthalmol Vis Sci* 1997; **38**: 253-257 [PMID: 9019458]

145 **Jhanji V**, Chan TC, Li EY, Agarwal K, Vajpayee RB. Adenoviral keratoconjunctivitis. *Surv Ophthalmol* 2015; **60**: 435-443 [PMID: 26077630 DOI: 10.1016/j.survophthal.2015.04.001]

146 **Ianchenko SV**, Sakhnov SN, Malyshev AV, Fedotova NV, Orekhova OIu, Grishchenko IV. [Treatment of chronic allergic blepharoconjunctivitis]. *Vestn Oftalmol* 2014; **130**: 78, 80-84 [PMID: 25711068]

147 **Brusini P**, Salvetat ML, Parisi L, Zeppieri M, Tosoni C. Discrimination between normal and early glaucomatous eyes with scanning laser polarimeter with fixed and variable corneal compensator settings. *Eur J Ophthalmol* 2005; **15**: 468-476 [PMID: 16001380 DOI: 10.1177/112067210501500409]

148 **Ferreira ACZ**, Mocelin LP, Zanini F, Santos MSD, Chong-Neto HJ, Mallozi MC, Solé D. Translation, adaptation, and psychometric properties of the Brazilian-Portuguese version of the Quality of Life in Children with Vernal Keratoconjunctivitis questionnaire. *Arq Bras Oftalmol* 2024; **87**: e2023 [PMID: 38656024 DOI: 10.5935/0004-2749.2023-0054]

149 **Christman JE**, Gram D, Wellehan JFX, Craft WF, Scrivener J, Crevasse S, Kepley FA, Alexander AB. The use of intradermal allergy testing for allergic dermatitis in pteropid bats and treatment with allergen specific immunotherapy: A case series. *J Zoo Wildl Med* 2021; **52**: 1298-1308 [PMID: 34998303 DOI: 10.1638/2020-0202]

150 **Levine RM**, Tattersall IW, Gaudio PA, King BA. Cicatrizing Blepharoconjunctivitis Occurring During Dupilumab Treatment and a Proposed Algorithm for Its Management. *JAMA Dermatol* 2018; **154**: 1485-1486 [PMID: 30347029 DOI: 10.1001/jamadermatol.2018.3427]

151 **Kanukollu VM**, Patel BC. Herpes Simplex Ophthalmicus. 2023 Apr 17. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [PMID: 32644620]

152 **Murphy O**, O' Dwyer V, Lloyd-McKernan A. The effect of lid hygiene on the tear film and ocular surface, and the prevalence of Demodex blepharitis in university students. *Cont Lens Anterior Eye* 2020; **43**: 159-168 [PMID: 31548151 DOI: 10.1016/j.clae.2019.09.003]

153 **Yanchenko SV**, Malyshev AV, Sakhnov SN, Fedotova NV, Orekhova OY. [Eye lid hygiene in chronic allergic blepharoconjunctivitis patients before laser refractive surgery]. *Vestn Oftalmol* 2016; **132**: 86-92 [PMID: 27911432 DOI: 10.17116/oftalma2016132586-92]

154 **Polunin GS**, Zabegaĭlo AO, Makarov IA, Safonova TN, Polunina EG. [Efficacy of lid hygiene in treatment of patients with blepharoconjunctival form of dry eye syndrome]. *Vestn Oftalmol* 2012; **128**: 37-40 [PMID: 22741294]

155 **Delelegn D**, Tolcha A, Beyene H, Tsegaye B. Status of active trachoma infection among school children who live in villages of open field defecation: a comparative cross-sectional study. *BMC Public Health* 2021; **21**: 2051 [PMID: 34753484 DOI: 10.1186/s12889-021-12106-8]

156 **Gammoh Y**, Abdu M. Contact lens procurement and usage habits among adults in Sudan. *PLoS One* 2021; **16**: e0251987 [PMID: 34010356 DOI: 10.1371/journal.pone.0251987]

157 **Hart KM**, Stapleton F, Carnt N, Arundel L, Lian KY. Optometry Australia's infection control guidelines 2020. *Clin Exp Optom* 2021; **104**: 267-284 [PMID: 33769228 DOI: 10.1080/08164622.2021.1887704]

158 **Shamim MA**, Satapathy P, Padhi BK, Veeramachaneni SD, Akhtar N, Pradhan A, Agrawal A, Dwivedi P, Mohanty A, Pradhan KB, Kabir R, Rabaan AA, Alotaibi J, Al Ismail ZA, Alsoliabi ZA, Al Fraij A, Sah R, Rodriguez-Morales AJ. Pharmacological treatment and vaccines in monkeypox virus: a narrative review and bibliometric analysis. *Front Pharmacol* 2023; **14**: 1149909 [PMID: 37214444 DOI: 10.3389/fphar.2023.1149909]

159 **Cinatl J**, Bechtel M, Reus P, Ott M, Rothweiler F, Michaelis M, Ciesek S, Bojkova D. Trifluridine for treatment of mpox infection in drug combinations in ophthalmic cell models. *J Med Virol* 2024; **96**: e29354 [PMID: 38180134 DOI: 10.1002/jmv.29354]

160 **Gujjar P**, Chaudhay R, Verma I, Bansal N, Gupta S, Bansal S. Recent Advances in the Prevention and Management of Monkeypox Viral Infection in Humans. *Curr Drug Targets* 2023; **24**: 1032-1045 [PMID: 37842888 DOI: 10.2174/0113894501258154231008194028]

161 **Al-Dwairi RA**, Aleshawi A, Adi S, Abu-Zreig L. Reactivation of Herpes Simplex Keratitis on a Corneal Graft Following SARS-CoV-2 mRNA Vaccination. *Med Arch* 2022; **76**: 146-148 [PMID: 35774041 DOI: 10.5455/medarh.2022.76.146-148]

162 **Naidu SK**, Nabi R, Cheemarla NR, Stanfield BA, Rider PJ, Jambunathan N, Chouljenko VN, Carter R, Del Piero F, Langohr I, Kousoulas KG. Intramuscular vaccination of mice with the human herpes simplex virus type-1(HSV-1) VC2 vaccine, but not its parental strain HSV-1(F) confers full protection against lethal ocular HSV-1 (McKrae) pathogenesis. *PLoS One* 2020; **15**: e0228252 [PMID: 32027675 DOI: 10.1371/journal.pone.0228252]

163 **Nesburn AB**, Slanina S, Burke RL, Ghiasi H, Bahri S, Wechsler SL. Local periocular vaccination protects against eye disease more effectively than systemic vaccination following primary ocular herpes simplex virus infection in rabbits. *J Virol* 1998; **72**: 7715-7721 [PMID: 9733807 DOI: 10.1128/JVI.72.10.7715-7721.1998]

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LETTER TO THE EDITOR

Climate-driven dengue fever outbreaks in Nepal: Trends, challenges, and strategies

Chandan Kumar Thakur, Samita Adhikari, Meghnath Dhimal

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Abstract

Dengue fever (DF) has become a major public health concern in Nepal, with increasing outbreaks in recent years. Transmitted by Aedes mosquitoes, this climate-sensitive viral disease presents a significant challenge for healthcare providers and policymakers. Since 2004, Nepal has experienced a sharp increase in DF cases, peaking in 2022 with 54784 cases and 88 deaths. The surge, driven mainly by serotypes 1, 2, and 3, is exacerbated by climate change, which prolongs mosquito breeding seasons due to warmer temperatures and increased rainfall. This trend has even impacted previously unaffected hilly regions. Effective dengue control strategies must focus on climate change adaptation, strengthening healthcare system reinforcement, raising public awareness, and enhancing vector control measures. Government initiatives, like the national dengue control program, play a critical role, but research and community engagement are also vital for prevention and early detection. Integrating climate resilience into public health efforts is essential to reducing the dengue burden in Nepal.

Key Words: Climate change; Dengue fever; Dengue outbreaks; Dengue control; Nepal; Public health; Vector-borne diseases

Core Tip: Given the escalating threat of dengue fever in Nepal, characterized by recurrent outbreaks exacerbated by climate change, prioritizing proactive measures is essential. Healthcare providers and policymakers should focus on bolstering the healthcare system, raising public awareness, and implementing effective vector control measures. Government initiatives, such as investing in research and fostering community engagement, are critical for early detection and prevention. By integrating climate resilience into public health strategies, Nepal can effectively mitigate the burden of dengue fever on its population and safeguard against future outbreaks.

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TO THE EDITOR

Dengue fever (DF) has become an escalating public health threat in Nepal, with rising morbidity and mortality rates and several outbreaks reported in recent years[1]. This climate-sensitive viral disease, transmitted by Aedes mosquitoes, presents a significant challenge for healthcare providers and policymakers in the country[1]. DF is caused by the dengue virus (DENV), a single-stranded RNA virus of the Flavivirus genus in the Flaviviridae family. DF can cause a range of health issues, from mild symptoms to life-threatening conditions such as dengue hemorrhagic fever (DHF), a severe form characterized by bleeding, blood plasma leakage, and low platelet count, or dengue shock syndrome (DSS), the most severe form, that occurs when the circulatory system fails due to severe plasma leakage. Both DHF and DSS are caused by one of the four serotypes (DENV1-4)[2]. Globally, the prevalence of DF has been increasing, with nearly half of the world's population at risk. It is estimated that 390 million people are infected annually, with 96 million developing severe clinical manifestations[3]. Several factors associated with mosquito invasion and increased travel within the population are believed to drive the epidemic's expansion, elucidating the dissemination of the DENV to new locations[4]. This study investigated the impact of climate change on DF outbreaks in Nepal.

CURRENT STATUS AND TRENDS OF DENGUE IN NEPAL

Since the first report in 2004, Nepal has witnessed a sharp increase in DF cases, with the geometric mean calculated rising by 503 between 2006-2023 (Figure 1)[1,5]. In 2019, the Ministry of Health and Population (MoHP) documented 17992 confirmed dengue cases, resulting in six deaths. However, by 2022, the situation worsened dramatically, with 54784 cases and 88 fatalities reported across all seven provinces and 77 districts of Nepal[6]. All four serotypes of DENV have been in circulation since 2006, and the 2022 epidemic was primarily attributed to serotypes 1, 2, and 3[7]. This 2022 outbreak was the worst the country had experienced since the first outbreak in 2006, nearly triple the number of cases reported in 2019. By December 15, 2023, a total of 51243 cases of dengue have been recorded across 77 districts, resulting in 20 deaths. Notably, Koshi Province reported the highest count (26021), followed by Gandaki Province (12688) and Bagmati Province (7704) along with other provinces (Figure 2)[8]. While dengue was previously confined to the warmer lowland regions of Nepal, recent data show its prevalence in upland hilly regions, a shift attributed to climate change and rapid urbanization [9].

Climate change is one of the greatest threats to global public health, particularly through its effects on the spread of vector-borne diseases like DF. Nepal, located in the Himalayan region, is particularly vulnerable to the impacts of climate change, raising concerns about its influence on dengue outbreaks[10]. Changes in temperature, precipitation patterns, and extreme weather events are altering the ecology of Aedes mosquitoes, the primary vectors for dengue transmission[11]. Over the past 40 years, Nepal's average annual maximum temperature has increased by 0.056 °C, with more pronounced warming at higher altitudes[12]. Rising temperatures accelerate mosquito development, enhance virus replication, and facilitate dengue transmission in areas previously unaffected by the disease. Changes in precipitation, including increased rainfall and erratic weather patterns, provide more breeding sites, particularly in urban areas with poor drainage, while higher humidity levels extend mosquito lifespan and activity. These climatic changes have extended the dengue transmission season and expanded the spread of the disease, complicating public health efforts to control outbreaks[4,13]. Recent studies indicate that alterations in the diurnal temperature range (DTR) hold greater significance than shifts in average temperature concerning the transmission of dengue[4]. A wider DTR can reduce transmission by shortening mosquito lifespans and lowering infection rates, while optimal transmission occurs within a narrow temperature range of 27-31°C. Seasonal variations in DTR influence mosquito survival and infection dynamics, which, in turn affect outbreak patterns[14,15]. To address the impacts of climate change on dengue in Nepal, effective strategies must include both climate change adaptation and mitigation efforts. Climate change adaptation measures involve strengthening the healthcare system to better manage dengue cases, increasing public awareness about dengue prevention, and enhancing vector control measures. Mitigation efforts target the root causes of climate change.

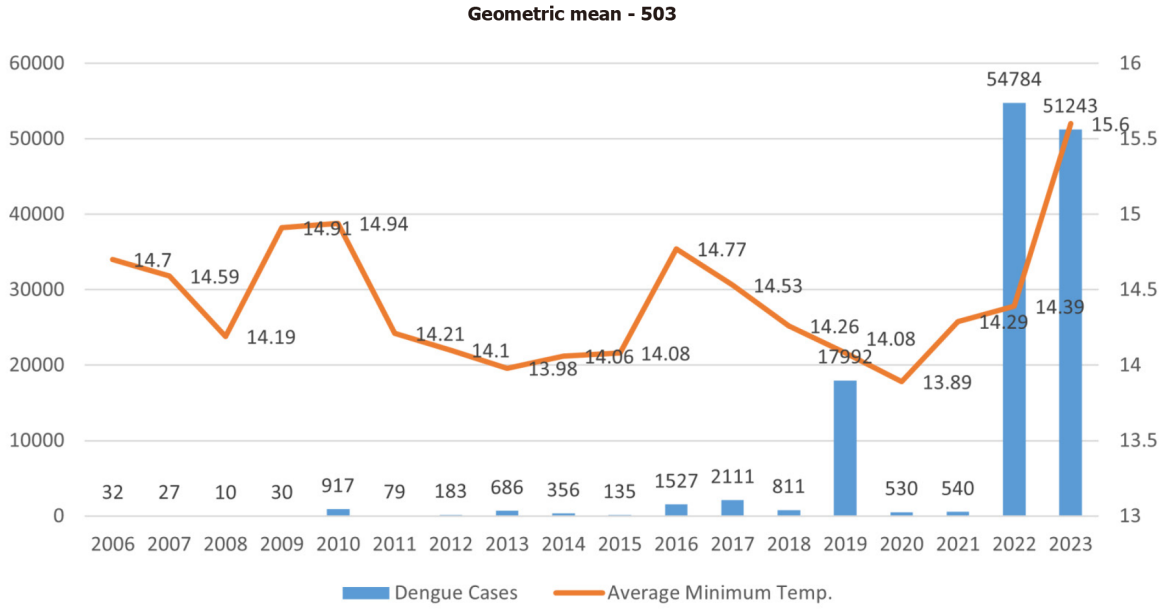

Figure 1 Annual dengue cases and climatic temperature shifts in Nepal (2006-2023). Data source: https://climateknowledgeportal.worldbank.org/country/nepal/climate-data-historical, https://edcd.gov.np/section/dengue-control-program.

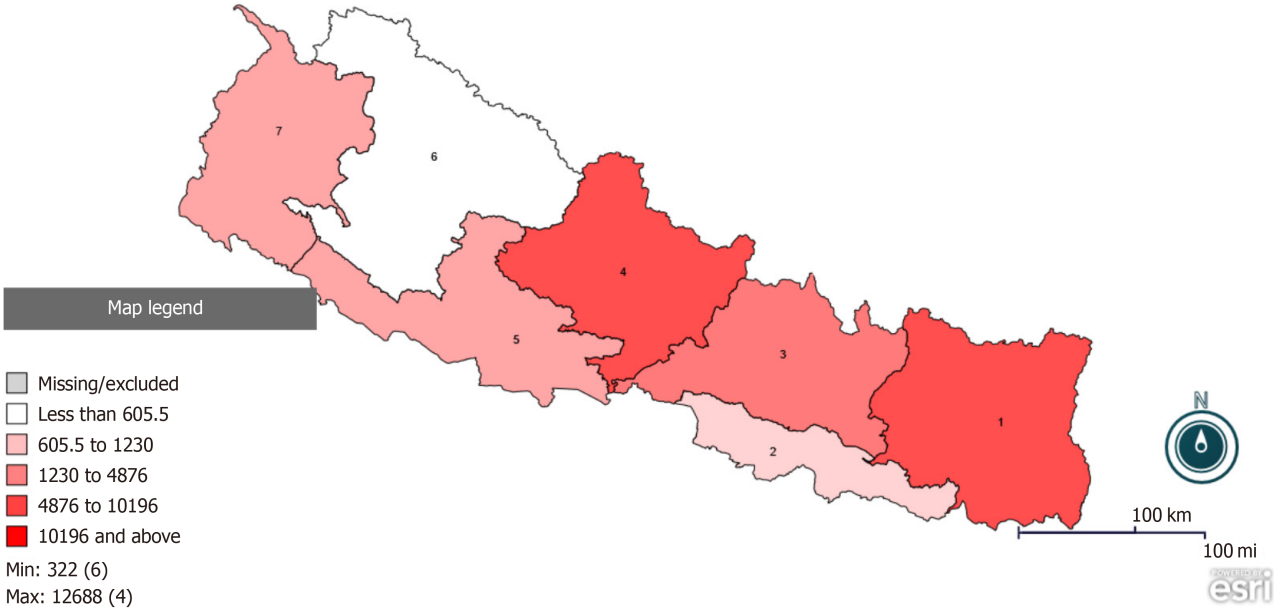

Figure 2 Distribution of dengue cases in Nepal in 2023. 1: Koshi Province; 2: Madhesh Province; 3: Bagmati Province; 4: Gandaki Province; 5: Lumbini Province; 6: Karnali Province; 7: Sudurpashchim Province. Data source: https://edcd.gov.np/section/dengue-control-program.

The government of Nepal has initiated several programs to combat dengue, including public awareness campaigns, healthcare provider training on dengue diagnosis and management, and strengthening vector control measures such as conducting search-and-destroy drives targeting mosquito breeding sites. The MoHP has also established a national dengue control program to coordinate among stakeholders (EDCD | Dengue Control Program). Despite these initiatives, the spread of dengue continues to increase annually, highlighting the need for improved strategies at the national level [16]. The failure to control the annual spread of dengue in Nepal can be attributed to inadequate community participation, inconsistent vector control efforts, and the challenges posed by rapid urbanization and climate change. To improve dengue control in Nepal, future plans should focus on enhancing community engagement through sustained education campaigns, ensuring consistent and integrated vector control efforts across districts, and addressing the impacts of urbanization and climate change. Strengthening surveillance systems and fostering collaboration between local authorities and health agencies will also be critical for improving management and prevention efforts.

Additionally, research and innovations are crucial in developing effective vaccines and antiviral drugs for dengue. Investigating the genetic diversity of the DENV and the Aedes mosquito population in Nepal can aid in the creation of targeted prevention and control strategies.

CONCLUSION

Dengue outbreaks are an increasing public health threat in Nepal, with increasing morbidity and mortality. Since 2004, cases have surged, with serotypes 1, 2, and 3, being the most prevalent, highlighting the need for urgent action. The government, healthcare providers, policymakers, researchers, and communities must collaborate to develop and implement comprehensive strategies that integrate climate change adaptation and mitigation efforts to reduce the burden of dengue. Together, we can address the escalating dengue burden and mitigate its impact on public health, working toward a future where dengue is effectively controlled and the health of the population is safeguarded in Nepal.

FOOTNOTES

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REFERENCES

1 **Adhikari N**, Subedi D. The alarming outbreaks of dengue in Nepal. *Trop Med Health* 2020; **48**: 5 [PMID: 32055230 DOI: 10.1186/s41182-020-0194-1]
2 **Wilder-Smith A**, Ooi EE, Horstick O, Wills B. Dengue. *Lancet* 2019; **393**: 350-363 [PMID: 30696575 DOI: 10.1016/S0140-6736(18)32560-1]
3 **Bhatt S**, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SI. The global distribution and burden of dengue. *Nature* 2013; **496**: 504-507 [PMID: 23563266 DOI: 10.1038/nature12060]
4 **Tozan Y**, Sjödin H, Muñoz ÁG, Rocklöv J. Transmission dynamics of dengue and chikungunya in a changing climate: do we understand the eco-evolutionary response? *Expert Rev Anti Infect Ther* 2020; **18**: 1187-1193 [PMID: 32741233 DOI: 10.1080/14787210.2020.1794814]
5 **Pandey BD**, Rai SK, Morita K, Kurane I. First case of Dengue virus infection in Nepal. *Nepal Med Coll J* 2004; **6**: 157-159 [PMID: 16295753]
6 Situation update of Dengue 2022 (As of December 31, 2022). Available from: https://edcd.ekbana.info/news/download/situation-updates-of-dengue-as-of-30-nov-2022
7 **Bagcchi S**. Nepal faces an outbreak of dengue. *Lancet Infect Dis* 2023; **23**: 35 [PMID: 36549322 DOI: 10.1016/S1473-3099(22)00821-0]
8 **Government of Nepal**; Ministry of Health and Population; Department of Health Services; Epidemiology and Disease Control Division. Situation Report on Dengue In Nepal- 2023. Available from: https://edcd.gov.np/news/20231215-dengue-situation-update
9 **Dhimal M**, Ahrens B, Kuch U. Climate Change and Spatiotemporal Distributions of Vector-Borne Diseases in Nepal--A Systematic Synthesis of Literature. *PLoS One* 2015; **10**: e0129869 [PMID: 26086887 DOI: 10.1371/journal.pone.0129869]
10 **Dhimal M**, Dhimal ML, Pote-Shrestha RR, Groneberg DA, Kuch U. Health-sector responses to address the impacts of climate change in Nepal. *WHO South East Asia J Public Health* 2017; **6**: 9-14 [PMID: 28857057 DOI: 10.4103/2224-3151.213795]
11 **World Bank Group**. Climate Change Knowledge Portal. Available from: https://climateknowledgeportal.worldbank.org/
12 **Kantipur Media Group**. Some Tarai districts face extreme heat as temperatures cross 40 degrees. Available from: https://kathmandupost.com/climate-environment/2023/06/02/some-tarai-districts-face-extreme-heat-as-temperatures-cross-40-degrees
13 **Pandey BD**, Costello A. The dengue epidemic and climate change in Nepal. *Lancet* 2019; **394**: 2150-2151 [PMID: 31839187 DOI: 10.1016/S0140-6736(19)32689-3]
14 **Lambrechts L**, Paaijmans KP, Fansiri T, Carrington LB, Kramer LD, Thomas MB, Scott TW. Impact of daily temperature fluctuations on dengue virus transmission by Aedes aegypti. *Proc Natl Acad Sci USA* 2011; **108**: 7460-7465 [PMID: 21502510 DOI: 10.1073/pnas.1101377108]
15 **Trejo I**, Barnard M, Spencer JA, Keithley J, Martinez KM, Crooker I, Hengartner N, Romero-severson EO, Manore C. Changing temperature profiles and the risk of dengue outbreaks. *PLOS Clim* 2023; **2**: e0000115 [DOI: 10.1371/journal.pclm.0000115]
16 **Rijal KR**, Adhikari B, Ghimire B, Dhungel B, Pyakurel UR, Shah P, Bastola A, Lekhak B, Banjara MR, Pandey BD, Parker DM, Ghimire P. Epidemiology of dengue virus infections in Nepal, 2006-2019. *Infect Dis Poverty* 2021; **10**: 52 [PMID: 33858508 DOI: 10.1186/s40249-021-00837-0]

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LETTER TO THE EDITOR

Understanding rhabdomyolysis induced acute kidney injury in patients with COVID-19

Alexander Ikanović, Karan Varshney

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Abstract

This work comments on an article published in the recent issue of the *World Journal of Virology*. Rhabdomyolysis is a complex condition with symptoms such as myalgia, changes to urination, and weakness. With the potential for substantial kidney impairment, it has also been shown to be a severe complication of coronavirus disease 2019 (COVID-19). To date, various theoretical explanations exist for the development of rhabdomyolysis induced acute kidney injury (RIAKI) in COVID-19 infection, including the accumulation of released striated muscle myoglobin in the urine (myoglobinuria). In their article, they (2024) demonstrate in a retrospective study that RIAKI in COVID-19 patients tended to have elevated levels of C-reactive protein, ferritin, and procalcitonin. These patients also had poorer overall prognoses when compared to COVID-19 patients who have acute kidney injury (AKI) due to other causes. It is clear from these findings that clinicians must closely monitor and assess for the presence of rhabdomyolysis in COVID-19 patients who have developed AKIs. Moreover, additional research is required to further understand the mechanisms behind the development of RIAKI in COVID-19 patients in order to better inform treatment guidelines and protocols.

Key Words: COVID-19; Rhabdomyolysis; Acute kidney injury; Mortality; Complication

Core Tip: The article published in the recent issue of the *World Journal of Virology* (2024) has shown that patients with rhabdomyolysis induced acute kidney injury (RIAKI) as a complication of coronavirus disease 2019 (COVID-19) infection tend to show elevated levels of specific biomarkers. Additionally, patients with RIAKI in COVID-19 infection have worse outcomes compared to patients who have acute kidney injury due to other causes in COVID-19 infection. To improve overall outcomes, there is a need to better understand the mechanism for RIAKI development in COVID-19, and to create evidence-based treatment protocols for this condition. To determine which approaches are most effective, more research on the outcomes for patients with differing treatment regimens is required.

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TO THE EDITOR

Rhabdomyolysis is a complex disease characterised by extensive skeletal muscle damage and the sequestration of intracellular contents into the bloodstream. Increased levels of contents such as uridine acid, myoglobin, creatine kinase (CK), and potassium reflect early structural changes and complications of rhabdomyolysis[1]. Whilst common symptoms include myalgia, muscle weakness, and red/brown urine, they are typically absent in almost 50% of patients[2]. Diagnosis is instead defined as per biochemical markers, namely, a CK level more than 5 times the upper limit of normal with an associated increased in transaminase and lactate dehydrogenase (LDH)[3]. Trauma, strenuous exercise, hyperthermia, toxin exposure, infection, and sepsis, as well as hypoxaemia-induced metabolic disequilibrium are predominating aetiologies[4]. One such recent infectious agent contributing in 2% of hospitalised patients is coronavirus disease 2019 (COVID-19) disease, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[5]. With a postulated incidence of 15%-20% of rhabdomyolysis in the course of COVID-19, a possible contributor to the poor prognosis and over 40% mortality seen in this cohort is the development of an acute kidney injury (AKI)[6]. Different theoretical mechanisms explain rhabdomyolysis induced AKI (RIAKI) in the course of COVID-19. One explanation for RIAKIs are the accumulation of released striated muscle myoglobin in the urine (myoglobinuria), resulting in the formation of pigment casts. These casts deposit within the nephron resulting in intratubular obstruction, with the resulting endothelial damage ultimately causing an acute tubular injury[7]. Whilst this is considered the main aetiology, additional pathological mechanisms of AKIs in COVID-19 include pre-renal azotaemia, glomerular disease, thrombotic microangiopathy, and treatment related AKI[8].

AKI IN COVID-19 PROGNOSTICATION

Whilst RIAKIs may be associated with higher rates of morbidity and mortality, Murt and Altiparmak[3], sought to compare the outcomes of COVID-19 patients with RIAKIs and AKIs of differing aetiologies. Previously, due to the difficulty in accurately determining the exact aetiology of AKIs particularly in COVID-19, there has been a scant presence in the literature of studies comparing the prognostic outcomes of varying AKI aetiologies.

In determining the mortality and morbidity of these groups, physicians treating AKIs may be better placed to accurately prognosticate patients experiencing an AKI or rhabdomyolysis as a result of a viral illness. Their paper found that patients when comparing aetiologies of AKIs in COVID-19 patients, those with RIAKIs had higher inflammatory and hypercoagulopathy markers as well as poorer prognoses. Through understanding these markers, challenge current treatment paradigms of RIAKIs in COVID-19 patients which currently struggle to rationalise potential stigmata of fluid restriction protocols such as pulmonary oedema could be challenged[9]. Commonly rhabdomyolysis treatment prioritises the removal of the cause and the reversal of any associated metabolic aberrations such as hypokalaemia, hypophosphatasemia, hypocalcaemia, hyponatraemia or hypernatraemia, and hyperglycaemic states[10]. Similar to much of the surrounding literature outlining the treatment challenges which are posed by poorly understanding the pathophysiology of RIAKIs in COVID-19, Murt and Altiparmak[3], look to provide an explanation for the additional factors which increased RIAKI mortality. Therefore, to substantiate alternative targeted treatment paradigms, monitoring biomarkers may prove useful.

Their paper sought to closely monitor biomarkers due to the possibility for rapid deterioration-RIAKI patients had higher mean creatinine levels and more patients with Kidney Diseases Improving Global Outcomes stage II and stage III AKIs than those of other aetiologies. Interestingly, the higher mortality in RIAKI patients could not be attributed to hyperkalaemia due to the similarities observed in the groups levels. COVID-19 may however uniquely provide additional drivers of muscular injury through and an inflammatory state with increased coagulation as higher peak ferritin and D-dimer levels were observed in the RIAKI group. Other increased markers such as C-reactive protein, LDH and ferritin were found to corroborate these findings of the RIAKI group. Additionally, researchers observed higher pro-brain natriuretic peptide levels in the RIAKI group which may reflect additional cardiac stress resulting from pulmonary tension or volume overload. As rhabdomyolysis independently causes more severe AKIs, this may account for the higher

levels observed. However, they too are indicator of a poorer prognosis in COVID-19[11].

CLINICAL IMPLICATIONS

With concerns for exacerbation of stigmata of COVID-19 such as pulmonary oedema hampering RIAKI treatment mainstays-it becomes increasingly difficult to accurately account for the poorer prognosis in COVID-19 patients complicated by AKIs. Treatments such as fluid prescription to decrease the propensity for intratubular cast formation are foregone here as instead hypoxaemia is touted as the main driver of acute tubular necrosis. Regardless, clearer evidence-based treatment guidelines and protocols need to be developed for patients with COVID-19 who develop RIAKI.

Currently, like the much of the existing literature, with an overreliance on clinical signs, symptoms, and laboratory findings due to patients being too unwell to biopsy, the lack of definition of exact aetiology further impedes our understanding of RIAKI disease progression[3,12]. As a result, additional confounding variables make it increasingly difficult to account for differences in morbidity and mortality when typically indicative biomarkers such as hyperkalaemia are not found to be dissimilar. A call may therefore be made for others to investigate alternative treatment paradigms in COVID-19 when RIAKI occurs, as this may entail deviating from regular treatment mainstays to improve overall health outcomes. This needs to occur alongside further research to more clearly determine the basis of the development of RIAKI in COVID-19. This will allow for faster diagnosing of this life-threatening complication, which can further improve the outcomes for patients with RIAKI.

CONCLUSION

In their work, Murt and Altiparmak[3] have shown that RIAKI is a major, life-threatening complication that can occur in a proportion of COVID-19 patients. These patients tend to have some biomarkers that may indicate the presence of RIAKI. Concerningly, patients with RIAKI during COVID-19 infection tend to have worse outcomes compared to patients who have developed AKI due to other causes in COVID-19 infection. More research is needed to determine the mechanisms for RIAKI development. Furthermore, to improve overall outcomes, there remains a clear need to determine the most effective treatment regimens for this life-threatening problem.

FOOTNOTES

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REFERENCES

1 **Stahl K**, Rastelli E, Schoser B. A systematic review on the definition of rhabdomyolysis. *J Neurol* 2020; **267**: 877-882 [PMID: 30617905 DOI: 10.1007/s00415-019-09185-4]
2 **McKenna MC**, Kelly M, Boran G, Lavin P. Spectrum of rhabdomyolysis in an acute hospital. *Ir J Med Sci* 2019; **188**: 1423-1426 [PMID: 30680491 DOI: 10.1007/s11845-019-01968-y]
3 **Murt A**, Altiparmak MR. Rhabdomyolysis-related acute kidney injury in patients with COVID-19. *World J Virol* 2024; **13**: 91107 [PMID: 39323452 DOI: 10.5501/wjv.v13.i3.91107]
4 **Giannoglou GD**, Chatzizisis YS, Misirli G. The syndrome of rhabdomyolysis: Pathophysiology and diagnosis. *Eur J Intern Med* 2007; **18**: 90-100 [PMID: 17338959 DOI: 10.1016/j.ejim.2006.09.020]
5 **Hannah JR**, Ali SS, Nagra D, Adas MA, Buazon AD, Galloway JB, Gordon PA. Skeletal muscles and Covid-19: a systematic review of

rhabdomyolysis and myositis in SARS-CoV-2 infection. *Clin Exp Rheumatol* 2022; **40**: 329-338 [PMID: 35225218 DOI: 10.55563/clinexprheumatol/mkfmxt]

6 **Haroun MW**, Dieiev V, Kang J, Barbi M, Marashi Nia SF, Gabr M, Eman G, Kajita G, Swedish K. Rhabdomyolysis in COVID-19 Patients: A Retrospective Observational Study. *Cureus* 2021; **13**: e12552 [PMID: 33575135 DOI: 10.7759/cureus.12552]

7 **Somagutta MR**, Pagad S, Sridharan S, Nanthakumaran S, Arnold AA, May V, Malik BH. Role of Bicarbonates and Mannitol in Rhabdomyolysis: A Comprehensive Review. *Cureus* 2020; **12**: e9742 [PMID: 32944457 DOI: 10.7759/cureus.9742]

8 **Ng JH**, Bijol V, Sparks MA, Sise ME, Izzedine H, Jhaveri KD. Pathophysiology and Pathology of Acute Kidney Injury in Patients With COVID-19. *Adv Chronic Kidney Dis* 2020; **27**: 365-376 [PMID: 33308501 DOI: 10.1053/j.ackd.2020.09.003]

9 **Matsumura Y**, Sugiyama T, Kondo N, Miyahara M, Hanaoka N, Nagashima H, Kasahara Y, Fujiyoshi N, Inada A, Inaba S. Fluid restriction management in the treatment of COVID-19: a single-center observational study. *Sci Rep* 2022; **12**: 17339 [PMID: 36243779 DOI: 10.1038/s41598-022-22389-5]

10 **Long B**, Koyfman A, Gottlieb M. An evidence-based narrative review of the emergency department evaluation and management of rhabdomyolysis. *Am J Emerg Med* 2019; **37**: 518-523 [PMID: 30630682 DOI: 10.1016/j.ajem.2018.12.061]

11 **Robbins-Juarez SY**, Qian L, King KL, Stevens JS, Husain SA, Radhakrishnan J, Mohan S. Outcomes for Patients With COVID-19 and Acute Kidney Injury: A Systematic Review and Meta-Analysis. *Kidney Int Rep* 2020; **5**: 1149-1160 [PMID: 32775814 DOI: 10.1016/j.ekir.2020.06.013]

12 **Nance JR**, Mammen AL. Diagnostic evaluation of rhabdomyolysis. *Muscle Nerve* 2015; **51**: 793-810 [PMID: 25678154 DOI: 10.1002/mus.24606]

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