OSONG PUBLIC HEALTH
AND RESEARCH PERSPECTIVES
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OSONG PUBLIC HEALTH
AND RESEARCH PERSPECTIVES

Vol. 14, No. 5, October 2023

Pages 329-438

phrp
OSONG PUBLIC HEALTH
AND RESEARCH PERSPECTIVES

pISSN 2210-9099
eISSN 2233-6052

Vol. 14, No. 5, October 2023

Korea Disease Control and
Prevention Agency

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

https://ophrp.org

pISSN 2210-9099
eISSN 2233-6052

Vol. 14, No. 5, October 2023

Published on 31 October 2023

Aims & Scope

Osong Public Health and Research Perspectives (PHRP) is the international bimonthly (published at the end of February, April, June, August, October, and December) journal founded in 2010 by the Korea Disease Control and Prevention Agency (KDCA). With the mission of the KDCA, to create a disease-free world, PHRP encourages sharing medical information and knowledge in the areas of public health.
PHRP publishes original articles, review articles, guidelines, data profiles (including cohort profiles), special articles, short communications, viewpoints, editorials, commentaries, and correspondence, and book reviews, with a focus on the following areas of expertise: emerging infectious diseases, vaccinology, zoonotic diseases, non-communicable diseases, intractable and rare diseases, and human genomics.

Abstracting and Indexing Services

A part of articles, metadata, or full text is available from CrossRef metadata (2010–), PubMed Central (2010–), PubMed (2010–), Scopus (2010–), Embase (2010–), EBSCO Host (2010–), Directory of Open Access Journals (2018–), Korea Citation Index (2011–), and Emerging Sources Citation Index (2020–).

Open Access

Publisher: The Korea Disease Control and Prevention Agency

Editor-in-Chief: Jong-Koo Lee, MD, PhD

Editorial Office:

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

https://ophrp.org
pISSN 2210-9099 eISSN 2233-6052

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https://doi.org/10.24171/j.phrp.2023.0317
Osong Public Health Res Perspect 2023;14(5):329-332

Editorial

To combat COVID-19 and prepare for future pandemics, public trust and communication are essential

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As of October 18, 2023, there have been 771,407,825 confirmed cases of coronavirus disease 2019 (COVID-19) worldwide, resulting in 6,972,152 deaths [1]. The swift development of vaccines mitigated the death toll. Now, as this Public Health Emergency of International Concern draws to an end, it is crucial to reflect on the lessons learned.

First and foremost, overcoming a disease is only possible through proper communication and an evidence-based scientific approach. Media during the COVID-19 pandemic underscored the importance of discovering scientific truth, as policies grounded in this truth garner public support. Evidence-based scientific communication is of fundamental importance in this regard. However, what we perceive as truth does not always align with scientific fact. Our decision-making has frequently been skewed due to false beliefs in uncertain situations, resulting in frequent errors. Unfortunately, scholars and journalists, who are primarily interested in novelty, often suppress or distort facts due to intense competition for coverage. Moreover, individuals frequently present false beliefs, which conflict with established theories, as truths due to political biases or religious faith [2]. Even some scientists have discussed unproven and potentially dangerous ideas as alternatives for new diseases based on past experiences, often presenting them to the public under the guise of science. This misleads the public. For instance, they have proposed managing the disease through herd immunity by exposing the younger population to protect the elderly, or advocate for the use of malaria medication, claiming it to be highly effective. Such propositions have been openly discussed in the media by some medical professionals. As a result, individuals with religious backgrounds who support the politician have refused to wear masks or rejected vaccines.

Secondly, we need to establish an research and development (R&D) system that can quickly discover scientific facts. How can we expediently verify scientific evidence and facts in anticipation of a new "Disease X" and devise a response strategy? Prompt efforts are required to identify the gap between our current knowledge and the unknown, but only well-prepared countries can accomplish this. In the initial stages of COVID-19, an external evaluation team from the World Health Organization (WHO) visited China and underscored the need to develop alternatives through rapid research. They discussed the nature and transmission route of the virus, as well as strategies to prevent its spread. Systems should be pre-established to efficiently execute a series of processes, including regular surveillance, enhanced surveillance, natural history and other clinical studies, observation of drug administration and responses, clinical intervention trials for vaccines and treatments, and large-scale clinical trials in the community.

Received: October 22, 2023
Accepted: October 26, 2023

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With such preparations for evidence-based science, we can triumph over this disease and future infectious diseases.

Thirdly, for a victorious battle, it is crucial to establish a prototype for the virus and have preparations in place. This approach can save time and effort in real-world scenarios. As Sun Tzu states in The Art of War, "If you know the enemy and know yourself, you need not fear the result of a hundred battles." Dr. Anthony Fauci, wrote an insightful article discussing the top 10 lessons from a scientist's perspective [3]. Some key points are: "1. Expect the unexpected," "6. The prototype pathogen approach to pandemic preparedness and response should be implemented," "9. Misinformation and disinformation are the enemy of public health and pandemic control," and "10. Emerging infections are a constant threat." It is challenging to predict what will emerge, and responding is difficult as viruses mutate. Fauci and Folkers [3] also emphasized the importance of preparing in advance with a prototype virus, a perspective shared by many scholars and WHO officials. Experience has shown that new infectious diseases re-emerge due to changes in the human-animal interface, alterations in bacterial antibiotic resistance, issues within the healthcare system, or the resurgence of diseases on the brink of eradication like poliomyelitis. Therefore, being prepared for these situations is vitally important. I had the privilege of meeting Dr. Fauci when I discussed the Republic of Korea's experience during the middle east respiratory syndrome outbreak in 2015 at Georgetown University. After a lecture on the theme "Emerging Infections: A Perpetual Challenge," I asked him what he thought the next epidemic would be. His response was, "I don't know." At the time, many scholars considered influenza as a likely candidate, and I found myself questioning his expertise due to his uncertainty. However, since most scholars failed to accurately predict the COVID-19 pandemic, it appears that Dr. Fauci's admission of uncertainty was indeed correct. The question of how to prepare for Disease X when we do not know what it is might seem paradoxical. Therefore, the current realistic approach for Disease X, particularly in vaccine development, appears to be preparation through a prototype [4].

Fourthly, we need to devise a strategy for rapid vaccine production in a winner-takes-all market. Is it truly possible to identify Disease X and develop a vaccine for it? Are we prepared as a nation? Scholars argue that the government's policy will and the enthusiastic expansion of corporate investments are crucial. However, we seem to be reverting to the pre-COVID-19 era. There seems to be a lack of participants for clinical trials, and due to market monopolization by pre-secured products, emerging companies appear to lack the incentive to invest in R&D. At the 2022 G7 meeting, a declaration was made to develop and distribute a vaccine within 100 days for any new infectious disease. This is a significant political statement. The goal for a Disease X vaccine is to further reduce the development time, building on the experience of the COVID-19 vaccine, which took 1.5 years. This strategy was outlined in the *New England Journal of Medicine*'s roadmap, which theoretically details how to cut the 250-day development time [5]. Extraordinary measures would seem to be needed for our country to emulate a successful model like that of the United States. In the COVID-19 vaccine market, much like the sentiment in ABBA's song "The Winner Takes It All," Moderna and Pfizer emerged as leaders. What facilitated their rapid progress? Several points remain uncertain for us: (1) Can we, with our distinct ecosystem, can overcome intellectual property barriers? (2) Can we self-sustain or procure essential raw materials? (3) Is swift scaled-up production feasible? (4) Is there a budget for phase 3 clinical trials? (5) Is there a robust coordinating body like the U.S. National Institutes of Health's Biomedical Advanced Research and Development Authority? (6) How will regulatory rigidity (e.g., emergency use authorization) be scientifically addressed? (7) Will companies invest in vaccine development considering the risks? (8) Will the government prioritize the use of developed products?

For the Republic of Korea, transitioning from a "fast follower" to a "fast mover" in terms of unique rapid product production requires strong leadership. Nonetheless, there seems to be a lack of willingness to invest and a reluctance to conduct research to improve processes without infringing on existing patents. As the pandemic subsides and R&D momentum decreases, the government's commitment to R&D investment becomes especially important.

Fifthly, meticulous measures are required concerning the efficacy and side effects of vaccines. The public's fatigue due to the prolonged COVID-19 pandemic has heightened skepticism about the effectiveness and adverse reactions of vaccines. The aim of vaccination is not to prevent and eradicate infections, but rather to prevent severe illness, with a focus on high-risk groups instead of the general population. Convincing the public of the necessity for multiple additional doses and annual vaccinations is becoming increasingly difficult. Concerns related to adverse reactions and compensation for vaccine-related injuries also deter people from getting vaccinated. Therefore, it is crucial to persuade the public using accurate data. The imported vaccines we use appear to be no different from those in other countries. There does not seem to be a significant difference in the reported frequency of severe cases or deaths. Therefore, the fact that we have approximately 50 times more claims temporally for adverse

https://doi.org/10.24171/j.phrp.2023.0317

reaction compensation than neighboring Japan suggests that the crux of the problem may lie in the explanations provided by healthcare professionals.

The government's lackluster investigation into injuries and the mechanical responses of frontline public servants is exacerbating the problem. Furthermore, it seems that healthcare professionals are not providing sufficient comprehensive explanations to those receiving vaccinations. Population-based studies have identified adverse reactions including acute flaccid myelitis, abnormal uterine bleeding, lymphadenitis, anaphylaxis, facial paralysis, encephalitis/encephalopathy, and meningitis, which largely align with preexisting knowledge [6]. The National Academy of Korea of Medicine, tasked with investigating these adverse reactions, is diligently reviewing statistical associations and mechanistic validity. Concurrently, it stays abreast of international trends to promptly provide research feedback and apply equal effort to compensation issues related to adverse reactions. However, compensation for vaccination-induced injuries, unlike population-based association studies, necessitates a comprehensive evaluation of individual cases, taking into account factors such as underlying diseases, overall health assessment, epidemiological investigations, and autopsies. There appears to be a lack of societal consensus on this matter. To overcome the limitations in investigating individual cases, it may be beneficial to establish centers for each type of adverse reaction, where all similar cases can be collected and investigated to enable causality assessment. In other words, we should actively consider improving the framework for disease-specific prospective and retrospective cohorts, active surveillance networks, and causality assessment. However, compensation for damages extends beyond causality assessment, necessitating different approaches within societal norms and on a social integrative level.

Sixthly, managing a disease requires, above all, a bold determination to eradicate it. Political will must be mobilized. However, there is a paradoxical phenomenon that occurs as a disease approaches eradication: the program itself first disappears, meaning the budget, organization, and institutional memory fade away [7]. This fall, even before the start of the vaccination campaign, next year's program budget has been slashed. With the government's policy on R&D changing, research funds are shrinking and the desire to eradicate the disease is disappearing. Therefore, it is crucial to actively persuade civil servants in charge of policy, as well as members of the national assembly who handle laws and budgets.

Finally, the above-discussed policies require public support, which can be achieved through effective communication. It is crucial to actively engage the public in the creation of scientific knowledge, the development and use of vaccines, the management of adverse reactions, and the securing of program budgets. As the COVID-19 pandemic shifts from a crisis stage to a regular management phase (i.e., becoming endemic), and as society begins to return to normal, this fall's vaccination rate will probably be lower than that of last year. Many have noted that the government's communication with the public regarding the vaccine's efficacy, the scientific rationale and cost-effectiveness due to changes in the target vaccination group, and compensation for adverse reactions, has been inadequate. Communicating with the public based on evidence fosters trust and establishes a support base for the policy, an important lesson we must remember in our efforts to eradicate disease. The principles for effective communication outlined in "The Lancet COVID-19 Commission Task Force on Public Health Measures to Suppress the Pandemic" review (Table 1) [8] should be actively referenced.

Table 1. Principles of effective communication

1	Communicate broadly and early to shaping public opinion
2	Use trusted sources and networks to deliver localized messages, in addition to mass media
3	Engage end-users in message design
4	Emphasize positive descriptive norms
5	Emphasize collective efficacy
6	Highlight alternative behaviors (e.g. food delivery services)
7	Use clear, concise, consistent and frequently repeated messages
8	In multiethnic and multilingual countries, ensure that all population groups are reached through customized messages
9	Be honest about uncertainty and failure
10	Emphasize benefits to the recipient and others
11	Align with the recipient's moral values
12	Highlight the prospect of social group approval
13	Avoid stigmatizing groups for not adhering to recommendations

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
Jong-Koo Lee has been the editor-in-chief of Osong Public Health and Research Perspectives since October 2021.

Funding
None.

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https://doi.org/10.24171/j.phrp.2023.0317

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2022.0323
Osong Public Health Res Perspect 2023;14(5):333-346

Review Article

Strategies to combat Gram-negative bacterial resistance to conventional antibacterial drugs: a review

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ABSTRACT

The emergence of antimicrobial resistance raises the fear of untreatable diseases. Antimicrobial resistance is a multifaceted and dynamic phenomenon that is the cumulative result of different factors. While Gram-positive pathogens, such as methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile*, were previously the most concerning issues in the field of public health, Gram-negative pathogens are now of prime importance. The World Health Organization's priority list of pathogens mostly includes multidrug-resistant Gram-negative organisms particularly carbapenem-resistant Enterobacterales, carbapenem-resistant *Pseudomonas aeruginosa*, and extensively drug-resistant *Acinetobacter baumannii*. The spread of Gram-negative bacterial resistance is a global issue, involving a variety of mechanisms. Several strategies have been proposed to control resistant Gram-negative bacteria, such as the development of antimicrobial auxiliary agents and research into chemical compounds with new modes of action. Another emerging trend is the development of naturally derived antibacterial compounds that aim for targets novel areas, including engineered bacteriophages, probiotics, metal-based antibacterial agents, odilorhabdins, quorum sensing inhibitors, and microbiome-modifying agents. This review focuses on the current status of alternative treatment regimens against multidrug-resistant Gram-negative bacteria, aiming to provide a snapshot of the situation and some information on the broader context.

Keywords: Alternative therapies; Antibiotic resistance; Antimicrobial; Gram-negative bacteria; Multidrug drug resistance

Received: December 27, 2022
Revised: July 11, 2023
Accepted: August 15, 2023

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Introduction

Antibiotic resistance is a global public health threat caused by the imprudent use and widespread overdosing of antibiotics [1,2]. Antimicrobial resistance poses a significant challenge in clinical settings, leading to devastating infections. In 2017, the World Health Organization (WHO) released a list of priority pathogens (Figure 1) [3], for which new medicines

are urgently needed. These pathogens were categorized as critical, high, or medium priority [4,5]. The majority of the pathogens listed by the WHO are Gram-negative bacteria. It has been clearly demonstrated that Gram-negative bacteria have a higher potential to cause serious diseases in humans, particularly among immunocompromised individuals, due to their specialized cellular structure [1,3]. The outer membrane (OM) of Gram-negative bacteria has been identified as the primary factor promoting resistance against a broad range of antibiotics. The OM functions as a permeability barrier, effectively blocking many current antibiotics, and it exerts its effect in 2 ways: by reducing influx and increasing efflux.

Reduced Influx

Membrane proteins permit the selective entry of various molecules. Numerous antibiotics, including penicillin, carbapenems, cephalosporins, and fluoroquinolones, gain access to the bacterial cell via these proteins. Changes in membrane permeability can influence the entry of these antibiotics, leading to resistance [6]. OM porin proteins are particularly significant in the context of antibiotic resistance, as they can limit the entry of several antibiotics, such as β-lactams and fluoroquinolones, into the cell [7,8].

Increased Efflux

Furthermore, even when an antibiotic successfully penetrates the OM, it can be swiftly expelled from the cell by a variety of broadly acting efflux pumps, thereby inducing resistance. These pumps utilize either the energy derived from ATP hydrolysis or the proton motive force to expel chemicals from the cell. For instance, an ABC (ATP-binding cassette) superfamily pump has been demonstrated to be linked with heightened resistance to aminoglycosides and polymyxins in *Serratia marcescens* [9].

Efforts to combat clinically relevant drug resistance have involved modifying existing antibiotic classes or introducing new antibiotics. Following the initial "golden era" of antibiotics, large pharmaceutical companies began to encounter significant scientific challenges in their search for new antibiotics, particularly those effective against Gram-negative bacterial drug resistance. This led to a waning interest in a field that no longer promised ever-increasing profits. However, the emergence of extreme drug resistance has prompted global public health authorities to seek alternatives to antibiotics. In this review, we have categorized antibiotic substitutes into 2 broad groups: (a) antibiotic adjuvants and (b) antibiotic alternatives. Antibiotic alternatives are further classified into (1) phages or phage-derived proteins, (2) direct-acting compounds, (3) repurposed approved drugs, (4) anti-virulence therapies, (5) RNA-based therapeutics, (6) nanomaterial-based therapeutics, and (7) miscellaneous.

HIGHLIGHTS

Antibiotic resistance poses a significant public health concern. This review explores various antibiotic alternatives and auxiliary agents that could potentially address the issue of antibiotic resistance. The primary organisms of focus are Gram-negative pathogens, specifically those on the World Health Organization priority list. The new approaches under consideration are conceptually diverse and innovative, with particular emphasis on nontraditional approaches such as the use of predatory bacteria or engineered phages. While the potential of these innovative ideas is vast, their link to clinical application remains tenuous. Increased global effort, focus, and funding are essential to combat the silent epidemic of antibiotic resistance.

Resistant Gram-Negative Bacteria and the Mechanisms of Their Resistance

In 2017, the WHO released a list of 12 antibiotic-resistant priority pathogens (Figure 1), which are considered to be the most significant threats to public health. All the critical priority list pathogens are Gram-negative bacteria, namely carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *Pseudomonas aeruginosa*, and carbapenem and third-generation cephalosporin-resistant Enterobacteriaceae (*Escherichia coli*, *Enterobacter* spp., and *Klebsiella pneumoniae*). Apart from these pathogens, clarithromycin-resistant *Helicobacter pylori*, fluoroquinolone-resistant *Campylobacter* sp. and *Salmonella* sp., and third-generation cephalosporin and fluoroquinolone-resistant *Neisseria gonorrhoeae* are also included in the WHO's list of high-priority pathogens, along with Gram-positive *Staphylococcus aureus* and *Enterococcus faecium* [4,10]. Recently Gram-negative bacteria have also recently acquired resistance toward the last-resort drugs, polymyxins. The following section briefly discusses the different mechanisms of resistance. Figure 2 provides a visual representation of these resistance mechanisms.

Mechanism of Resistance

Enzymes

Bacteria employ different enzymes to exert resistance

https://doi.org/10.24171/j.phrp.2022.0323

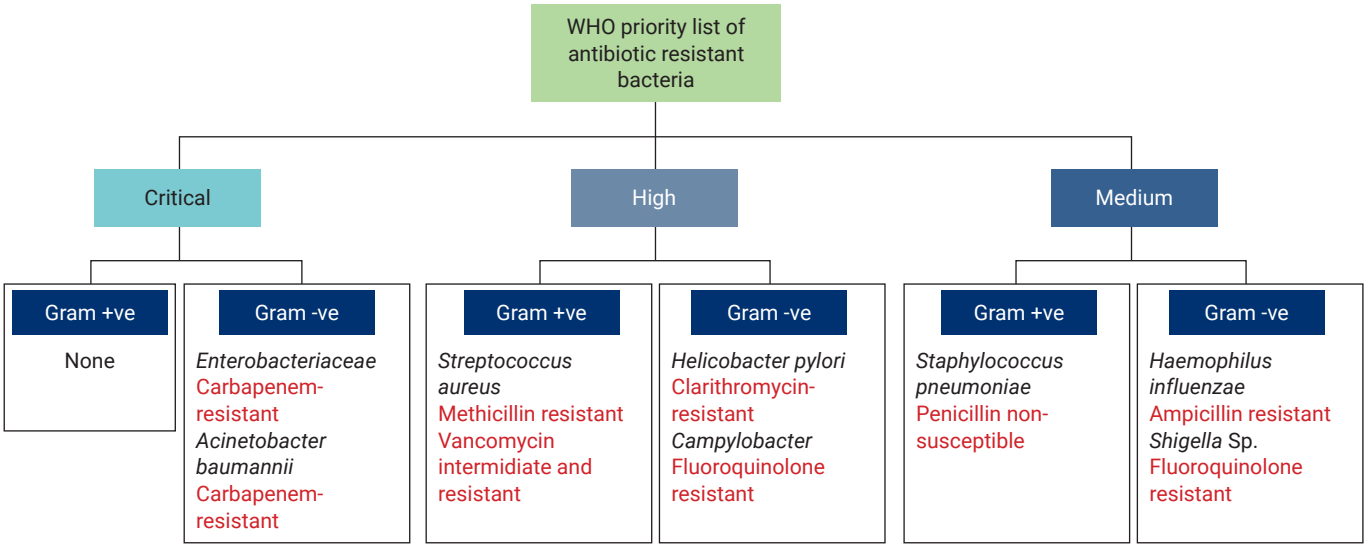

Figure 1. The priority list of pathogens declared by the World Health Organization (WHO). Breijyeh et al. Molecules 2020;25:1340, according to the Creative Commons license [3].

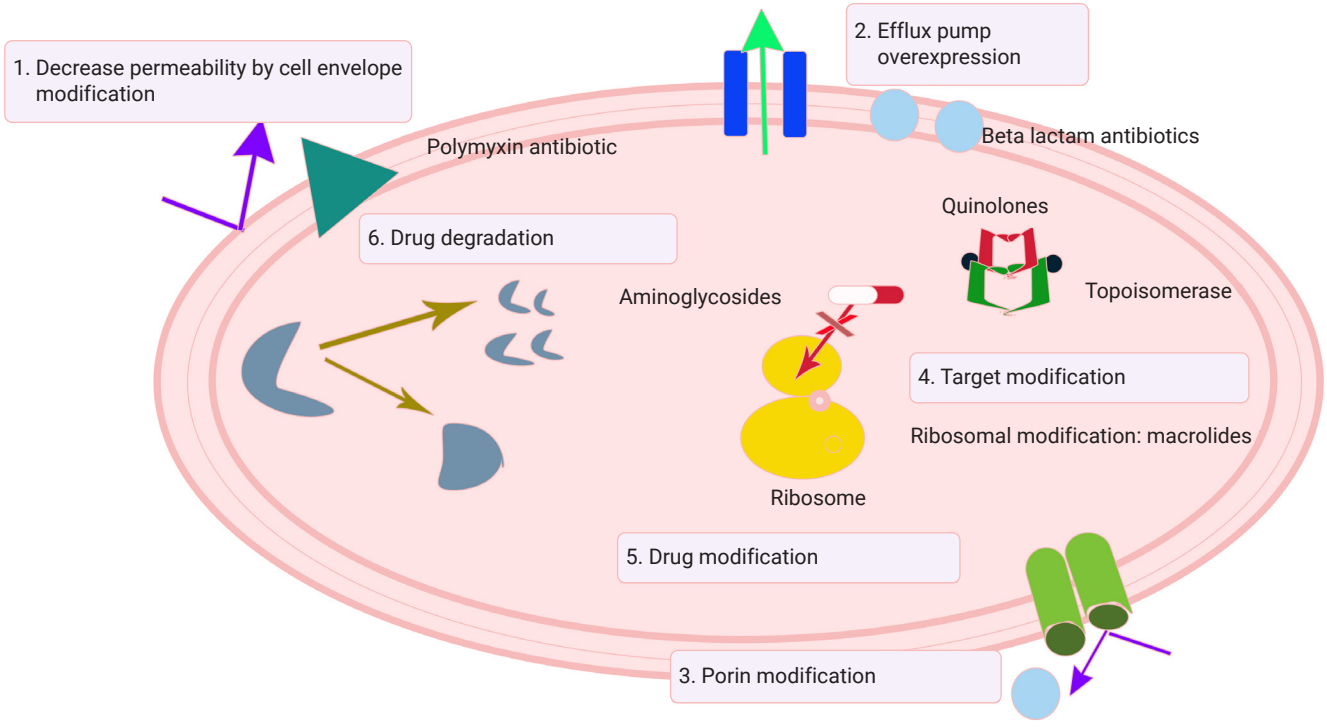

Figure 2. Different mechanisms of antibiotic resistance.

towards different antibiotics, either by degrading the antibiotics or by interfering with their activities. The mechanisms of action of these enzymes are shown in Figure 3.

β-Lactamases

The β-lactam antibiotics hold prime importance. β-lactamases are enzymes that hydrolyze the amide bond of the 4-membered β-lactam ring, which leads to the inactivation of the antibiotic.

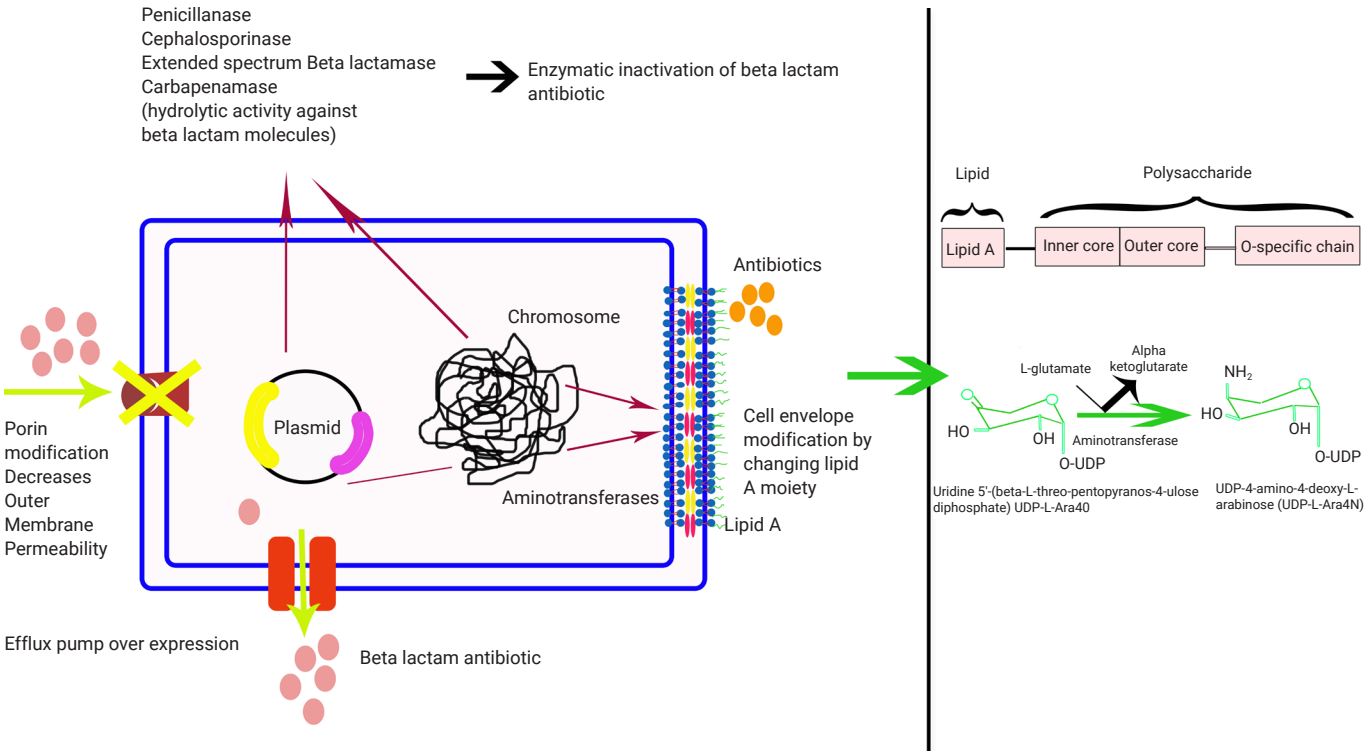

Figure 3. Schematic depiction of β-lactamase and aminotransferase activity.

The subsequent result is resistance.

Carbapenem resistance: Resistance to carbapenems typically arises from 2 factors: (1) non-carbapenemase-mediated carbapenem resistance, and (2) the production of carbapenemases, which are enzymes that hydrolyze carbapenem antibiotics. The production of an extended-spectrum β-lactamase or AmpC enzymes, coupled with a decrease in cell membrane permeability due to modifications in porin proteins, has been reported to contribute to carbapenem resistance in Enterobacteriaceae [11]. The most common carbapenamase enzymes are *K. pneumoniae* carbapenemases (KPCs), which circulate in Enterobacteriaceae worldwide. Strains harboring KPCs are resistant to all β-lactams and often develop resistance to other classes of antibiotics, such as fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole. The global prevalence of KPC-producing Enterobacteriaceae is increasing, leading to a worldwide multidrug resistance pandemic. OXA-48 and NDM are among the most prevalent types of carbapenemases found in *K. pneumoniae* [12]. The acquisition of carbapenemases has also been well-documented in *A. baumannii* [13].

TEM, CTX-M, and extended-spectrum β-lactamase (ESBL)—cephalosporin resistance: These enzymes are broadly distributed via plasmids and other mobile genetic elements. The acquisition of point mutations in these enzymes allows them to hydrolyze oxyiminocephalosporins, such as cefotaxime and ceftazidime. This confers the so-called "extended-spectrum" phenotype, also known as ESBLs [14].

Aminotransferase

The primary cause of resistance to polymyxins, specifically colistin, is generally attributed to chemical alterations of the lipid A moiety. These alterations result in a net decrease in negative charge, which in turn leads to a diminished affinity for the positively charged colistin molecule. The pmrHFIJKLM operon (also known as arnBCADTEF or pbgPE) has been identified as being associated with colistin-resistant bacteria. In a similar vein, plasmid-borne *mcr* genes, which possess phosphatidyl aminotransferase properties, facilitate the transfer of the phosphoethanolamine moiety to lipid A in the OM, thereby altering the structure of lipid A. Consequently, colistin is unable to penetrate into the periplasmic space, resulting in resistance to colistin [15].

Porin proteins

Porins are the most abundant proteins in the OM of Gram-negative bacteria. These water-filled open channels allow the selective entry of certain chemicals and are closely

https://doi.org/10.24171/j.phrp.2022.0323

associated with resistance to various antibiotics. For instance, β-lactams and fluoroquinolones penetrate the cell through the non-specific porin OmpF [16,17]. Some Gram-negative bacteria with mutations in OmpF have been reported to be resistant to several β-lactam antibiotics, such as *E. coli*, *K. pneumoniae*, *S. marcescens*, *P. aeruginosa*, and *Enterobacter aerogenes*. Carbapenem sensitivity in *A. baumannii* is associated with the reduced expression of many porins, such as Caro and Omp [18]. Colistin tolerance in *A. baumannii* is promoted by the loss of lipopolysaccharide, as well as OM proteins, resulting in a reduction in membrane integrity [19].

Target site modifications

Antibiotic target sites, including penicillin-binding proteins and DNA gyrase, can undergo modifications. Alterations have been found in fluoroquinolone antibiotic targets such as *parC* and *gyrA* in *P. aeruginosa* isolates, which confer resistance to fluoroquinolone. A single point mutation, C257T, in the *gyrA* gene results in an amino acid substitution in the gyrase A subunit. This mutation has been identified in fluoroquinolone-resistant *Campylobacter* strains and appears to be horizontally transferred among these strains. The emergence of the fluoroquinolone-resistant *Shigella sonnei* population, which first appeared in South Asia, was facilitated by the sequential accumulation of mutations (gyrA-S83L, parC-S80I, and gyrA-D87G) [20]. In contrast, resistance to aminoglycosides, which exert their antibacterial effect by binding and inhibiting bacterial 16S ribosomal RNA (rRNA), emerges through modification of the antibiotic target 16S rRNA [21–24]. Mutations in genes such as *pmrAB* and *phoPQ*, which are associated with OM lipid A modification, can lead to colistin resistance in *P. aeruginosa* [25]. Clarithromycin resistance can develop in bacteria owing to mutations in the 23S rRNA gene, such as A2142G, A2142C, or A2143G. These mutations decrease the drug's affinity, which is very important in the treatment of *H. pylori* infection [3,26].

Efflux pumps

A high level of intrinsic resistance in Gram-negative bacteria is largely mediated by resistance-nodulation-division efflux pumps, which are capable of transporting antibiotics out of the bacterial cell. Various experiments have demonstrated that the inactivation of 1 or more components of efflux pumps is associated with increased susceptibility to antibiotics. High levels of efflux pump expression are associated with strains isolated in clinical settings. Evidence suggests that other resistance mechanisms also depend on efflux pump-mediated resistance. For example, the loss of efflux activity can alter the expression of other genes. The deletion of acrB or tolC has been correlated with decreased expression of the OM porin OmpF in *Salmonella* [27]. Additionally, the presence or absence of efflux can influence the rate at which antibiotic resistance mutations occur in a population [28,29].

Treatment

Antibiotic resistance is a grave and escalating issue. The Centers for Disease Control and Prevention report that antimicrobial resistance claims over 700,000 lives annually, a figure projected to surge to 10 million by 2055. Consequently, the urgent discovery of new antimicrobial drugs is deemed a top priority. Recent advancements have begun to concentrate more on natural products, reintroducing natural product screening in the quest for innovative therapeutics to tackle resistant bacterial diseases. In this section, we will explore several ground-breaking alternative strategies that have surfaced from research and development programs, specifically for combating drug-resistant Gram-negative bacteria.

Antibiotic Adjuvants

Antibiotic adjuvants, also referred to as "resistance breakers" or "antibacterial potentiators," are chemicals that possess little to no antibiotic activity on their own. However, when these adjuvants are combined with other antibiotics in a treatment regimen, they enhance the efficacy of the antibiotic. The use of antibiotic adjuvants has revitalized the application of several antibiotics against resistant organisms, thereby reducing the need for the discovery of new, complex, and costly antibiotics, as their efficacy can be restored simply by adding adjuvants [1].

Thus far, 3 types of antibiotic adjuvants have been investigated or developed: lactamase antagonists, efflux pump antagonists, and OM permeabilizers (Table 1). These antibiotic adjuvants function via 1 of 4 mechanisms to aid antibiotics in overcoming bacterial resistance: inhibiting drug enzymes, facilitating drug efflux through efflux pumps, reducing absorption due to alterations in membrane permeability, and modifying drug targets [30].

β-Lactamase inhibitors

In clinical practice, the most commonly used antibiotic adjuvants are lactamase inhibitors [14,30]. They are employed to counteract resistance to β-lactam antibiotics [1]. Despite their long-term use, for over 70 years, β-lactamase inhibitors have been recognized as effective antibiotic adjuvants. These inhibitors work by hydrolyzing the amide bond of the 4-membered β-lactam ring in β-lactam antibiotics

Table 1. A list of adjuvants that are routinely used and their mechanisms and target bacteria

Adjuvants types	Compound name	Mechanism	Target bacteria
β-Lactamase inhibitors	Clavulanic acid	Used with β-lactamase-sensitive penicillins to protect them against the hydrolysis of their beta-lactam ring.	*Acinetobacter*
	Zidebactam	Inhibits penicillin-binding protein resulting in the formation of spheroplasts	Gram-negative multidrug-resistant microorganisms
	Cyclic boronate ester RPX7009 (vabrobactam)	Cyclic boronates potently inhibit both nucleophilic serine and zinc-dependent β-lactamases by a mechanism involving mimicking of the common tetrahedral intermediate	Enterobacteriaceae
Efflux pump inhibitors	Quinoline	Mechanism includes suppressing the expression of the genes that code for these pumps, hindering the assembly of pump components at the membrane level, blocking the expression of genes encoding these pumps or decreasing the required energy for these pumps to operate.	*Enterobacter aerogenes*
	7-Nitro-8-methyl-4-(2'(piperidino) ethyl)-aminoquinoline		*Escherichia coli*
	Conessine	It reduced the MIC of all antibiotics.	*Pseudomonas aeruginosa*
	3,4-Dibromopyrrole-2,5-dione compound	Reduced MIC of various antibiotics like chloramphenicol,aminoglycoside, macrolide etc.	*P. aeruginosa*
Drugs targeting bacterial cell permeability	Glycine-based peptides	Membrane barrier and ion-channel is disrupted resulting in leakage of ions Ca^{2+}, K^{+}, and Mg^{2+}, which cause cell death by leakage of ions, affect the surface tension of the membranes and disrupt ionic channels causing increase membrane permeabilisation.	*E. coli*
	Endogenous antimicrobial peptides	Forms pores	*Prokaryotes*
	Menadione	Causes damage to the integrity of the membrane	*Pseudomonas aeruginosa and Escherichia coli,*
	Naphthylacetylspermine	OM permeabilizer	*E. coli*
	Methoctramine (N,N'-[6-[[(2-methoxyphenyl) methyl] amino] hexyl]-1,8-octanediamine)	OM permeabilizer	*E. coli*
	Novobiocin and erythromycin	Increase in cell permeability	Gram-negative bacteria
	Squalamine	Membrane permeabilizer	*E. coli*

[1,14]. They can be divided into 2 distinct groups: (1) serine β-lactamases (SBLs) and (2) metallo-β-lactamases (MBLs). In SBLs, the lactamase's nucleophilic serine forms a covalent bond with a hydrolyzed β-lactam. These are classified as Ambler categories A, C, or D. Enzymes in this group are inhibited by sulbactam, tazobactam, and clavulanic acid. In contrast, MBLs, which contain 1 or 2 active site zinc ions, attack β-lactams via polarized water molecules. MBLs are classified as B1, B2, or B3, based on the number of bonded zinc ions and sequence identity. MBLs are effective against all β-lactam antibiotics, except for monobactams. Currently, there are no approved inhibitors for class B MBLs [31–33]. β-lactamase inhibitors are typically administered in conjunction with β-lactam antibiotics to inhibit bacterial β-lactamases. Generally, the antimicrobial spectrum of these combinations is determined by the activity of the β-lactam and the characteristics of the β-lactamase inhibitor.

Clavulanic acid and penicillin-based sulfones

Clavulanic acid is a β-lactam chemical produced by *Streptomyces clavuligerus*. It was discovered in 1976 that *S. clavuligerus* could produce clavulanic acid, making it the first agent used in conjunction with amoxicillin to inhibit β-lactamase. Structurally similar to penicillin, clavulanic acid forms a covalent bond with β-lactamase through a catalytic serine, creating a stable adduct. This binding occurs near the β-lactamase active site, effectively blocking enzymatic activity and enhancing the antibiotic's effects.

https://doi.org/10.24171/j.phrp.2022.0323

Currently, clavulanic acid is combined with amoxicillin and ticarcillin. However, it should be noted that clavulanic acid has no clinical effect against class B, C, and D β-lactamases.

Sulfones based on penicillin, such as sulbactam and tazobactam, among others, are highly effective in combating antimicrobial resistance. Sulbactam is a semi-synthetic β-lactamase inhibitor. It binds irreversibly to a β-lactamase in proximity to its active site, thereby inhibiting these enzymes and preventing the degradation of β-lactam antibiotics.

Tazobactam belongs to the class of penicillanic acids. It irreversibly attaches itself to the β-lactamase enzyme near its active site, thereby shielding β-lactam antibiotics from the enzyme's activity [34,35].

Zidebactam is a β-lactamase inhibitor classified as a bicyclo-acyl hydrazide [34,36]. Currently, WCK5222, a combination of zidebactam and cefepime, is undergoing phase 1 clinical trials investigating whether it could be used to treat severe infections caused by Gram-negative multidrug-resistant microorganisms [37].

Boronic acids as transition state analogues

The target enzymes of β-lactam antibiotics facilitate transpeptidase and carboxypeptidase reactions during the biosynthesis of bacterial cell walls. Both the formation and deacylation of acyl-enzyme complexes in transpeptidase and SBL catalysis occur via a high-energy tetrahedral (sp^3-hybridised) intermediate. Boronic acids and boronate esters, particularly cyclic ones, can effectively inhibit both SBLs and MBLs. They do this by rapidly reacting with SBLs and MBLs to form stable enzyme-inhibitor complexes that mimic the common high-energy tetrahedral intermediates in SBL/MBL catalysis [38].

The cyclic boronate ester RPX7009 (vaborbactam), which has been shown to restore carbapenem activity against KPC25, is the most promising homologue to date [39]. Carbavance, a combination of RPX7009 and biapenem, is currently in phase 3 clinical trials for the treatment of KPC-producing Enterobacteriaceae infections [38]. Despite their effectiveness, no standalone (cyclic) boronates have been developed for clinical use that can compare to the currently used β-lactam antibiotics. However, there are reports suggesting that monocyclic vaborbactam and, in particular, bicyclic boronates (VNRX-5133 and related compounds) hold potential for use as broad-spectrum β-lactamase inhibitors when used in conjunction with suitable β-lactam antibiotics.

Efflux pump inhibitors

Given the role of efflux pumps in antibiotic resistance, the importance of their inhibitors cannot be overstated. Most efflux pump inhibitors (EPIs) function by physically blocking substrate molecules from passing through the transporter. There are several EPIs commonly used in therapy, including reserpine, which is used to combat resistance to tetracycline and norfloxacin, and berberine, which is used to counteract imipenem resistance caused by *P. aeruginosa* [40]. Berberine, although not as effective alone, has been shown to potentiate ciprofloxacin against multidrug-resistant *K. pneumoniae* isolates [41]. PaβN also has shown broad effectiveness against antibiotic-resistant bacteria [42]. Alkylaminoquinazoline derivates have been found to be able to restore antibiotic activity in Gram-negative resistant isolates [43,44]. PAβN, 1-(1-naphthylmethyl)-piperazine (NMP) and carbonyl cyanide 3-chlorophenylhydrazone (CCCP) are the most studied synthetic EPIs studied with *A. baumannii* [43].

Membrane permeabilizers

Membrane permeabilizers increase the permeability of the Gram-negative OM, facilitating the increased entry of antibiotics. These substances can chelate and eliminate positively charged divalent ions from the OM, leading to a disruption in the structural organization of the OM [45].

Antibiotic Alternatives

Bacteriophages

Bacteriophages are bactericidal viruses that infect bacteria. Phage therapy, an alternative to antibiotics, is regaining interest worldwide due to the rise of broad-spectrum antibiotic resistance among various bacterial species. Lytic phages, which lyse their host bacterial cells, are particularly intriguing as potential antimicrobial agents. Numerous proteins assist in this process, including the widely studied holin protein. These small hydrophobic proteins create holes in the bacterial membrane, leading to a subsequent loss of proton motive force across the membrane. For instance, the *S. aureus* phage GH15 holin protein HolGH15 has demonstrated activity against *Listeria monocytogenes* in addition to *S. aureus* [46]. Another phage-encoded protein, endolysin, which is involved in peptidoglycan degradation, is also being explored as a potential antimicrobial agent. It has been reported that extracellularly supplied endolysin can cause lysis of the cell to which it has been applied, through a mechanism known as "lysis from without." Many endolysins have shown broad-spectrum activity. For example, Salmonella phage BSPM4 M4Lys endolysin has demonstrated *in vitro* activity against numerous Gram-negative bacteria, such as *Salmonella enterica*, *E. coli*, *P. aeruginosa*, and *Cronobacter sakazakii* [47,48]. LysSAP26 endolysin can inhibit many drug-resistant bacteria, such as *A. baumannii*, *E. coli*, *K.*

pneumoniae, *P. aeruginosa*, *S. aureus*, and *E. faecium* [49]. However, endolysin activity against Gram-negative bacteria is somewhat limited by the presence of an OM, which inhibits the synergistic effect of endolysin with antibiotics. A study demonstrated that the combination of Cp-711 with the β-lactam antibiotic amoxicillin or cefotaxime can be effective against drug-resistant *Streptococcus pneumoniae*. Endolysin LysECD7 was found to be effective against biofilms formed by *K. pneumoniae* [50]. Endolysins are also being used to create "enzybiotics," which can be modified by adding extra domains to enhance their peptidoglycan-degrading ability. These fusions have shown activity against many Gram-negative pathogens [51]. The OM, which presents a significant resistance to phage endolysins, can be destroyed by another phage protein, called spanin. Holin, endolysin, and spanin together form the "lytic cassette." As such, these proteins could also be exploited as potential tools against drug-resistant Gram-negative bacteria. Another protein, amurin, mimics the mode of action of β-lactam antibiotics and could be used as an alternative to antibiotics. While phage resistance in bacteria is not uncommon, phages can mutate and counter the phage resistance of bacteria.

One benefit of utilizing phages as alternatives to antibiotics is their host specificity. Unlike antibiotics, phages do not harm the normal microflora. Additionally, phages do not carry the typical side effects associated with antibiotics, such as allergic reactions and anaphylactic shock.

However, bacterial resistance to phages is inevitable. This resistance can hinder phage therapy due to the potential emergence of phage-resistant bacterial variants. A continuous "arms race" exists between the phage and its host, resulting in the co-evolution of both the virus and the bacteria. This co-evolution and resulting adaptability pose concerns for the development of phage therapy. However, the issue of phage resistance can be mitigated by using "phage cocktails," which involve the use of more than 1 type of phage [52].

The most recent clinical trial involving bacteriophages was a phase 1/2 randomized, controlled, double-blind study. The aim was to determine the effectiveness and tolerability of Phago-Burn, a combination of 12 naturally lytic *P. aeruginosa* bacteriophages in an alginate template, for treating *P. aeruginosa*-infected burn wounds [53,54]. In this trial, Phago-Burn was immediately applied to the wound. It was observed that the concentration of the phage cocktail had decreased after production, resulting in patients receiving a lower dosage than initially anticipated. Several commercial applications of phage therapy have been demonstrated, including Agriphage (Omnilytics Ltd.), Listex (Micreos Ltd.), SalmFresh (Intralytix Ltd.), ListShield (Intralytix Ltd.), and EcoShield (Intralytix Ltd.). Further research is required to establish a standard protocol that ensures public safety.

Direct-acting compounds

Plant-derived antimicrobial compounds

Plants are rich sources of various bioactive molecules, such as polyphenols, phenols, terpenoids, numerous phytochemicals, and essential oils. Many of these have significant antimicrobial effects. For instance, allicin, derived from garlic, can inhibit a broad range of bacteria, including *Escherichia* (including enterohaemorrhagic *E. coli* 0157 and enterotoxigenic *E. coli*), *Salmonella*, *Streptococcus*, *Staphylococcus*, *Klebsiella*, *Proteus*, and *H. pylori*. Many plant-derived compounds have been reported to inhibit quorum sensing in bacteria, resulting in reduced motility and biofilm formation, factors directly related to antibiotic resistance. Many phytochemicals are currently being studied as potential alternatives to antibiotics, particularly in antibiotic-resistant bacteria, including multidrug-resistant strains [55–57]. Extracts from thyme, lemongrass, tulsi, neem, aloe vera, oregano, and rosemary have been shown to be effective against multidrug-resistant clinical isolates [58]. Tannins and flavonoids from plant extracts were even found to inhibit methicillin-resistant *S. aureus* (MRSA). Extracts of various plants like *Lawsonia, Curcuma longa, Zingiber officinale*, and *Tinospora cordifolia* were shown to be capable of inhibiting clinical isolates. The synergistic action of antibiotics and plant chemicals has also been proven to be effective against a range of resistant pathogens, supporting the use of plant-derived chemicals against drug-resistant bacteria [59]. Boswellic acid, derived from *Boswellia* sp., has been reported to be effective against *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *E. coli* biofilms when used along with antibiotics.

Antimicrobial peptides

Antimicrobial peptides (AMPs) are mostly cationic short peptides that can inhibit ion channels, protein transport and enzymatic activities. They are diverse in their nature, activities, and sources.

Plant-derived AMPs: Plants produce AMPs as a component of their innate defence mechanism against pathogens. Some of the significant plant-derived AMPs include defensins, 2S albumins, glycine-rich proteins, lipid transfer proteins, snakins, thionins, cyclotides, and napins [60]. For instance, a recombinant plant AMP from tomatoes, when overexpressed in *E. coli*, was shown to be active against a host of fungi and bacteria [61].

Insect-derived AMPs: Insect-derived AMPs present excellent alternatives to traditional antibiotics. Four distinct categories of insect AMPs exist: defensins, cecropins, drococins, and

https://doi.org/10.24171/j.phrp.2022.0323

attacins. These have been found to be effective against bacteria such as *E. coli* [62]. Insect-derived AMPs play a significant role in combating many Gram-negative pathogens, for example, cecropins produced by *Diptera* and *Lepidoptera* [63].

Bacterial AMPs (bacteriocins): Bacteriocins are bacterial AMPs. They are highly effective at inhibiting a variety of bacterial species. For example, pyocin, a phage-derived protein synthesized by *P. aeruginosa*, provides a selective advantage to its host organism within a mixed population [64]. Another bacterial AMP, nisin, which is produced by *Lactococcus lactis*, has been approved as an alternative to antibiotics for combating Gram-negative pathogens [65].

Animal AMPs: Tachyplesin III, an AMP derived from the horseshoe crab, has been demonstrated to effectively clear infections of *P. aeruginosa* and *A. baumannii* in mouse models [66]. Esculentin-1, an AMP derived from frogs of the genus *Rana*, was found to be potent against Gram-negative pathogens such as *P. aeruginosa* and *E. coli*. Likewise, Brevinin-2Ta displayed potency against *Klebsiella* [67,68]. Additionally, temporin-1Ja derived from the skin secretions of *Rana japonica* was able to neutralize endotoxin [69]. Recently, mammalian AMPs are found to be very important in combatting antibiotic resistant bacteria. The mammalian AMPs are categorized into two groups namely: Cathelicidins and Defensins. Cathelicidins have shown broad spectrum antimicrobial action. Bovine defensins were shown to be active against various Gram-negative pathogens such as *E.coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Equine α and β Defensins also were observed to show broad spectrum antimicrobial action against Gram-negative pathogens. Furthermore, these peptides can stimulate the immune system to act against evading bacteria. Certain mammals can produce proline rich antimicrobial peptides those show profound antimicrobial activity by inhibiting bacterial protein synthesis. Mammalian milk is an important source of AMPs. Lactalbumin, β-Lactoglobulin, lactoferrin and many others function as potent antibacterial compound. For example, β-Casein 211–225 from human milk was found to show high activity against *E.coli* and *Y. enterocolitica*.

DCAP (2-((3,6-dichloro-9H-carbazol-9-yl)-2-hydroxypropyl) amino)-2-(hydroxymethyl)propane-1,3-diol

DCAP is a broad-spectrum antibiotic that exhibits potential bactericidal effects against various strains of both Gram-positive and Gram-negative bacteria, including *E. coli* and *P. aeruginosa*. As a membrane-targeting drug, DCAP inflicts cellular damage on both Gram-positive and Gram-negative bacteria through 2 primary mechanisms. First, it enhances ion transport across the membrane, which reduces the membrane potential and results in the mislocalization of 2 crucial membrane proteins, MinD and FtsA. Second, it decreases the permeability of the lipid bilayer [70,71]. DCAP is a membrane-active antibiotic that also possesses the added benefit of eliminating dormant bacteria and biofilms.

Odilorhabdins

Odilorhabdins can effectively kill both Gram-positive and Gram-negative bacteria, particularly ceftazidime-resistant Enterobacteriaceae. They function as inhibitors by binding to specific subunits of bacterial ribosomes and interacting with rRNA and transfer RNA (tRNA). This interaction decodes the translation machinery and enhances the binding affinity of non-cognate aminoacyl tRNAs for the ribosome [3,72].

Use of repurposed drugs

Repurposed marketed drugs can provide an effective solution to the issue of antibiotic resistance. Ciclopirox, an antifungal drug, has demonstrated significant antibacterial activity against high-priority, multidrug-resistant Gram-negative bacteria such as *A. baumannii*, *E. coli*, and *Klebsiella*. It has been found to inhibit the synthesis of lipopolysaccharide in Gram-negative bacteria and chelate iron, leading to bacterial inhibition. The anti-diarrheal drug loperamide, when used synergistically with various antibiotics like tetracycline, cephalosporin, and polymyxin B, has been shown to sensitize Gram-negative bacteria to Gram-positive antibiotics such as novobiocin. This may result in changes to the shape of the bacterial cell, leading to dysregulation of the cell's influx and efflux mechanisms. Berberine, another anti-diarrheal drug, has shown efficacy against multidrug-resistant *Mycobacterium tuberculosis* and MRSA. A prime example of a repurposed drug is niclosamide, an anti-helminthic drug. Niclosamide has been found to inhibit quorum sensing, leading to the subsequent inhibition of virulence factors and biofilm formation in *P. aeruginosa* [73]. Clofoctol, a drug used for treating upper respiratory tract and tracheobronchial infections, has also been found to significantly reduce biofilm formation in *P. aeruginosa* [74].

Anti-virulence therapy

Quorum sensing inhibitors

Microbial chemical communication, or quorum sensing, refers to the coordinated regulation of gene clusters by a community. This regulation governs processes such as the production of virulence factors, antibiotic sensitivity, and biofilm formation. Autoinducer-2 (AI-2) is a so-called universal autoinducer that controls intraspecies and interspecies bacterial communication [75]. In Gram-positive bacteria, oligopeptides are the most common quorum sensing mediators. However, in Gram-negative bacteria, N-acyl homoserine lactones

are the most prevalent. Autoinducer analogues have been demonstrated to be effective quorum sensing inhibitors. For instance, thiolactone analogues of autoinducers have been shown to be potent quorum sensing inhibitors in *E. coli*, while 3-amino-2-oxazolidinone analogues were found to be effective against *P. aeruginosa* [76]. Tests have also been conducted on non-analogue quorum sensing inhibitors. For example, halogenated furanones have been reported to reduce quorum sensing in *Pseudomonas* [76].

Fimbriae antagonists (glycomimetic-based therapy)

Glycomimetics, which emulate carbohydrate structures, can act as inhibitors of many natural ligands involved in the bacterial pathogenesis pathway. Urinary tract infections caused by uropathogenic *E. coli* are a major concern, as these bacteria are typically multidrug-resistant and have recently developed resistance to last-resort antibiotics such as colistin and carbapenems. These bacteria produce type 1 fimbriae, which are necessary for their colonization, biofilm formation, and pathogenicity. The FimH adhesin, located at the distal tip of these appendages, binds to mannosylated glycoproteins on urinary tract epithelial cells, promoting bacterial adhesion. Small-molecule mannoside ligand antagonists, a type of glycomimetic, target FimH and subsequently inhibit bacterial colonization [77].

RNA-based therapeutics

Short antisense oligonucleotides (ASOs) are currently being explored as a powerful alternative to antibiotics for treating Gram-negative bacteria. These ASOs target the RNA products of specific essential genes within the bacteria. These "programmable RNA antibiotics" are undergoing testing against several different pathogens, including *E. coli*, *Acinetobacter*, *Campylobacter*, *Haemophilus*, *Klebsiella*, *Pseudomonas*, and *Salmonella* [78,79]. The inherent instability of RNAs is addressed by creating synthetic modified polymers, often attached to a polypeptide backbone. These polymers are also engineered to resist nucleases and proteases. Various types of these modified synthetic ASOs are available, such as locked nucleic acids (LNA), phosphorodiamidate morpholino oligomers (PMO), and peptide nucleic acids (PNA) [80]. However, this method has a significant limitation. To date, it has been targeted towards a limited set of well-defined pathogens, as listed above. Yet, information about the vast majority of microbiota remains unknown. Consequently, ASO-based targeting is challenging for many other human pathogens, such as the human commensal turned pathogen *Fusobacterium nucleatum*. This bacterium has garnered significant attention due to its association with human diseases such as colorectal cancer, making it a potential target for ASO-based therapy, which could selectively target *F. nucleatum* in the colon. However, due to the sparse transcriptomic data available for this bacterium, pursuing ASO therapy for this specific purpose is difficult [81].

Nanomaterial-based therapy

Nanoparticles (NPs) which are organic, inorganic, or hybrid particles ≤500 nm in size, are currently under evaluation as an alternative treatment for drug-resistant bacteria. Nanomaterials target a broad spectrum, including the cell wall/cell membrane, reactive oxygen species (ROS) generation, and intracellular component damage. Various types of metal-based and carbon-based NPs, such as quantum dots, polymeric NPs, nanocomposites, and smart nanomaterials, are being utilized against bacteria. These are effective against both planktonic forms and bacterial biofilms. Compared to conventional antibiotics, NPs are less susceptible to existing bacterial resistance mechanisms [82]. Carbon-based NPs have been found to inhibit *Klebsiella oxytoca* and *P. aeruginosa*–mediated biofilm formation [83]. Polymeric NPs, such as glycopeptide dendrimers, have also been found to inhibit *P. aeruginosa* biofilms [84]. Star-shaped polymeric peptide NPs (SNAPPs) have been reported to exert activity against ESKAPE pathogens, which comprise *E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* species and are violent nosocomial pathogens [85]. SNAPPs employ a range of bactericidal mechanisms, from membrane disruption and ion exchange to apoptosis-like death mechanisms. Most notably, SNAPPs have been successful in inhibiting colistin-resistant Gram-negative infections and remain potent against multidrug-resistant *A. baumannii*, which has yet to develop resistance against SNAPPs. Liposome-based NPs have been found to restore the potency of several antibiotics against certain drug-resistant Gram-negative pathogens [86,87].

Miscellaneous

Other antibiotic alternatives are currently under study and have shown efficacy.

Carbon dots/carbon quantum dots

Carbon dots are utilized as drugs or drug carriers, demonstrating exceptional efficiency in real-time monitoring of treatment processes. Their antibacterial action is light-driven, and the side effects of their actions are minimal. The mechanism of action involves the photo-induced production of ROS, which damage bacterial DNA, RNA, and proteins. This process also disrupts the bacterial membrane. Broad-spectrum antibacterial action by carbon dots has been observed in cases

https://doi.org/10.24171/j.phrp.2022.0323

involving multidrug-resistant *E. coli* [88]. Given their relative affordability and minimal side effects, these materials could potentially serve as an alternative to antibiotics.

Silver-doped phosphate coacervates

Silver-doped phosphate coacervates serve as effective alternatives to antibiotics due to their ability to sustainably release silver, which exhibits a significant antimicrobial effect. These coacervates have demonstrated potent activity against wound-related infections, particularly those associated with *E. coli* and *P. aeruginosa* [89].

Royal jelly nutraceutical

Royal jelly (RJ) is a yellowish-white viscous fluid formed by worker honeybees. It is known for its ability to inhibit disease-causing bacteria, particularly those associated with periodontal diseases, specifically targeting anaerobic ones [90]. RJ has also been proven to be a potent anti-biofilm agent, capable of inhibiting clinical isolates of *P. aeruginosa* as well [91]. Another advantage of RJ treatment is its relative lack of side effects.

Predatory bacteria

Multidrug-resistant Gram-negative pathogens can be targeted by predatory bacteria such as *Bdellovibrio* spp. and *Micavibrio* spp. These are Gram-negative bacteria and exhibit a growth phase within the host bacteria. Therefore, they can be used as alternatives to antibiotics and can be used as tools against multidrug-resistant bacteria. *Bdellovibrio bacteriovorus* HD100 has been reported to reduce the growth of multidrug-resistant Gram-negative bacteria, such as *A. baumannii*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. *Micavibrio aeruginosavorus* ARL-13 was found to prey on *P. aeruginosa* and *K. pneumoniae*.

Nanobodies

Nanobodies are heavy chain-only antibodies of the IgG subtype, possessing a highly stable antigen-binding region. This region allows the nanobodies to bind to and inactivate pathogen proteins, thereby inhibiting the pathogens. A study involving mice demonstrated that nanobodies can protect these animals from infection by enterotoxigenic *E. coli* [92].

Conclusion

Multidrug-resistant Gram-negative pathogens, specifically those on the WHO priority bacteria list, are a major focus in public health. Promising new techniques are being developed to overcome the innate and acquired resistance of Gram-negative bacteria. These approaches are characterized by a diverse range of scientific concepts. Therapies such as the use of β-lactamase-inhibiting adjuvants in combination with antibiotics have proven effective in inhibiting the growth of resistant strains of Gram-negative bacteria. Another promising approach is to explore nature for antibacterial agents that can be used as targeted therapeutics, such as bacteriophages, RNA-based therapies, fimbriae antagonists, nanobodies, and AMPs. These methods show promise in combating the silent pandemic of antimicrobial resistance. Indeed, a vast number of alternative approaches are currently under investigation. However, there are several challenges. Translational hurdles are not being adequately addressed. There is a clear trend towards narrow-spectrum or even pathogen-specific approaches, which require a highly developed diagnostic infrastructure that may not be accessible in developing and underdeveloped nations. Adjunctive therapies require an active antibacterial drug and therefore do not fully address the problem of current antibiotic resistance.

Misuse and overreliance on antibiotics have contributed to the global spread of antibiotic resistance. It is crucial to educate people on the proper use and dispensation of antibiotics, and to implement further regulations and controls. The WHO's list of priority diseases serves as both an incentive and a framework for developing new antimicrobials and combining new and older medications to combat the rise in multidrug-resistant Gram-negative infections. The success of these novel antibacterial drugs hinges on increased efforts by global governments to stimulate research and development, alongside comprehensive antimicrobial stewardship initiatives and specific local resistance knowledge. To ensure the efficacy of future antimicrobial therapies, protocols must be established to prevent the incorrect prescribing and misuse of antimicrobial drugs in agriculture.

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Funding
None.

Availability of Data
All data generated or analyzed during this study are included in this published article. For other data, these may be requested through the corresponding author.

Authors' Contributions
Conceptualization: PB; Data curation: all authors; Formal analysis: PB, BM;

Funding acquisition: NA; Investigation: PB, BM; Methodology: BM, PR, AC; Project administration: PB; Resources: PB; Software: PB; Supervision: PB; Validation: PB, BM; Visualization: BM, AC, PR; Writing–original draft: PB, BM; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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https://doi.org/10.24171/j.phrp.2022.0323

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0124
Osong Public Health Res Perspect 2023;14(5):347-355

Original Article

Drug resistance and the genotypic characteristics of *rpoB* and *katG* in rifampicin- and/or isoniazid-resistant *Mycobacterium tuberculosis* isolates in central Vietnam

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ABSTRACT

Objectives: Tuberculosis (TB) and drug-resistant TB (DR-TB) are national health burdens in Vietnam. In this study, we investigated the prevalence of rifampicin (RIF) and/or isoniazid (isonicotinic acid hydrazide, INH) resistance in patients with suspected TB, and applied appropriate techniques to help rapidly target DR-TB.
Methods: In total, 1,547 clinical specimens were collected and cultured using the BACTEC MGIT system (Becton Dickinson and Co.). A resazurin microtiter assay (REMA) was used to determine the proportions of RIF and/or INH resistance. A real-time polymerase chain reaction panel with TaqMan probes was employed to identify the mutations of *rpoB* and *katG* associated with DR-TB in clinical isolates. Genotyping of the identified mutations was also performed.
Results: A total of 468 *Mycobacterium tuberculosis* isolates were identified using the REMA. Of these isolates, 106 (22.6%) were found to be resistant to 1 or both antibiotics. Of the resistant isolates, 74 isolates (69.8%) were resistant to isoniazid (INH) only, while 1 isolate (0.94%) was resistant to RIF only. Notably, 31 isolates (29.24%) were resistant to both antibiotics. Of the 41 phenotypically INH-resistant isolates, 19 (46.3%) had the Ser315Thr mutation. There were 8 different *rpoB* mutations in 22 (68.8%) of the RIF-resistant isolates. The most frequently detected mutations were at codons 531 (37.5%), 526 (18.8%), and 516 (6.3%).
Conclusion: To help prevent new cases of DR-TB in Vietnam, it is crucial to gain a comprehensive understanding of the genotypic DR-TB isolates.

Keywords: Drug resistance; Genotypic characteristics in *rpoB* and *katG*; Real-time polymerase chain reaction TaqMan; Resazurin microtiter assay; Tuberculosis in central Vietnam

Received: May 9, 2023
Revised: July 31, 2023
Accepted: August 1, 2023

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Introduction

Tuberculosis (TB) poses a significant health burden worldwide, and drug-resistant TB (DR-TB) is one of the biggest challenges to TB treatment [1–3]. Therefore, developing diagnostic methodologies for TB and DR-TB is a critical aspect of TB management. In 2019, the World Health Organization (WHO) estimated that 10.4 million patients had TB, and 1.5 million deaths were attributed to the disease. Vietnam is currently ranked 16th among the 30 countries with high burdens of TB and 13th among the 30 countries with the highest prevalence of DR-TB [4]. Although the Vietnam National Tuberculosis Control Program has nationwide coverage, diagnosing, treating, and managing TB and DR-TB remains a significant public health challenge [5,6]. Commercial drug susceptibility testing (DST) systems such as the liquid-medium BACTEC MGIT 960 (Becton Dickinson and Co.) and molecular DST methods such as the GeneXpert MTB/RIF (Cepheid) provide rapid results and reduce turnaround time [1,7]. However, they require costly reagents, modern equipment, and only detect some drugs, limiting their availability in Vietnamese laboratories. Therefore, developing phenotypic DST for *Mycobacterium tuberculosis* that determines the minimum inhibitory concentration (MIC) values of many TB drugs will help doctors make better treatment decisions by considering the level of resistance of each drug. These methods are simple, reliable, and cost-effective, making them necessary in countries with a high prevalence of DR-TB [8–10]. Moreover, there are limited genetic studies that characterize the genotype of *M. tuberculosis* isolates in central Vietnam. A thorough understanding of the genotypic DR-TB isolates helps support a focus on infection control and surveillance to prevent new cases of DR-TB in this region. To reduce the morbidity and mortality of DR-TB, accurate and rapid diagnosis in the early stages of TB is important. This requires implementing a molecular assay that can detect the wide variety of drug-resistant mutations in clinical isolates of *M. tuberculosis*. Evaluating the molecular assays that can detect a wide variety of *M. tuberculosis*-resistant mutations in clinical isolates is vital in the effort to reduce DR-TB morbidity and mortality by enabling accurate and rapid diagnosis in the early stages [11,12]. Therefore, to assist in decreasing DR-TB cases in Vietnam, it is necessary to combine phenotypic and molecular methods until more is understood about the clinical relevance of phenotypic susceptibility in isolates with drug-resistant mutation mechanisms. This study evaluated the frequency of TB resistance to rifampicin (RIF) and isoniazid (isonicotinic acid hydrazide, INH), which are the most effective first-line drugs in TB treatment.

HIGHLIGHTS

Tuberculosis (TB) and drug-resistant TB (DR-TB) are serious public health problems. New strategies are urgently needed to manage and reduce the mortality related to this disease, especially in resource-limited settings. To prevent new cases of DR-TB in Vietnam, a comprehensive understanding of the genotypes of DR-TB isolates is crucial so that the most appropriate and effective diagnostic methods for detecting DR-TB can be developed and implemented.

Materials and Methods

Sample Collection

From June 2019 to June 2020, samples were collected from patients suspected of TB, and *M. tuberculosis* isolates were cultured for DST at various locations in central Vietnam, including Da Nang Lung Hospital, Central Hospital 71, Thanh Hoa, and the Department of Microbiology at Hue Central Hospital in Hue city. The Carlo Urbani Center's Department of Microbiology at Hue University of Medicine and Pharmacy conducted the real-time (RT)-polymerase chain reaction (PCR) TaqMan allelic discrimination assays.

Drug Susceptibility Testing

DST was performed using the resazurin microtiter assay (REMA) method. A total of 1,547 clinical samples were inoculated in the BACTEC MGIT 960 (Becton Dickinson and Co.), of which 500 were found to be positive for *M. tuberculosis*. The *M. tuberculosis* strain H37Rv (ATCC 27294) was utilized as the susceptible control strain, while a previously identified multidrug-resistant (MDR)-TB strain from the microbiology department at Da Nang Lung Hospital was employed as the resistant control strain.

Middlebrook 7H9 broth was dispensed into each well of a Corning 96-well plate for the REMA. All drugs (HiMedia Laboratories Private Ltd.) were used in lyophilized form and subsequently rehydrated and filtered to make the stock solution. INH was diluted with sterile water to reach a concentration of 1 mg/mL, while RIF was diluted with methanol to achieve a concentration of 10 mg/mL. The INH concentration ranged from 1.00 to 0.031 μg/mL, and the RIF concentration ranged from 2.00 to 0.061 μg/mL. To determine the resistance or susceptibility of *M. tuberculosis*, a strain was considered resistant to INH if the MIC was ≥0.25 μg/mL, while a strain was deemed resistant to RIF if the MIC was ≥0.5 μg/mL. This method, developed by Palomino et al. [9] in 2002 and Martin et al. [13] in 2005 using the colorimetric

https://doi.org/10.24171/j.phrp.2023.0124

indicator resazurin, has been proposed for the DST of *M. tuberculosis*. To confirm continued susceptibility or resistance, independent of the MIC, the results of the REMA were compared with those of the reference method using a liquid culture in the BACTEC MGIT mycobacterial detection system (Becton Dickinson and Co.). The commercial kits that we utilized were supplied with fixed concentrations of 0.1 μg/mL and 1.0 μg/mL, for INH and RIF, respectively (Figure 1) [9,13].

RT-PCR TaqMan Allelic Discrimination Assay

DNA extraction

A total of 52 clinical isolates of *M. tuberculosis* (31 MDR, 1 RIF mono-resistant, 10 INH mono-resistant, and 10 drug-sensitive isolates) were used to extract genomic DNA. A 200-μL sample was placed in an Eppendorf tube and 400 μL of InstaGene Matrix (Bio-Rad Laboratories) was added. This was followed by vortexing and incubating the mixture at 100°C for 10 minutes. The mixture was then centrifuged at 14,000 rpm for 2 minutes, and the DNA in the supernatant was collected and stored at −20°C.

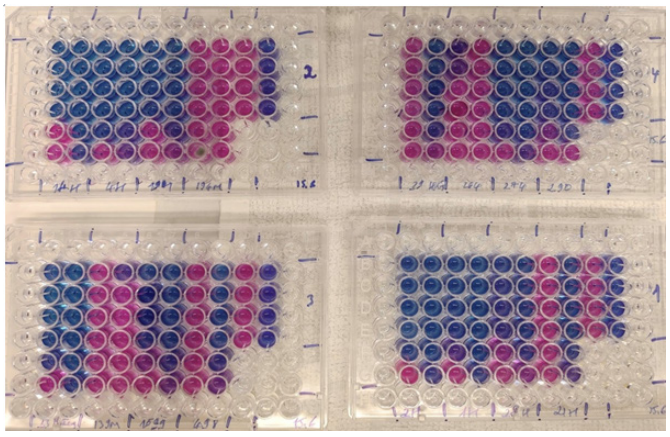

Figure 1. The resazurin microtiter assay results in a study of *Mycobacterium tuberculosis* isolates for analysis of drug-resistant tuberculosis in Vietnam.

Primers and probes

To conduct the allelic discrimination test, primers were designed to amplify a sequence of 208 base pairs (bp) in the *rpoB* gene and 110 bp in the *katG* gene. Four TaqMan probes, which could distinguish one-base mismatches, were used to detect genetic variation in the wild-type *M. tuberculosis* strain (H37Rv) in both *rpoB* and *katG*. All primers and probes used in this study were manufactured by Integrated DNA Technologies Inc. (Table 1) [14,15].

Procedure

To detect mutations in the selected regions of the *rpoB* and *katG* genes, 2 separate reactions were conducted in tube A (for *rpoB* mutation detection) and tube B (for *katG* mutation detection) with a final reaction volume of 25 μL. The master mix used in this study was produced by Integrated DNA Technologies Inc. An optimized multiplex-probe RT-PCR reaction was established, and PCR amplification was performed as follows: initial denaturation at 94°C for 10 minutes, followed by 40 cycles at 94°C for 25 seconds and 60°C for 55 seconds, using the Mx3000P qPCR System (Agilent Technologies Inc.).

Table 1. Primers and probes used in the RT-PCR TaqMan allelic discrimination assay

Primer or probe	Target region	Concentration (μM)	Oligonucleotide	Product size (bp)	Design
RT-PCR					
Primers	*rpoB*	1.0	F: 5′-TCACACCGCAGACGTTGATC-3′ R: 5′-CGTAGTGCGACGGGTGC-3′	208	[14]
	KatG	1.0	F: 5′-GGGCTTGGGCTGGAAGA-3′ R: 5′-GGAAACTGTTGTCCCATTTCG-3′	110	
Probes					[14]
	rpoB TB control	0.5	5′-HEX-CGATCAAGGAGTTCTTCGGCACCA-BHQ-3′		
	rpoB1 510-516	0.5	5′-FAM-CAGCTGAGCCAATTCATGGACCAGA-BHQ-1-3′		
	rpoB2 526-531	0.5	5′-HEX-CACAAGCGCCGACTGTCGGC-BHQ-1-3′		
	katG 311-316	0.5	5′-FAM-ACGCGATCACCAGCGGCA-BHQ-1-3′		
Sequencing primers	*rpoB*	1.0 1.0	F: 5′- GTCAGACCACGATGACCGTT-3′ R: 5′- GAGCCGATCAGACCGATGTT-3′	445	This study
	KatG	1.0 1.0	F: 5′- CCCATGTCTCGGTGGATCAG-3′ R: 5′- GGCGGTCACACTTTCGGTAA-3′	475	

RT, real-time; PCR, polymerase chain reaction; bp, base pairs; TB, tuberculosis.

Allelic discrimination data analysis

We determined the cycle threshold (Ct) value from the TB control probe that bound to the 81 bp hot spots outside the target regions in the *rpoB* gene, and calculated the ΔCt value, which represented the difference between the control TB probe and each specific probe (ΔCt = mutant Ct−contr ol TB Ct). In the mutant genotypes, a single base variation in the target sequence of *rpoB* or *katG* could lead to mismatching of the corresponding probe and failure to combine with the target, resulting in probe dropout from the sequence. This would generate negative fluorescence signals during amplification, and the Ct values of the mutant sequences would be recorded as zero (Ct = 0). Ultimately, *M. tuberculosis* was identified using the TB control probe.

rpoB and *katG* sequencing

DNA samples extracted from the 52 isolates that had been identified as *M. tuberculosis* by RT-PCR, were used to amplify *rpoB* and *katG* using the primer sequences presented in Table 1. PCR amplification was performed as follows: an initial denaturation step at 95°C for 10 minutes, followed by 36 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and a final extension step at 72°C for 5 minutes in the Veriti Thermal Cycler (Thermo Fisher Scientific Inc.). The PCR products were then sent to Apical Scientific Sdn Bhd for Sanger sequencing. The sequencing data were initially analyzed using Sequencing Analysis Software ver. 6.0, and quality control was checked by Sequence Scanner Software (both from Thermo Fisher Scientific Inc.).

Ethics Approval

Ethical approval for this study was granted by The Ethical Committee in Biomedical Research of the University of Medicine and Pharmacy-Hue University (No: H2019/350).

The director of each hospital granted permission to conduct the study. Before specimen collection, patients/guardians were provided with information about the study and asked to provide written informed consent. Participants were informed of their right to voluntarily participate in the study.

Results

Among the 1,547 clinical specimens collected from patients suspected of having TB, 32.3% (500/1,547 samples) were identified as positive through the BACTEC MGIT 960 system (Becton Dickinson and Co.). RT-PCR was also able to detect all *M. tuberculosis* isolates. Of the 500 isolates, 6.4% (32/500 samples) were excluded from the DST because they were heavily contaminated.

Phenotypic DST by REMA

A total of 468 *M. tuberculosis* isolates underwent REMA testing, which revealed that 106 (22.6%) were drug-resistant. Specifically, 74 (69.8%; 95% confidence interval [CI], 60.1%–78.4%) wer e resistant to INH, while 1 (0.94%; 95% CI, 0.02%–5.1%) was r esistant to RIF, and 31 (29.24%; 95% CI, 20.8%–38.9%) wer e resistant to both antibiotics (MDR-TB strains). When the REMA results were compared to those independently obtained using the BACTEC MGIT 960 system (Becton Dickinson and Co.), the sensitivity and specificity for INH resistance were 100% and 99.19%, respectively. The sensitivity and specificity for RIF resistance were both 100%, the overall concordance for MDR-TB was 99.78%, and the positive predictive value and negative predictive value for MDR-TB detection were 96.77% and 100%, respectively (Table 2).

Genotypic Detection of Drug-Resistant Isolates

Of the phenotypic INH-resistant isolates, 46.3% (19/41) had a Ser315Thr mutation (AGC > ACC). Among the 31 MDR and 1 RIF-resistant isolates, 22/32 (68.8%) had 8 types of *rpoB* mutations. The most frequent *rpoB* mutations were found at codons 531, 526, and 516, accounting for 37.5% (12/32), 18.8% (6/32), and 6.3% (2/32), respectively. Sequencing analysis detected the point mutation Ser522Leu (TCG > TTG) outside of the 2 probes used to determine mutations in *rpoB*. None of the 11/11 INH-susceptible isolates and 20/20 RIF-susceptible isolates had mutations in *katG* and *rpoB* (Figure 2).

The RT-PCR allelic and DNA sequencing results showed a sensitivity and specificity of 95.5% and 100%, respectively, for detecting INH resistance through mutation analysis of *katG* codons 311– 316. Compared to the sequencing for *katG*, this method had an accuracy of 98.08%. For the detection of RIF resistance, the RT-PCR allelic and DNA sequencing results had a sensitivity and specificity of 95.00% and 100%, respectively, for mutation analysis in the *rpoB* codons 510–531 (Figure 3).

Table 2. Proportion of drug-resistant *Mycobacterium tuberculosis* according to patient groups in Vietnam

History	Overall (%)	INH (%)	RIF	MDR-TB (%)
New TB cases	413 (88.2)	89 (21.6)	0	23 (5.6)
Previous TB cases	55 (11.8)	16 (29.1)	1	8 (14.5)
p	0.006	0.208	NA	0.012

TB, tuberculosis; INH, isoniazid (isonicotinic acid hydrazide); RIF, rifampicin; MDR-TB, multidrug-resistant tuberculosis; NA, not available.

https://doi.org/10.24171/j.phrp.2023.0124

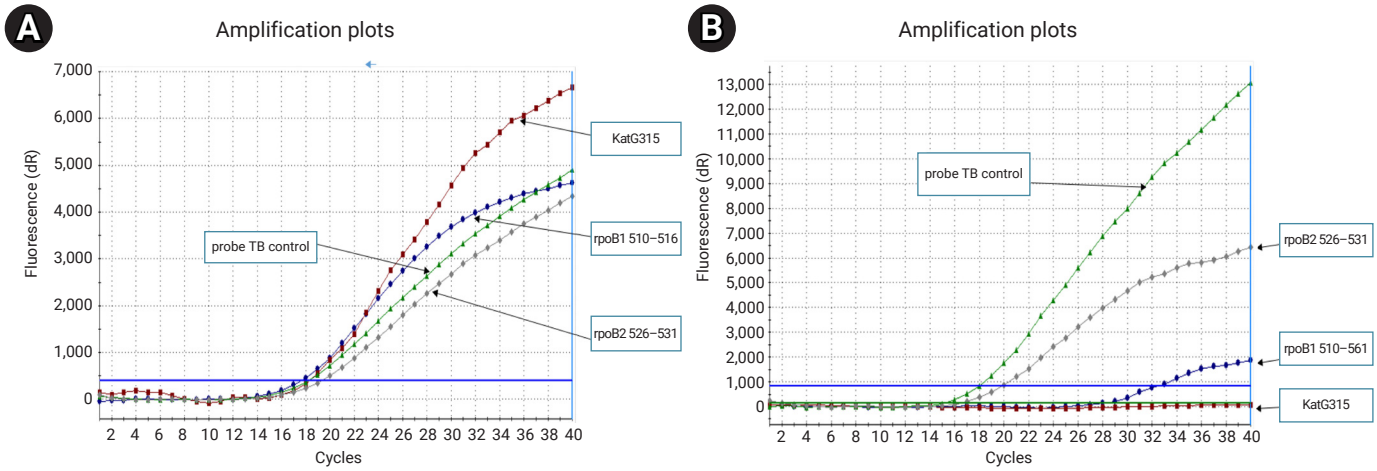

Figure 2. Analysis of the DNA from *Mycobacterium tuberculosis* isolates with 4 Taqman probes by multi-fluorescence real-time polymerase chain reaction.
(A) No mutation. (B) *rpoB*1510-516 ΔCt>10 Ct, KatG315 Ct=0. Ct, cycle threshold; dR, baseline subtracted fluorescence reading.

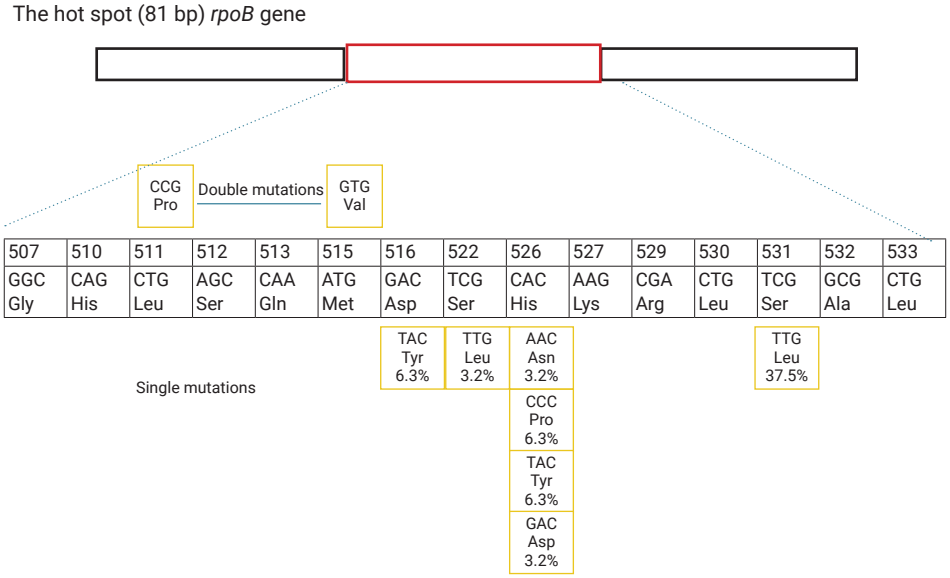

Figure 3. Schematic representations of *rpoB* gene mutations in RIF-resistant *Mycobacterium tuberculosis* isolates.
Bp, base pairs; RIF, rifampicin.

Discussion

This study evaluated the rate of phenotypic INH and/or RIF resistance in *M. tuberculosis* isolates from 3 hospitals in central Vietnam using REMA as a DST method. The REMA plate method, which was developed by Palomino et al. [9] in 2002 and Martin et al. [16] in 2003 and uses the colorimetric indicator resazurin, was employed for this purpose. REMA is a useful tool for the DST of both first-line and second-line TB drugs and can also determine resistance levels by assessing the MIC value, thus assisting clinicians in making better treatment decisions.

In this study, REMA was used to test the drug susceptibility of 500 *M. tuberculosis* isolates. Although 468 isolates gave interpretable results, 32 strains (6.4%) provided invalid results due to suspected contamination, likely resulting from poor storage. This outcome is consistent with the WHO's 2020 epidemiological report on DR-TB in Vietnam, which reported a rate of 3.6% for new MDR-TB cases and 17% for previously treated cases. INH resistance can be a precursor to the emergence of MDR-TB, and the drug plays a crucial role in treating TB and latent TB infection [17,18]. However, despite the effectiveness of the GeneXpert MTB/RIF test in detecting *M. tuberculosis* and RIF resistance in

the process of identifying MDR-TB cases, many pulmonary hospital laboratories in Vietnam may miss cases of INH mono-resistance, which are difficult to treat in patients with TB and latent TB infection. Our study suggests that this strategy may not be adequate since a high proportion (99%) of RIF-resistant isolates are also resistant to INH. In addition, REMA is a cost-effective alternative to commercial phenotypic DST and cultures, with an estimated cost of 3 United States dollars (USD) per strain tested, whereas the cultures and DST of many drugs in Vietnam by the BACTEC MGIT 960 can cost up to 35 USD for each strain tested. Therefore, REMA can be an effective method for detecting MDR-TB cases, especially in countries with a high prevalence of TB and limited resources. Its low cost, simplicity, and ease of execution do not demand highly technical skills [16,19]. Finally, the prompt screening of drug resistance in pulmonary hospital laboratories and the quick availability of results will facilitate the detection of MDR strains in these hospitals and enable the prescription of suitable treatment for the patient [8]. Since it demonstrates higher sensitivities and specificities than other methods, REMA can help reduce the incidence of TB and DR-TB. In addition, we plan to use REMA for the DST of other first-line and second-line anti-TB drugs to detect extensively drug-resistant TB at an early stage. The results of the present study may contribute to reducing the rate of TB and drug-resistant TB by demonstrating the high sensitivities and specificities of REMA. Our results are compared to the results of other authors in Table 3 [8,9,13,20–23].

Genotypic DST by RT-PCR TaqMan Allelic Discrimination Assay

Although conventional DST is considered the "gold standard" for assessing DR-TB, its disadvantages include slow turnaround times and high rates of contamination. In contrast, molecular assays offer faster results without compromising sensitivity and specificity [24,25]. In this study, we used the RT-PCR TaqMan allelic discrimination assay to test 52 *M. tuberculosis* clinical isolates, including 31 MDR, 1 RIF mono-resistant, 10 INH mono-resistant, and 10 drug-sensitive isolates. Previous research has shown that drug-resistant strains of *M. tuberculosis* can be detected by observing curve patterns or Ct values using 3 TaqMan probes (without minor groove binder) in an RT-PCR format [14]. The proposed method was optimized and evaluated for analytical sensitivity and specificity in the clinical isolates. When compared with DNA sequencing, this method provided good sensitivity and specificity in the results of our study and of other studies [26,27]. Since there is a lack of information on the genetic characteristics of *M. tuberculosis* isolates in central Vietnam, the use of molecular DST could aid in identifying the diversity of drug resistance-associated mutation patterns and provide insights into the genotypic DR-TB isolates in Vietnam. This will support a focus on infection control and surveillance to prevent new cases of MDR-TB in the region.

In this study, sequencing of the *katG* gene confirmed the presence of the Ser315Thr (AGC>ACC) mutation in 19 of 41 (46.3%) INH-resistant isolates, including 15 of 31

Table 3. The sensitivities and specificities of REMA results in the present study compared to the DST results of previous studies

Study	Country	No. of clinical isolates	Reference test	Sample size (no. of resistant/ no. of susceptible)	Sensitivity (95% CI)	Specificity (95% CI)
INH						
Palomino et al. [9] (2002)	Belgium	80	LJ	54/26	1.00 (0.93–1.00)	0.96 (0.80–1.00)
Martin et al. [13] (2005)	Belgium	203	LJ	82/212	0.98 (0.91–1.00)	0.98 (0.93–0.99)
Nateche et al. [8] (2006)	Algeria	136	LJ	17/119	1.00 (0.80–1.00)	0.99 (0.95–1.00)
Coban et al. [21] (2006)	Turkey	73	BACTEC	35/38	1.00 (0.85–1.00)	0.95 (0.76–0.99)
Dixit et al. [22] (2012)	India	105	LJ	51/54	0.93	0.98
Nour et al. [23] (2013)	Egypt	30	PM	20/10	1.00	0.98
This study	Vietnam	468	BACTEC	105/363	100 (0.96–1.00)	0.99 (0.98–1.00)
RIF						
Palomino et al. [9] (2002)	Belgium	80	LJ	49/31	1.00 (0.93–1.00)	1.00 (0.89–1.00)
Martin et al. [13] (2005)	Belgium	203	LJ	102/101	0.98 (0.93–1.00)	0.99 (0.95–1.00)
Nateche et al. [8] (2006)	Algeria	136	LJ	12/124	0.92 (0.62–1.00)	0.99 (0.96–1.00)
Coban et al. [21] (2006)	Turkey	73	BACTEC	21/52	1.00 (0.81–1.00)	0.94 (0.89–1.00)
Dixit et al. [22] (2012)	India	105	LJ	52/53	0.95	1.00
Nour et al. [23] (2013)	Egypt	30	PM	13/17	0.95	0.93
This study	Vietnam	468	BACTEC	32/436	0.99 (0.88–1.00)	0.94 (0.98–0.99)

REMA, resazurin microtiter assay; DST, drug susceptibility testing; CI, confidence interval; INH, isoniazid (isonicotinic acid hydrazide); RIF, rifampicin.

https://doi.org/10.24171/j.phrp.2023.0124

(48.4%) MDR-TB isolates and 4 of 10 (40.0%) INH mono-resistant isolates. This study only investigated mutations from codon 256 to codon 420 of the *katG* gene, and Ser315 was found to be the most frequently encountered mutation associated with INH resistance. This is consistent with other research findings such as a systematic review of 118 publications analyzing 11,411 *M. tuberculosis* isolates from 49 countries, which found that 64% of all observed phenotypic INH resistance was associated with mutation *katG*315 [28]. A study by Chikamatsu et al. [29] reported that 54.5% of isolates harbored the *katG*315 mutation in Japan. In addition, van Doorn et al. [30] found that 55% of INH-resistant strains had a mutation in codon 315 of *katG*, while Riahi et al. [27], reported approximately 53.55%.

In a recent study by Sadri et al. [31] in Iran, of 125 *M. tuberculosis* clinical isolates, 34 strains were INH-resistant, and 91 strains were INH-sensitive. Of the 34 INH-resistant strains, 32% of the mutations were identified in *katG*315 (Ser→Thr), 14% in *katG*315 (Ser→ Asn), 52% in *InhA* 15 (C→T), and 2.9% in *InhA* 17 (G→T).

The question remains, which genetic variants in INH-resistant bacteria are related to the prevalence of genetic mutations conferring *M. tuberculosis* drug resistance in these different geographic regions. INH resistance was found to be more difficult to detect because the mutations were associated with multiple genes. In our study, we only investigated *katG* at codon 315. Further studies are needed to gain a complete understanding of the genetic variations in *M. tuberculosis* resistance to INH drugs, and to determine the prevalence of resistant mutations among *M. tuberculosis* isolates in the different geographic regions of Vietnam.

We utilized 2 wild-type probes for the *rpoB* gene to determine RIF resistance in this region, including the point mutations mentioned above. The most frequently detected mutation was *rpoB* Ser531Leu (TCG > TTG) at 37.5%. We identified 4 different types of amino acid substitution in codon 526, with His526Asp (CAC > GAC) mutations present in 2 isolates, and His526Tyr (CAC > TAC) mutations present in 2 isolates. One isolate had a His256Asn (CAC > AAC) mutation, and 2 isolates had His256Pro (CAC > CCC) mutations. Only 2 strains (6.25%) had mutations at codon Asp516Tyr (GAC > TAC). One strain had a double point mutation in L511Pro (CTG > CCG) and Met515Val (ATG > GTG). Only 1 case of RIF mono-resistance (0.21%) was detected, with a mutation in *rpoB* S531L. In a recent study in southern Vietnam, 56 isolates (approximately 76%) had mutations in the rifampin resistance-determining region (RRDR) of the *rpoB* gene, at codons 531 (37.8%), 526 (23%), and 516 (9.46%). In other research, 104 RIF-resistant isolates had mutations in the 81bp RRDR of the *rpoB* gene; the most prevalent mutations were at codons 531 (43%), 526 (31%), and 516 (15%) [32–34]. In the west of Iran, a study by Hamed et al. [35] found 32 MDR isolates among 54 *M. tuberculosis* samples, while the other 22 isolates were INH and RIF sensitive. Among the 32 MDR isolates, 22 strains (70%) had *katG* codon 315 mutations, 1 strain (3%) had *inhA* mutations, and 9 strains (28%) had no mutations at all. Regarding RIF, mutations associated with the *rpoB* gene in codons 531, 516 and 626 comprised 21 strains (66%), 7 strains (22%) and 1 strain (3%) respectively, and no mutations were found in 3 strains (9%).

In another study by Mohajeri et al. [36], 35 (28.0%) of 125 *M. tuberculosis* isolates were found to be RIF-resistant with S512T (AGC > ACC; 20%), D516V (GAC > GTC; 20%), H526D (CAC > GAC; 6%), H526R (CAC > CGC; 20%), H526Y (CAC > TAC; 23%), and S531W (TCG > TGG; 8%), and the most frequent site mutations were L511P (CCG > CTG; 46%), followed by S531l (TCG > TTG; 40%) and D516Y (GAC > TAC; 26%). This study detected resistance in strains with mutations within the hotspot at 81 bp but did not find cases with mutations located outside the hot spot of *rpoB*. Our study could only examine a short segment of the *rpoB* and did not observe the *rpoA* and *rpoC* of *M. tuberculosis*.

Conclusion

This study highlights the importance of developing molecular tests that allow quick screening for INH and RIF resistance-related mutations in Vietnam, where a significant percentage of patients with TB remain undiagnosed and untreated. The use of the RT-PCR TaqMan allelic assays, followed by the determination of MIC values using REMA, can assist clinicians when deciding on effective drug resistance regimens for their patients with DR-TB. This approach can help control the spread of MDR-TB, not only in Vietnam but globally as well. Therefore, it is recommended that laboratories in pulmonary hospitals adopt this approach to improve the diagnosis and treatment of DR-TB patients.

Notes

Ethics Approval

Ethical approval for this study was granted by The Ethical Committee in Biomedical Research of the University of Medicine and Pharmacy-Hue University (No: H2019/350). The director of each hospital granted permission to conduct the study. Before specimen collection, patients/guardians were provided with information about the study and asked to provide written informed consent. Participants were informed of their right to voluntarily participate in the study.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding
This study was also supported by project No. DHH 2020-04-111 of Hue University, Hue, Vietnam.

Availability of Data
All data generated or analyzed during this study are included in this published article. Other data may be requested through the corresponding author.

Authors' Contributions
Conceptualization: TBNN, VQTN, PM; Data curation: HBN, vBTP, TKDN; Formal analysis: VQTN, VTM, TBNN; Funding acquisition: all authors; Methodology: PM, VQTN, TBNN; Project administration: TBNN, PM, TTN; Visualization: PM, VQTN, VTM, TBNN; Writing–original draft:TBNN, VQTN; Writing–review & editing: all authors. All authors read and approved the final manuscript.

Acknowledgements
This work was also supported by professors at Sassari University, Department of Microbiology and Clinical Microbiology, Hospital University Sassari, Italy.

Additional Contributions
The authors would like to thank all faculty members, residents, and technical staff members at the Department of Microbiology and Clinical Microbiology, Hospital University Sassari, Italy.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0052
Osong Public Health Res Perspect 2023;14(5):356-367

Original Article

The associations of health behaviors and working hours with high-sensitivity C-reactive protein levels in Korean wage workers: a cross-sectional study

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ABSTRACT

Objectives: We investigated differences in high-sensitivity C-reactive protein (hs-CRP) levels by age group according to working hours, socioeconomic level, health behavior and status, and occupational class, and aimed to identify factors affecting hs-CRP levels in various age groups using data from the Korean National Health and Nutrition Examination from 2016 to 2018.
Methods: The study included a total of 4,786 male wage workers across the nation, aged between 19 and 65. Data from 4,674 workers were analyzed using multiple logistic regression analysis.
Results: Obesity, metabolic syndrome, and weekly working hours were associated with hs-CRP, a biomarker of inflammation. Participants with a body mass index (BMI) ≥25.0 kg/m^2 showed significantly higher hs-CRP levels than those with a BMI 23.0 to 25.0 kg/m^2. Workers with high-risk drinking and metabolic syndrome showed significantly higher hs-CRP levels in the 50 to 65 years group. Obesity, walking 0 to 149 min/wk, and working ≥61 hours a week were associated with significantly higher hs-CRP levels in the 35 to 49 years group. The factors that significantly affected hs-CRP levels were different among age groups.
Conclusion: Plans to adjust working hours should be considered health behaviors, such as drinking and physical activity, and health conditions, such as metabolic syndrome and obesity, according to workers' age.

Keywords: High-sensitivity C-reactive protein; Metabolic syndrome; Obesity; Working hours

Received: March 3, 2023
Revised: July 10, 2023
Accepted: July 17, 2023

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Introduction

In 1919, the International Labor Organization introduced the first agreement to limit daily working hours to 8 hours and weekly working hours to 48 hours in the manufacturing sector. A century has passed since then, and many countries have gradually reduced statutory working hours, with many now limiting them to 40 hours per week [1]. When Korea implemented a reduction in working hours on July 1, 2018, there was a significant decrease in working hours.

However, it was reported that the proportion of employed individuals (28%) who still worked more than 48 hours per week was 12%p higher than the 16% reported in Europe in 2015 [2].

Previous studies have suggested that long working hours have various impacts. In the short term, extended working hours can increase fatigue due to increased job demands, interference with personal life, and reduced sleep time. This can lead to negative health behaviors such as smoking and alcohol abuse [3]. These factors can increase the incidence of workplace accidents [4], mental illnesses [5], cerebro cardiovascular diseases [6], stress, and problems with the gastrointestinal, musculoskeletal, and immune systems [7]. On a societal level, these factors can lead to decreased productivity [8] and increased absenteeism in the long term [9]. Spurgeon et al. [10] suggested that working more than 48 hours a week can negatively impact mental health due to increased job stress. They also found that unhealthy behaviors such as smoking and poor eating habits, as well as a rise in physical diseases, increase with longer working hours. This is because extended labor hours not only lead to the accumulation of fatigue by reducing sufficient recovery time, but also rapidly deplete energy [11]. A recent study found that in men, working 61 hours or more per week was significantly associated with elevated aspartate transaminase and alanine transaminase levels compared to working 35 to 52 hours per week [12]. The study by Lee [13] found little difference in health status among management jobs, except for sales positions. However, health problems were more prevalent among service workers, functional workers, device assembly workers, and simple labor jobs. It was concluded that service workers, who perform emotional labor, and functional, device assembly, and simple laborers, who perform high-intensity physical labor, experience substantial health impacts from their work content [13].

Nevertheless, more than 50 hours of work per week has been reported by 16.7% of managers, 10.0% of professionals and related workers, 8.3% of office workers, 32.0% of service workers, 33.7% of salespeople, 24.5% of skilled workers and agricultural and forestry workers, 27.6% of device/machine operators and assembly workers, and 16.8% of unskilled laborers [14]. According to the 4th Korean Working Conditions Survey, Baik [15] found that service workers worked an average of 51.24 hours per week, sales workers worked 53 hours, experts worked 41.67 hours, semi-professional workers worked 42.88 hours, office workers worked 42.55 hours, agricultural, forestry, and fisheries workers worked 39.71 hours, and unskilled laborers worked 39.96 hours. Generally, workers tend to work longer hours when there is an increased demand for work or labor, when their employment status is unstable, and when there is increased pressure to perform at work [7].

HIGHLIGHTS

- Participants with a body mass index (BMI) ≥25.0 kg/m^2 showed significantly higher high-sensitivity C-reactive protein (hs-CRP) levels than those with a BMI of 23.0 to 25.0 kg/m^2.
- Workers with high-risk drinking and metabolic syndrome showed significantly higher hs-CRP levels in the 50 to 65 years group.
- Obesity, walking 0 to 149 min/wk, and working ≥61 hours a week were associated with significantly higher hs-CRP levels in the 35 to 49 years group.

Inflammation is recognized as a significant factor in the process of arteriosclerosis, which leads to cardiovascular disease. Numerous studies have demonstrated that high-sensitivity C-reactive protein (hs-CRP) serves as an inflammation biomarker, even in individuals without high blood pressure, diabetes, or hyperlipidemia. Elevated hs-CRP levels have been linked to an increased likelihood of cardiovascular disease [16–18]. Furthermore, hs-CRP levels can forecast the future risk of myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death, even in individuals without any existing diseases. These levels have also been reported to have a strong correlation with the development of metabolic syndrome and diabetes [19,20]. Since 1990, more sensitive hs-CRP measurements have been developed, and their use has been recommended as an index for risk assessment and prognosis estimation [21]. Notably, the hs-CRP assay is a more powerful predictor of cardiovascular disease than low-density lipoprotein cholesterol [22]. It is also a well-established risk marker for future cardiovascular disease [23–25] and mortality.

An association between hs-CRP levels and occupational classification has been identified [26]. After adjusting for all covariates, white-collar workers exhibited significantly higher levels of hs-CRP (β = 0.16; 95% confidence interval [CI], 0.02–0.30) than blue-collar workers. This pattern was particularly pronounced among professionals. However, the association was not significant for unemployed and pink-collar workers.

The Korean government has recently been attempting to increase the weekly working hours to 52 hours [27]. The proposed extension of weekly working hours necessitates a

thorough review in light of both national and international laws and institutions, as well as the correlation between a worker's age, occupation, health status, and overall health. However, there is a dearth of studies that have taken into account the hs-CRP levels of workers, considering their age, occupation, and working hours. Consequently, this study explored the variations in hs-CRP levels across different age groups, taking into account factors such as working hours, socioeconomic status, health behaviors and conditions, and occupational class. Furthermore, it aimed to identify the factors that influenced hs-CRP levels within each age group.

Materials and Methods

Study Participants

Data were obtained from the Korea National Health and Nutrition Examination Survey in 2016–2018 (KNHANES VII). Briefly, the Korea Centers for Disease Control and Prevention (now known as the Korea Disease Control and Prevention Agency) has conducted the KNHANES since 1998, measuring population health profiles of noninstitutionalized Koreans aged 1 year or older through health examinations, nutrition surveys, and health interviews. The survey employed a stratified multistage cluster probability sampling design to draw a nationally representative sample of Koreans. As shown in Figure 1, from the initially targeted individuals in KNHANES VII, (n=24,269), we excluded participants younger than 19 years (n=2,513) or older than 65 years (n=1,991), those who did not report their current occupation (n=1,781), those with a missing hs-CRP level (n=71), and those with missing information on any other variables used in this analysis (n=41), resulting in a total sample of 4,674 men (Figure 1). Women were not included because it has been reported that the use of hormone replacement therapy affects hs-CRP levels, but the dataset in this study did not contain information on whether female subjects had received hormone replacement therapy [19].

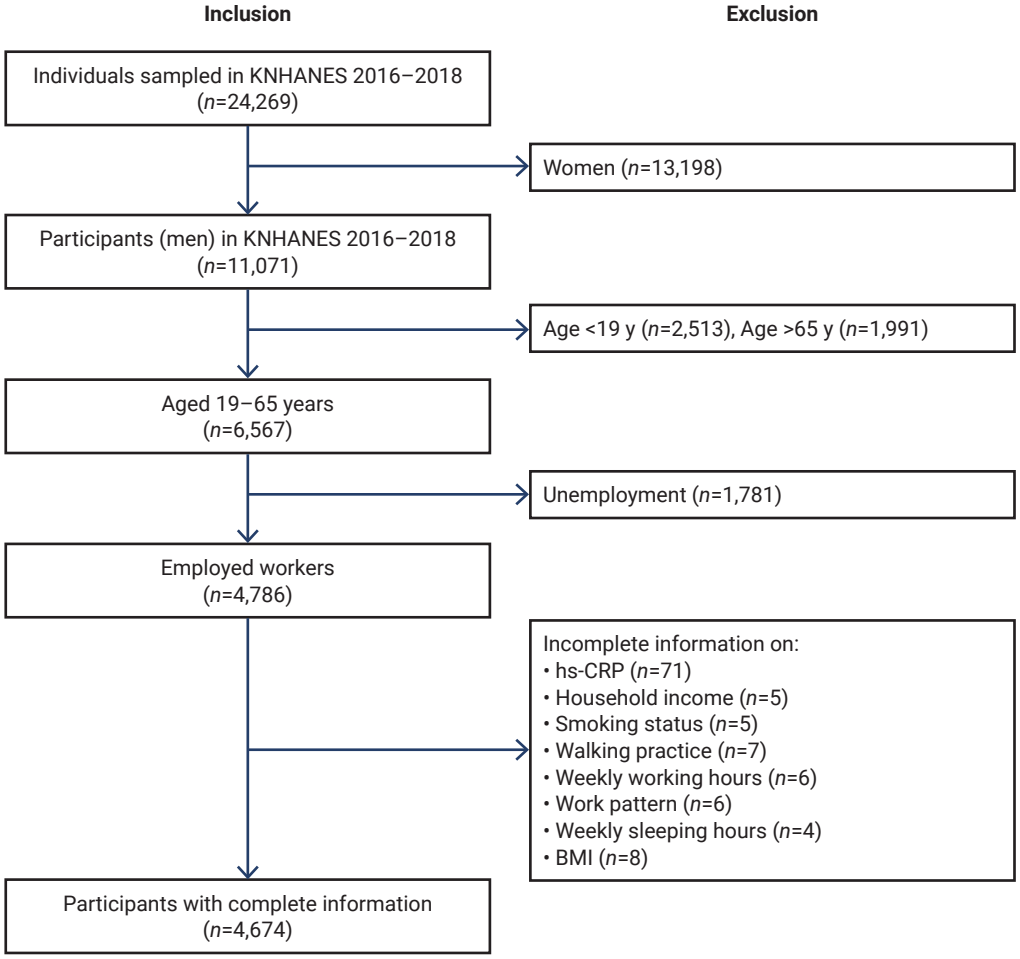

Figure 1. Flow of participants this study.
KNHANES, Korea National Health and Nutrition Examination Survey; hs-CRP, high-sensitivity C-reactive protein; BMI, body mass index.

https://doi.org/10.24171/j.phrp.2023.0052

Variables

Participants were grouped into 3 age categories: 19 to 34, 35 to 49, and 50 to 65 years. Education levels were divided into 2 groups: those with a high school diploma or less, and those with a college degree or higher. Marital status was categorized as unmarried, married, or separated/divorced/widowed. Household economic status was classified into 4 levels: high, mid-high, mid-low, and low. Smoking habits were categorized as current smoking, former smoking, and non-smoking. High-risk drinking was defined as consuming more than 7 drinks at a time for men, and drinking more than twice a week. Regular walking was defined as walking for more than 30 minutes, at least 5 days a week, in the previous week.

The data contained items on conditions such as hypertension, dyslipidemia (hyperlipidemia), stroke, myocardial infarction, angina, rheumatoid arthritis, diabetes, cancer, elderly macular degeneration, and renal failure. These variables, as identified in a previous study [28], have a high correlation with hs-CRP. Consequently, if any of these diseases were present, participants were considered to have a history of cancer or cerebrocardiovascular disease. Participants were categorized based on their body mass index (BMI), with a threshold set at 25.0 kg/m^2. The World Health Organization's revised criteria for Asian-Pacific populations were used to classify BMI. The classifications were as follows: underweight (less than 18.5 kg/m^2), normal weight (18.5 to less than 23.0 kg/m^2), overweight (23.0 to less than 25.0 kg/m^2), and obese (25.0 and above kg/m^2).

Low-density cholesterol was calculated using the formula by Friedewald et al. [29] and participants were classified into groups of ≤130 mg/dL and >130 mg/dL.

Metabolic syndrome was defined by the presence of 3 cardiovascular risk markers. These include an elevated waist circumference for Asian-Pacific populations (≥90 cm for males), increased blood pressure (≥135/85 mmHg or the use of antihypertensive medication), high fasting plasma glucose levels (≥100 mg/L or the use of antihyperglycemic medication), high triglyceride levels (≥150 mg/dL), and low high-density lipoprotein cholesterol levels (<40 mg/dL for males). This definition was based on the Joint Scientific Statement by the American Heart Association, National Heart, Lung, and Blood Institute, and International Diabetes Federation [30].

Perceived stress was classified as present if participants felt some or very much stress and as absent if they felt little or very little stress. Working hours were classified into an average of <40 hours per week, 40 to 49 hours, 50 to 53 hours, 54 to 61 hours, and >61 hours per week.

Based on previous studies, occupational groups were classified into 3 classes: (1) white-collar workers (i.e., managers and professionals); (2) pink-collar workers (i.e., clerks, service, and salespeople); and (3) blue-collar workers (i.e., technicians, craftworkers, and device/machine operators/assemblers, skilled agricultural/fishery workers, and elementary-level laborers).

The work pattern was segmented into day shifts and adjusted tasks, taking into account the hours and frequency of work. Adjusted tasks encompassed the evening shift (14:00–24:00), night shift (21:00–8:00 the following day), day and night shift, 24-hour shift, split shift (2 or more daily shifts), and irregular shift. Regarding sleep duration, the average sleep time was determined by summing the sleep hours during the week and the weekend. Weekly sleep hours were categorized into 42 hours (an average of 6 hours per day), 42 to 56 hours (an average of 6 to 8 hours per day), and more than 56 hours (an average of 8 hours or more per day).

hs-CRP levels were classified into less than or equal to 3 mg/L and more than 3 mg/L. Plasma levels of hs-CRP 1 mg/L, 1 to 3 mg/L, and 3 mg/L were established as representing lower, average, or higher relative vascular risk when added to traditional risk factors [31]. This classification was based on the findings of a study that the risk of developing vascular disease was at its peak when hs-CRP levels reached or exceeded 3 mg/L in a clinical setting [17]. The hs-CRP level analysis in this study was conducted using a Hitachi 7600 biochemical analyzer (Hitachi).

Statistical Analysis

A statistical analysis was conducted to compare hs-CRP levels according to working hours, segmented by age groups (19 to 34, 35 to 49, and 50 to 65 years) among male workers in Korea. The analysis was conducted in accordance with the guidelines for using the National Health and Nutrition Survey, and it incorporated a complex sample analysis. Cross-tabulation tests and mean comparisons were utilized for the intergroup analyses. The results were presented using actual observations for frequency and composite weights for percentages. To examine the relationship between working hours and hs-CRP levels, a multiple logistic regression analysis was performed, applying composite weights. The analysis was conducted using SAS ver. 9.2 (SAS Institute Inc.). A p-value of less than 0.05 (2-sided) was considered statistically significant.

Results

Characteristics of Participants

Table 1 shows the characteristics of the study sample.

Table 1. Characteristics of participants

Characteristic	Category	Total (*n* = 4,674)	19–34 y (*n* = 964)	35–49 y (*n* = 1,926)	50–65 y (*n* = 1,784)
Education	≤ High school	2,212 (46.5)	402 (43.2)	665 (35.4)	1,145 (62.5)
	≥ College	2,462 (53.5)	562 (56.78)	1,261 (64.6)	639 (37.5)
Marital status	Unmarried	954 (24.7)	661 (70.5)	255 (14.5)	38 (1.6)
	Married	3,522 (71.5)	298 (29.1)	1,618 (82.8)	1,606 (90.5)
	Separated/divorced/widowed	198 (3.8)	5 (0.4)	53 (2.7)	140 (7.9)
Household income	1st (lowest) quartile	252 (5.5)	66 (7.7)	66 (3.4)	120 (6.2)
	2nd quartile	1,016 (21.6)	206 (22.0)	424 (22.2)	386 (20.5)
	3rd quartile	1,576 (34.4)	335 (35.3)	712 (37.2)	529 (30.3)
	4th (highest) quartile	1,830 (38.6)	357 (35.0)	724 (37.2)	749 (42.9)
Smoking status	Current smokers	1,911 (41.4)	405 (42.8)	876 (45.4)	630 (35.3)
	Former smokers	1,696 (34.7)	186 (18.4)	663 (34.7)	847 (47.4)
	Never smokers	1,067 (23.9)	373 (38.8)	387 (19.9)	307 (17.3)
High-risk drinking	Yes	3,534 (76.2)	758 (79.1)	1,419 (74.6)	1,357 (76.0)
	No	1,140 (23.8)	206 (20.9)	507 (25.4)	427 (24.0)
Walking activity (min/wk)	0–149	2,942 (61.9)	507 (52.3)	1,259 (64.8)	1,176 (65.9)
	≥ 150	1,732 (38.1)	457 (47.8)	667 (35.2)	608 (34.1)
History of cancer/cerebro cardiovascular disease	No	3,306 (73.6)	919 (95.7)	1,549 (80.8)	838 (47.8)
	Yes	1,368 (26.4)	45 (4.3)	377 (19.2)	946 (52.2)
BMI category (kg/m^2)	< 23.0	1,366 (29.8)	330 (33.9)	524 (27.8)	512 (29.1)
	23.0–25.0	1,234 (25.9)	226 (23.1)	482 (25.2)	526 (28.9)
	≥ 25.0	2,074 (44.3)	408 (43.0)	920 (47.0)	746 (42.1)
LDL (mg/dL)	≤ 130	3,089 (66.4)	695 (72.5)	1,192 (62.3)	1,202 (66.6)
	> 130	1,585 (33.6)	269 (27.5)	734 (37.7)	582 (33.4)
Metabolic syndrome	No	3,181 (69.7)	822 (85.4)	1,288 (67.3)	1,071 (60.6)
	Yes	1,493 (30.3)	142 (14.6)	638 (32.7)	713 (39.4)
Perceived stress level	No	3,359 (71.5)	637 (66.1)	1,324 (69.2)	1,398 (78.4)
	Yes	1,315 (28.6)	327 (33.9)	602 (30.8)	386 (21.7)
Weekly working hours	< 40	1,040 (21.9)	253 (26.0)	275 (14.9)	512 (27.4)
	≥ 40, < 49	1,999 (42.6)	412 (42.4)	916 (46.9)	671 (37.6)
	≥ 49, < 53	440 (9.7)	96 (10.2)	219 (11.5)	12 5 (7.2)
	≥ 53, < 61	685 (15.1)	120 (12.9)	311 (16.3)	254 (15.1)
	≥ 61	510 (10.7)	83 (8.6)	205 (10.4)	222 (12.6)
Occupational group	White-collar	1,190 (25.9)	276 (27.8)	585 (30.4)	329 (18.9)
	Pink-collar	1,580 (35.0)	447 (45.6)	676 (34.8)	457 (27.1)
	Blue-collar	1,904 (39.1)	241 (26.6)	665 (34.7)	998 (54.0)
Work pattern	Day work	3,984 (84.1)	746 (76.7)	1,680 (86.6)	1,558 (86.8)
	Shift work	690 (15.9)	218 (23.3)	246 (13.4)	226 (13.2)
Sleeping hours	< 6	551 (11.4)	99 (9.8)	228 (11.4)	224 (12.7)
	6–8	3,042 (65.8)	607 (63.4)	1,276 (67.2)	1,159 (65.9)
	≥ 8	1,081 (22.8)	258 (26.8)	422 (21.4)	401 (21.5)
hs-CRP level (mg/L)	< 3.0	4,291 (91.9)	895 (91.8)	1,774 (92.2)	1,622 (91.5)
	≥ 3.0	383 (8.11)	69 (8.2)	152 (7.8)	162 (8.5)

LDL cholesterol was estimated using the measured values of total cholesterol, HDL cholesterol, and triglycerides. LDL cholesterol (mg/dL) = total cholesterol (mg/dL) − HDL cholesterol (mg/dL) − triglycerides (mg/dL)/5. Reference range: < 130 mg/dL (desirable); 131–160 mg/dL (borderline high); > 160 mg/dL (high).

BMI, body mass index; LDL, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein.

A total of 20.62% (*n* = 964) of participants were 19 to 34 years old, 41.21% (*n* = 1,926) were 35 to 49 years old, and 38.17% (*n* = 1,784) were 50 to 65 years old. The percentage of participants with hs-CRP levels above ≥ 3.0 mg/L was 8.14%. The distribution of participants across the working hours groups was as follows: 21.93%, 42.63%, 9.71%, 15.05%, and 10.69% for the ≤ 40 hours, 40 to 49 hours, 49 to 53 hours, 53 to 61 hours, and ≥ 61 hours groups, respectively. The percentages of white-, pink-, and blue-collar workers were 25.89%, 35.03%, and 39.07%, respectively.

https://doi.org/10.24171/j.phrp.2023.0052

Distribution of hs-CRP Levels

Table 2 presents the results of a cross-tabulation analysis of the distribution of hs-CRP levels based on the general characteristics and health status of participants across various age groups. Significant differences in hs-CRP levels were observed across all age groups in relation to BMI category ($p < 0.001$), metabolic syndrome ($p = 0.004$), and weekly working hours ($p = 0.012$). More specifically, significant differences in the distribution of hs-CRP levels were found between BMI classes in both the 19 to 34 ($p = 0.005$) and the 35 to 49 age groups ($p = 0.001$). Furthermore, a significant difference was noted in the distribution of hs-CRP levels in relation to walking practices (min/week) within the 35 to 49 age group ($p = 0.046$).

Factors Affecting hs-CRP Levels

Table 3 presents the results of multiple logistic regression analysis, which was conducted to identify factors influencing hs-CRP levels across different age groups. When compared to the group with a BMI of 23.0 to 25.0 kg/m^2, the group with a BMI of ≥25.0 kg/m^2 exhibited significantly higher hs-CRP levels (odds ratio [OR], 1.473; 95% CI, 1.078–2.013; $p = 0.0015$). This trend was also observed in the 35 to 49 age group, where the BMI ≥25.0 kg/m^2 group had significantly higher hs-CRP levels (OR, 1.717; 95% CI, 1.041–2.834; $p = 0.001$). In comparison to the group with no high-risk drinking habits, the high-risk drinking group within the 50 to 65 years age bracket showed significantly higher hs-CRP levels (OR, 1.819; 95% CI, 1.172–2.824; $p = 0.008$). Regarding walking habits, participants aged 35 to 40 years who walked between 0 and 149 minutes per week had significantly higher hs-CRP levels (OR, 1.561; 95% CI, 1.055–2.308; $p = 0.026$) than those who did not walk at all in the 50 to 65 age group. Lastly, participants who worked more than 61 hours per week exhibited significantly higher hs-CRP levels (OR, 2.652; 95% CI, 1.393–5.046; $p = 0.007$) than those who worked between 40 to 49 hours per week in the 35 to 49 age group. However, we were unable to identify any factors that influenced hs-CRP levels among other variables, such as general characteristics, occupational groups, health-related behaviors, and history of cancer or cerebrocardiovascular disease.

Discussion

This study examined the distribution of hs-CRP level distributions in male workers according to working hours, socioeconomic level, health status and health behavior, and occupational class across the age groups of 19 to 34, 35 to 49, and 50 to 65 years, and identified factors affecting hs-CRP levels. Among the factors related to health status and

Table 2. The distribution of hs-CRP level according to various categories

Characteristic	Total			hs-CRP								
				19–34 y			35–49 y			50–64 y		
	<3.0 mg/L	≥3.0 mg/L	p	<3.0 mg/L	≥3.0 mg/L	p	<3.0 mg/L	≥3.0 mg/L	p	<3.0 mg/L	≥3.0 mg/L	p
Education												0.2920
≤High school	2,021 (91.1)	191 (8.9)	0.1347	375 (90.7)	27 (9.3)	0.4038	610 (91.7)	55 (8.3)	0.6034	1,036 (91.0)	109 (9.0)	
≥College	2,270 (92.5)	192 (7.5)		520 (92.6)	42 (7.4)		1,164 (92.5)	97 (7.5)		586 (92.5)	53 (7.5)	
Marital status												0.2206
Unmarried	871 (90.9)	83 (9.1)	0.4838	608 (90.7)	53 (9.3)	0.1212	231 (92.3)	24 (7.7)	0.4295	32 (83.6)	6 (16.4)	
Married	3,242 (92.2)	280 (7.8)		283 (94.5)	15 (5.5)		1,492 (92.0)	126 (8.0)		1,467 (91.9)	139 (8.1)	
Separated/divorced/widowed	178 (91.3)	20 (8.7)		4 (90.3)	1 (9.7)		51 (96.9)	2 (3.1)		123 (89.0)	17 (11.0)	
Household income												0.4064
1st (lowest) quartile	228 (90.4)	24 (9.6)	0.8595	63 (95.0)	3 (5.0)	0.3266	58 (87.0)	8 (13.0)	0.2797	107 (88.2)	13 (11.8)	
2nd quartile	925 (91.7)	91 (8.3)		196 (94.5)	10 (5.5)		385 (90.9)	39 (9.1)		344 (90.3)	42 (9.7)	
3rd quartile	1,449 (92.0)	127 (8.0)		311 (90.8)	24 (9.2)		657 (92.2)	55 (7.8)		481 (92.6)	48 (7.4)	
4th (highest) quartile	1,689 (92.1)	141 (7.9)		325 (90.3)	32 (9.7)		674 (93.3)	50 (6.7)		690 (91.9)	59 (8.1)	
Smoking status												0.1858
Current smokers	1,744 (91.4)	167 (8.6)	0.7255	376 (91.7)	29 (8.3)	0.9695	806 (91.9)	70 (8.1)	0.2347	562 (90.5)	68 (9.5)	
Former smokers	1,563 (92.2)	133 (7.8)		175 (92.3)	11 (7.7)		605 (91.3)	58 (8.7)		783 (93.0)	64 (7.0)	
Never smokers	984 (92.1)	83 (7.9)		344 (91.6)	29 (8.4)		363 (94.4)	24 (5.6)		277 (89.7)	30 (10.3)	

(Continued to the next page)

Table 2. Continued

Characteristic	hs-CRP											
	Total			19–34 y			35–49 y			50–64 y		
	<3.0 mg/L	≥3.0 mg/L	*p*	<3.0 mg/L	≥3.0 mg/L	*p*	<3.0 mg/L	≥3.0 mg/L	*p*	<3.0 mg/L	≥3.0 mg/L	*p*
High-risk drinking												0.0054
Yes	3,262 (92.3)	272 (7.7)	0.0974	703 (91.5)	55 (8.5)	0.5401	1,314 (92.5)	105 (7.5)	0.3948	1,245 (92.7)	112 (7.3)	
No	1,029 (90.5)	111 (9.5)		192 (93.1)	14 (6.9)		460 (91.2)	47 (8.8)		377 (87.9)	50 (12.1)	
Walking activity (min/wk)												0.4862
0–149	2,703 (92.4)	239 (7.6)	0.1041	473 (93.0)	34 (7.0)	0.1530	1,171 (93.2)	88 (6.8)	0.0459	1,059 (91.2)	117 (8.8)	
≥150	1,588 (91.0)	144 (9.0)		422 (90.5)	35 (9.5)		603 (90.4)	64 (9.6)		563 (92.2)	45 (7.8)	
History of cancer/cerebro- cardiovascular disease												0.4523
No	1,233 (90.4)	135 (9.6)		40 (91.1)	5 (8.9)		341 (89.5)	36 (10.5)		852 (90.8)	94 (9.2)	
Yes	1,274 (93.8)	92 (6.2)	<.0001	314 (95.1)	16 (4.9)	0.0050	501 (95.7)	23 (4.3)	0.0002	459 (90.3)	53 (9.7)	
BMI category (kg/m^2)												0.4523
<23.0	1,274 (93.8)	92 (6.2)	<.0001	314 (95.1)	16 (4.9)	0.0050	501 (95.7)	23 (4.3)	0.0002	459 (90.3)	53 (9.7)	
23.0–25.0	1,151 (93.3)	83 (6.7)		214 (94.0)	12 (6.0)		450 (93.6)	32 (6.4)		487 (92.7)	39 (7.3)	
≥25.0	1,866 (89.7)	208 (10.3)		367 (88.0)	41 (12.0)		823 (89.4)	97 (10.6)		676 (91.6)	70 (8.4)	
LDL (mg/dL)												0.3377
≤130	2,848 (92.3)	241 (7.75)	0.2463	650 (92.7)	45 (7.3)	0.1883	1,098 (92.1)	94 (7.9)	0.8398	1,100 (92.0)	102 (8.0)	
>130	1,443 (91.1)	142 (8.9)		245 (89.3)	24 (10.7)		676 (92.3)	58 (7.7)		522 (90.5)	60 (9.5)	
Metabolic syndrome												0.0509
No	2,951 (92.7)	230 (7.3)	0.0037	767 (92.4)	55 (7.6)	0.1297	1,198 (93.0)	90 (7.0)	0.0707	986 (92.7)	85 (7.3)	
Yes	1,340 (89.8)	153 (10.2)		128 (88.0)	14 (12.0)		576 (90.4)	62 (9.6)		636 (89.8)	77 (10.2)	
Perceived stress level												0.3472
No	3,094 (92.3)	265 (7.7)	0.1295	597 (93.2)	40 (6.8)	0.0512	1,221 (92.2)	103 (7.8)	0.9864	1,276 (91.9)	122 (8.1)	
Yes	1,197 (90.8)	118 (9.2)		298 (89.1)	29 (10.9)		553 (92.2)	49 (7.8)		346 (90.2)	40 (9.8)	
Weekly working hours												0.7857
<40	949 (91.6)	91 (8.4)	0.0119	231 (89.7)	22 (10.3)	0.1665	256 (93.1)	19 (6.9)	0.0521	462 (91.9)	50 (8.1)	
≥40, <49	1,857 (93.4)	142 (6.6)		387 (94.3)	25 (5.7)		854 (93.6)	62 (6.4)		616 (92.5)	55 (7.5)	
≥49, <53	401 (89.8)	39 (10.2)		87 (86.8)	9 (13.2)		201 (91.5)	18 (8.5)		113 (89.9)	12 (10.1)	
≥53, <61	628 (91.7)	57 (8.3)		114 (93.9)	6 (6.1)		284 (91.5)	27 (8.5)		230 (90.6)	24 (9.4)	
≥61	456 (88.3)	54 (11.7)		76 (88.4)	7 (11.6)		179 (86.5)	26 (13.5)		201 (90.0)	21 (10.0)	
Occupational group												0.4505
White-collar	1,095 (92.3)	95 (7.7)	0.2921	255 (92.1)	21 (7.9)	0.1310	533 (91.9)	52 (8.1)	0.9289	307 (93.5)	22 (6.5)	
Pink-collar	1,461 (92.5)	119 (7.5)		418 (93.5)	29 (6.5)		629 (92.5)	47 (7.5)		414 (91.1)	43 (8.9)	
Blue-collar	1,735 (91.0)	169 (9.0)		222 (88.6)	19 (11.4)		612 (92.1)	53 (7.9)		901 (91.1)	97 (8.9)	
Work pattern												0.9392
Day work	3,650 (92.0)	334 (8.0)	0.6601	694 (92.5)	52 (7.5)	0.1927	1,544 (92.0)	136 (8.0)	0.4161	1,412 (91.6)	146 (8.4)	
Shift work	641 (91.4)	49 (8.6)		201 (89.4)	17 (10.6)		230 (93.6)	16 (6.5)		210 (91.4)	16 (8.6)	
Sleeping hours												0.2215
<6	504 (91.4)	47 (8.6)	0.3014	90 (88.5)	9 (11.5)	0.318	207 (91.9)	21 (8.1)	0.9897	207 (92.4)	17 (7.6)	
6–8	2,785 (91.5)	257 (8.5)		560 (91.3)	47 (8.7)		1,179 (92.2)	97 (7.78)		1,046 (90.7)	113 (9.3)	
≥8	1,002 (93.2)	79 (6.8)		245 (94.1)	13 (5.9)		388 (92.1)	34 (7.9)		369 (93.6)	32 (6.4)	

LDL cholesterol was estimated using the measured values of total cholesterol, HDL cholesterol, and triglycerides. LDL cholesterol (mg/dL)=total cholesterol (mg/dL)−HDL cholesterol (mg/dL)−triglycerides (mg/dL)/5. Reference range: <130 mg/dL (desirable); 131–160 mg/dL (borderline high); >160 mg/dL (high).
hs-CRP, high-sensitivity C-reactive protein; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

https://doi.org/10.24171/j.phrp.2023.0052

Table 3. The factors affecting hs-CRP levels

Characteristic	Total		19–34 y		35–49 y		50–65 y	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Age (y)								
19–34	1 (ref.)							
35–49	1.049 (0.717–1.536)	0.9696						
50–65	1.090 (0.691–1.720)	0.7156						
Education								
≤High school	1 (ref.)							
≥College	0.919 (0.667–1.264)	0.6019	0.947 (0.481–1.863)	0.8743	0.967 (0.567–1.649)	0.9014	0.944 (0.588–1.515)	0.8111
Marital status								
Unmarried	1.359 (0.958–1.927)	0.2162	1.655 (0.811–3.379)	0.6395	1.122 (0.673–1.869)	0.2262	2.222 (0.667–7.400)	0.3368
Married	1 (ref.)							
Separated/divorced/widowed	1.063 (0.567–1.995)	0.7748	1.394 (0.090–21.658)	0.9534	0.387 (0.072–2.072)	0.24	1.462 (0.734–2.909)	0.9661
Household income	1 (ref.)							
1st (lowest) quartile	1.163 (0.655–2.066)	0.6331	0.446 (0.129–1.534)	0.2782	1.846 (0.694–4.911)	0.1865	1.476 (0.678–3.214)	0.4297
2nd quartile	1.040 (0.773–1.400)	0.9258	0.573 (0.273–1.202)	0.4658	1.167 (0.716–1.903)	0.9354	1.217 (0.719–2.060)	0.8796
3rd quartile								
4th (highest) quartile	1.011 (0.753–1.356)	0.7348	1.018 (0.496–2.090)	0.1906	0.811 (0.516–1.273)	0.0649	1.094 (0.675–1.772)	0.6586
Smoking status								
Current smokers	0.997 (0.713–1.393)	0.8082	0.910 (0.418–1.981)	0.8854	1.549 (0.885–2.713)	0.3808	0.721 (0.411–1.262)	0.7172
Former smokers	0.932 (0.649–1.338)	0.634	0.908 (0.337–2.451)	0.9061	1.661 (0.954–2.893)	0.1635	0.609 (0.358–1.038)	0.1102
Never smokers	1 (ref.)							
High-risk drinking								
Yes	1.212 (0.900–1.631)	0.2049	0.795 (0.353–1.788)	0.5778	1.064 (0.689–1.644)	0.7797	1.819 (1.172–2.824)	0.0077
No	1 (ref.)							
Walking activity (min/wk)								
0–149	1.254 (0.984–1.597)	0.067	1.254 (0.755–2.083)	0.3813	1.561 (1.055–2.308)	0.0259	0.890 (0.597–1.326)	0.5658
≥150	1 (ref.)							
History of cancer/cerebro-cardiovascular disease								
No	1 (ref.)							
Yes	1.229 (0.915–1.651)	0.1695	0.809 (0.195–3.359)	0.7698	1.362 (0.876–2.118)	0.1691	1.248 (0.824–1.889)	0.2949
BMI category (kg/m^2)								
<23.0	0.943 (0.650–1.367)	0.1105	0.770 (0.279–2.125)	0.1653	0.630 (0.339–1.171)	0.0075	1.482 (0.895–2.454)	0.0604
23.0–25.0	1 (ref.)							
≥25.0	1.473 (1.078–2.013)	0.0015	1.883 (0.844–4.202)	0.0151	1.717 (1.041–2.834)	0.0005	0.977 (0.617–1.549)	0.2604
LDL (mg/dL)								
≤130	1 (ref.)							
>130	1.236 (0.946–1.614)	0.12	1.396 (0.684–2.850)	0.3579	0.973 (0.662–1.431)	0.8896	1.492 (0.995–2.238)	0.0529
Metabolic syndrome								
No	1 (ref.)							
Yes	1.161 (0.849–1.588)	0.3501	1.192 (0.547–2.598)	0.6572	0.907 (0.564–1.457)	0.6846	1.578 (1.034–2.409)	0.0346
Perceived stress								
No	1 (ref.)							
Yes	1.170 (0.902–1.519)	0.2368	1.691 (0.997–2.867)	0.0511	0.865 (0.567–1.320)	0.5005	1.244 (0.806–1.919)	0.3234
Weekly working hours								
<40	1.281 (0.919–1.786)	0.6448	1.957 (1.049–3.652)	0.2509	1.136 (0.614–2.102)	0.359	0.953 (0.568–1.600)	0.3961
≥40, <49								
≥49, <53	1.561 (1.013–2.406)	0.4056	2.033 (0.818–5.049)	0.3704	1.342 (0.724–2.488)	0.8234	1.313 (0.561–3.071)	0.6311
≥53, <61	1.249 (0.852–1.830)	0.5666	0.955 (0.315–2.897)	0.3227	1.405 (0.783–2.522)	0.9733	1.182 (0.672–2.079)	0.81
≥61	1.834 (1.218–2.761)	0.0548	1.788 (0.615–5.196)	0.6289	2.652 (1.393–5.046)	0.0065	1.210 (0.634–2.311)	0.7633

(Continued to the next page)

Table 3. Continued

Characteristic	Total		19–34 y		35–49 y		50–65 y	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Occupational group								
White-collar								
Pink-collar	0.956 (0.687–1.331)	0.4816	0.903 (0.451–1.810)	0.328	0.812 (0.512–1.288)	0.611	1.343 (0.707–2.549)	0.3943
Blue-collar	1.108 (0.766–1.602)	0.4182	1.475 (0.697–3.121)	0.1788	0.812 (0.449–1.468)	0.693	1.220 (0.615–2.419)	0.8395
Work pattern								
Day work								
Shift work	1.027 (0.728–1.449)	0.8801	1.288 (0.727–2.282)	0.3847	0.647 (0.342–1.223)	0.1793	1.054 (0.551–2.016)	0.8725
Sleeping hours								
<6	0.888 (0.599–1.319)	0.976	1.307 (0.517–3.304)	0.3303	0.881 (0.507–1.532)	0.595	0.690 (0.372–1.280)	0.6954
6–8	1 (ref.)							
≥8	0.780 (0.556–1.093)	0.2959	0.664 (0.323–1.365)	0.187	1.054 (0.651–1.704)	0.6573	0.606 (0.377–0.974)	0.211

LDL cholesterol was estimated using the measured values of total cholesterol, HDL cholesterol, and triglycerides. LDL cholesterol (mg/dL) = total cholesterol (mg/dL)−HDL cholesterol (mg/dL)−triglycerides (mg/dL)/5. Reference range: <130 mg/dL (desirable); 131–160 mg/dL (borderline high); >160 mg/dL (high).

hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio; CI, confidence interval; ref., reference; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

behavior, obesity, metabolic syndrome, high-risk drinking, walking habits, and long working hours were shown to be significant factors influencing hs-CRP across all or specific age groups.

It has been consistently shown that individuals with hs-CRP levels exceeding 3 mg/L are at very high risk for vascular issues. Elevated hs-CRP levels play a crucial role in the onset and progression of cardiovascular disease, underscoring the importance of studying their association with diabetes, obesity, reduced physical activity, and smoking habits [17]. Yamada et al. [32] found that factors such as sex, age, systolic index, and lipoprotein were linked to elevated CRP levels. In the present study, metabolic syndrome, encompassing obesity, and reduced walking activity were associated with high hs-CRP levels, which are also implicated in the onset and progression of cardiovascular disease.

In a study examining the relationship between CRP levels and the risk of metabolic syndrome and diabetes in men, it was found that men with CRP levels exceeding 3 mg/L were several times more likely to develop metabolic syndrome or diabetes than those with CRP levels of 1.0 mg/L [20]. Numerous prior studies have found no correlation between age (ranging from 20 to 70 years) and CRP concentration [33,34], and age did not emerge as a significant factor in this study. However, in light of studies where a statistically significant increase in CRP was observed with age [32], it is clear that the factors influencing hs-CRP vary across different age groups. Therefore, it is crucial to consider each age group individually in future studies related to hs-CRP.

High-risk drinking was identified as a significant factor influencing hs-CRP levels in the 50 to 65-year-old group, while walking activity significantly influenced hs-CRP levels in the 35 to 49-year-old age group. This aligns with a previous report on healthy, middle-aged individuals. In that report, daily alcohol intake demonstrated a clear U-shaped association with hs-CRP and fibrinogen values in males. A proportional odds model analysis revealed that moderate alcohol consumption (20 to 70 g versus no drinking per day: OR, 0.32; 95% CI, 0.14–0.74), and regular exercise (≥3 times/wk versus no exercise: OR, 0.52; 95% CI, 0.35–0.77) were inversely correlated with elevated hs-CRP values [35].

In all participants, variations in the distribution of working hours across different age groups could potentially influence the significance of any observed differences. Therefore, working hours are not just a strong factor affecting hs-CRP levels, but it appears that the group aged 35 to 49 is more impacted by longer working hours. In Korea, individuals aged 35 to 49 have the highest rate of regular employment and the lowest rate of unemployment compared to other age groups, making it logical to focus on this demographic [36]. Consequently, when contemplating policies such as extending working hours, it is crucial to give sufficient consideration to workers' health behaviors and conditions according to their age, and to assess the impact of increased working hours on their health.

Exercise positively impacts serum CRP levels by causing a decrease in CRP. Studies have discovered that individuals with elevated CRP levels or dyslipidemia prior to starting an exercise regimen experienced more significant reductions in CRP [37]. Past interventional studies have demonstrated that interventions such as quitting smoking, engaging in regular exercise, and reducing weight can lower CRP levels among individuals at high risk of developing noncommunicable diseases. These diseases include

https://doi.org/10.24171/j.phrp.2023.0052

diabetes mellitus, cerebrovascular events, and ischemic cardiovascular disease [38,39].

A strength of this study lies in its use of the KNHANES, a nationwide survey that investigates large groups. This survey thoroughly examines individuals' socioeconomic statuses and locations, while also providing a comprehensive record of their lifestyles and health conditions. Furthermore, this study holds significant value as it represents the first attempt in the Republic of Korea to establish a relationship between hs-CRP levels, age, and working hours using such extensive data.

Nevertheless, this study does have certain limitations. First, our analysis was confined to working hours, without taking into account the intensity of labor. This is a limitation because it fails to consider that labor intensity often decreases with age, or that the proficiency of labor cannot be factored in. Second, our analysis of hs-CRP levels was restricted to a dichotomization between ≤3 mg/L and >3 mg/L, which prevented us from obtaining more detailed results. Lastly, as this study only targeted male workers, we recommend broadening the scope to include female workers in future research for a comparative study according to gender.

Conclusion

We identified factors affecting hs-CRP levels by age group among Korean wage workers using the KNHANES from 2016 to 2018.

There were significant differences in hs-CRP levels according to BMI, metabolic syndrome, and weekly working hours in all participants. Additionally, the amount of time spent walking each week significantly impacted the hs-CRP level, particularly between those who walked 0 to 149 minutes per week and those who walked 150 minutes or more per week in the 35 to 49 age group. According to the results of multiple logistic regression analysis, BMI influenced hs-CRP levels in all participants. In the 35 to 49 age group, those who walked less frequently, had a higher BMI, and worked longer hours each week showed significantly higher hs-CRP levels. Furthermore, in the 50 to 65 age group, those who engaged in high-risk drinking and had metabolic syndrome also showed significantly higher hs-CRP levels.

The Korean government intends to modify the legally mandated weekly working hours for employees, aiming to increase labor market flexibility. This study's findings indicate a correlation between obesity, metabolic syndrome, and weekly working hours with hs-CRP, an inflammation biomarker. Furthermore, the factors significantly impacting hs-CRP levels varied across different age groups. Consequently, any plans to adjust working hours should be thoroughly deliberated, taking into account health behaviors such as alcohol consumption and physical activity, as well as health conditions such as metabolic syndrome and obesity, which differ according to age.

Notes

Ethics Approval

Written informed consent was obtained for publication of this study and accompanying images.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

All data generated or analyzed during this study are included in this published article. For other data, these may be requested through the corresponding author.

Authors' Contributions

Conceptualization: all authors; Data curation: CWS, THY; Formal analysis: CWS; Investigation: CWS, THY; Methodology: CWS, THY; Project administration: CWS; Resources: CWS; Validation: CWS, Visualization: CWS, Writing–original draft: all authors; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0144
Osong Public Health Res Perspect 2023;14(5):368-378

Original Article

The risk associated with psychiatric disturbances in patients with diabetes in Indonesia (2018): a cross-sectional observational study

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ABSTRACT

Objectives: The global prevalence of psychiatric disturbances is rising, detrimentally affecting the quality of care and treatment outcomes for individuals, particularly those with diabetes.This study investigated the association of risk factors for psychiatric disturbances among productive-age patients with diabetes (ages 30–59 years), considering sociodemographic characteristics and co-existing diseases. The risk factors considered included sociodemographic factors (e.g., residence, age, sex, marital status, education, and occupation) and co-existing diseases (e.g., hypertension, heart disease, stroke, renal failure, rheumatism, asthma, and cancer).
Methods: This cross-sectional study utilized data from the 2018 Indonesian National Health Survey (Riskesdas). The study population comprised respondents aged between 30 and 59 years who had diabetes and had completed the 20-question self-reporting questionnaire (SRQ-20). After the exclusion of incomplete SRQ-20 data, the sample included 8,917 respondents. Data were analyzed using logistic regression.
Results: Approximately 18.29% of individuals with diabetes displayed symptoms indicative of psychiatric disturbances. After adjusting for sociodemographic factors such as age, sex, education level, occupation, marital status, and place of residence, patients with diabetes who had co-existing conditions such as hypertension, heart diseases, rheumatic disorders, asthma, or cancer had a higher risk for developing psychiatric disturbances than those with diabetes alone (adjusted odds ratio, 6.67; 95% confidence interval, 4.481–9.928; $p < 0.001$).
Conclusion: The elevated risk of psychiatric disturbances among patients with diabetes who had comorbidities underscores the importance of addressing mental health issues in the management of diabetes, especially in patients with concurrent disease conditions.

Keywords: Chronic disease; Diabetes mellitus; Emotional disturbance; Non-communicable diseases; Psychiatric disturbance; Riskesdas

Received: May 29, 2023
Revised: September 6, 2023
Accepted: September 10, 2023

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Introduction

Diabetes mellitus is a widespread chronic disease with substantial medical and economic consequences [1]. The International Diabetes Federation estimates that around 451 million adults globally are living with diabetes [2]. In Indonesia, it is the third leading cause of disability-adjusted life years [3]. The coexistence of comorbidities like cardiovascular diseases with diabetes can elevate the risk of premature mortality, underscoring the need for effective diabetes management [4].

Depression is a psychiatric disturbance that can profoundly impact an individual's functioning, contribute to physical health issues, and decrease life expectancy. The global prevalence of psychiatric disturbances, including depression, is substantial, affecting approximately 300 million people at present [5,6]. Individuals with diabetes are more likely to exhibit depressive symptoms compared to those without the condition [7]. Both conditions can impede treatment adherence, disrupt metabolic control, and result in poor compliance with medication and dietary plans. As a result, these conditions can lower the quality of life and escalate healthcare costs.

The concurrent presence of hypertension and diabetes alongside psychiatric disorders detrimentally impacts both healthcare outcomes and prognosis. A comprehensive survey carried out by the World Health Organization (WHO) across 60 countries reported that comorbid depression affects 9.3% to 23.0% of individuals with chronic diseases [8].

Understanding the impact of psychiatric disturbances on individuals with type 2 diabetes mellitus is essential for achieving treatment goals [1]. Prior research exploring the connection between diabetes and psychiatric disturbances in Indonesia has predominantly focused on healthcare environments [9,10]. This study investigated the risk association of psychiatric disturbances among patients with diabetes, considering sociodemographic factors and concurrent disease conditions. The insights gained from this analysis are beneficial for healthcare providers, as they can guide the creation of programs and interventions that encourage treatment compliance and self-care among individuals with diabetes.

HIGHLIGHTS

- An interdependent relationship exists between diabetes and psychiatric disturbances. The presence of diabetes can exacerbate psychiatric disturbances, and vice versa.
- We discovered that the risk associated with psychiatric disturbances was aggravated when diabetes co-existed with cardiovascular diseases and other chronic conditions.
- All participants had high COVID-19 vaccine confidence.
- It is essential to conduct screenings for psychiatric disturbances in patients with diabetes, especially those with co-existing diseases. These screenings should be performed using the 20-question self-reporting questionnaire tool in primary care settings (Posbindu) and community health centers (Puskesmas), as psychiatric disturbances can worsen treatment adherence and self-care.

Materials and Methods

Study Design

We analyzed data from the 2018 National Health Survey (NHS), which was carried out by the Ministry of Health in the Republic of Indonesia. The NHS employed a multistage systematic random sampling approach to select participants. The initial step involved identifying and designating census blocks as primary sampling units (PSUs). From each PSU, census blocks were chosen using the probability proportional to size method, which was based on the master sampling from the 2010 Indonesian Central Bureau of Statistics (Badan Pusat Statistik). This process resulted in the selection of 180,000 census blocks from a total of 720,000, representing both urban and rural areas within each sub-district at the district level. Researchers carried out face-to-face interviews with all members of the selected households who had resided there for a minimum of 6 months and were part of the same food management unit [11,12]. Our analysis focused on the age group of 30 to 59 years from the 387,542 respondents in the 2018 Indonesian NHS. This focus was due to the reported sharp increase in the prevalence of diabetes within this demographic. Among the participants, we identified 8,935 individuals (2.3%) with diabetes. However, we excluded 18 individuals (0.2%) from the analysis because they had not completed the 20-question self-reporting questionnaire (SRQ-20). Consequently, our study incorporated complete data from 8,917 individuals (Figure 1) [11,12].

Samples

Population

This study included participants in the Indonesian NHS of 2018. The inclusion criteria specified that participants must be Indonesian, have participated in the 2018 NHS, have diabetes, and be between the ages of 30 and 59 years. The

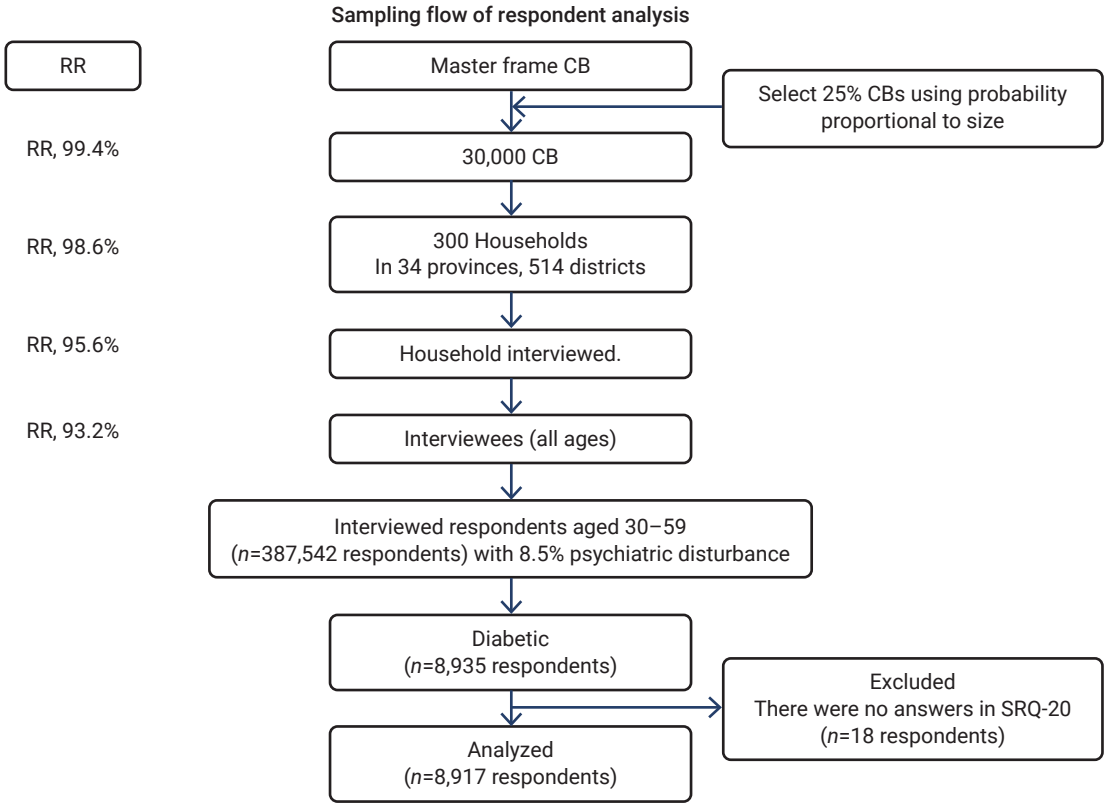

Figure 1. Flow chart of data selection. Modified from Report of national community health survey 2018 [11] and Idaiani and Indrawati. BMC Public Health 2021;21:2332, according to the Creative Commons license [12].
RR, response rate; CB, census blocks; SRQ-20, 20-question self-reporting questionnaire.

exclusion criteria were set to omit any participants with incomplete data, including those without the SRQ-20. The study sample comprised 8,917 participants, all of whom were between the ages of 30 and 59 and had diabetes.

Data collection

The data were gathered from the Health Development Policy Agency, adhering to specific requirements and procedures available at https://www.badankebijakan.kemkes.go.id/.

Variables

The outcome variable under investigation was psychiatric disturbances, which included anxiety and depression, and were determined based on the respondent's mental and emotional state over the past 30 days. The SRQ, developed by the WHO, was utilized for this purpose. This questionnaire is specifically designed to identify psychiatric disturbances, particularly in developing countries. It comprises 20 yes-or-no questions pertaining to the respondent's mental state over the previous 30 days (SRQ-20). The reliability of the SRQ-20 in this study was confirmed by a Cronbach α of 0.899 (Supplementary Material 1). This Cronbach α value of 0.899 (>0.60) indicates that all the questionnaire items related to psychiatric disturbances were reliable. Respondents were categorized as having a psychiatric disturbance if they responded affirmatively to at least 6 questions [13,14].

Explanatory

The primary explanatory variable in this study was diabetes status, which was categorized as either with or without co-existing disease conditions. These co-existing conditions encompassed a range of diseases such as heart disease, stroke, hypertension, renal failure, rheumatic disorders, asthma, or cancer, all of which were diagnosed by a medical professional. The multivariate analyses incorporated several covariates, including sex (categorized as male or female), age groups (divided into 30–39 years, 40–49 years, and 50–59 years), occupation (distinguished as working or not working), marital status (classified as single or married), education (categorized as either having a high school certificate or not), and place of residence (classified as either rural or urban).

https://doi.org/10.24171/j.phrp.2023.0144

Data Analysis

We conducted a series of univariate, bivariate, and multivariate analyses. The multivariate analysis involved the execution of logistic regression using IBM SPSS ver. 21.0 (IBM Corp.). Variables from the bivariate analysis that had a *p*-value of less than 0.2 were incorporated into the multivariate analysis.

Ethical Considerations

Ethical approval for Riskesdas 2018 was granted by the Ethical Committee of Health Research, National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia, under the reference number LB.02.01/2/KE.267/2017 [11].

Results

Table 1 illustrates the distribution of diabetes in Indonesia, both as a standalone condition and in conjunction with other diseases. Of the total, 4,652 cases (52.2%) were of diabetes alone, while 4,265 cases (47.8%) involved diabetes with co-existing disease conditions. The 3 most prevalent co-existing conditions were all cardiovascular-related diseases: 17.0% of cases involved diabetes with hypertension, 6.2% involved diabetes with hypertension and other conditions, and 2.5% involved diabetes with heart diseases. In terms of chronic diseases co-existing with diabetes, the highest prevalence was seen in cases of diabetes with rheumatic diseases at 9.1%, followed by diabetes with asthma at 1.8%, and diabetes with cancer at 0.3%.

Table 2 presents the demographic data of the participants. The majority, 5,253 (58.9%), resided in urban areas. There were 5,248 participants (58.9%) in the age group of 50 to 59 years old. Females constituted a significant portion of the participants, with 5,590 (62.7%) in total. Regarding education, 5,407 (60.6%) had not completed high school. Most of the participants were married, with a total of 7,773 (87.2%). Additionally, 5,768 (64.7%) of the participants were employed.

Among the participants with diabetes, 1,631 individuals, or 18.3%, exhibited psychiatric disturbances. The socioeconomic factors associated with these disturbances included living in urban areas (crude odds ratio [cOR], 0.69), being between the ages of 50 and 59 (cOR, 0.83), and being unemployed (cOR, 1.73). Conversely, the risk of psychiatric disturbances was higher for female participants (cOR, 1.77), single individuals (cOR, 1.65), and those who did not complete high school (cOR, 2.28). Diabetes combined with other chronic diseases displayed an increasing association with psychiatric disturbances. This was observed in cases of diabetes with rheumatic conditions (cOR, 1.81), diabetes with asthma (cOR, 1.90), and diabetes with cancer (cOR, 2.09). The association was even stronger when diabetes was combined with 1 or more of these conditions: rheumatic conditions, asthma, or cancer (cOR, 4.47). The strongest association was found in cases of diabetes, hypertension, and heart disease, combined with a variety of rheumatic conditions, asthma, and/or cancer (cOR, 6.40) (Table 2).

Table 1. Distribution of diabetes and co-existing disease conditions in Indonesia in 2018 (*n* = 8,917)

Variable	*n* (%)
Diabetes	4,652 (52.2)
Diabetes+HT	1,516 (17.0)
Diabetes+HT+other	550 (6.2)
Diabetes+HD	226 (2.5)
Diabetes+HD+other	93 (1.0)
Diabetes+stroke	85 (1.0)
Diabetes+stroke+other	29 (0.3)
Diabetes+RF	49 (0.5)
Diabetes+RF+other	25 (0.3)
Diabetes+HT+HD	163 (1.8)
Diabetes+HT+HD+other	108 (1.2)
Diabetes+HT+HD+stroke, diabetes+HT+HD+stroke+RF, diabetes+HD+stroke, or diabetes+stroke+RF	224 (2.5)
Diabetes+HT+HD+stroke+other, diabetes+HT+HD+stroke+RF+other, diabetes+HD+stroke+other, or diabetes+stroke+RF+other	113 (1.3)
Diabetes+rheumatic	815 (9.1)
Diabetes+asthma	162 (1.8)
Diabetes+cancer	25 (0.3)
Diabetes+combination with 2 or 3 others	82 (0.9)
Total	8,917 (100.0)

HT, hypertension; HD, heart disease; RF, renal failure; other, rheumatic and/or asthma and/or cancer.

Table 2. Sociodemographic characteristics of respondents with diabetes, their co-existing diseases, and associations with psychiatric disturbances (*n* = 8,917)

Variable	Total	No	Yes	cOR (95% CI)	*p*
Place of residence					
Rural	3,664	2,873 (78.4)	791 (21.6)	Ref.	
Urban	5,253	4,413 (84.0)	840 (16.0)	0.69 (0.62–0.77)	<0.001
Age (y)					
30–39	694	554 (79.8)	140 (20.2)	Ref.	
40–49	2,975	2,390 (80.3)	585 (19.7)	1.00 (0.79–1.19)	0.08
50–59	5,248	4,342 (82.7)	906 (17.3)	0.83 (0.68–1.00)	0.06
Sex					
Male	3,327	2,886 (86.7)	441 (13.3)	Ref.	
Female	5,590	4,400 (78.7)	1,190 (21.3)	1.77 (1.57–1.99)	<0.001
Marital status					
Currently married	7,773	6,435 (82.8)	1,338(17.2)	Ref.	
Single	1,144	851 (74.4)	293 (25.6)	1.66 (1.43–1.91)	<0.001
Education					
High school graduate	3,510	3,109 (88.6)	401 (11.4)	Ref.	
Below high school graduate	5,407	4,177 (77.3)	1,230(22.7)	2.28 (2.20–2.58)	<0.001
Occupation					
Employed	5,768	4,886 (84.7)	882 (15.3)	Ref.	
Not employed	3,149	2,400 (76.2)	749 (23.8)	1.73 (1.55–1.93)	<0.001
Diabetes with comorbidities					
Diabetes	4,652	4,043 (86.9)	609 (13.1)	Ref.	
Diabetes+HT	1,516	1,239 (81.7)	277 (18.3)	1.48 (1.27–1.73)	<0.001
Diabetes+HT+other	550	384 (69.8)	166 (30.2)	2.87 (2.35–3.51)	<0.001
Diabetes+HD	226	193 (85.4)	33 (14.6)	1.13 (0.78–1.66)	0.51
Diabetes+HD+other	93	59 (63.4)	34 (36.6)	3.83 (2.49–5.88)	<0.001
Diabetes+stroke	85	59 (69.4)	26 (30.6)	2.93 (1.83–4.68)	<0.001
Diabetes+stroke+other	29	23 (79.3)	6 (20.7)	1.73 (0.70–4.27)	0.23
Diabetes+RF	49	33 (67.3)	16 (32.7)	3.22 (1.76–5.88)	<0.001
Diabetes+RF+other	25	15 (60.0)	10 (40.0)	4.43 (2.00–10.00)	<0.001
Diabetes+HT+HD	163	130 (79.8)	33 (20.2)	1.68 (1.14–2.50)	0.009
Diabetes+HT+HD+other	108	55 (50.9)	53 (49.1)	6.40 (4.34–9.42)	<0.001
Diabetes+HT+HD+stroke; and diabetes+HT +HD+stroke+RF; and diabetes+HD+stroke; and diabetes+stroke+RF	224	159 (71.0)	65 (29.0)	2.71 (2.01–3.67)	<0.001
Diabetes+HT+HD+stroke+other; and diabetes+HT +HD+stroke+RF+other; and diabetes+HD+stroke +other; and diabetes+stroke+RF+other	113	60 (53.1)	53 (46.9)	5.86 (4.01–8.57)	<0.001
Diabetes+rheumatic	815	640 (78.5)	175 (21.5)	1.81 (1.50–2.19)	<0.001
Diabetes+asthma	162	126 (77.8)	36 (22.2)	1.90 (1.30–2.77)	0.001
Diabetes+cancer	25	19 (76.0)	6 (24.0)	2.09 (0.83–5.27)	0.12
Diabetes+combination with 2 or 3 other conditions	82	49 (59.8)	33 (40.2)	4.47 (2.85–7.01)	<0.001
Total	8,917	7,286 (81.7)	1,631 (18.3)		

Data are presented as *n* (%).
cOR, crude odds ratio; CI, confidence interval; ref., reference; HT, hypertension; HD, heart disease; RF, renal failure; other, rheumatic and/or asthma and/or cancer.

Table 3 illustrates the adjusted ORs (aORs) of the associations of sociodemographic characteristics, diabetes, and co-existing disease conditions with psychiatric disturbances among individuals with diabetes. This table presents the same pattern as depicted in Table 2, but with a different degree of influence.

Discussion

Prior research has consistently shown that when individuals with diabetes and/or hypertension also exhibit depression, they tend to exhibit decreased self-care practices, diminished adherence to treatment, less than optimal glycemic control,

https://doi.org/10.24171/j.phrp.2023.0144

Table 3. Multivariate analysis of psychiatric disturbances among individuals with diabetes, Riskesdas 2018

Variable	B	SE (B)	aOR (95% CI)	*p*
Place of residence				
Rural			Ref.	
Urban	0.210	0.059	0.81 (0.72–0.91)	< 0.001
Age (y)				
30–39			Ref.	
40–49	−0.147	0.110	0.86 (0.70 –1.07)	0.18
50–59	−0.459	0.107	0.63 (0.51–0.78)	< 0.001
Sex				
Male			Ref.	
Female	0.189	0.071	1.21 (1.05–1.39)	0.008
Marital status				
Married			Ref.	
Single	0.410	0.079	1.51 (1.29–1.76)	< 0.001
Education				
High school graduate			Ref.	
Below high school graduate	0.649	0.067	1.91 (1.68–2.18)	< 0.001
Occupation				
Employed			Ref.	
Not employed	0.308	0.064	1.36 (1.20–1.54)	< 0.001
Diabetes with comorbidity				
Diabetes			Ref.	
Diabetes+HT	0.350	0.081	1.42 (1.21–1.66)	< 0.001
Diabetes+HT+other	0.983	0.106	2.67 (2.17–3.29)	< 0.001
Diabetes+HD	0.231	0.197	1.26 (0.86–1.85)	0.24
Diabetes+HD+other	1.337	0.226	3.81 (2.45–5.93)	< 0.001
Diabetes+stroke	1.193	0.247	3.29 (2.03–5.35)	< 0.001
Diabetes+stroke+other	0.565	0.472	1.76 (0.70–4.43)	0.23
Diabetes+RF	1.249	0.315	3.49 (1.88–6.46)	< 0.001
Diabetes+RF+other	1.501	0.422	4.49 (1.96–10.26)	< 0.001
Diabetes+HT+HD	0.443	0.204	1.56 (1.04–2.32)	0.03
Diabetes+HT+HD+other	1.898	0.203	6.67 (4.48–9.93)	< 0.001
Diabetes+HT+HD+stroke, diabetes+HT+HD+stroke+RF, diabetes +HD+stroke, or diabetes+stroke+RF	1.033	0.158	2.81 (2.06–3.83)	< 0.001
Diabetes+HT+HD+stroke+other or diabetes+HT+HD+stroke+RF+other or diabetes+HD+stroke+other or diabetes+stroke+RF+other	1.762	0.201	5.82 (3.93–8.63)	< 0.001
Diabetes+rheumatic	0.559	0.098	1.75 (1.44–2.12)	< 0.001
Diabetes+asthma	0.596	0.197	1.81 (1.23–2.67)	0.003
Diabetes+cancer	0.800	0.481	2.23 (0.87–5.72)	0.1
Diabetes+combination with 2 or 3 other conditions	1.345	0.235	3.84 (2.42–6.09)	< 0.001

SE, standard error; aOR, adjusted odds ratio; CI, confidence interval; ref., reference; HT, hypertension; HD, heart disease; RF, renal failure; other, rheumatic and/or asthma and/or cancer.

heightened complication rates, increased healthcare costs, reduced quality of life, and higher mortality rates. Unfortunately, many individuals within these populations who have undiagnosed depression do not receive the requisite treatment [15–17].

The relationship between psychiatric disturbances and chronic diseases such as diabetes is mutually influential. Psychiatric disturbances can exacerbate the conditions of individuals with diabetes, and conversely, diabetes can intensify psychiatric disturbances, impacting overall well-being [18]. In this context, "diabetic distress" is defined as the emotional response marked by fear, discomfort, or disappointment that arises from the perceived inability to effectively manage the challenges of living with diabetes [19]. Our analysis revealed that among Indonesians aged 30 to 59 (387,542 respondents), the prevalence of psychiatric disturbances was 8.5%, while the prevalence of diagnosed diabetes was 2.3% (Figure 1). Furthermore, nearly 18.3% of diabetes patients (8,917 respondents) displayed symptoms of nonspecific psychological distress indicative of psychiatric disturbances.

Our findings did not reveal a significant impact of age on

the incidence of psychiatric disturbances. It is possible that older adults have already developed coping mechanisms and resources, unlike their younger counterparts who are still grappling with various challenges [20]. Our study also found that women were 1.21 times more likely to experience psychiatric disturbances than men. This finding is consistent with the research by Deischinger et al. [21], which suggests that major depressive disorder is more prevalent in women, especially those between the ages of 40 and 49. The higher incidence of psychiatric disturbances in women could be due to biological factors and psychological burdens. Conversely, men may be less likely to openly express depressive symptoms [21]. In the case of patients with diabetes, those with co-existing disease conditions were more common among younger individuals, females, and single individuals. These individuals had a 1.51 times higher risk compared to married individuals. Furthermore, individuals with lower education levels had a 1.91 times higher risk than those with higher education. These findings align with the 2018 Canadian clinical practice guideline, which identifies risk factors for developing diabetes distress. These risk factors include being younger, being female, having a lower level of education, and living alone [22].

Our study revealed that the incidence of psychiatric disturbances in individuals with both diabetes and hypertension was 1.42 times greater than in those with only diabetes and no comorbidities. AlKhathami et al. [23] found a high prevalence of depression and anxiety in patients with diabetes and/or hypertension, with these mental health disorders affecting 57.3% of the 368 participants. The participants had an average age of 50.9 ± 11.7 years. Among those diagnosed with depression, 39.8% exhibited mild symptoms, 7.1% showed moderate symptoms, and 1.8% displayed severe symptoms. In terms of anxiety, 25.1% had mild symptoms, 8.8% had moderate symptoms, and 4.4% had severe symptoms. The simultaneous presence of depression and anxiety was noted in 29.5% of the participants. Sleep disturbances, weight fluctuations, and low income were identified as significant independent predictors of depression and anxiety [23]. Fisher et al. [24] conducted a study that showed a common association between a combination of depression, anxiety, and other psychological distress with type 2 diabetes.

Our analysis indicated that individuals with diabetes who also have co-existing conditions such as hypertension, heart disease, rheumatic disease, asthma, or cancer are at the highest risk of experiencing psychiatric disturbances. The OR was significantly high at 6.67 (95% confidence interval [CI], 4.48–9.93; $p < 0.001$) compared to those with diabetes alone. This finding aligns with a previous study by Widakdo and Besral [18], which established a statistically significant relationship between psychological stress and diabetes, along with other chronic diseases, with an OR of 1.6 (95% CI, 1.5–1.7). However, their study did not provide a detailed breakdown of co-existing diseases within the diabetic population, which could account for the lower OR observed in comparison to our study.

We noted that the risk correlation between diabetes and concurrent heart disease on psychiatric disturbances did not statistically differ from that of diabetic individuals without co-existing diseases ($p = 0.24$). This comparable risk could be due to our study not distinguishing the type of psychiatric disturbance, such as depression, anxiety, or neuroticism. Hu et al. [25] reported similar findings, stating that the cause of mental disorders in patients with coronary artery disease (CAD) and diabetes remains unclear. There was no definitive causal link between CAD and anxiety or neuroticism in patients with diabetes. Given these findings, healthcare providers should prioritize mental health treatments to prevent CAD in patients with diabetes [25]. The disruption of cortisol, a stress hormone, is associated with both short-term and long-term stress factors [26]. Inflammation is another potential biological mechanism that links psychosocial factors to cardiovascular disease and diabetes. Individuals under high levels of psychosocial stress often show increased concentrations of inflammatory cytokines [27].

Our research suggests that individuals who have both diabetes and rheumatic conditions are 1.75 times more likely to experience psychiatric disturbances compared to those who only have diabetes and no co-existing diseases. This observation is consistent with the study conducted by Nazarinasab et al. [28], which discovered that individuals suffering from both knee osteoarthritis (OA) and diabetes experience more severe pain and higher incidences of physical and mental health disorders than those without diabetes. The linear regression models used in the study further established that diabetes, independent of other factors such as higher body mass index and the severity of radiographic OA, is a risk factor for increased pain [28].

In addition, our analysis revealed that individuals with both diabetes and asthma had a 1.81-fold higher risk of psychiatric disturbances than those who had diabetes but did not have any co-existing diseases. It is important to note that diabetes is associated with an increased risk of schizophrenia and type 2 diabetes, which in turn further escalates the risk of cardiovascular complications and reduces life expectancy.

Imbalances in Ca^{2+} signaling may establish a link between these diseases and the cAMP signaling pathways. Both the Ca^{2+} and cAMP signaling pathways play a role in regulating

https://doi.org/10.24171/j.phrp.2023.0144

the release of neurotransmitters and hormones, as well as controlling the contractility of airway smooth muscles. Any disruptions in these cellular processes could potentially contribute to the dysregulation observed in these diseases [29].

In this study, the incidence of psychiatric disturbances in individuals with both diabetes and cancer was not significantly different from those with diabetes alone. The risk of psychiatric disturbances in individuals with both diabetes and cancer was represented by an OR of 2.23, but this association was not statistically significant. This could be due to the fact that the study did not differentiate between types of cancer. Furthermore, the number of individuals with both diabetes and cancer was relatively small, which may have contributed to the unstable association observed in our analysis due to the limited number of cases. The research of Vissers et al. [30] suggests that individuals diagnosed with both cancer and diabetes tend to have a poorer health-related quality of life compared to those diagnosed with either disease alone. Other studies have proposed that patient empowerment and self-management strategies can help to lessen the impact of these conditions on patient-reported outcomes, such as depression [30].

Individuals who have diabetes and multiple concurrent diseases may experience more severe psychiatric disturbances than those with diabetes and fewer co-existing conditions. For instance, the combination of diabetes, hypertension, heart disease, and other conditions was more significant than just diabetes, hypertension, and heart disease, with an aOR of 6.67 (95% CI, 4.48–9.93). However, it is crucial to consider other factors such as the duration of the illness, the quality of sleep, family income, and specific types of psychiatric disturbances. These factors also impact cognitive and emotional disorders.

Diagnosing psychiatric disorders in individuals with diabetes often presents a challenge due to the absence of specific guidelines for screening and diagnosis within the healthcare system. This leads to a frequent underdiagnosis of psychiatric disturbances in patients with diabetes [31]. In India, data on the prevalence of non-communicable diseases (NCDs) and psychiatric disturbances are limited. Rajan et al. [32] found that anxiety disorders and depression were present in 3.9% to 44.0% and 8.0% to 46.0% of individuals with NCDs, respectively. These mental health issues were strongly associated with poor quality of life, overall health status, and physical function. Therefore, it is crucial to focus on integrated healthcare approaches to improve the screening and identification of NCDs and psychiatric disturbances [32].

Depression, anxiety, and schizophrenia are commonly observed in individuals with diabetes. These observations underscore the importance of addressing mental health issues within the realm of diabetes care. This is particularly crucial for those with co-existing disease conditions. Providing such information would be beneficial for local policymakers.

Effective diabetes management should engage patients in modifying their lifestyle, monitoring their condition independently, and adhering to prescribed medication. The diagnosis and treatment of mental disorders represent critical elements of diabetes therapy. This is because addressing both physical and psychological health components is vital for providing comprehensive care [33].

After accounting for sociodemographic factors, our analysis suggests that individuals with uncomplicated diabetes or any co-existing diseases exhibit the lowest risk of developing psychiatric disturbances. While the relationship between diabetes and psychiatric disturbances remains uncertain, community health studies have reported a higher prevalence of psychiatric disturbances among individuals with diabetes compared to those without diabetes. Psychiatric disturbances in patients with diabetes can impede treatment adherence and deteriorate treatment outcomes [34].

At the national level, the Indonesian government has enacted the GERMAS program, a healthy lifestyle movement initiated by Indonesian Ministry of Health. This policy intervention aims to prevent and identify NCDs at an early stage. However, there is a current deficiency in mental health interventions for individuals diagnosed with NCDs, including diabetes, within Indonesia. The WHO advocates for the personalization of mental health services for patients with NCDs. This includes conducting mental health screenings for those with diabetes and managing NCDs as a whole [35].

Under the Indonesian Universal Health Insurance (Jaminan Kesehatan Nasional), the management of NCDs is a critical performance indicator with a focus on prevention. Regular screening programs for prevalent NCDs, including blood pressure checks and glucose tests for diabetes, are carried out in primary care environments via initiatives such as Posbindu, program initiated by Indonesian Ministry of Health, which aim for early detection and treatment. Nonetheless, the success of these programs is dependent on the resources and capabilities available in each district.

Our analysis reveals varying risk associations of psychiatric disturbances in patients with diabetes, contingent upon their concurrent diseases and demographic traits. This underscores the necessity of customizing mental health services for individuals with diabetes, considering their demographic attributes and comorbidities. In the holistic healthcare management of type 2 diabetes, specific strategies for the early identification and treatment of psychiatric disturbances should be incorporated to lessen their effect on health outcomes [36].

Conclusion

Patients with diabetes who also have multiple diseases seem to exhibit a higher prevalence of mental disorders. Beyond the presence of co-existing diseases, several sociodemographic factors appear to influence the occurrence of cognitive and emotional disorders in individuals with diabetes. These factors include residing in rural areas, being female, being unmarried, having a lower level of education, and being unemployed.

Recommendation

These findings underscore the necessity of customizing mental health services within diabetic treatment plans, which should involve both health policymakers and specialists. The Indonesian government, in collaboration with internal medicine organizations, has implemented several programs to manage diabetes. These programs include promoting a healthy lifestyle and diet as preventative measures.

Given that psychiatric disturbances can exacerbate treatment adherence and self-care issues among individuals with diabetes, it is crucial for healthcare providers and facilities to implement mental health screenings and support systems. Additionally, promoting mental health literacy among patients with diabetes and other NCDs can help prevent treatment non-compliance, which can lead to complications, and minimize stigmatization. It is now necessary for Indonesian healthcare providers to conduct psychiatric disturbance assessments in diabetes patients using the SRQ in primary care settings (Posbindu) and community health centers (Puskesmas). Integrated management for conditions such as diabetes, hypertension, asthma, arthritis, cancer, and others may aid in delaying or preventing the onset of diseases and mental disorders.

Strengths and Limitations

One advantage of our analysis is that it is grounded in the Indonesian NHS. The findings provide a comprehensive picture of the health status of Indonesians. Our analysis further dissects diabetes into specific comorbidities, thereby representing the range of comorbidities experienced by Indonesians with diabetes. The results indicate a significantly increased risk of psychiatric disturbances among Indonesian diabetes patients who have multiple chronic diseases. This insight prompts the Indonesian health system and practitioners to consider the integration of mental health services for patients with diabetes.

A limitation of this study was that the information collected during the interviews was not derived from medical examinations, but rather depended on self-reported data. Despite our specific inquiries about whether a medical doctor had ever diagnosed the participants, some respondents may have either forgotten or been unaware of their diagnosis. It is crucial to acknowledge that the significant attrition of subjects with stroke or cancer could potentially introduce bias into our findings. Although our analytical approach does not permit us to make causal assertions, the fundamental assumption of our study was that various aspects of diabetes status could impact mental health status. Conversely, it is also conceivable that the relationship between psychiatric disturbances and diabetes with chronic diseases could operate in the reverse direction. That is to say, issues with psychiatric disturbances could contribute to the onset of diabetes with co-existing diseases. The data regarding diabetes and its co-existing conditions were gathered through the question, "Have you ever been diagnosed by a doctor?" Some respondents may need to recall or comprehend the medical term used, especially if a local term for the disease exists. Moreover, the estimates could be underreported, particularly in rural or remote areas and regions with limited resources, where access to doctors is often restricted to larger urban cities.

Supplementary Material

Supplementary Material 1. 20-question self-reporting questionnaire (SRQ-20) Indonesian translation. Supplementary data are available at https://doi.org/10.24171/j.phrp.2023.0144.

Notes

Ethics Approval

Ethical approval for Riskesdas 2018 was granted by the Ethical Committee of Health Research, National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia, under the reference number LB.02.01/2/KE.267/2017.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

This published article and its supplementary files include all data generated or analyzed during this study. The data that support the findings of this study can be obtained from the Data Management Laboratory of the National Institute of Health Research and Development (NIHRD), which is a part of the Ministry of Health of Indonesia. The data will be made available upon approval of a written request to the Data Management Laboratory—NIHRD at the following email address: datin.bkpk@kemkes.go.id.

Authors' Contributions

Conceptualization: SI, BR, LKM; Data curation: all authors; Formal analysis: SI, BR, ST; Investigation: all authors; Methodology: SI, ST;

Supervision: all authors; Validation: all authors; Visualization: ST; Writing–original draft: all authors; Writing–review & editing: all authors. All authors read and approved the final manuscript.

Acknowledgements
The authors would like to express our gratitude to Aliza Hunt, MA, for her assistance in refining the English language and contributing to the analysis in this manuscript. We also extend our gratitude to the technical staff from the Data Management Laboratory of NIHRD, Ministry of Health, Indonesia, and other individuals who facilitated the realization of this research.

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https://doi.org/10.24171/j.phrp.2023.0144

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0089
Osong Public Health Res Perspect 2023;14(5):379-387

Original Article

Factors associated with the combination of general and abdominal obesity in middle-aged and older Korean women: a cross-sectional study

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ABSTRACT

Objectives: To identify factors associated with general and abdominal obesity in middle-aged and older Korean women based on the biopsychosocial model.
Methods: Data from 4,076 women aged ≥45 years who participated in the Korea National Health and Nutrition Examination Survey from 2015 to 2020 were analyzed. Complex sampling analysis was performed using IBM SPSS ver. 26.0.
Results: The combination of general and abdominal obesity was positively associated with age ≥65 years, postmenopausal status, and without breastfeeding experience among biomedical factors; depressive symptoms and prolonged (≥8 hours a day) sedentary behavior among psychosocial factors; and an educational level lower than middle or high school graduation and the first and second income quantiles among biosocial factors.
Conclusion: Healthcare providers in communities and public societies should screen for risk factors for the combination of general and abdominal obesity while considering non-modifiable biomedical (e.g., age) and biosocial factors (e.g., educational level). In addition, intervention strategies should be developed by considering modifiable psychosocial factors such as sedentary behavior.

Keywords: Abdominal obesity; Middle aged; Obesity; Women

Received: April 3, 2023
Revised: June 1, 2023
Accepted: June 29, 2023

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Introduction

Obesity is a major health problem. The prevalence rates of general obesity based on body mass index (BMI) and abdominal obesity based on waist circumference (WC) have steadily increased over time [1]. According to a report on Korean national trends, the prevalence of general obesity in adults increased from 29.7% in 2009 to 36.3% in 2019, and that of abdominal obesity in adults increased from 19.0% in 2009 to 23.9% in 2019 [2]. Furthermore, middle-aged and older women aged >50 years may have higher risks than men of developing general and abdominal obesity,

owing to menopause with decreased ovarian function and related aging processes [2]. According to previous studies, in middle-aged and older Korean women aged >45 years, 37.3% of those aged between 45 and 80 years were obese (BMI ≥25 kg/m^2) [3], and 28.9% of those aged between 45 and 65 years had abdominal obesity with (WC ≥85 cm) [4]. Additionally, a study of Brazilian middle-aged women aged 45 to 64 years reported the prevalence rates of general obesity and abdominal obesity as 68.2% to 73.8% and 56.4% to 63.1%, respectively [5]. Thus, general and abdominal obesity may present major health problems for middle-aged and older women.

General obesity is independently associated with metabolic syndrome, cardiovascular disease (CVD), and cancer in adults, including middle-aged and older women [6,7]. Abdominal obesity is also strongly associated with a high risk of developing metabolic abnormalities (e.g., dyslipidemia, hypertension, and diabetes), CVD, cancer, and all-cause mortality [6–10]. However, previous studies have reported that the development of diabetes and CVD was associated with the combination of general and abdominal obesity [11,12]. Considering their individual effects, this combination might have synergetic effects on health, such as the development of diabetes [12]. Additionally, the risk of developing diabetes increased significantly only with the combination of general and abdominal obesity, but not with other patterns such as general obesity without abdominal obesity and vice versa [12]. Individuals with both general and abdominal obesity have a higher risk of cancer and stroke than those with other obesity patterns [7,13]. Particularly in women, the combination of general and abdominal obesity, and not other patterns of obesity alone, is significantly associated with an elevated risk of stroke [13]. Furthermore, in individuals aged >50 years, the combination of general and abdominal obesity is associated with a high risk of CVD [13]. Similarly, postmenopausal women with the combination of general and abdominal obesity have a notably higher risk of cancer (e.g., breast and endometrial cancer) than postmenopausal women with other patterns of obesity (general obesity without abdominal obesity) and premenopausal women with the combination of general and abdominal obesity [7]. Thus, the combination of general and abdominal obesity is considered a better predictor of CVD and cancer in middle-aged women [7,13]. Considering that this combination is the most common pattern of obesity in adults [13], preventing it may be important for promoting good health in middle-aged and older women. To prevent this combination, the identification of associated factors at multiple levels may be the first step in the development of health promotion strategies. However, awareness of the factors associated with the combination of general and abdominal obesity is limited, although they differ according to obesity patterns [12]. Middle-aged and older Korean women grew up in a Confucian culture that imposed stricter social norms on women than men and attributes superiority to men [14]. Thus, these women might have lower smoking and alcohol consumption than same-aged Korean men [14]. They might also be less educated than same-aged Korean men, resulting in low information about how to engage in a healthy lifestyle [15]. Therefore, the factors associated with the combination of general and abdominal obesity in middle-aged and older Korean women are different from those in same-aged Korean men and same-aged women in other countries with different cultural norms.

HIGHLIGHTS

- Age >65 years, postmenopausal status, and without breastfeeding experience were positively correlated with the combination of general and abdominal obesity.
- Depressive symptoms and prolonged sedentary behavior were positively correlated with the combination of general and abdominal obesity.
- Lower educational level and lower income were positively correlated with the combination of general and abdominal obesity.

Several factors contribute to the development of obesity, with various underlying mechanisms. Straub [16] proposed that obesity as a complex phenomenon is influenced by biological, social, and psychological factors. According to Hoffman and Driscoll [17], the biopsychosocial model might be an appropriate framework to evaluate the effects of multivariate factors on metabolic health (e.g., abdominal obesity). Thus, the biopsychosocial model can provide a framework for a comprehensive understanding of distinctive individual characteristics associated with health status, including obesity [18].

According to the biopsychosocial model, biomedical (e.g., age and sex), psychosocial (e.g., mood and health-related behaviors), and biosocial factors (e.g., educational level and socioeconomic household status) interact to influence people's health. Biomedical factors associated with general and abdominal obesity include age [10,19], postmenopausal status, and breastfeeding experience [20]. Psychosocial factors include depressive symptoms [21], skipping breakfast [22], eating out [22], sedentary behavior [22], physical activity (days of walking per week) [22–24], current and past smoking [10,19], and current alcohol

https://doi.org/10.24171/j.phrp.2023.0089

consumption [19,25]. Among biosocial factors, educational level [10], employment [26], and household socioeconomic status [10] have been associated with abdominal obesity. Therefore, this study aimed to identify factors associated with the combination of general and abdominal obesity in middle-aged and older Korean women based on the biopsychosocial model.

Materials and Methods

Study Design and Samples

Using a cross-sectional design, secondary data analysis was conducted using the Korea National Health and Nutrition Examination Survey (KNHANES) data of 44,951 Korean individuals (20,473 men and 24,478 women) 1 to 80 years old who participated in the survey from 2015 to 2020. Of 20,176 Korean women aged ≥20 years old, 13,725 middle-aged and older women aged 45 to 80 years were selected. Finally, after excluding individuals who did not complete the questionnaires and physical examinations (height, body weight, and WC), data from 4,076 women aged 45 to 80 years old were analyzed (Figure 1).

Measurements

Dependent variables

To evaluate the combination of general and abdominal obesity, BMI and WC were measured. Obesity patterns were categorized into 4 groups: combination of general and abdominal obesity, general obesity without abdominal obesity, abdominal obesity without general obesity, and neither general nor abdominal obesity.

General obesity

To evaluate adiposity, BMI was calculated by dividing weight (kg) by height squared (m^2). BMI was classified into the following categories: underweight (<18.5 kg/m^2), normal weight (≥18.5 kg/m^2 and <23 kg/m^2), overweight (≥23 kg/m^2 and <25 kg/m^2), or obese (≥25 kg/m^2) [27]. Regarding adiposity, participants were classified into non-obese (underweight and normal weight) and obese (overweight and obese) groups.

Abdominal obesity

Abdominal obesity was assessed using WC. A WC ≥85 cm in women indicates abdominal obesity [28].

Independent variables

Independent variables and measurements are described in Table 1 [29,30].

Data Analyses

Following the guidelines for the statistical analysis of the KNHANES data, a complex sampling analysis was applied using IBM SPSS ver. 26.0 (IBM Corp.). The prevalence of obesity according to the patterns and variables of biomedical, psychosocial, and biosocial factors was analyzed using frequencies and percentages. Logistic regression analysis was applied for identification of the factors associated with the combination of general and abdominal obesity.

IRB/IACUC Approval

Since this study used the KNHANES results for secondary data analysis, the institutional review board of Chungnam National University approved the exemption of review

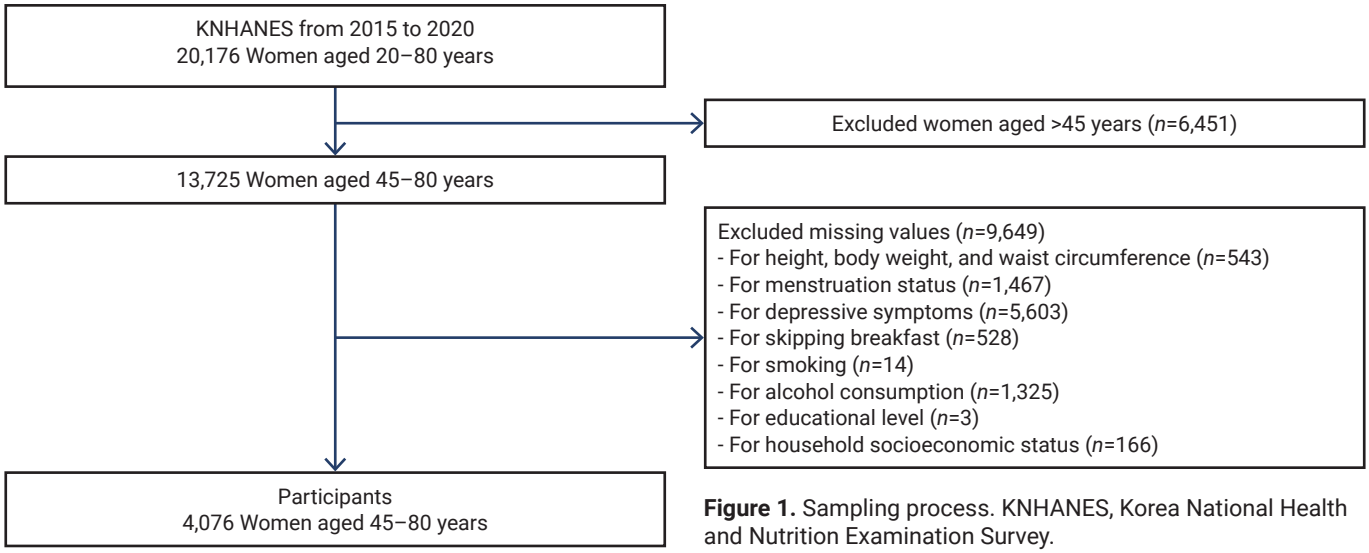

Figure 1. Sampling process. KNHANES, Korea National Health and Nutrition Examination Survey.

Table 1. Measurement of independent variables

Variable	Measurements
Biomedical factors	
Age (y)	Categorized into 45 to 64 years and ≥65 years
Post-menopause	Assessed by a single question asking whether the respondent had stopped regularly menstruating Answers were classified as post-menopause (yes) or pre-menopause (no).
Breastfeeding experience	Assessed by a single question asking whether the respondent had prior breastfeeding experience for more than 1 month. Answers were categorized as yes or no.
Psychosocial factors	
Depression symptoms	Assessed by a single question asking whether the respondent had experienced depressed moods lasting more than 2 weeks in total over the preceding year. Answers were classified as yes or no.
Skipping breakfast (1 wk)	Frequency of skipping breakfast was assessed by a single question asking about the respondent's frequency of having breakfast over the preceding week. Answers were categorized as ≤2 days in 1 week, 3 to 6 days in 1 week, and every day (all 7 days).
Eating out	Assessed by a single question asking about the respondent's average frequency of eating at restaurants over the preceding year. Answers were classified as ≤3 times/mo, 1 to 6 times/wk, and ≥1 time/d.
Sedentary behaviors	Assessed by a single question asking about the respondent's number of hours spent sitting or lying down per day. Answers were categorized as appropriate (<8 h/d) or prolonged (≥8 h/d) [29].
Physical activity (walking, 1 wk)	Assessed by a single question asking about the number of days the respondent walked for at least 10 minutes over the preceding week (7 days). Answers were categorized as none, 1 to 2 days, 3 to 4 days, and 5 to 7 days [30].
Smoking	Assessed by a single question asking the respondent about current or past smoking habits over the preceding year. Answers were classified as yes or no.
Alcohol consumption	Assessed by a single question asking the respondent about their average frequency of alcohol consumption over the preceding year. Answers were classified into <1 time/mo, 1 to 4 times/mo, 2 to 3 times/wk, and ≥4 times/wk.
Biosocial factors	
Education level	Assessed by a single question asking about the highest level of education attained by the respondent. Answers were categorized into ≤completion of middle school, graduation from high school, and ≥graduation from college.
Employment	Assessed by a single question asking about whether the respondent had worked for income over the preceding week. Answers were classified as yes or no.
Socioeconomic household status	Assessed using equalized monthly household income. Income levels were divided into 4 quartiles (first quartile, second quartile, third quartile, and fourth quartile).

(202210-SB-147-01). It was performed in accordance with the principles of the Declaration of Helsinki.

Results

Prevalence of Obesity by Patterns and Biomedical, Psychosocial, and Biosocial Factors

Table 2 shows that 33.1% of the participants had the combination of general and abdominal obesity. Regarding biomedical factors, 71.7% of the participants were aged 45 to 64 years old, 76.7% were postmenopausal, and 83.5% had breastfeeding experience. Regarding psychological factors, 14.7% had depressive symptoms; 75.2% and 9.1% skipped breakfast <2 days a week and 7 days a week (daily), respectively; 51.2% and 10.9% ate out 1 to 6 times a week and >1 time a day; 49.8% had prolonged (>8 hours a day) sedentary behavior, 45.4% walked >10 minutes 5 to 7 days a week, and 17.6% did not walk at all in a week; 4.9% and 4.2% were current and past smokers, respectively; and 57.5% currently consumed alcohol <1 time a month. Regarding biosocial factors, 41.6% of the participants graduated from middle school, and 51.5% were employed; regarding socioeconomic status, 22.3% reported that their monthly income was in the first quantile (lowest).

Factors Associated with the Combination of General and Abdominal Obesity

The associated factors included age and postmenopausal status, depressive symptoms and sedentary behavior, educational level, and household socioeconomic status (Table 3).

Regarding biomedical factors, participants aged ≥65 years had a 1.35-fold higher likelihood of developing the combination of general and abdominal obesity than 45- to 64-year-olds (95% confidence interval [CI], 1.09–1.69; $p=0.01$). In addition, women aged >65 years had a higher likelihood of developing general obesity without abdominal obesity and vice versa than women aged 45 to 64 years. Postmenopausal

https://doi.org/10.24171/j.phrp.2023.0089

Table 2. Characteristics of obesity by patterns and biomedical, psychosocial, and biosocial factors among participants (n = 4,076)

Variable	Category	n[a] (%)[b]
Obesity patterns		
Combination of general obesity and abdominal obesity		1,416 (33.1)
General obesity without abdominal obesity		1,002 (24.9)
Abdominal obesity without general obesity		180 (4.3)
Neither general nor abdominal obesity		1,478 (37.7)
Biomedical factors		
Age (y)	45–64	2,714 (71.7)
	≥65	1,362 (28.3)
Post-menopause	Yes	3,106 (76.7)
	No	970 (23.3)
Breastfeeding experience	Yes	3,489 (83.5)
	No	587 (16.5)
Psychosocial factors		
Depressive symptoms	Yes	615 (14.7)
	No	3,461 (85.3)
Skipping breakfast (a week)	≤2 days	3,170 (75.2)
	3 to 6 days	572 (15.7)
	7 days (daily)	334 (9.1)
Eating out	≤3 times/mo	1,672 (37.9)
	1 to 6 times/wk	1,989 (51.2)
	≥1 time/d	415 (10.9)
Sedentary behaviors	Appropriate (<8 h/d)	2,097 (50.2)
	Prolonged (≥8 h/d)	1,979 (49.8)
Physical activity (days of walking per week)	Never	765 (17.6)
	1–2 days	630 (14.9)
	3–4 days	870 (22.1)
	5–7 days	1,811 (45.4)
Smoking consumption	Never	3,718 (90.9)
	Past	148 (4.2)
	Current	210 (4.9)
Alcohol consumption	<1 time/mo	2,418 (57.5)
	1–4 times/mo	1,267 (32.5)
	2–3 times/wk	288 (7.6)
	≥4 times/wk	103 (2.4)
Biosocial factors		
Educational level	≤Middle school	1,918 (41.6)
	High school	1,320 (35.8)
	≥College	838 (22.6)
Employment	Employed	2,053 (51.5)
	Unemployed	2,023 (48.5)
Socioeconomic household status	1st quantile (lowest)	928 (22.3)
	2nd quantile	1,028 (24.8)
	3rd quantile	1,069 (26.3)
	4th quantile (highest)	1,051 (26.6)

[a]Unweighted, [b]weighted.

status was associated with a 1.91-fold increased likelihood of developing the combination of general and abdominal obesity, compared with premenopausal status (95% CI, 1.68–2.17; p < 0.001). A lack of breastfeeding experience was associated with a 1.55-fold increased likelihood of developing the combination of general and abdominal obesity, compared with having breastfed (95% CI, 1.22–1.97; p < 0.001) (Table 3).

Regarding psychosocial factors, depressive symptoms were associated with a 1.25-fold increased likelihood of developing the combination of general and abdominal obesity, compared with the absence of depressive symptoms (95% CI, 1.01–1.55; p = 0.04). In addition, prolonged (≥8 hours a day) sedentary behavior was associated with a 1.30-fold increased likelihood of developing the combination of general and abdominal obesity, compared with appropriate (<8 hours a day) sedentary behavior (95% CI, 1.09–1.56; p = 0.003) (Table 3).

Regarding biosocial factors, having an educational level lower than middle school and high school graduation was associated, respectively, with 3.85-fold (95% CI, 2.86–5.26, p < 0.001) and 2.56-fold (95% CI, 2.04–3.23; p < 0.001) increased likelihoods of developing the combination of general and abdominal obesity, compared with college graduation. Regarding household socioeconomic status, the first and second quantiles of monthly income were associated with 1.17-fold (95% CI, 1.07–1.29; p = 0.001) and 1.11-fold (95% CI, 1.01–1.22; p = 0.03) increased likelihoods of developing the combination of general and abdominal obesity, respectively, compared with the fourth quantile (Table 3).

Discussion

This study was conducted to identify factors associated with the combination of general and abdominal obesity among middle-aged and older Korean women. According to the results of this study, age, postmenopausal status, and breastfeeding experience were positively associated with the combination of general and abdominal obesity. A previous study on Norwegian and Russian individuals aged 40 to 69 years showed that older women had a higher likelihood of developing general and abdominal obesity [19]. This may be linked to metabolic changes associated with aging and hormonal changes during menopause in women [31,32]. During normal aging, white adipose tissue in the abdominal cavity and fat deposition in skeletal muscle increase [33,34]. In addition, middle-aged and older women gain weight with decreased energy expenditure via increased sedentary behavior and decreased physical activity due to reduced muscle strength and physical

Table 3. Factors associated with the combination of general and abdominal obesity

Variable	Category	Combination of general and abdominal obesity	
		AOR (95% CI)	*p*
Biomedical factors			
Age (y) (ref. 45–64)	≥65	1.35 (1.09–1.69)	0.01
Post-menopause (ref. no)	Yes	1.91 (1.68–2.17)	<0.001
Breastfeeding experience (ref. yes)	No	1.55 (1.22–1.97)	<0.001
Psychosocial factors			
Depressive symptoms (ref. no)	Yes	1.25 (1.01–1.55)	0.04
Skipping breakfast (a week) (ref. ≤2 days)	3–6 days	0.97 (0.83–1.13)	0.67
	7 days (daily)	1.23 (0.92–1.64)	0.16
Eating out (ref. ≤3 times/mo)	1–6 times/wk	0.95 (0.77–1.18)	0.64
	≥1 time/d	0.99 (0.80–1.23)	0.95
Sedentary behaviors (ref. appropriate, <8 h/d)	Prolonged (≥8 h/d)	1.30 (1.09–1.56)	0.003
Physical activity (days of walking per week) (ref. 5–7 days)	None	0.85 (0.62–1.17)	0.32
	1–2 days	0.83 (0.62–1.10)	0.19
	3–4 days	0.89 (0.68–1.17)	0.40
Smoking consumption (ref. never)	Past	1.33 (0.89–1.99)	0.16
	Current	0.76 (0.43–1.34)	0.34
Alcohol consumption (ref. <1 time/mo)	1–4 times/mo	0.73 (0.42–1.25)	0.25
	2–3 times/wk	0.67 (0.38–1.16)	0.15
	≥4 times/wk	1.05 (0.80–1.37)	0.75
Biosocial factors			
Education level (ref. ≥college)	≤Middle school	3.85 (2.86–5.26)	<0.001
	High school	2.56 (2.04–3.23)	<0.001
Employment (ref. ≥employed)	Unemployed	0.96 (0.79–1.17)	0.68
Socioeconomic household status (ref. 4th quantile)	1st quantile	1.17 (1.07–1.29)	0.001
	2nd quantile	1.11 (1.01–1.22)	0.03
	3rd quantile	1.12 (0.98–1.29)	0.10

AOR, adjusted odds ratio; CI, confidence interval; Ref, reference.

endurance [31,35]. Lower postmenopausal estrogen levels also result in increased accumulation of adipose tissue in the abdominal cavity [32]. Thus, middle-aged and older women may be at risk of increased weight gain and WC.

In addition, middle-aged women who experienced 1–6 months of breastfeeding have shown lower general and abdominal obesity rates [36,37]. A total breastfeeding duration >3 months has been associated with a lower abdominal obesity rate in postmenopausal Korean women aged >40 years [38]. Bobrow et al. [39] reported that every 6 months of breastfeeding was associated with a 1% decrease in the mean BMI. Furthermore, a longer duration of breastfeeding was associated with a lower BMI among women with obesity 6 years postpartum [40]. In a previous study on Filipino, Caucasian, and African-American women aged 55 to 80 years [41], breastfeeding duration >3 months was associated with less visceral fat than in women with no history of breastfeeding. Since breastfeeding improves lipid metabolism [42], adipose tissue deposition in the visceral cavity during pregnancy decreases when breastmilk containing high-calorie fat is generated [43]. Thus, a longer duration of breastfeeding is associated with a greater decrease in general and abdominal obesity rates [36,44].

According to a meta-analysis, depressive symptoms are associated with general obesity in women [45,46]. Another study reported that depressive symptoms were associated among middle-aged women, though not among middle-aged men, with an increased likelihood of developing general and central obesity [21]. Furthermore, depressive symptoms have been associated with general obesity in adults who have abdominal obesity [47]. However, a previous study on middle-aged and older Chinese participants reported that obesity was not associated with depressive symptoms in women [48]. Since obesity and depressive symptoms are interrelated, they cause emotional problems (e.g., depression), resulting in an increase in emotion-driven eating (eating in response to negative emotions rather than a physical need). An increase in energy intake promotes obesity with decreased energy expenditure via low physical activity levels, due to reduced self-efficacy [49]. In particular, since middle-aged women seem vulnerable to depressive symptoms [49], they may experience increased

https://doi.org/10.24171/j.phrp.2023.0089

weight and WC.

A sedentary lifestyle is associated with general and abdominal obesity in middle-aged women [50]. According to a national study, watching television and videos for 2 hours or more per day is associated with a 1.66-fold increase in general and abdominal obesity among women [22]. In addition, sitting for 8 hours or more per day has been associated with a 1.38-fold increase in the prevalence of obesity and a 1.05-fold increase in the prevalence of abdominal obesity in a population-based study [51]. Sedentary behavior is defined as participation in minimal activities requiring a resting level of energy expenditure (1.0–1.5 times the basal metabolic rate), such as watching television [52]. Thus, prolonged sedentary behavior might be associated with an overall decrease in energy expenditure, which may result in a positive energy balance, even when energy intake does not increase by eating. Therefore, interventions should be developed to decrease sedentary behavior in middle-aged and older women.

Educational level and socioeconomic status are crucial factors associated with obesity [53]. According to KNHANES data gathered from 1998 to 2018, among women, a high educational level and socioeconomic class were inversely associated with increased general and abdominal obesity rates [54]. Educational level is positively associated with access to resources to assist in a healthy lifestyle. Thus, individuals with higher educational levels have greater access to health-related resources that provide information and assistance for following healthy lifestyles [15]. A previous study reported that women with low educational levels did not follow recommendations for obesity prevention [15]. In addition, individuals currently spend more money to maintain a healthy lifestyle, including the consumption of foods without junk calories (e.g., fresh fruits and vegetables), which is beneficial for preventing excessive weight gain [55]. Thus, it may be easier for individuals with a high socioeconomic status to maintain a healthy lifestyle, which costs more. In particular, Korean women who experience social pressure to be slender (culturally ideal body weight) may try to maintain a normal weight and slender body shape with a healthy lifestyle [54]. Thus, women with a high socioeconomic status can actively maintain a healthy lifestyle, which contributes to the prevention of general and abdominal obesity, while those with low socioeconomic status and educational levels may need public support to maintain healthy lifestyles and prevent general and abdominal obesity.

In a secondary analysis of Korean national data, this study identified factors associated with the combination of general and abdominal obesity in middle-aged and older Korean women. In particular, although Lu et al. [13] reported a few factors associated with the combination of general and abdominal obesity, such as age, smoking, and alcohol consumption, the current study identified multiple factors (biomedical, psychosocial, and biosocial) associated with the combination of general and abdominal obesity based on the biopsychosocial model. The results showed that comprehensive intervention strategies should be developed to prevent and manage the combination of general and abdominal obesity in middle-aged and older Korean women. In addition, cohort studies could be proposed to identify the cumulative effects of aging with associated factors on developing the combination of general and abdominal obesity.

However, this study had some limitations. First, a cross-sectional study design was applied, which limited the verification of causal relationships among the potential factors associated with the combination of general and abdominal obesity. Further cohort studies are needed to identify causal associations. Second, the factors associated with the combination of general and abdominal obesity may differ according to sex and ethnicity. Thus, sex-based and ethnic differences need to be identified in factors associated with general and abdominal obesity. Finally, this study focused on identifying factors associated with general and abdominal obesity. Since other obesity patterns (e.g., abdominal obesity without general obesity) are also associated with obesity-related complications, further studies are needed to identify and compare the factors associated with obesity patterns.

Conclusion

According to the results of this study, women older than 65 years, with postmenopausal status, without breastfeeding experience, with a low educational level, and/or with a low socioeconomic household status may be at risk for the combination of general and abdominal obesity. Prevention and early management of depressive symptoms and decreased sedentary behavior may be important to prevent general and abdominal obesity in middle-aged and older women. Thus, healthcare providers in communities and public health clinics should screen for risk factors for the combination of general and abdominal obesity with non-modifiable biomedical (e.g., age) and biosocial factors (e.g., educational level). In addition, prevention strategies for obesity (the combination of general and abdominal obesity) should be developed with a focus on modifiable psychosocial factors (e.g., sedentary behavior).

Notes

Ethics Approval
This study was approved by the institutional review board of Chungnam National University and was exempted from review because it was a secondary analysis (202210-SB-147-01). It was performed in accordance with the principles of the Declaration of Helsinki.

Conflicts of Interest
The author has no conflicts of interest to declare.

Funding
This study was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean Ministry of Science and ICT (2021R1A2C100682811).

Availability of Data
The datasets are not publicly available but are available from the corresponding author upon reasonable request.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0033
Osong Public Health Res Perspect 2023;14(5):388-417

Original Article

Estimating the prevalence of oral manifestations in COVID-19 patients: a systematic review

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ABSTRACT

Objectives: Patients with coronavirus disease 2019 (COVID-19) present with a variety of oral manifestations. Therefore, we conducted a systematic review to estimate the prevalence of oral lesions among COVID-19 patients.

Methods: An extensive literature search of several electronic bibliographic databases (PubMed, Scopus, Science Direct, Litcovid) was conducted to retrieve all articles published in the English language from January 1, 2020 to March 31, 2023 that reported the prevalence of oral manifestations among COVID-19 patients. A meta-analysis of pooled prevalence was performed using Jamovi ver. 2.3 (2022). The I^2 and Q statistics were used to assess heterogeneity between studies, and p-values < 0.01 were considered statistically significant.

Results: In total, 79 studies with data from 13,252 patients were included. The articles were predominantly published in 2020 (n = 33), and Italy was the most common country (n = 14). Most of the affected patients more than 50 years old and women (56.6%). The most common sites of involvement were the tongue (n = 65), followed by the oral mucosa (n = 37) and lips (n = 19). High heterogeneity was found between studies. The most common oral manifestation was taste alteration, followed by xerostomia and ulceration, showing pooled prevalence rates of 48%, 35%, and 21%, respectively.

Conclusion: COVID-19 patients show various oral manifestations that may help clinicians identify the disease promptly. Recognition of the signs and symptoms of COVID-19 is critical for an early diagnosis and better prognosis.

Keywords: COVID-19; Oral manifestations; Oral ulcer; SARS-CoV-2

Received: February 6, 2023
Revised: June 9, 2023
Accepted: July 10, 2023

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Introduction

The novel coronavirus disease 2019 (COVID-19) has rapidly evolved into a global crisis, posing a

significant challenge to public health due to its swift spread and high mortality rate. Initially identified in December 2019 in China's Hubei Province, the disease quickly spread across the globe. By March 2020, the World Health Organization (WHO) had declared it a 'pandemic emergency'. As of April 2023, the outbreak has resulted in over 762,201,169 confirmed cases and 6,893,190 deaths worldwide [1]. The disease's incubation period spans from 1 to 14 days, with the most frequently observed symptoms being fever, cough, shortness of breath or difficulty breathing, and fatigue. Other less common symptoms, such as headache, loss of taste or smell, sore throat, diarrhea, and nausea or vomiting, may also manifest [2]. The severity of these symptoms can vary greatly among individuals, as it is influenced by factors such as the timing of exposure to the virus, the patient's age and gender, and any pre-existing health conditions.

Research has shown that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infiltrates human cells using receptors known as angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (also referred to as transmembrane serine protease or TMPRSS2) [3]. Of these 2, the ACE2 receptor is primarily located in the cells of the lungs, liver, kidneys, and gastrointestinal (GI) tract, as well as the salivary glands and the dorsum of the tongue within the oral cavity [4]. These cells, equipped with the receptors, serve as host cells for the virus. The virus penetrates these cells and triggers an inflammatory response in the affected organs [4].

Previously, COVID-19 was not thought to have oral symptoms, unlike other viral rash. However, the subsequent detection of SARS-CoV-2 in patients' saliva suggested that oral manifestations could indeed be clinical characteristics of the disease [3]. The presence of the ACE2 receptor in specific oral organs, such as the tongue and salivary glands, further supports the potential involvement of the oral cavity in COVID-19 infection [3]. The prevalence of oral manifestations among COVID-19 patients is currently unknown, but several studies have attempted to determine their incidence and prevalence [5–10]. A large-scale study by Nuno-Gonzalez et al. [5] involving 666 patients found oral cavity findings in 25.65% of cases. The most frequently observed oral symptoms, as reported in a case series by Sinadinos and Shelswell [6], were blisters, ulcerations, and desquamative gingivitis. Within the oral cavity, the palate and tongue are the sites most commonly affected by COVID-19, followed by the gums and lips [7]. On the tongue, ulcerations are particularly common, especially on the dorsal surface or sides. However, only 15% of patients develop ulcerations on the ventral surface. Other possible tongue symptoms include multiple pinpoint yellowish ulcers and white plaque [3]. The presence of white plaque on the tongue's dorsal surface is often due to fungal infections, another common oral manifestation of SARS-CoV-2, likely resulting from reduced immunity. Dima et al. [8] reported a case of a neonate with COVID-19 who developed oral cavity candidiasis. These oral symptoms are often painful, with 75% of patients reporting discomfort [7]. In another study, 25% of patients reported taste impairment, 15% experienced burning sensations, and 20% had difficulty swallowing. Taste disorders were observed in 24% of patients (ageusia), 35% (hypogeusia), and 38% (dysgeusia). These taste disorders were more prevalent in women than in men [9].

It is crucial for dentists to understand the oral manifestations of COVID-19, as this knowledge aids in early disease diagnosis and consequently, prevents transmission. This systematic review aims to summarize the findings from existing literature on the oral manifestations of COVID-19, highlighting the role of the dentist in mitigating the severity of this deadly pandemic.

HIGHLIGHTS

- The present systematic review shows a higher prevalence of oral manifestations among COVID-19 patients, specifically taste alterations, followed by xerostomia, ulceration, and red and white lesions.
- COVID-19 patients show various oral manifestations that may help clinicians detect the disease early in its course.
- Identifying the oral signs and symptoms of COVID-19 is crucial for initiating early diagnosis and treatment of this deadly disease; therefore, increasing awareness of these symptoms is importa

Materials and Methods

A systematic assessment and description of the currently reported cases and studies related to oral manifestations associated with SARS-CoV-2 infection was conducted. This review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines [10].

Eligibility Criteria

We conducted a search for clinical evidence in the form of original, peer-reviewed journal articles. These included observational and cross-sectional studies that investigated

the prevalence of oral disorders in patients with COVID-19. In addition to these, we also incorporated case reports and case series into our systematic review. The data publication range was restricted from January 1, 2020 to June 30, 2022. We further updated our research on March 31, 2023 across these databases. We did not utilize conference papers, book reviews, book chapters, letters to the editor and replies, newspaper and newsletter articles, expert opinions, or theses and dissertations. Any articles not published in English were also excluded.

Data Sources and Search Strategy

We carried out a comprehensive search of various electronic bibliographic databases, including PubMed, Scopus, Science Direct, and Litcovid. We gathered all articles published between January 1, 2020, and March 31, 2023. We then screened observational cross-sectional, case-control and cohort studies, case reports/series that reported on multisystem inflammatory syndrome in children, as well as letters to the editor. The 2 independent reviewers (A.G. and K.S.) conducted an electronic search of all cross-sectional studies, case reports, and case series up until March 31, 2023. They used a combination of relevant keywords, appropriately linked by Boolean operators. (1) COVID-19 OR SARS-CoV-2 OR Coronavirus disease 2019 OR novel coronavirus; (2) AND oral manifestations OR oral involvement OR oral lesions; (3) AND cross-sectional studies OR case reports OR case series.

Selection Process

The inclusion of studies was done in 2 phases. During the first phase, the titles of all studies were initially screened, followed by a review of their abstracts using the established inclusion and exclusion criteria. Two authors (A.G. and K.S.) independently performed this screening. If a title and abstract appeared to meet the criteria, the full article was then read and assessed for eligibility by these same 2 authors (A.G. and K.S.). Any disagreements between the authors were resolved through discussion and consensus, with the involvement of a third author (A.A.) if necessary. Duplicates were removed and irrelevant articles were excluded from the systematic review. We obtained and evaluated the full-text articles of all potentially relevant studies. In the second phase, we screened the references of all the included studies, case reports, and case series once more to identify any additional potentially eligible studies.

Data Collection

Three authors (A.G., K.S., and A.P.) independently extracted data from the eligible studies. In the event of disagreements, a 4th author (A.A.) was included to facilitate consensus through discussion. We included all studies that reported orofacial manifestations in patients with COVID-19. To systematically review these studies, we assessed the included studies based on demographic details such as author, year, country, study type, sample size, gender, age, study duration, medical history, intensive care unit (ICU) admission, and disease severity. In addition, we recorded details related to oral manifestations, including the affected site, onset of orofacial manifestations, general symptoms, any special investigations conducted, treatment of oral lesions, and disease outcome. The flow diagram for article inclusion is depicted in Figure 1.

Assessment of Risk of Bias of the Included Studies

The Joanna Briggs Institute (JBI) Critical Appraisal Tools for use in systematic reviews of cross-sectional studies, case-control studies, case reports, and case series were used to assess the risk of bias and the individual quality of the selected studies [11]. Each type of study was assessed using its respective checklist, with each question offering 3 possible responses: yes, no, or unclear. Two blinded reviewers (A.G. and K.S.) evaluated the risk of bias in each study, using a scoring system agreed upon by all reviewers. Following the assessment, studies were categorized based on their scores: high bias (if the study scored up to 49% "yes"), moderate bias (if the study scored between 50% and 69% "yes"), and low bias (if the study scored more than 70% "yes").

Statistical Analysis

Qualitative data were reorganized by grouping and comparing the information reported in the studies. Conditions affecting the oral and mucosal areas were summarized using schematic diagrams. The primary outcome of interest was the prevalence of oral symptoms in COVID-19 patients. The prevalence of oral lesions was categorized into subgroups such as taste alteration, red and white lesions, vesiculobullous lesions, xerostomia, ulceration, burning sensation, and salivary gland disorders. A meta-analysis of the combined prevalence was then conducted using Jamovi ver. 2.3 (2022; https:// www.jamovi.org). To evaluate heterogeneity between studies, the I^2 and Q statistics were utilized, with p-values less than 0.01 considered statistically significant. The analyses were conducted using a random model. Oral lesions in COVID-19 patients, as reported in case reports and case series, were not included in the meta-analysis.

Results

After conducting the initial search, a total of 275 articles

https://doi.org/10.24171/j.phrp.2023.0033

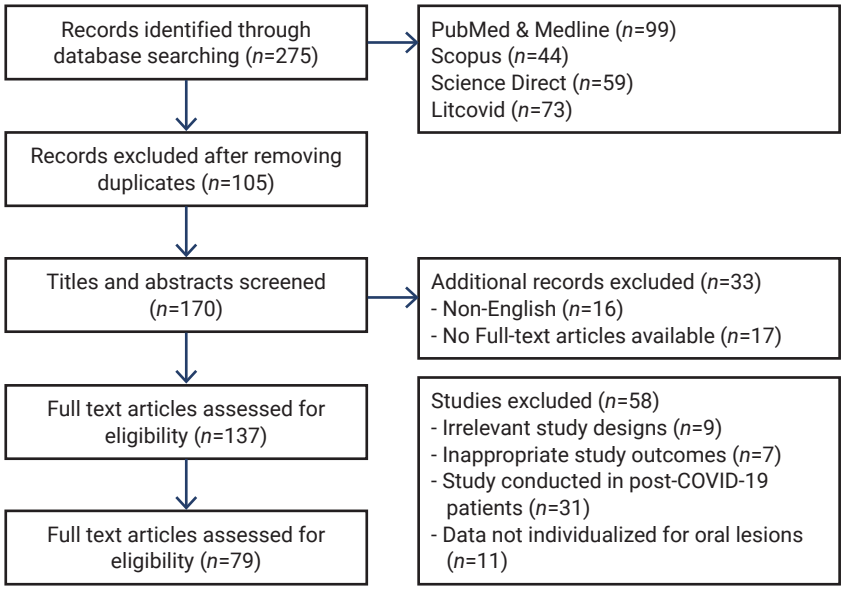

Figure 1. Flow diagram of literature search and selection criteria of the included studies (*n* = 79).

were found. Of these, 79 articles were selected for inclusion in the final analysis [5,6,8,9,12–86].

Characteristics of the Studies

The demographic characteristics of the populations in the studies included in our analysis (*n* = 79) are detailed in Table 1. We extracted data from these 79 studies [5,6,8,9,12–86], which encompassed a total of 13,252 patients. The individual sample sizes within these studies varied, ranging from as few as 14 [47] to as many as 1,172 [35] patients. All of the studies (*n* = 79) were published between the years 2020 [6,8,9,12–27,56–69] and 2023 [83–86]. The majority of these studies (*n* = 33) were published in 2020, followed by 2021 (*n* = 31), 2022 (*n* = 15), and 2023 (*n* = 4).

The 79 articles analyzed presented data from various countries worldwide. Italy accounted for the most articles, with 14 studies [16,19,20,22,23,28,29,36,37,58,66,72,81,86]. This was followed by India with 8 studies [17,33,44,45,50,55,80,83], United States with 7 studies [21,25,30,31,54,62,79], Brazil with 7 studies [9,47,49,52,56,75,77], and both Egypt and Turkey with 5 studies each [34,38,41,68,71] and [26,39,42,48,64], respectively. Iran [5,53,59,60], Iraq [12,43,84,85], and Spain [27,57,65,67] contributed to 4 studies each. Saudi Arabia was represented by 3 studies [40,46,51], while China [15,35] and Israel [13,74] each had 2 studies. Two studies contain data from multiple European countries [18,70]. The remaining countries—Denmark [14], France [24], Qatar [32], Romania [8], the United Kingdom [6], Colombia [61], Norway [63], Indonesia [69], Afghanistan [73], the Czech Republic [76], Ukraine [78], and Poland [82]—each contributed 1 study, respectively.

Most of the 79 studies were cross-sectional studies (*n* = 41) [5,12–26,29–31,33,34,37–46,48–51,53–55,84–86] followed by case reports (*n* = 20) [9,61–75,78–81], case series (*n* = 11) [6,8,56–60,76,77,82,83], retrospective studies (*n* = 6) [27,28,35,36,47,52], and case-control studies (*n* = 1) [32].

The studies contained data from 13,252, of whom 7,509 (56.6%) were females. In most of the studies (*n* = 40), the mean age of the patients was 58.26 ± 11.50 years followed by 41.42 ± 17.32 years (23 studies) and 26.18 ± 18.42 years (12 studies). Three studies did not report the patients' ages [5,34,35] and 1 study was conducted among newborns [8].

Only 34 studies have documented patients' medical history. The majority of these studies documented a history of hypertension (*n* = 26), followed by diabetes (*n* = 20), respiratory diseases and asthma (*n* = 7), cardiovascular disease (*n* = 6), allergies (*n* = 4), and other conditions. Twenty-one studies indicated that patients with COVID-19 were admitted to the hospital. Additionally, 11 studies reported that patients were hospitalized in the ICU, with 5 studies revealing that the patients required ventilation.

Oral signs and symptoms can be broadly categorized into the following: oral ulcerations, redness and burning sensation, xerostomia, red and white lesions, vesiculobullous lesions, morphological changes of the tongue, taste alteration, gingival and periodontal lesions, and salivary gland disorders. Many patients exhibited multiple signs and symptoms affecting various parts of the oral cavity. Therefore, we have assessed each oral manifestation individually. The most prevalent oral manifestation, observed in 23.8% (*n* = 3,157) of patients, was taste alteration (46 studies). This was followed by oral ulceration in 8.1% (*n* = 1,082 patients in 41 studies),

redness and burning sensation in 2.2% (*n*=297 patients in 33 studies), xerostomia in 12.7% (1,694 patients in 24 studies), red and white lesions in 2.4% (326 patients in 18 studies), vesiculobullous lesions in 0.55% (*n*=73 patients in 13 studies), morphological changes of the tongue in 2.7% (*n*=360 patients in 27 studies), gingival and periodontal changes in 3.2% (*n*=430 patients in 12 studies), and salivary gland disorder in 1.07% (*n*=143 patients in 3 studies). In the majority of patients (23.6%), the tongue was the most commonly affected area, followed by the oral mucosa (14.7%), lips (4.9%), gingiva and periodontium (3.2%), palate (1.9%), and salivary glands (1.1%).

Risk of Bias Assessment

JBI critical appraisal checklists were utilized to assess the risk of bias in cross-sectional studies, case-control studies, case reports, and case series. Of the 79 studies evaluated, 50 (63.7%) demonstrated a low risk of bias, while 23 (29.3%) exhibited moderate bias, and a mere 6 studies (7%) showed a high risk of bias (Table 1) [5,6,8,9,12–86]. The detailed computation of the risk of bias, using the JBI Critical Appraisal Tools, is presented in Tables S1–S4 [5,6,8,9,12–86].

Descriptive Characteristics of the Oral Lesions

Taste disorders and tongue manifestation

The prevalence of taste disorders and tongue manifestations was evaluated using data from 46 and 27 studies, respectively. We further subdivided the taste disorders into additional categories. These include complete loss of taste, or ageusia, as reported in 22 studies [12,14,20,23,35,40–42,44,49,53,55,56,58,63,65,67,70,72,74,85], taste alteration or dysfunction, as reported in 19 studies [5,13,16–19,21,22,29,36,38,46,51,54,66,78,84–86], dysgeusia, as reported in 7 studies [24,28,37,50,54,59,60], and amblygeustia, as reported in only one study [15].

Xerostomia

Xerostomia was noted in 22 cross-sectional studies [13,15,22,28,34,37,38,40–42,44–46,48,50–55,84,85] and 2 case reports [60,78].

Vesiculobullous lesions

Thirteen studies, comprising case reports and case series, reported vesiculobullous lesions [6,9,24,26,29,50,57,59,61,62,70,77,81].

Ulceration

Ulceration was reported in 41 studies [5,6,24,27–29,31,33,34,37,39–51,53,55,56,61,64,69,73,75–85].

Table 1. Demographic characteristics of the included studies (*n*=79)

No.	Study	Year of publication	Study location	Study design	Sample size (*n*)	Sex	Mean age (range, y)	Study duration	Medical history	Admission in the ICU	Risk of bias
1	Nuno-Gonzalez et al. [5]	2021	Spain	Cross-sectional	666	-	55.7 (40–70)	April 10–25, 2020	-	History of hospitalization	Low
2	Al-Zaidi and Badr [12]	2020	Iraq	Cross-sectional	65	M, 41.6%; F, 58.4%	41.2 (11–80)	April 5, 2020–May 17, 2020	-	-	Moderate
3	Biadsee et al. [13]	2020	Israel	Cross-sectional	140	M, 58; F, 70	36.5 (18–73)	March 25, 2020–April 15, 2020	-	-	Moderate
4	Bodnia and Katzenstein [14]	2020	Copenhagen, Denmark	Cross-sectional	51	F, 22; M, 28	45 (16–62)	March 2020	-	-	Moderate
5	Chen et al. [15]	2020	China	Cross-sectional	31	M, 15; F, 16	60.6 (18–86)	February 28, 2020–March 4, 2020	-	-	Moderate
6	Dell'Era et al. [16]	2020	Italy	Cross-sectional	355	M, 54%	45 (51–60)	March 10–30, 2020	Cardiovascular disease, allergic (sinusitis)	-	Low
7	Kumar et al. [17]	2021	India	Cross-sectional	141	M, 58.9%; F, 41.1%	15.2 (10–19)	May–August 2020	-	-	Low
8	Lechien et al. [18]	2020	Europe (multi center)	Cross-sectional	417	F, 263; M, 154	36.9 (19–77)	-	Allergic rhinitis, asthma, hypertension, hypothyroidism	Hospitalization of severe cases	Low

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https://doi.org/10.24171/j.phrp.2023.0033

https://doi.org/10.24171/j.phrp.2023.0033

Table 1. Continued

No.	Study	Year of publication	Study location	Study design	Sample size (*n*)	Sex	Mean age (range, y)	Study duration	Medical history	Admission in the ICU	Risk of bias
9	Paderno et al. [19]	2020	Italy	Cross-sectional	508	M, 56%; F, 44% (55±15 y)[a]	55 (40–70)	March 27–April 1, 2020	-	Hospitalization of severe cases	Low
10	Boscolo-Rizzo et al. [20]	2020	Italy	Cross-sectional	202	F, 55.1%; M, 44.9%	56 (20–89)	March 19–22, 2020	-	-	Low
11	Yan et al. [21]	2020	California, USA	Cross-sectional	59 and 203 (COVID-19 +ve and –ve)	M & F, 49.2% (COVID-19 +ve): M, 34%; F, 65% (COVID-19 –ve)	54 (18–80)	March 3–29, 2020	Allergic rhinitis, immunocompromised state, hypertension, DM, cardiac disorders, cancer, CLD, history of head trauma, neurological disease	Hospitalization of severe cases	Low
12	Sinjari et al. [22]	2020	Italy	Cross-sectional	20	-	69.2 (39–81)	May 2020–June 2020	DM, cardiovascular conditions	-	Low
13	Giacomelli et al. [23]	2020	Italy	Cross-sectional	59	M, 40%; F, 60%	60 (40–74)	March 19, 2020	-	-	Moderate
14	Mascitti et al. [24]	2020	France	Cross-sectional	59	M:F, 3:1	57.6 (49–69)	March 31, 2020	-	-	Moderate
15	Salehi et al. [25]	2020	Iran	Cross-sectional	53	M, 43.4%; F, 56.6%	63.1 (27–90)	March 1, 2020–April 30, 2020	Cardiovascular diseases (52.83%), DM (37.7%), chronic kidney disease (20.7%)	-	Low
16	Askin et al. [26]	2020	Turkey	Cross-sectional	210	M, 58.6%; F, 41.4%	57.4 (20–75)	April 2020	Comorbidities	29 in ICU, 129 in wards	Moderate
17	Katz and Yue [27]	2021	USA	Retrospective study	889	F, 509; M, 386	18–34	Registry study	-	-	Moderate
18	Fantozzi et al. [28]	2020	Italy	Retrospective study	326	M, 52.3%; F, 47.7%	57 (48–67)	March 6, 2020–April 30, 2020	Hypertension (*n*=29), chronic pulmonary disease (*n*=11), DM (*n*=10), cardiovascular disease (*n*=9), cancer (*n*=5)	Hospitalized (median, 12.5 d)	High
19	Favia et al. [29]	2021	Bari, Italy	Cross-sectional	123	M:F, 1.3:1	Median, 72	October 2020–December 2020	-	History of hospitalization and ICU	Moderate
20	Halepas et al. [30]	2021	New York, USA	Cross-sectional	47	M, 51.1%; F, 48.9%	9.0 (1.3–20)	March 15–June 1, 2020	-	History of hospitalization, ICU	Moderate

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Table 1. Continued

No.	Study	Year of publication	Study location	Study design	Sample size (*n*)	Sex	Mean age (range, y)	Study duration	Medical history	Admission in the ICU	Risk of bias
21	Rekhtman et al. [31]	2021	New York, USA	Cross-sectional	296	M, 71%; F, 29%	64 (50–77)	May 11, 2020–June 15, 2020	CAD, 23%; congestive heart failure, 14%; asthma, 9%; COPD, 14%; DM, 34%; hypertension, 71%	History of hospitalization	Low
22	Marouf et al. [32]	2021	Qatar	Case control	Case, 40; control, 528	Case: M, 50%; F, 50% Control: M, 54.9%; F, 45.1%	Case, 53.6; control, 41.5	February–July 2020	DM: case, 42.5%; control, 27.8%	Hospitalization and ICU admission	Low
23	Subramaniam et al. [33]	2021	India	Cross-sectional	713	M:F, 6:3	69 (60–81)	May 2020–June 2020	DM, hypertension	-	Moderate
24	Abubakr et al. [34]	2021	Egypt	Cross-sectional	573	F, 408; M, 165	36.19 (30–45)	May 1, 2020–July 1, 2020	-	-	Low
25	Song et al. [35]	2021	China	Retrospective	1172	-	-	December 2019	-	History of hospitalization	Low
26	Bardellini et al. [36]	2021	Italy	Retrospective	27	M:F, 19:8	4.2 y (3 mo–14 y)	March–April 2020	-	-	High
27	Gherlone et al. [37]	2021	Italy	Cross-sectional	122	M, 75.4%; F, 24.6%	62.5 (53.9–74.1)	July 23, 2020–September 7, 2020	CAD, DM, chronic kidney disease, active neoplasia, COPD	History of hospitalization and ICU and ventilation	High
28	El Kady et al. [38]	2021	Egypt	Cross-sectional	58	M, 53.4%; F, 46.6%	18–46	May 15, 2020–June 10, 2020	-	History of hospitalization	High
29	Fidan et al. [39]	2021	Turkey	Cross-sectional	74	M, 66.2%; F, 33.8%	51.6 (28–68)	April–October 2020	-	Hospitalized	High
30	Natto et al. [40]	2021	Saudi Arabia	Cross-sectional	109	M, 67%; F, 33%	39.3 (18–56)	July–October 2020	DM (10.1%), hypertension (7.3%), asthma and arthritis (1.7%)	-	Moderate
31	Elamrousy et al. [41]	2021	Egypt	Cross-sectional	124	M, 74.2%; F, 25.8%	50.32±12.47[a]	September 2, 2020–June 10, 2021	DM (*n*=52), hypertension (*n*=16), cardiac disease (*n*=8), renal disease (*n*=4), liver disease (*n*=4)	Hospitalized	Moderate
32	Bulut et al. [42]	2021	Turkey	Cross-sectional	200	M, 75; F, 125	38 (20–70)	September 2020–March 2021	-	Hospitalized (11.5%)	Low
33	Naser et al. [43]	2021	Iraq	Cross-sectional	338	M, 59%; F, 41%	45	August 2020–March 2021	Respiratory diseases, DM, hypertension, heart disease, urogenital diseases	Hospitalized	Moderate

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Table 1. Continued

No.	Study	Year of publication	Study location	Study design	Sample size (*n*)	Sex	Mean age (range, y)	Study duration	Medical history	Admission in the ICU	Risk of bias
34	Muthyam et al. [44]	2022	India	Cross-sectional	100	M, 51%; F, 49%	More than 35 y, 54%; less than 35 y, 46%	-	Immunocompromised state, multidrug therapy	Hospitalization	Low
35	Ganesan et al. [45]	2022	India	Cross-sectional	500	M, 73.4%; F, 26.6%	53.46±17.50[a]	-	-	-	Low
36	El Tantawi et al. [46]	2022	Multicountry study (Saudi Arabia)	Cross-sectional	434	M, 41.5%; F, 58.5%	18–23	August 2020–January 2021	Cancer, COPD	-	Moderate
37	Soares et al. [47]	2022	Brazil	Retrospective	14	M, 71.5%; F, 38.5%	58 (20–65)	-	-	-	Low
38	Tuter et al. [48]	2022	Turkey	Cross-sectional	204	M, 37.3%; F, 62.7%	53.3 (18–70)	February–March 2021	DM, hypertension, immunosuppression	Hospitalization, ICU	Low
39	Schwab et al. [49]	2022	Brazil	Cross-sectional	154	M, 59.7%; F, 40.3%	54.60 (20–88)	January 13, 2021–May 28, 2021	-	Hospitalization, ICU, ventilation	Moderate
40	Chawla et al. [50]	2022	India	Cross-sectional	217	M, 70%; F, 30%	56 (18–60)	September–December 2020	DM, hypertension, CAD, bronchial asthma	-	High
41	Binmadi et al. [51]	2022	Saudi Arabia	Cross-sectional	195	M, 25%; F, 75%	26 (18–34)	March 2020–March 2022	Immunosuppression, hormonal modulation	Hospitalization, ICU, ventilation	Moderate
42	Eduardo et al. [52]	2022	Brazil	Retrospective	519	M, 68.2%; F, 31.8%	51–80	May 2020–February 2021	-	ICU	Moderate
43	Villarroel-Dorrego et al. [53]	2022	Spain	Cross-sectional	55	M, 54.5%; F, 45.5%	51 (1–89)	-	-	-	Moderate
44	Manifar et al. [54]	2022	Iran	Cross-sectional	140	M, 44.2%; F, 55.8%	53.78 (15–92)	September 1, 2020–October 17, 2020	-	Hospitalization	Moderate
45	Bhuyan et al. [55]	2022	India	Cross-sectional	169 (1st wave), 211 (2nd wave)	1st wave: M, 35.5%; F, 64.5% 2nd wave: M, 45.5%; F, 55.5%	63±17 and 57±18 (1st and 2nd wave)[a]	-	Comorbidities	Hospitalization, ventilator	Moderate
46	Mohammad et al. [84]	2023	Iraq	Cross-sectional	200	M, 81; F, 119	36.69 (16–78)	September–December 2021	-	-	Moderate
47	Al-Magsoosi et al. [85]	2023	Iraq	Cross-sectional	574	M, 196; F, 378	18–78	October 2021–April 2022	-	-	Low
48	Cazzolla et al. [86]	2023	Italy	Cross-sectional	1,155	M, 57%; F, 43%	M, 59±13; F, 56±16[a]	March 15, 2020–April 15, 2021	-	-	Moderate
49	Sinadinos and Shelswell [6]	2020	United Kingdom	Case series	3	M:F, 2:1	56 (45–61)		DM, hypertension (case 2); obesity (case 3)	-	Low
50	Dima et al. [8]	2020	Romania	Case series	3	M:F, 2:1	Newborns	May 2020	Diaper erythema	Neonatology ward	Low

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Table 1. Continued

No.	Study	Year of publication	Study location	Study design	Sample size (*n*)	Sex	Mean age (range, y)	Study duration	Medical history	Admission in the ICU	Risk of bias
51	Brandao et al. [56]	2021	Brazil	Case series	8	M, 5; F, 3	53 (28–83)	-	Hypertension, COPD (case 1); DM, obesity, renal failure, bariatric surgery, fibromyalgia (case 2); obesity, Parkinson disease, hypertension, COPD (case 3); DM, hypertension (case 4)	Hospitalization	Low
52	Cruz Tapia et al. [57]	2020	Latin America	Case series	4	F:M, 3:1	47.2 (41–54)	-	-	Case 2, hospitalized	Low
53	Vaira et al. [58]	2020	Italy	Case series	72	M, 27; F, 45	49.2 (18–67)	March 31, 2020–April 6, 2020	History of head trauma, allergic rhinitis, chronic rhino sinusitis, psychiatric or neurological disorders	-	Low
54	Martin Carreras-Presas et al. [59]	2021	Spain	Case series	3	M:F, 2:1	55 (56–65)	Last week of March–First week of April 2020	DM, hypertension (case 2); obesity, hypertension (case 3)	Case 3, hospitalization	Low
55	Rodriguez et al. [60]	2022	Spain	Case series	3	F:M, 2:1	68 (53–78)	-	-	Case 1, home quarantine; cases 2 & 3, hospitalization	Low
56	Corchuelo and Ulloa [61]	2020	Colombia	Case report	1	F	40	-	-	-	Low
57	Amorim Dos Santos et al. [9]	2020	Brazil	Case report	1	M	67	March 31, 2020	CAD, autosomal dominant polycystic kidney disease, and kidney transplant, immunosuppression, venous thromboembolism	Hospitalization in ICU	Low
58	Eghbali Zarch and Hosseinzadeh [62]	2021	Iran	Case report	1	F	56	October 2020	-	-	Low
59	Hjelmesaeth and Skaare [63]	2020	Norway	Case report	1	F	60	-	-	-	Low
60	Cebeci Kahraman and Caskurlu [64]	2020	Turkey	Case report	1	M	51	March 18, 2020	-	-	Low
61	Smith et al. [65]	2020	USA	Case report	1	M	21	March 19, 2020	-	-	Low
62	Maniaci et al. [66]	2020	Italy	Case report	1	M	15	-	-	-	Low

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Table 1. Continued

No.	Study	Year of publication	Study location	Study design	Sample size (*n*)	Sex	Mean age (range, y)	Study duration	Medical history	Admission in the ICU	Risk of bias
63	Melley et al. [67]	2020	Pennsylvania, USA	Case report	1	F	59	May 2020	-	-	Low
64	Riad et al. [68]	2022	Egypt	Case report	1	F	47	-	Cardiovascular disease, DM	-	Low
65	Putra et al. [69]	2020	Indonesia	Case report	1	M	29	-	Cardiovascular diseases	-	Low
66	Dalipi et al. [70]	2021	Europe	Case report	1	M	17	-	-	-	Low
67	Eita [71]	2021	Egypt	Case report	1	F	31	-	Irritable bowel syndrome, atopy	-	Low
68	Cirillo and Colella [72]	2021	Italy	Case report	1	F	36	March 2020	-	-	Low
69	Nejabi et al. [73]	2021	Afghanistan	Case report	1	M	62	-	-	-	Low
70	Klein et al. [74]	2021	Israel	Case report	1	F (pregnant)	40	-	-	-	Low
71	Ramires et al. [75]	2021	Brazil	Case report	1	F	50	-	Obesity, hypertension, type 2 DM	Hospitalization, ventilation	Low
72	Hockova et al. [76]	2021	Czech Republic	Case series	3	M:F, 3:0	62		Arterial hypertension, hypercholesterolemia, GERD (case 1); arterial hypertension, history of MI, septic shock (case 2)	ICU	Low
73	Teixeira et al. [77]	2021	Brazil	Case series	4	M:F, 1:3	68.75 (57–84)	-	Hypertension, hypothyroidism, rectal tumor (case 2); hypertension, hypothyroidism (case 3); bipolar disorder (case 4)	-	Low
74	Emelyanova et al. [78]	2021	Ukraine	Case report	1	F	38	-	-	-	Low
75	Fathi et al. [79]	2021	Iran	Case report	1	F	22	April 2020	-	Hospitalization (2nd day)	Low
76	Shenoy et al. [80]	2022	India	Case report	1	F	55	-	-	-	Low
77	Palaia et al. [81]	2022	Italy	Case report	1	F	30	-	-	-	Low
78	Rafalowicz et al. [82]	2022	Poland	Case series	6	M, 4; F, 2	58.8 (43–72)	January–June 2021	Hypertension, insulin resistance (case 2)	No	Low
79	Jogdand et al. [83]	2023	India	Case series	2	M, 1; F, 1	50 and 60	June 2020	Diabetes, hypertension (case 1)	No	Low

ICU, intensive care unit; M, male; F, female; DM, diabetes mellitus; CLD, chronic lung disease; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; GERD, gastroesophageal reflux disease; MI, myocardial infarction; -, not mentioned in article.

[a]Mean ± standard deviation.

Red and white lesions

Eighteen studies reported red and white lesions in COVID-19-positive patients [8,24–26,29,33,36,37,39,43,49,51,53,57,61,68,80,82].

Periodontal involvement

Twelve studies described the involvement of gingiva and periodontium among patients with COVID-19 [6,26,29,32,38,39,42,44,51,55,59,78].

Redness and burning sensation

In total, 33 studies reported patients who complained of redness and burning sensation [5,6,22,26,28,30,31,33,34,38–44,46,48,50–55,57,59,60,69,78,79,81,82,85].

Salivary gland involvement

Salivary gland disorders [37,38,52] were found in 3 studies.

The latency time between the emergence of systemic symptoms and oral lesions ranged from 2 weeks prior to 10 days following the onset of systemic symptoms. In the majority of the studies (n=14), systemic symptoms occurred after the appearance of oral symptoms (Table 2) [5,6,8,9,12–86].

The general treatment protocol, as well as the specific treatment for oral lesions in COVID-19 patients, is outlined in Table S5 [6,8,9,29,43,47,56,57,59–61,64,65,67–71,73,75,77,79,80,82]. Oral lesions typically healed between 7 and 21 days post-emergence. Depending on the severity and cause of the oral lesions, various therapies were prescribed. These included chlorhexidine mouthwash, nystatin, oral fluconazole, topical or systemic corticosteroids, systemic antibiotics, systemic acyclovir, artificial saliva, and photobiomodulation therapy.

Results of the Meta-Analysis

Data from 48 studies were meta-analyzed to determine the prevalence of taste alteration, xerostomia, red and white lesions, vesiculobullous lesions, ulceration, burning sensation, and salivary gland involvement.

Among the prevalence studies, 28 separate studies [12–14,16–20,22–24,28,29,35,36,38,40–44,46,51,53–55,85,86] investigated the occurrence of taste alteration in a total of 3,157 COVID-19 patients. The meta-analysis of these studies revealed a combined prevalence rate of 48% (95% confidence interval [CI], 39%–57%). The heterogeneity of these studies was assessed using Cochran Q test and the I^2 index, indicating a high degree of heterogeneity with an I^2 value of 98.7 (Figure 2).

A total of 327 COVID-19 patients from 14 different studies reported experiencing red and white lesions [5,13,24,25,29,36,37,39,43,48,51–53,84]. These studies revealed a pooled

Table 2. Oral manifestations of the included studies (n=79)

No.	Study	Year of publication	Presenting symptoms given by the patient	Site	Oral signs and symptoms	Occurrence/duration of oral manifestation	Systemic manifestation
1	Nuno-Gonzalez et al. [5]	2021	Oral mucosal changes (11.7%), transient anterior U-shaped lingual papillitis (11.5%), tongue swelling (6.6%), aphthous stomatitis (6.9%), burning sensation in the mouth (5.3%), mucositis (3.9%), glossitis with patchy depapillation (3.9%), white tongue (1.6%), and enanthema (0.5%), taste disturbances	Tongue, oral mucosa	Redness and burning sensation, oral ulceration, red and white lesions, morphological changes of tongue, taste alteration	-	-
2	Sinadinos and Shelswell [6]	2020	Pain in palate (case 1); pain and ulcerations in palate (case 2), pain in tongue, blisters of the labial mucosa; desquamative gingivitis (case 3)	Palate, tongue, gums, lips	Oral ulceration, vesiculobullous lesions, redness and burning sensation, gingival and periodontal changes	-	Sore throat (case 1), pneumonia (case 3)
3	Dima et al. [8]	2020	Oral candidiasis	Oral mucosa	Red and white lesions/ fungal	-	Epistaxis and diaper erythema (all 3 cases); palpebral edema (newborn 2)

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https://doi.org/10.24171/j.phrp.2023.0033

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Table 2. Continued

No.	Study	Year of publication	Presenting symptoms given by the patient	Site	Oral signs and symptoms	Occurrence/duration of oral manifestation	Systemic manifestation
4	Amorim Dos Santos et al. [9]	2020	Hypogeusia, white plaque, multiple pinpoint yellowish ulcers in the tongue, nodule in lower lip (1 cm)	Tongue, lower lip	Vesiculobullous lesions, red and white lesions, taste alteration	Mean duration, 14 d	Respiratory symptoms and progressive dyspnea on exertion, fever and diarrhea
5	Al-Zaidi and Badr [12]	2020	Loss of taste (83%)	Tongue	Taste alteration	1 wk before systemic symptoms	Fever (63.08%), cough (60.00%), dyspnea (47.69%), sore throat, diarrhea (32.31%), chest pain (30.77%)
6	Biadsee et al. [13]	2020	Taste alteration (n = 67), dry mouth (n = 72), plaque-like changes in the tongue (n = 9), swelling in the oral cavity (n = 10)	Tongue, oral mucosa	Red and white lesions, taste alteration, xerostomia	Along with systemic symptoms	Cough and runny nose (p = 0.018), olfactory dysfunction
7	Bodnia and Katzenstein [14]	2020	Total loss of taste (70%)	Tongue	Taste alteration	1–3 wk (78%), 3–6 wk (22%)	Fatigue, headache, fever, dry cough, disturbance of the sense of smell
8	Chen et al. [15]	2020	Amblygeustia (47.2%), dry mouth (11.1%)	Tongue, oral mucosa	Taste alteration, xerostomia	Along with systemic symptoms	Submandibular lymph node enlargement (n = 1), cough (n = 21), fever (n = 20), diarrhea (n = 4), chest tightness (n = 13)
9	Dell'Era et al. [16]	2020	Taste disorders (65.5%)	Tongue	Taste alteration	Mean duration, 10 d	Fever (72.1%), cough (47.9%), fatigue (40.3%), dyspnea (21.7%), diarrhea (19.7%)
10	Kumar et al. [17]	2021	Taste, dysfunction (28.4%)	Tongue	Taste alteration	Duration, 2–15 d	Malaise (14.2%), sore throat (19.9%), cough (20.6%), fever (48.2%), diarrhea (5.7%), nasal discharge (3.5%), headache (5.7%)
11	Lechien et al. [18]	2020	Gustatory dysfunction (88.8%)	Tongue	Taste alteration	Mean duration, 9.2 ± 6.2 d	Olfactory dysfunction (85.6%)
12	Paderno et al. [19]	2020	Gustatory dysfunction (group A, 51.9%; group B, 78.9%); partial, 36.8%; total, 60.1%; unable to assess, 3.1%	Tongue	Taste alteration	First symptom in 11.9% (group A) and 10.2% (group B); mean duration, 9.2 ± 5.4	Olfactory dysfunction, fever, cough, headache, dyspnea, asthenia, diarrhea, nausea, nasal congestion, pharyngodynia
13	Boscolo-Rizzo et al. [20]	2020	Loss of taste (n = 113)	Tongue	Taste alteration	Mean duration, 9.5 d	Dry cough, fever, headache, sore throat, chest pain, nausea, abdominal pain
14	Yan et al. [21]	2020	Gustatory impairment (71%) (p < 0.001)	Tongue	Taste alteration	-	Fatigue (81%), fever (70%), anosmia (68%), myalgia or arthralgia (63%), diarrhea (48%), nausea (27%)
15	Sinjari et al. [22]	2020	Impaired taste (25%), burning sensation (15%), difficulty in swallowing (20%), dry mouth (30%) (p = 0.02)	Oral mucosa, tongue	Redness and burning sensation, taste alteration, xerostomia	-	-

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Table 2. Continued

No.	Study	Year of publication	Presenting symptoms given by the patient	Site	Oral signs and symptoms	Occurrence/duration of oral manifestation	Systemic manifestation
16	Giacomelli et al. [23]	2020	Dysgeusia (8.5%), ageusia (1.7%)	Tongue	Taste alteration	Before hospitalization (91%)	Fever (72.8%), cough (37.3%), dyspnea (25.4%), sore throat (1.7%), arthralgia (5.1%), headache (3.4%), asthenia (1.7%), abdominal symptoms (8.5%)
17	Mascitti et al. [24]	2020	Oral lichenoid reaction (32.5%), oral enanthema (27.5%), macroglossia (25.0%), cheilitis (12.5%), ageusia (20.5%), extensive ulcerations of the tongue (2.5%)	Lips, tongue, oral mucosa	Oral ulceration, vesiculobullous lesions. taste alteration	-	Macular exanthema (80%), face edema (32%), livedo (13%), urticarial rash (8%), purpura (5%), oral lichenoid lesions (33%), conjunctivitis (18%)
18	Salehi et al. [25]	2020	White plaques on the intraoral mucous layer of oral mucosa	Oral mucosa	Red and white lesions	-	-
19	Askin et al. [26]	2020	Aphthous stomatitis (5.8%), rash and erythema, aphthous lesion on side of tongue	Tongue, oral mucosa	Vesiculobullous lesions, redness and burning sensation	-	Cutaneous findings (36.1%)
20	Katz and Yue [27]	2021	Recurrent aphthous stomatitis (0.64%)	Oral mucosa	Oral ulceration	-	-
21	Fantozzi et al. [28]	2020	Dry mouth (45.9%), swallowing difficulties (39.2%), dysgeusia (59.5%)	Tongue, oral mucosa	Redness and burning sensation, oral ulceration, taste alteration, xerostomia	First symptom (xerostomia) (19.6%); dysgeusia (87.9%), duration (xerostomia), 7 d; dysgeusia 6 d	Fever (90.9%), cough (46.8%), dyspnea (34.3%), diarrhea (4.5%), sore throat (3.6%), fatigue (3.6%), myalgia/ arthralgia (2.7%), vomiting (2.7%)
22	Favia et al. [29]	2021	Geographic tongue (n = 7), fissured tongue (n = 5), ulcerative lesion (n = 65), blisters (n = 19), hyperplasia of papillae (n = 48), angina bullosa (n = 11), candidiasis (n = 28), ulceronecrotic gingivitis (n = 7), petechiae (n = 14), oral haemorrhage (n = 1), taste disorders (90%)	Tongue, oral mucosa, lips	Red and white lesions, morphological changes of tongue, vesiculobullous lesions, oral ulceration, taste alteration	Together with general symptoms (26.2%); duration, 1 wk (41%); after 1 wk of general symptoms (32.6%)	Fever, anosmia, cough, sore throat, congestion, runny nose, nausea or vomiting, muscle and body aches, dermatologic manifestation, pneumonia, dyspnea, hypoxia (SpO_2 < 90%)
23	Halepas et al. 2021 [30]	2021	Red and/or swollen lips (48.9%), strawberry tongue (10.6%)	Lips, tongue	Redness and burning sensation, morphological changes of tongue	-	Fever
24	Rekhtman et al. [31]	2021	Rashes on lips and tongue (5.7% and 2.9%), ulcers on lips and tongue	Lips and tongue	Redness and burning sensation, oral ulceration		Generalized rashes
25	Marouf et al. [32]	2021	Periodontitis (258/568)	Periodontium	Gingival and periodontal changes	-	-

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Table 2. Continued

No.	Study	Year of publication	Presenting symptoms given by the patient	Site	Oral signs and symptoms	Occurrence/duration of oral manifestation	Systemic manifestation
26	Subramaniam et al. [33]	2021	Ulcers on oral mucosa; burning mouth and mucositis on lower labial mucosa, papillary atrophy; reddish-white spots on the palate; ulcers on lower lip; pallor of lip	Oral mucosa palate, lips, tongue	Oral ulceration, petechiae, redness and burning sensation, morphological changes of tongue	-	Fever, cough, dyspnea, runny nose, chest tightness, loss of smell
27	Abubakr et al. [34]	2021	Dental pain (23%), pain in jaw bones or joint (12.0%), halitosis (10.5%), ulcerations (20.4%), dry mouth (47.6%)	Oral mucosa	Oral ulceration, redness and burning sensation, xerostomia	-	Fever, myalgia, dysphagia, and hyposmia, loss of smell, nasal itching
28	Song et al. [35]	2021	Loss of taste (20.6%)	Tongue	Taste alteration	First symptom (0.4%), recovery time, 7 d	Nasal obstruction (8.6%), rhinorrhea (10.3%), nasal itching (4.9%), sneezing (11.0%), loss of smell (11.4%)
29	Bardellini et al. [36]	2021	Oral pseudomembranous candidiasis (7.4%), geographic tongue (3.7%), coated tongue (7.4%), taste alteration (11.1%)	Tongue, oral mucosa	Red and white lesions, morphological changes of tongue, taste alteration	-	Fever, cough, rhinorrhoea, breathing difficulty
30	Gherlone et al. [37]	2021	Salivary gland ectasia (38%), dry mouth (30%), dysgeusia (17%), white plaque (28%), oral ulcers (12%)	Salivary glands, tongue, oral mucosa	Red and white lesions, oral ulceration, xerostomia, salivary gland disorder	-	-
31	El Kady et al. [38]	2021	Dry mouth (39.7%), loss of salt sensation (34.5%), loss of sweet sensation (29.3%), altered food taste (25.9%), tongue redness (8.8%), gingival bleeding (7%), salivary glands infection (22.4%), swellings in the salivary gland or cheek (13.8%), pain or swelling below mandible (10.8%), burning mouth sensation (22.4%), ulcers (17.2%)	Tongue, salivary glands, gingiva, oral mucosa	Redness and burning sensation, taste alteration, xerostomia, gingival and periodontal changes, salivary gland disorder	-	-
32	Fidan et al. [39]	2021	Aphthous-like ulcer (36.5%), erythema (25.7%), lichen planus (16.2%), tongue (31.8%), oral mucosa (27.0%), gingiva (18.9%), palate (5.4%)	Tongue (39.7%), oral mucosa (34.5%), gingiva (18.9%), palate (6.9%)	Oral ulceration, redness and burning sensation, red and white lesions	Oral lesions prior COVID-19 diagnosis	-
33	Natto et al. [40]	2021	Loss of taste (43.4%), erythema/desquamated gingivitis and coated tongue (7.3%), ulcers/blisters (6.4%), pain and soreness (2.8%), dry mouth (0.9%)	Tongue, gingiva, oral mucosa	Oral ulceration, redness and burning sensation, taste alteration, xerostomia, gingival changes	After systemic symptoms	Cough, fever, sore throat, runny nose, muscle pain, headaches, nausea, diarrhea

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Table 2. Continued

No.	Study	Year of publication	Presenting symptoms given by the patient	Site	Oral signs and symptoms	Occurrence/duration of oral manifestation	Systemic manifestation
34	Elamrousy et al. [41]	2021	Oral ulcers (92.8%), dry mouth (84%), loss of taste (55%), hemorrhagic ulcers with crust on lips	Lip (42.3%), tongue (38.5%), labial mucosa (34.6%)	Oral ulceration, redness and burning sensation, taste alteration, xerostomia	-	Asthenia (67.7%), breath problems (67.7%), cough (67.7%), fatigue (19.4%), abdominal symptoms (12.9%)
35	Bulut et al. [42]	2021	Taste loss (53%), halitosis (21%), oropharyngeal wound and pain (18%), pain in the chewing muscles (16%), gum bleeding (17.5%), dry mouth (38%, after recovery 12.0%), aphthous ulcer (14.5%), sensitivity and/or pain in teeth (12%), herpes labialis (8.5%), burning in the tongue (7.5%)	Tongue, gingiva, lips	Oral ulceration, redness and burning sensation, taste alteration, xerostomia	-	Presence of symptoms (87.5%)
36	Naser et al. [43]	2021	Burning sensation (6%), numbness or tingling of the tongue (2%), white coat of the tongue, gingiva, palate (31.6%, 22.4%, 15.6%), loss of taste (79.5%), aphthous ulcers (24.8%), black discoloration of oral cavity, lips and tongue (4.7%, 6.8%), yellow coating on lips (5.3%)	Tongue, palate, lips, oral mucosa	Oral ulceration, redness and burning sensation, red and white lesions, taste alteration	-	-
37	Muthyam et al. [44]	2022	Dry mouth (44%) followed by swallowing difficulty, mouth ulcerations, chewing problems, gum bleeding, and burning sensation, altered taste (72%); fissured tongue, halitosis, and loss of taste, 2%	Gums, tongue, oral mucosa	Oral ulceration, redness and burning sensation, morphological changes of tongue, taste alteration, xerostomia	Altered taste lasted more than 1 wk (53%)	Weakness (8%), cough and cold (4%), body pain (2%)
38	Ganesan et al. [45]	2022	Gustatory disturbance (51.2%); dry mouth (28%); erythema, ulcers and depapillation of tongue (15.5%); A statistically significant correlation between oral manifestations and disease severity ($p \leq 0.001$).	Tongue, oral mucosa	Morphological changes of tongue, oral ulceration, taste alteration, xerostomia	-	-
39	El Tantawi et al. [46]	2022	Dry mouth (11.1% vs. 7.5%, p = 0.009) and change in taste (11.5% vs. 2.7%, $p < 0.001$) were greater in COVID-19 person; leukoplakia (4.6%); ulcers & hairy tongue (2.3%); gingival redness and burning sensation (13.1%)	Oral mucosa tongue, gingiva	Morphological changes of tongue, oral ulceration, redness and burning sensation, taste alteration, xerostomia, gingival and periodontal changes	-	-

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https://doi.org/10.24171/j.phrp.2023.0033

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Table 2. Continued

No.	Study	Year of publication	Presenting symptoms given by the patient	Site	Oral signs and symptoms	Occurrence/duration of oral manifestation	Systemic manifestation
40	Soares et al. [47]	2022	Ulcerative lesions in the palate (57.1%), tongue (29%), lips or palate (14.3%)	Tongue, lips, palate	Oral ulceration	-	Anosmia, fever, headache
41	Tuter et al. [48]	2022	Dry mouth (44.2%), oral ulceration (22.4%), oral mucosa (15.2%), tongue (10.8%)	Tongue, oral mucosa	Oral ulceration, redness and burning sensation	-	-
42	Schwab et al. [49]	2022	Ageusia (11.0%); opportunistic oral infections such as pseudomembranous candidiasis and herpes simplex (4.5%)	Tongue	Red and white lesions/ fungal, taste alteration, oral ulceration	-	Cough (72.7%), dyspnoea (63.0%), fever (53.9%), anosmia (14.3%)
43	Chawla et al. [50]	2022	Dry mouth (38%) ($p = 0.03$), dysgeusia (32%) ($p = 0.04$), vesiculobullous lesion (13%), oral ulcers (3.7%)	Oral mucosa tongue	Oral ulceration, redness and burning sensation, vesiculobullous lesions, taste alteration, xerostomia	-	Cough (30%), sore throat (20%), shortness of breath (7%), running nose (11%)
44	Binmadi et al. [51]	2022	Taste disturbance (60%); dry mouth (42%); oral ulcerations (11%); gingivitis/petechiae/candidiasis (6%); necrotizing periodontal disease/vesiculobullous lesions/ erythema migrans/geographic tongue (4%)	Gingiva, tongue, oral mucosa	Oral ulceration, redness and burning sensation, vesiculobullous lesions, morphological changes of tongue, red and white lesions, taste alteration, xerostomia	Concurrently (47%), after the general symptoms (43%), before the general symptoms (9%)	Fever (95%), headache (65%), fatigue (65%), cough (63%), myalgia/arthralgia (53%), loss of smell (53%), sore throat (50%), shortness of breath or dyspnea (40%), nausea or vomiting (21%), diarrhea (15%)
45	Eduardo et al. [52]	2022	Saliva alterations (24.4%), dryness (9.9%), tongue coating (3%), sialorrhea (3.3%), petechiae (10.5%), oral bleeding (7.5%)	Oral mucosa tongue, salivary glands	Red and white lesions, redness and burning sensation, salivary gland disorder, xerostomia	-	-
46	Villarroel-Dorrego et al. [53]	2022	Hemorrhagic ulcerative lesions (7.3%), erythematous and pseudomembranous forms of candidiasis (12.7%), angular cheilitis (1.5%), total loss of taste (60%), burning mouth (36.4%), dry mouth (27.3%)	Tongue, lips, oral mucosa	Oral ulceration, redness and burning sensation, red and white, lesions, xerostomia	-	-
47	Manifar et al. [54]	2022	Dry mouth (68.6%) ($p < 0.001$), dysgeusia (51.4%) ($p < 0.001$), hypogeusia (49.3%), halitosis (31.4%), metallic taste (29.3%)	Tongue, oral mucosa	Redness and burning sensation, taste alteration, xerostomia	-	Gastrointestinal symptoms, smell defects, asthma, skin rashes, cough, malaise, myalgia, anorexia, respiratory distress, olfactory dysfunction
48	Bhuyan et al. [55]	2022	Burning sensation (2.4%), dry mouth (2.4%), loss of taste (31%) ($p < 0.001$), mouth ulcer (2.4%), bleeding gum (2.4%)	Oral mucosa gums, tongue	Oral ulceration, redness and burning sensation, taste alteration, xerostomia, gingival changes	-	-

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Table 2. Continued

No.	Study	Year of publication	Presenting symptoms given by the patient	Site	Oral signs and symptoms	Occurrence/duration of oral manifestation	Systemic manifestation
49	Brandao et al. [56]	2021	Multiple aphthous-like ulcers covered with mucopurulent membrane in the upper and lower lip mucosa and tongue (cases 1, 2, 4, 5); ulcers on tongue and hard palate (case 3); ulcers on tongue and ageusia (cases 6, 7, 8)	Lips, tongue, palate	Oral ulceration, taste alteration	6–10 d	Chest tightness, fever, cough (cases 1, 5, 7, 8); cough, fever, dyspnea (cases 2, 6); abdominal distension, fever, mild dyspnea (cases 3, 4)
50	Cruz Tapia et al. [57]	2020	Bulla on the hard palate (×6 mm) (case 1); diffuse purple macule (×12 mm) and papule-plaque (×8 mm) on the left and right palatal mucosa (case 2); tongue enlargement (case 3); Burning mouth sensation and reddish macules on hard palate (case 4)	Palate, tongue	Redness and burning sensation, vesiculobullous lesions, morphological changes of tongue		Fever, myalgia, dysphagia, hyposmia
51	Vaira et al. [58]	2020	Hypogeusia (33 cases); complete ageusia (1 case)	Tongue	Taste alteration		Fever, cough, nasal obstruction, sore throat, hyposmia, anosmia, pneumonia
52	Martin Carreras-Presas et al. [59]	2021	Dysgeusia (case 1); multiple ulcers On palate (case 2); pain on tongue, blisters in lip mucosa and Desquamative gingivitis (case 3)	Tongue, lips	Oral ulceration, redness and burning sensation, vesiculobullous lesions, taste alteration, gingival changes	Along with systemic symptoms	Asthenia, hyposmia, enlargement of lymph nodes in the neck (cases 1, 3); fever, diarrhea (case 2)
53	Rodriguez et al. [60]	2022	Dysgeusia, aphthous-like lesions, burning sensation, and tongue depapillation (case 1); burning mouth sensation and unilateral commissural fissures (case 2); dry mouth, lesions on the tongue, palate, and commissure (case 3)	Tongue, palate, oral mucosa	Redness and burning sensation, morphological changes of tongue, taste alteration, xerostomia	Before presentation (case 1); after discharge (case 2); with systemic symptoms (case 3)	Fever, malaise, anosmia, diarrhea, pneumonia (cases 1, 3)
54	Corchuelo and Ulloa [61]	2020	Reddish plaques on the lower lip, dark brown pigmentation and aphthous ulcers in the gums, whitish area in tongue	Lower lips, gums	Oral ulceration, vesiculobullous lesions, red and white lesions	Mean duration, 8–10 d	-
55	Eghbali Zarch and Hosseinzadeh [62]	2021	Vesicles on lower lip mucosa	Lip	Vesiculobullous lesions	2 d before systemic symptoms	High fever, fatigue, lack of appetite
56	Hjelmesaeth and Skaare [63]	2020	Total ageusia	Tongue	Taste alteration	-	-
57	Cebeci Kahraman and Caskurlu [64]	2020	Erythematous surface (hard palate), few petechiae in the midline and numerous pustular enanthema near the soft palate border	Palate	Red and white lesions, oral ulceration	Mean duration, 10 d	Sore throat; fever, fatigue, severe dry cough, inability to taste or smell

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https://doi.org/10.24171/j.phrp.2023.0033

https://doi.org/10.24171/j.phrp.2023.0033

Table 2. Continued

No.	Study	Year of publication	Presenting symptoms given by the patient	Site	Oral signs and symptoms	Occurrence/duration of oral manifestation	Systemic manifestation
58	Smith et al. [65]	2020	Loss of taste	Tongue	Taste alteration	Before general symptoms	Frontal headache, loss of smell, headache, loose stools
59	Maniaci et al. [66]	2020	Transient loss of taste	Tongue	Taste alteration	Mean duration, 12 d	Fever, sore throat, runny nose, presence of erythematous skin lesions on the lower limbs, asthenia
60	Melley et al. [67]	2020	Loss of taste	Tongue	Taste alteration	1 wk before systemic presentation	Shortness of breath, fatigue, loss of appetite
61	Riad et al. [68]	2022	Painful white patches on the dorsal surface of the tongue and palate, mild tongue pain	Tongue, palate	Red and white lesions	2 wk before diagnosis	Sore throat, generalized myalgia, fatigue with intermittent fever
62	Putra et al. [69]	2020	Stomatitis aphthous	Oral mucosa	Oral ulceration, redness and burning sensation	Day 7	Fever, back pain, myalgia, sore throat, dry cough, rhinorrhea, anosmia
63	Dalipi et al. [70]	2021	Loss of taste Bullous and erosive erythematous lesions of lips and oral mucosa	Tongue, lips	Vesiculobullous lesions, taste alteration	Loss of taste, 2 wk before diagnosis	Fever, cough, headache, muscle pain, loss of smell, dark red, purpuric, irregular maculopapular lesions on abdomen
64	Eita [71]	2021	Dysgeusia and greasy tongue coat	Tongue	Morphological changes of tongue, taste alteration	Before systemic symptoms	Sore throat, fever (38 °C), nasal congestion, conjunctivitis, nausea; abdominal pain, diarrhea, fatigue, severe joint pain
65	Cirillo and Colella [72]	2021	Loss of taste	Tongue	Taste alteration	1 wk before presentation	Loss of smell, headache, fatigue, muscle pain
66	Nejabi et al. [73]	2021	Fissured tongue, white scars and painful erosive ulcer on the dorsal surface of the tongue	Tongue	Morphological changes of tongue, oral ulceration	After 1 wk of general symptoms	Fever, cough, taste alterations, olfactory dysfunction, chest tightness
67	Klein et al. [74]	2021	Loss of taste	Tongue	Taste alteration	From 4th to 6 wk	Fever, dry cough, chest pain, sore throat, diarrhea, nausea, headache, back pain
68	Ramires et al. [75]	2021	Crusted ulcers on lip vermilion (both upper and lower lips)	Lip	Oral ulceration	2 wk after the onset of fever	Flu-like syndrome: severe and progressive dyspnea (SpO_2 = 88%)
69	Hockova et al. [76]	2021	Oral lesions at the dorsal surface of the tongue (case 1); multiple lesions located on the tongue dorsum and labial mucosa (case 2); lesions on upper and lower lip (case 3)	Tongue, lips	Oral ulceration	After the diagnosis (all 3 cases)	Headache, fever, dry cough, dyspnoea

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Table 2. Continued

No.	Study	Year of publication	Presenting symptoms given by the patient	Site	Oral signs and symptoms	Occurrence/duration of oral manifestation	Systemic manifestation
70	Teixeira et al. [77]	2021	Painful vesiculobullous lip lesions	Lips	Vesiculobullous lesions	After 4 d (case 1); after 10 d (case 2); after 11 d (case 3); after 19 d (case 4)	Headache, myalgia, dyspnea
71	Emelyanova et al. [78]	2021	Unusual tongue appearance and burning sensation, intermittent bleeding of gums, severe dryness in the oral cavity and persistent distortion of taste	Tongue, gums, oral mucosa	Redness and burning sensation, morphological changes of tongue, xerostomia	3rd day (dysgeusia) and 5th day (xerostomia) after systemic symptoms	Rhinorrhea, coughing and increased body temperature to 38.5 °C
72	Fathi et al. [79]	2021	Oral pain, ulcerative lesions on oral mucosa, hemorrhagic crusts on lips	Oral mucosa, lips	Oral ulceration, redness and burning sensation	3rd day (oral pain)	Fever, abdominal pain, nausea, occasional vomiting
73	Shenoy et al. [80]	2022	Ulcer with irregular borders on the dorsum of the tongue surrounded by a scrapable whitish plaque	Tongue	Oral ulceration, red and white lesions	Systemic symptoms, 3 wk prior	Fever, cough, chest tightness
74	Palaia et al. [81]	2022	Extensive erosions involving lips, ulcers on the hard palate, blisters and ulcers on the dorsal surface of the tongue cheek mucosa	Palate, lips, oral mucosa	Oral ulceration, vesiculobullous lesions, redness and burning sensation	7 d prior to general symptoms (duration of oral lesions, 14 d)	Bilateral cutaneous lesions were also evident on the hands. Low-grade fever
75	Rafalowiczet al. [82]	2022	Unilateral aphthous-like lesions on the left side of the hard palate (case 1 & 5); hemorrhagic changes on the palate and cheilitis (case 2); smooth tongue with intensely red-purple mucosa (case 3); angioma-type lesion on the right side of the palate (case 4); mycosis of the tongue, extensive lesions on the palate, cheilitis (case 6)	Hard palate, tongue, lips	Oral ulceration, redness and burning sensation, red and white/fungal, morphological changes of tongue	-	Fever, malaise, taste disorders, anosmia, and pneumonia (case 1); dyspnea, persistent diarrhea, and vomiting (case 2); loss of smell and taste and fever for 9 d (cases 4, 5)
76	Jogdand et al. [83]	2023	Ulcers with yellowish gray pseudo-membrane on oral mucosa and palate	Oral mucosa, palate	Oral ulceration, red and white lesions	-	-
77	Mohammad et al. [84]	2023	Dry mouth (50%), gustatory dysfunction (37%), burning mouth sensation (22.5%), oral pain (17%), aphthous lesions, fissural cheilitis and tongue depapillation (9.5%), candidiasis (7.5%), gingival bleeding (2.5%)	Gingiva, oral mucosa, tongue	Oral ulceration, red and white lesions, morphological changes of tongue, taste alteration, xerostomia, gingival changes	-	Fever (83.5%), weakness (80%), myalgia (73%), headache (70%), cough (65%), loss of smell sensation (54%), loss of taste sensation (48.5%), sore throat (38.5%), nasal congestion (26.5%), runny nose (25%), gastrointestinal symptom (24.5%)

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Table 2. Continued

No.	Study	Year of publication	Presenting symptoms given by the patient	Site	Oral signs and symptoms	Occurrence/duration of oral manifestation	Systemic manifestation
78	Al-Magsoosi et al. [85]	2023	Ageusia (66.8%), dry mouth (59%), gustatory changes (46%), dysphagia (40.5%), burning sensation (20.8%), oral ulceration (14.5%), gingival bleeding (3.3%)	Tongue, oral mucosa, gingiva	Redness and burning sensation, taste alteration, xerostomia	-	-
79	Cazzolla et al. [86]	2023	Taste dysfunction (208/25%)	Tongue	Taste alteration	1 wk before general symptoms	Fever, breathing, asthenia, rhinorrhea, headache, abdominal symptoms, sore throat, chest pain, cough

-, not mentioned in article.

prevalence of these symptoms at 17% (95% CI, 9%–26%), with an I^2 value of 98.2% (Figure 3).

Xerostomia was reported in 22 studies [13,15,22,28,34,37,38,40–42,44–46,48,50–55,84,85] involving 1,694 COVID-19 patients, showing a pooled prevalence of 35% (95% CI, 26%–44%) and I^2 = 98.7% (Figure 4).

Ulceration was reported in 23 studies [5,24,27–29,31,33,34,37,39–48,50,51,53,85] involving 1,086 patients, with a a prevalence of 21% (95% CI, 12%–30%) and I^2 of 99.62% (Figure 5).

The pooled prevalence of a burning sensation was reported to be 12% (95% CI, 6%–18%) with I^2 = 98.3%; this symptom was found in 297 patients in 11 studies [5,22,33,38,42–44,53,55,84,85] (Figure 6).

Vesiculobullous lesions were reported by 73 participants from 5 studies [26,29,36,50,51], with a pooled prevalence of 10% (95% CI, 5%–16%) and an I^2 of 98.5% (Figure 7A).

Salivary gland involvement was reported only in 3 studies [37,38,52] involving 193 patients, showing a pooled prevalence of 32% (95% CI, 22%–41%) and I^2 = 98.7% (Figure 7B).

Funnel plots demonstrated asymmetry, indicating the presence of high publication bias in the studies (Figure 8).

Discussion

COVID-19 has emerged as a global public health issue. Initially, it was believed that the absence of oral mucosa involvement distinguished COVID-19 from other viral infections. However, in April 2020, a case report by Chaux-Bodard et al. [87] demonstrated a link between COVID-19 and oral mucosa. The report detailed a 45-year-old woman who experienced painful inflammation of the tongue's papilla, which eventually healed into an asymptomatic ulcer within 10 days, leaving no scar. This patient also developed a skin lesion on her toe and tested positive for COVID-19 on the eighth day. Since this report, numerous observational studies and case reports have been published, highlighting the involvement of oral mucosa in COVID-19 patients. This systematic review was undertaken to determine the prevalence of oral manifestations in patients with COVID-19.

SARS-CoV-2 infiltrates human cells in the lower respiratory system using receptors known as ACE2 and transmembrane protease serine 2 [3]. Of these 2, the ACE2 receptor is primarily located in the cells of the lung, liver, kidney, GI tract, and even in the cells of the nasal epithelium and oral mucosa [4]. These cells serve as host cells for the virus, which invades these body cells and triggers an inflammatory response in these organs. This response, in turn, leads to early smell and taste dysfunctions during the disease's progression [15]. Therefore, the development of oral lesions can occur directly

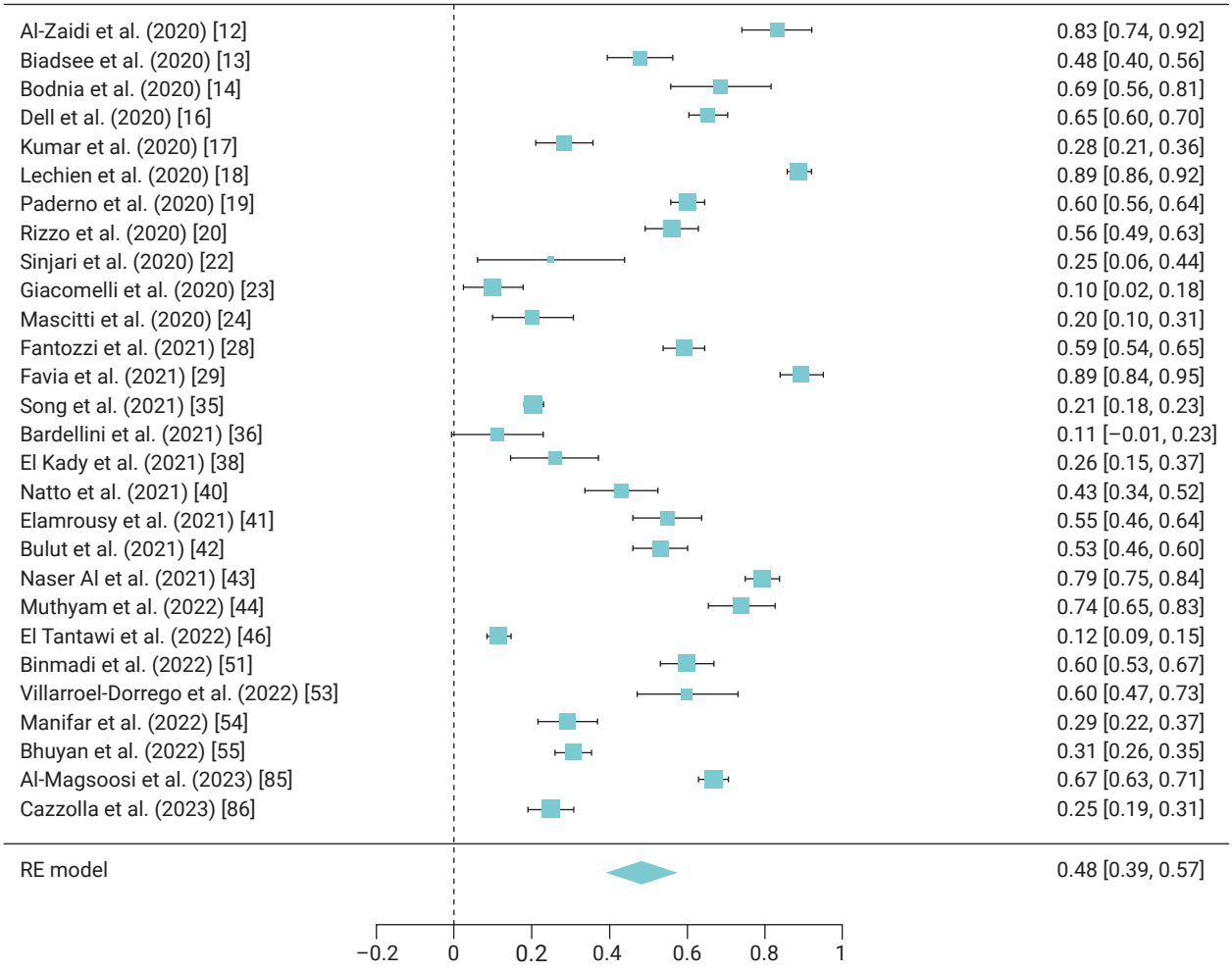

Figure 2. Forest plot showing the pooled prevalence of taste disorders in patients with COVID-19 (28 studies). RE, random effects.

through the effects of the virus replicating in these cells (resulting in SARS-CoV-2-specific lesions) and indirectly as a consequence of potential drug reactions that may occur during the latency period, viral exanthem, due to the physical and psychological stress of COVID-19 or its treatment, or co-infection with other bacterial infections that exacerbate the severity of COVID-19 [59,88]. The involvement of the oral cavity becomes a unique characteristic of COVID-19 [89]. According to Amorim dos Santos et al. [4], the general health deterioration of COVID-19 patients, coupled with extended hospitalization periods and numerous treatment procedures, also increases the likelihood of oral lesions. Chaux-Bodard et al. [87] proposed that oral lesions might emerge as a result of various inflammatory reactions that induce vascular inflammation. Previous reports from Italy and the United Kingdom have noted a temporary association between pediatric inflammatory multisystem syndrome and SARS-COV-2 cases [90]. Certain diseases, such as Kawasaki disease and erythema multiforme, can predispose individuals to oral manifestations. Consequently, we have excluded such conditions from our systematic review.

Regarding oral lesions, the tongue was the most frequently affected area (n=65), followed by the oral mucosa (n=37), and then the lips (n=19). de Sousa and Paradella [7] identified the palate and tongue, followed by the gums and lips, as the areas most commonly affected in COVID-19 patients. The oral manifestations among COVID-19 patients are described below:

Taste Disorders

Numerous studies have indicated that alterations in smell and taste can serve as early signs of COVID-19 infection, playing a crucial role in early diagnosis and decision-

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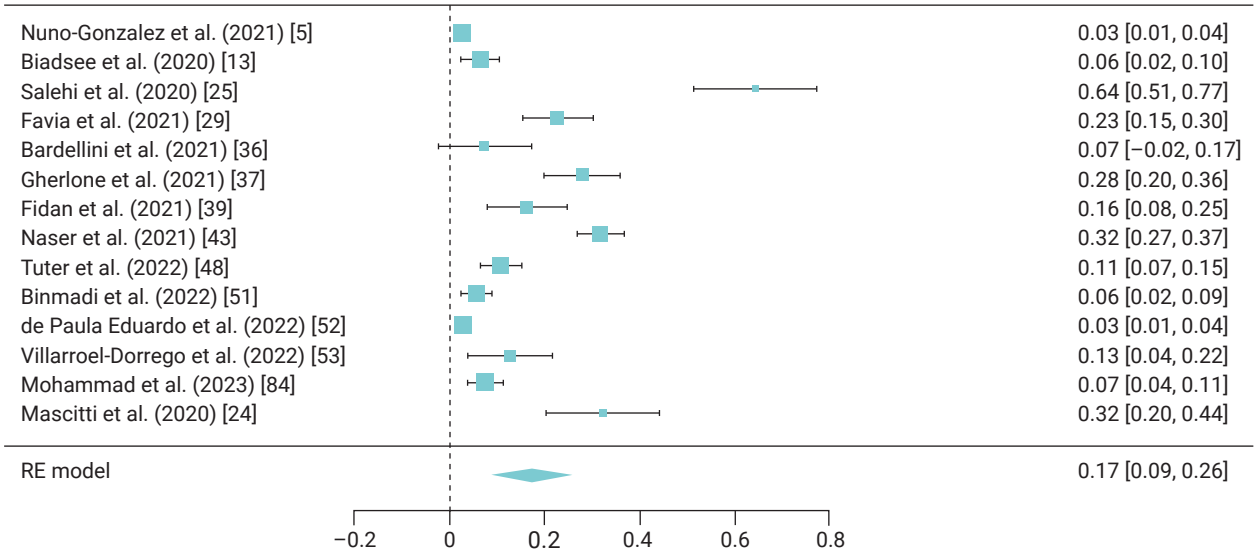

Figure 3. Forest plot showing the pooled prevalence of red and white lesions in patients with COVID-19 (14 studies). RE, random effects.

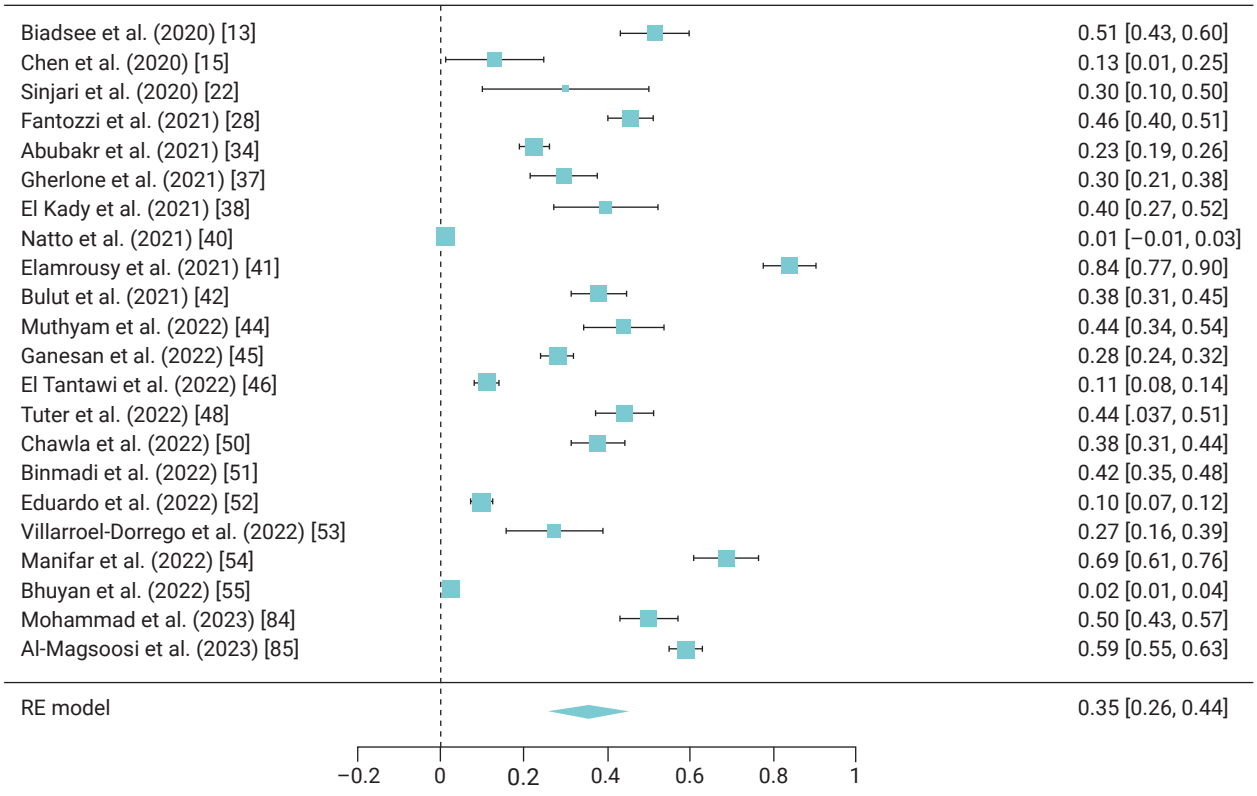

Figure 4. Forest plot showing the pooled prevalence of xerostomia in patients with COVID-19 (22 studies). RE, random effects.

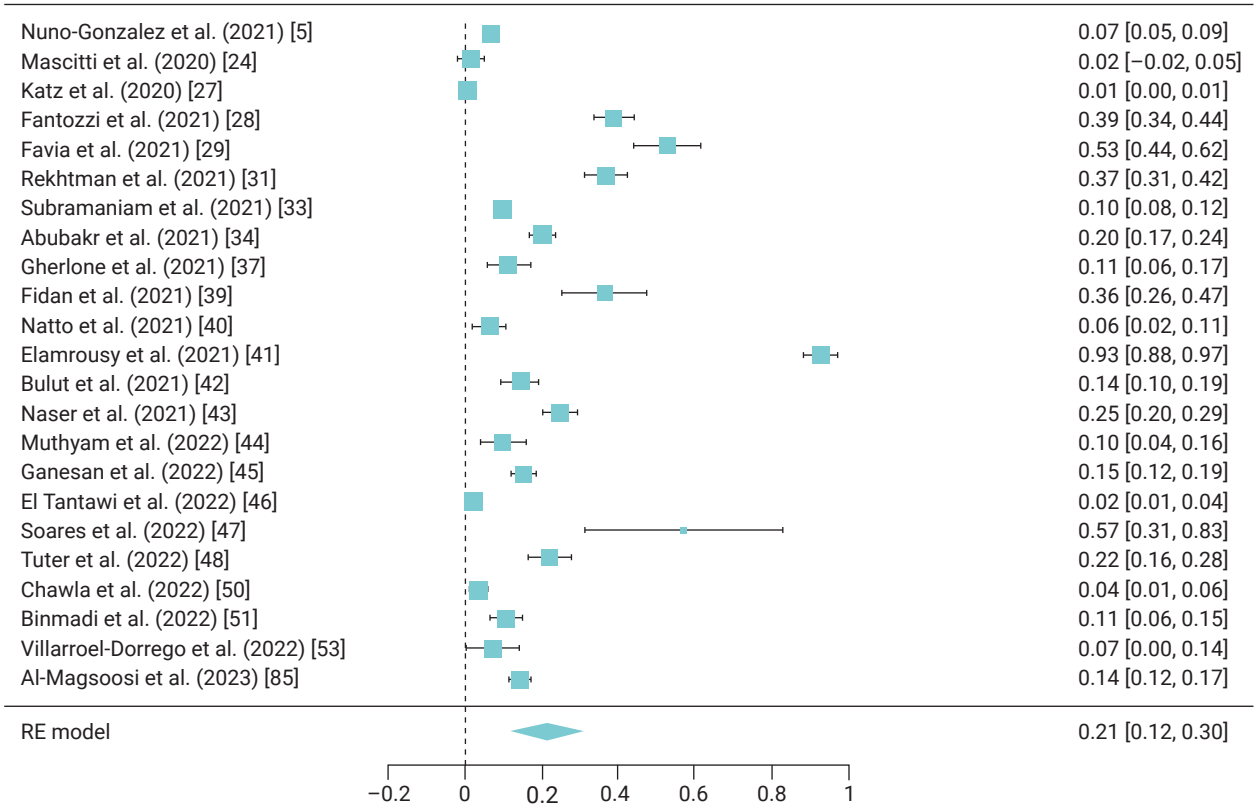

Figure 5. Forest plot showing the pooled prevalence of ulceration in patients with COVID-19 (23 studies). RE, random effects.

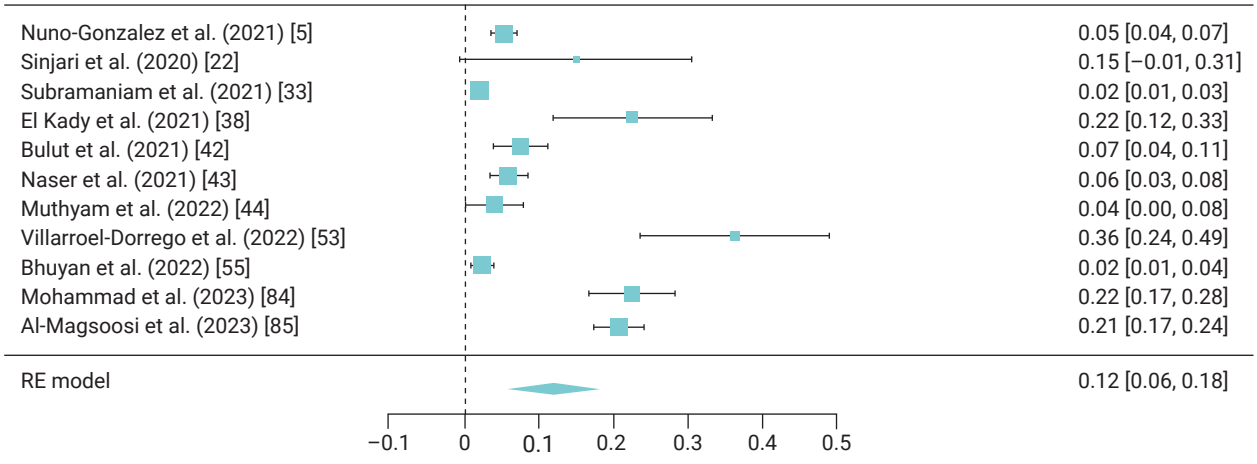

Figure 6. Forest plot showing the pooled prevalence of burning sensation in patients with COVID-19 (11 studies). RE, random effects.

making. While these symptoms are not life-threatening, they can significantly impact a patient's quality of life. Professor C. Hopkins, President of the British Rhinological Society, has noted that the loss of smell or taste may be the sole symptom of COVID-19 [91]. Several public health surveillance organizations, including the European Centre for Disease Prevention and Control, the Centers for Disease Control and Prevention, the WHO [92], and Public Health England, have incorporated the sudden onset of anosmia, ageusia, or dysgeusia into their primary clinical criteria for defining a COVID-19 case [93]. The present systematic review also found that general symptoms typically follow

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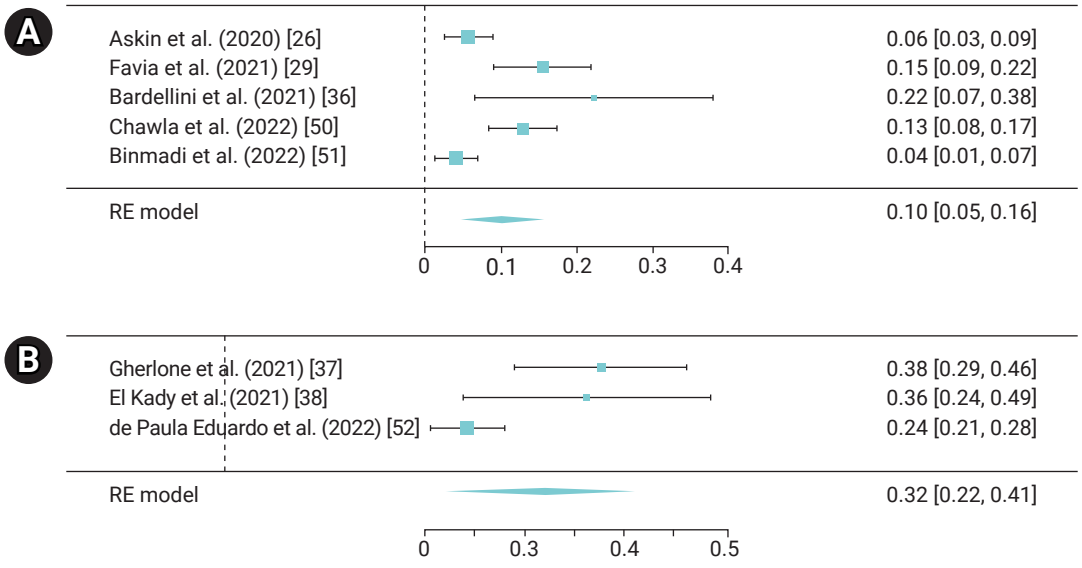

Figure 7. Forest plot showing the pooled prevalence of (A) vesiculobullous lesions (5 studies) and (B) salivary gland involvement (3 studies) in patients with COVID-19. RE, random effects.

oral symptoms, particularly the loss of taste. The likely reason for taste alteration in COVID-19 patients is the higher expression of the ACE2 receptor in the tongue than in the buccal and gingival tissues. This results in damage to the mucosal epithelial cells of the oral cavity [94,95].

In the present systematic review, the pooled prevalence of taste alteration was 48%. A recent review by Scotto et al. [89] indicated that the prevalence of taste disorders varied widely across studies, ranging from 1.0% to 93.0%. In a cross-sectional study by Al-Zaidi and Badr [12], 83.08% of COVID-19 patients experienced taste dysfunction. For 50% of these patients, taste returned within a week, while for 25% it took less than a week, for 18.75% it took within 2 weeks, and for 6.25% it took within 3 weeks. In their living systematic review (LSR), Amorim dos Santos et al. [4] identified taste disorders as the most common oral symptom in this population, with a prevalence of 45%. However, in their subsequent LSR, the prevalence dropped to 38%. They noted that the prevalence of taste disorders among COVID-19 patients varies geographically, from 14% in Africa to 49% in Europe [96]. Yan et al. [21] reported taste loss in 71% of COVID-19-positive subjects, and found a strong association between taste loss and COVID-19 positivity (odds ratio, 10.2; 95% CI, 4.74–22.1). In a study by Biadsee et al. [13], 52% of participants reported changes in taste sensation, with 52 patients noting a change in spicy taste perception, 54 in salty taste, 53 in sour taste, and 61 in sweet taste. Bodnia and Katzenstein [14] found that 70% of patients experienced a total loss of taste, which resolved within 1 to 3 weeks for 78% of patients and within 3 to 6 weeks for 22%. A meta-analysis by Tong et al. [97] revealed that 43.93% of individuals noted changes in taste. Another meta-analysis by Nijakowski et al. [98] estimated the prevalence of taste alterations to be around 54.73% (95% CI, 46.28%– 63.04%).

Three studies in our systematic review, conducted by Favia et al. [29], Bardellini et al. [36], and Binmadi et al. [51], reported geographic tongue. Bardellini et al. [36] carried out a retrospective cohort study on pediatric patients and identified the most common oral lesions as oral pseudomembranous candidiasis (n=2), coated tongue (n=2), taste alteration (n=3), and geographic tongue (n=1). Notably, the occurrence of geographic tongue coincided with a high fever, as reported by the patient's mother. The etiopathogenesis of geographic tongue remains unclear, but some researchers have suggested a link with several non-genetic multifactorial factors, including viral infections [99].

Vesiculobullous Lesions and Ulceration

Thirteen studies, encompassing both case reports and case series [6,9,24,26,29,50,57,59,61,62,70,77,81], documented vesiculobullous lesions. Among these, 5 cross-sectional studies revealed a combined prevalence of 10%.

Ulceration was reported by 23 studies [5,24,27–29,31,33,34,37,39–48,50,51,53,85], involving 1,086 patients with a pooled prevalence of 21%. This is slightly higher than the value reported in the meta-analysis by Aragoneses et al.

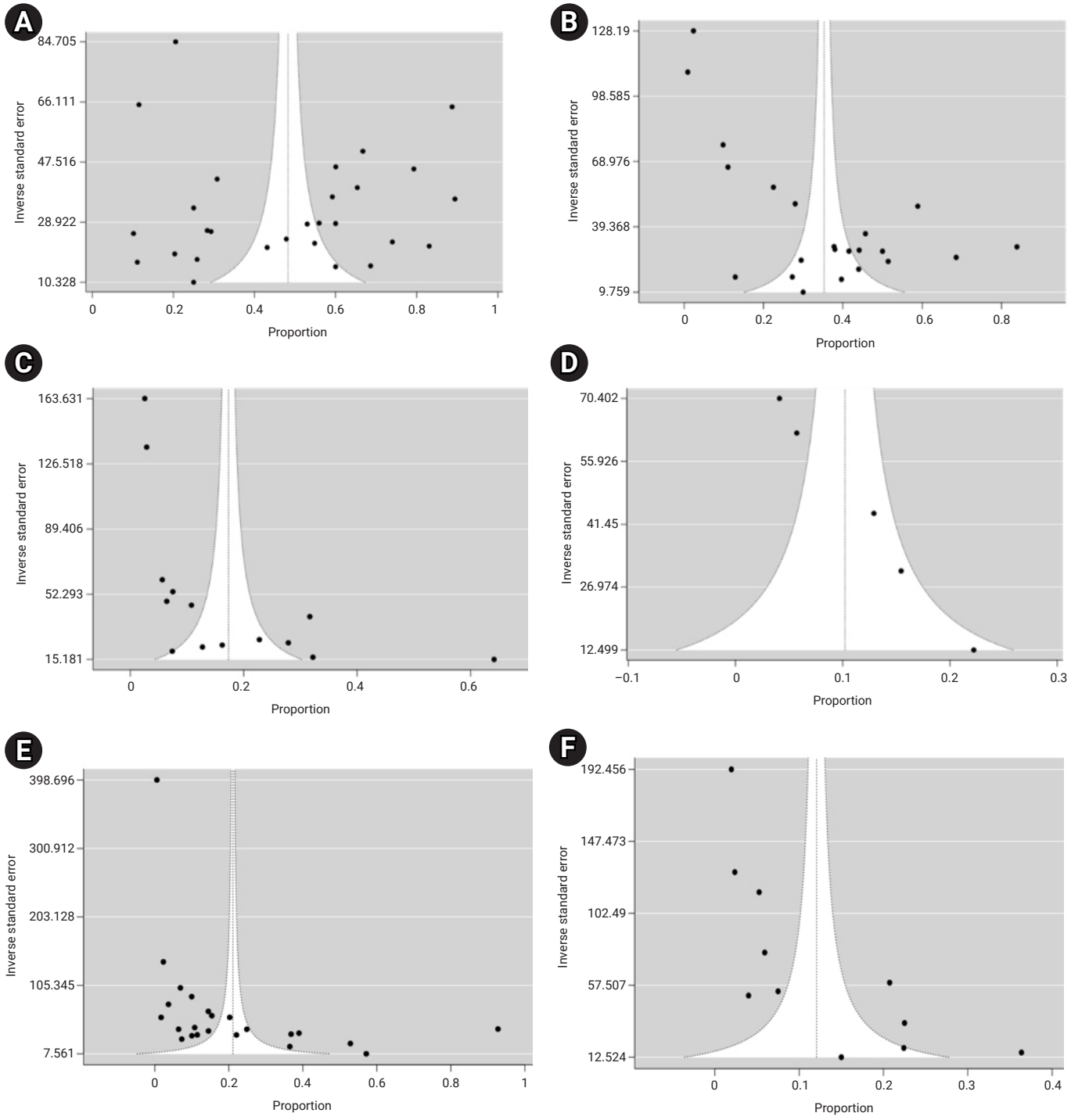

Figure 8. Funnel plot of studies on the prevalence of (A) taste alteration, (B) xerostomia, (C) red and white lesions, (D) vesiculobullous lesions, (E) ulceration, and (F) burning sensation.

[100] (10%). Favia et al. [29] provided a detailed description of the histological aspects of oral SARS-CoV-2-related lesions, identifying ulcers as the most common oral manifestation, in 52.8% of patients. Martin Carreras-Presas et al. [59] reported 3 cases of ulcers, 1 of which was confirmed to be infected with SARS-CoV-2, while the other 2 were suspected cases. These lesions were similar in appearance to herpes simplex infection, but this was not confirmed by biopsy. Halboub

https://doi.org/10.24171/j.phrp.2023.0033

et al. [101] conducted a review and found that painful oral ulcers were the most common orofacial manifestation in patients with COVID-19. Bullous lesions on the palate and oral mucosa were found by Cruz Tapia et al. [57] and Dalipi et al. [70], respectively. Orilisi et al. [102] conducted a systematic review and reported that oral ulcers, cheilitis, and tongue lesions were more common in patients prior to hospitalization. In contrast, perioral pressure ulcers, macroglossia, blisters, and oral candidiasis were more frequently observed in patients during their hospital stay.

The mechanism behind ulcer formation is thought to involve an elevated level of tumor necrosis factor-α in COVID-19 patients, which can trigger the chemotaxis of neutrophils to the oral mucosa, leading to the development of aphthous-like lesions. Other potential causes for these lesions in COVID-19 patients could be stress and immunosuppression, both secondary effects of the infection [9].

Red and white lesions

Eighteen studies have reported the presence of red and white lesions in patients who tested positive for COVID-19, with a pooled prevalence of 17% [8,24–26,29,33,36,37,39,43,49,51,53,57,61,68,80,82]. The variety of red and white lesions documented in these studies include cheilitis and oral lichenoid reactions [24], white plaques on the inner oral mucous layer [25,37,43,68,80], rashes and erythema [26], candidiasis [9,29,36,49,51,53], reddish-white spots on the palate [33,57], erythema and lichen planus [39], angular cheilitis [53,82], and reddish plaques on the lower lip [61].

Xerostomia

Xerostomia was observed in 22 cross-sectional studies [13,15,22,28,34,37,38,40–42,44–46,48,50–55,84,85] and 2 case reports [60,78]. These studies reported a combined prevalence of 35%, a figure slightly lower than the 44% prevalence (95% CI, 36%–52%) found in a recent meta-analysis by Aragoneses et al. [100]. In research conducted by Biadsee et al. [13], 56% of patients reported experiencing xerostomia, as determined by the question, "Do you feel the need to drink more (dry mouth)?". In the revised version of the LSR by Amorim Dos Santos et al. [96], xerostomia was the most frequently reported oral symptom in COVID-19 patients, whereas tassate disturbances were the primary feature in the original LSR [4]. In a meta-analysis by Nijakowski et al. [98], xerostomia was prevalent in 37.58% (95% CI, 26.35%–49.53%) of COVID-19 patients.

Gingival and periodontal involvement

Twelve studies (15%) [6,26,29,32,38,39,42,44,51,55,59,78] reported the prevalence of gingivitis and periodontitis. The gingival symptoms identified in COVID-19 patients from our systematic review included gingivitis [29], desquamative gingivitis [6,59], ulceronecrotic gingivitis [29], and gingival bleeding [6,38,42,44,55,78]. Two studies reported instances of periodontitis [32] and necrotizing periodontal disease [51].

Other findings

Red and/or swollen lips was observed by Halepas et al. [30] in 48.9% of patients. Other findings related to lip involvement in COVID-19 patients included pale lips [33], reddish plaques on the lower lip [61], nodules on the lower lip [10], and reddish macules [42]. In terms of palatal findings among COVID-19 patients, there were reddish-white spots on the palate [33], palate ulcerations [6,39,56,59,60,81,82], a white coating on the palate [43,68], bullae on both the left and right palatal mucosa [57], an erythematous surface on the hard palate [64], and an angioma-type lesion on the right side of the palate [82].

Limitations

Although we have attempted to summarize the findings of studies that report oral manifestations in COVID-19 patients, a significant limitation of this systematic review is the absence of a temporal dimension. We cannot definitively state that these oral manifestations are directly linked to COVID-19, or if they are indirect manifestations of other factors such as stress, immunosuppression, and/or medications. Another limitation is the absence of a definitive diagnosis, as most of the cases included in the review did not undergo a biopsy to confirm the diagnosis.

Conclusion

Our systematic review showed a relatively high prevalence of oral manifestations, specifically taste alteration (48%), followed by dry mouth (35%), ulceration (21%), and red and white lesions (17%). These patients exhibit a variety of oral symptoms that could aid clinicians in the early detection of the disease. It is crucial to recognize the signs and symptoms of COVID-19 for a prompt diagnosis and an improved prognosis. Dental practitioners can play a significant role not only in preventing the transmission of COVID-19 but also in interrupting the disease's progression. Increasing awareness of these symptoms is vital for the early diagnosis and treatment of this deadly disease.

Supplementary Material

Table S1. Risk of bias assessed using the JBI Critical Appraisal Tools for use in JBI systematic reviews of case reports (n=20);

Table S2. Risk of bias assessed using the JBI Critical Appraisal Tools for use in JBI systematic reviews of case series (n=11); **Table S3.** Risk of bias assessed using the JBI Critical Appraisal Tools for use in JBI systematic reviews of cross-sectional studies/retrospective/prospective studies (n=47); **Table S4.** Risk of bias assessed using the JBI Critical Appraisal Tools for use in JBI systematic reviews of case control study (n=1); **Table S5.** Treatment of oral lesions in COVID-19 patients. Supplementary data are available at https://doi.org/10.24171/j.phrp.2023.0033.

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Funding
None.

Availability of Data
The datasets are not publicly available but are available from the corresponding author upon reasonable request.

Authors' Contributions
Conceptualization: AG, KS, AP; Data curation: AG, AA; Formal analysis: AG, AP; Investigation: AG, KS, AA; Methodology: AG, KS, AA, AP, RC; Project administration: AG, KS; Software: AG, AP; Supervision: AG, AA; Validation: AG, AP; Visualization: AG, RC; Writing–original draft: AG; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0188
Osong Public Health Res Perspect 2023;14(5):418-426

Original Article

Evaluation of COVID-19 vaccine effectiveness in different high-risk facility types during a period of Delta variant dominance in the Republic of Korea: a cross-sectional study

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ABSTRACT

Objectives: We evaluated the effectiveness of coronavirus disease 2019 vaccination in high-risk facilities in the Republic of Korea during the period when the highly transmissible Delta variant was prevalent. Additionally, we aimed to explore any disparities in vaccine effectiveness (VE) across various types of institutions, specifically distinguishing between non-medical and medical establishments.

Methods: We examined 8 outbreak clusters covering 243 cases and 895 contacts from 8 high-risk facilities divided into 2 groups: group A (4 non-medical institutions) and group B (4 medical institutions). These clusters were observed from July 27, 2021 to October 16, 2021 for the attack rate (AR) and VE with respect to disease severity. A generalized linear model with a binomial distribution was used to determine the odds ratio (OR) for disease severity and death.

Results: AR was notably lower in group B (medical institutions). Furthermore, VE analysis revealed that group A exhibited higher effectivity for disease severity and death than group B. The OR for disease severity was 0.24 (95% confidence interval [CI], 0.03–2.16) for group A and 0.27 (95% CI, 0.12–0.64) for group B, with the OR for death at 0.12 (95% CI, 0.01–1.32) in group A and 0.34 (95% CI, 0.14–0.87) in group B.

Conclusion: Although VE may vary across institutions, our findings underscore the importance of implementing vaccinations in high-risk facilities. Customized vaccination programs, tailored response plans, and competent management personnel are essential for effectively addressing and mitigating public health challenges.

Keywords: Communicable diseases; COVID-19; COVID-19 vaccines; SARS-CoV-2; Vaccine efficacy

Received: July 5, 2023
Revised: August 25, 2023
Accepted: September 6, 2023

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Introduction

Coronavirus disease 2019 (COVID-19) has become widespread since it was first reported in Wuhan, China in December 2019 [1]. COVID-19 spreads from person to person, mainly through direct contact with infected individuals or exposure to respiratory droplets [2]. Airborne infections are uncommon; however, in special circumstances such as aerosol-generating procedures in medical institutions and environments where droplets are produced for a long time in an enclosed space, the virus can spread beyond the typical 2-m range of respiratory droplets [3]. New variants of COVID-19 have emerged during the pandemic [4]. These variants are classified as those of concern, interest, or monitoring. In particular, B.1.617.2, also known as the Delta variant, was designated as a variant of concern by the World Health Organization on May 11, 2021 [5]. The risk of hospitalization doubled during infection with the Delta variant compared to infection with the Alpha variant [6].

Previous studies have shown that vaccination is an effective way to reduce the severity and mortality of COVID-19 [7]. On December 8, 2020, the United Kingdom became the first country to implement a COVID-19 vaccination program after emergency authorization [8]. In the Republic of Korea, the national vaccination program began on February 26, 2021, shortly after emergency authorization. Patients and healthcare workers in long-term care facilities and healthcare workers who directly cared for COVID-19 patients were prioritized for vaccination [9]. The preventive effects of COVID-19 vaccines have been demonstrated nationally and internationally [10].

Nursing institutions for older adults, where vulnerable aging populations with low immunity live in groups, are structurally susceptible to the spread of infectious diseases, which may cause fatalities in cases of severe infections [11]. It is difficult to maintain a safe distance between residents because of their spatial characteristics, as well as because residents and staff carry out activities in confined spaces with a high frequency of physical contact [12]. Therefore, there is a critical need to consistently provide scientific evidence that can be utilized to proactively respond to outbreaks of respiratory infectious diseases such as COVID-19 in high-risk facilities such as nursing homes.

In this study, we aimed to observe the epidemiological characteristics and vaccine effects of 8 high-risk facilities reported to the Korea Disease Control and Prevention Agency (KDCA) from July 27, 2021, to October 16, 2021 (when the Delta variant predominated), when both the incidence and mortality of COVID-19 increased [13]. In addition, we divided the 8 high-risk facilities into non-medical and medical institutions and evaluated the differences according to institutional characteristics. Based on reliable information from these 8 institutions (older adult day care centers, nursing homes, and nursing hospitals), the goal was to review past measures to prevent infection and reduce severity and death, thereby providing scientific evidence for future public health response strategies.

Although the Delta variant is no longer the dominant strain, new variants with potential resistance to existing immunity continue to emerge. Notably, as of the fourth week of July 2023, the number of daily confirmed cases has surged by 17% from the previous week, averaging 45,529 cases. This marks the fifth consecutive week of such escalation. In light of these developments, experts are increasingly concerned about the risk of COVID-19 infection in vulnerable populations, especially the elderly and those with underlying medical conditions. They recommend COVID-19 vaccination as a protective measure for these groups [14]. The ongoing emergence of these variants underscores the importance of strict surveillance of these high-risk groups. Our research provides crucial insights for the continued adoption of vaccine policies targeting these groups.

HIGHLIGHTS

- Vaccination significantly reduced disease severity and death in high-risk facilities during a period of Delta variant dominance.
- Variations in vaccine effectiveness were observed between non-medical and medical institution
- Customized vaccination programs and response plans are essential for protecting vulnerable populations and improving public health outcomes in high-risk facilities.

Materials and Methods

Data Source

This study was based on confirmed COVID-19 data from the mass outbreak reported to the National Notifiable Disease Surveillance System conducted by the KDCA from July 27, 2021 to October 16, 2021. A confirmed COVID-19 case was defined as a COVID-19 infection confirmed through a real-time reverse transcriptase-polymerase chain reaction test regardless of clinical symptoms. After a cluster outbreak was identified, a study was conducted on patients related to the cluster outbreak in 8 institutions (2 older adult day

care centers, 2 nursing homes, and 4 nursing hospitals), from which epidemiological information was collected through field epidemiological investigations. The criteria for enrollment in a cluster were based on 10 or more confirmed cases with epidemiological associations [15]. The data included the number of cases in the order of diagnosis, date of laboratory-confirmed COVID-19 diagnosis, vaccination, sex, and age. To evaluate severity according to vaccination status after case confirmation, we included patients in critical condition requiring high-flow nasal cannula oxygen therapy, mechanical ventilation, or extracorporeal membrane oxygenation; those treated with continuous renal replacement therapy; and those with multiple organ failure, based on information obtained from the Health Insurance Review and Assessment Service System [16]. During the study period, reported deaths were classified as deaths due to COVID-19.

Study Participants

There were 1,177 cases reported in the 8 high-risk facilities, including 278 confirmed cases related to nursing institutions and 899 contacts. Among them, 8 index cases, 14 confirmed cases with family contact, 6 confirmed cases at the place of index case transfer, and 11 other cases (changes in quarantine location during cohort quarantine, transfer and discharge cases, and previously confirmed cases, among others) were excluded. A total of 1,138 cases (243 confirmed cases and 895 contacts) were included in the study. Contacts other than confirmed cases were defined as those who had been subjected to self-quarantine or cohort quarantine after the occurrence of an index case and were classified as contacts after the exposure scale evaluation (Figure 1).

Study Design

Before evaluating vaccine effectiveness (VE), we divided the 8 high-risk facilities for older adults into 2 groups, A and B, corresponding to non-medical and medical facilities, respectively. Group A included 2 older adult day care centers and 2 nursing homes, and group B included 4 nursing hospitals. In this study, data on the history of COVID-19 vaccination in patients confirmed with COVID-19 were obtained from the Immunization Registry Information System. Patients were classified based on their COVID-19 vaccination status as follows: (1) completely vaccinated—14 days or more after completing vaccination; (2) incompletely vaccinated—14 days or less after at least 1 dose of vaccination; and (3) unvaccinated.

The attack rate (AR) was calculated as the percentage of the number of confirmed cases relative to the number of individuals studied. The severity rate was calculated as the percentage of the number of severe cases and the number of deaths among the confirmed cases. The fatality was calculated as the percentage of the number of deaths relative to the number of confirmed cases during the study period. Severity rates were calculated along with death status, because severity could be underestimated if critical patients died before reaching an intensive care unit, such as a nursing hospital, meaning that some severe patients would be excluded from consideration [17]. After evaluating the effect of vaccination on disease severity and fatality in the incompletely vaccinated (including unvaccinated) and completely vaccinated groups, the odds ratio (OR) was calculated.

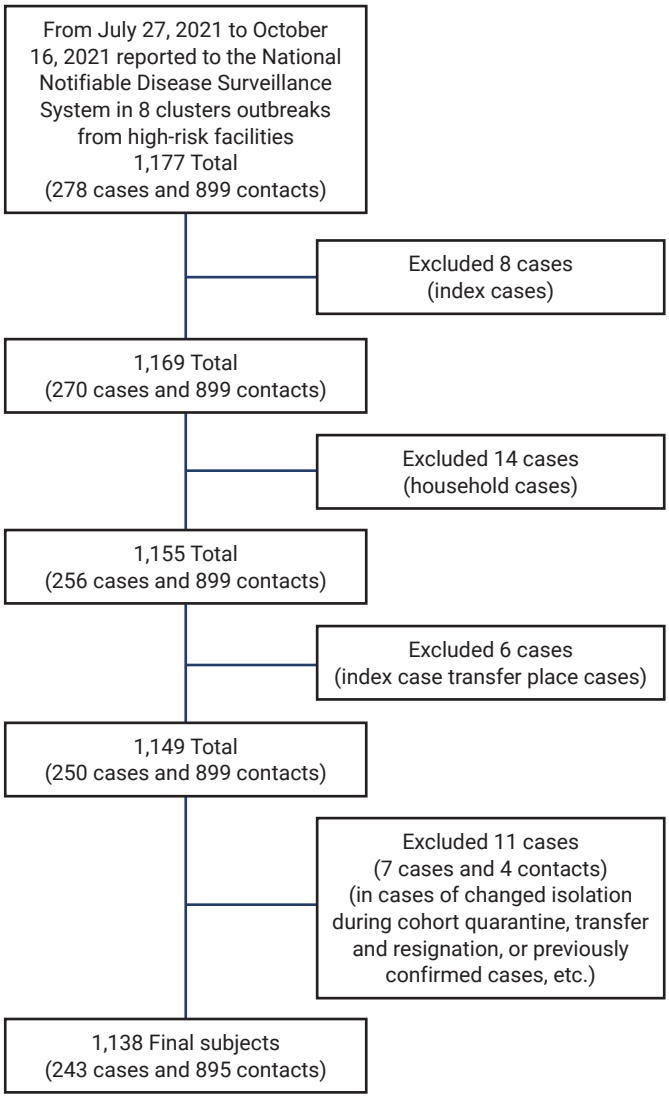

Figure 1. Flow chart of the study participants.

https://doi.org/10.24171/j.phrp.2023.0188

Statistical Analysis

To analyze the general characteristics of the 1,138 cases in this study, we divided the confirmed and non-confirmed cases (contacts) into 2 groups: A and B. The 243 confirmed cases were categorized by sex, age, status (resident or worker), and vaccination history, and were additionally categorized by severity (severe or death). The chi-square and Fisher's exact tests were used to determine differences in group composition. To evaluate confirmed COVID-19 cases, we calculated the AR as follows: AR = [(number of people with the disease)/(number of people at risk)]×100.

To evaluate the effectiveness of vaccination against COVID-19 severity and fatality, we calculated the VE as follows: VE = [1–(completed vaccination %/unvaccinated or incomplete vaccination %)]×100.

Additionally, to evaluate the effect of vaccination on the severity and death of COVID-19, we used generalized linear models (GLMs) with binomial distributions. Emphasizing the effect of vaccination, the results were displayed as ORs with 95% confidence intervals (CIs). We performed analyses of the overall study population and separately within the 2 institutional groups (A and B), enabling a comparison across different settings. R statistical software ver. 4.2.2 (The R Foundation) was used, and statistical significance was set at $p = 0.05$.

Ethics Approval

This study was approved by the Institutional Review Board (IRB) of the KDCA (IRB No: KDCA 2021-12–02-PE-A). The Board waived the requirement for informed consent.

Results

General Characteristics

Table 1 shows the general characteristics of the 8 high-risk facilities, including their vaccination histories. The facilities included 2 day care centers for older adults and 2 nursing homes in group A, and 4 nursing hospitals in group B. A total of 1,138 patients were included, with 137 in group A and 1,001 in group B. The proportion of confirmed patients in group A was 48.2%, whereas the proportion was 17.7% in group B ($p < 0.005$). Among the confirmed cases, most patients were aged ≥ 65 years (81.8% and 79.7% in groups A and B, respectively). Additionally, among the confirmed

Table 1. General characteristics, including vaccine history, among individuals at 8 high-risk faciliti

Characteristic	8 High-risk facilities			p[a]
	Group A	Group B	Total	
Total	137 (100.0)	1,001 (100.0)	1,138 (100.0)	
Status				< 0.005
Confirmed	66 (48.2)	177 (17.7)	243 (21.4)	
Non-confirmed	71 (51.8)	824 (82.3)	895 (78.6)	
Confirmed case				
Sex				1.000
Male	18 (27.3)	47 (26.6)	65 (26.7)	
Female	48 (72.7)	130 (73.4)	178 (73.3)	
Age (y)				0.846
< 65	12 (18.2)	36 (20.3)	48 (19.8)	
≥ 65	54 (81.8)	141 (79.7)	195 (80.2)	
Status				0.527
Residents (patients and older adults)[b]	51 (77.3)	145 (81.9)	196 (80.7)	
Worker	15 (22.7)	32 (18.1)	47 (19.3)	
Vaccine history				< 0.005
Unvaccinated	10 (15.2)	21 (11.9)	31 (12.8)	
Incomplete vaccination	4 (6.1)	16 (9.0)	20 (8.2)	
Complete vaccination	52 (78.8)	140 (79.1)	192 (79.0)	
Case severity				
Severity				
Total	4 (100.0)	31 (100.0)	35 (100.0)	
Severe[c]	1 (25.0)	5 (16.1)	6 (17.1)	0.546
Death	3 (75.0)	26 (83.9)	29 (82.9)	

Group A comprised 2 day care centers for older adults and 2 nursing homes, while group B comprised 4 nursing hospitals.
[a]*p*-values were obtained by comparing the groups using the chi-square or Fisher's exact tests. [b]The term "resident" refers to patients and older adults in day care centers for older adults, nursing homes, and nursing hospitals. [c]Severe was defined as excluding death.

cases, residents (patients and older adults) accounted for the majority of the cases (77.3% and 81.9% in groups A and B, respectively). A total of 35 severe cases (4 in group A and 31 in group B) were observed. There were 3 deaths (75.0%) in group A and 26 deaths (83.9%) in group B.

AR, Severity Rate, and Fatality in Eight High-Risk Facilities

Table 2 provides a comparative analysis of AR, severity, and fatality in the 8 high-risk facilities. AR was higher in group A than in group B; specifically, AR was higher in both males (47.4% vs. 17.8%) and females (48.5% vs. 17.6%). In group A, the ARs were higher in both age groups, 32.4% for those aged <65 years and 54.0% for those aged ≥65 years, which were both higher than the corresponding rates of 9.5% and 22.7% in group B. Moreover, the AR among residents, which included patients and older adults, was higher in group A (55.4%) than in group B (23.1%). Similarly, workers in group A experienced a higher AR (33.3%) than those in group B (8.6%). For vaccination history, the AR was higher in group A for unvaccinated patients (62.5% vs. 17.6%), followed by patients with incomplete vaccination (80.0% vs. 20.5%) and complete vaccination (44.8% vs. 17.4%) (Figure 2). The severity rate was lower in group A than in group B for both the total population and those aged ≥65 years. However, the severity rate was higher in both groups in those who were not vaccinated or

Table 2. Comparison of attack, severity, and fatality rates in the 8 high-risk facilities

Variable	8 High-risk facilities			*p*[a]
	Group A	Group B	Total	
Total	48.2 (40.0–56.5)	17.7 (15.4–20.1)	21.4 (19.1–23.8)	
Sex				1.000
Male	47.4 (32.5–62.7)	17.8 (13.7–22.9)	21.5 (17.3–26.5)	
Female	48.5 (38.9–58.2)	17.6 (15.1–20.6)	21.3 (18.7–24.2)	
Age (y)				0.547
<65	32.4 (19.6–48.5)	9.5 (6.9–12.8)	11.5 (8.8–14.9)	
≥65	54.0 (44.3–63.4)	22.7 (19.6–26.2)	27.0 (23.9–30.4)	
Status				0.399
Residents (patients and older adults)[b]	55.4 (45.3–65.2)	23.1 (20.0–26.5)	27.2 (24.1–30.6)	
Worker	33.3 (21.4–47.9)	8.6 (19.6–26.2)	11.2 (8.6–14.6)	
Vaccine history				0.523
Unvaccinated	62.5 (38.6–81.5)	17.6 (11.8–25.5)	23.0 (16.7–30.7)	
Incomplete vaccination	80.0 (37.6–96.4)	20.5 (13.0–30.8)	24.1 (16.2–34.3)	
Complete vaccination	44.8 (36.1–53.4)	17.4 (14.9–20.2)	20.9 (18.4–23.6)	
Severity and fatality rates[c]				
Severity				
Total	6.1 (2.4–14.6)	17.5 (12.6–23.8)	14.4 (10.5–19.4)	
Age (y)				1.000
<65	0	2.8 (0.5–14.2)	2.1 (0.4–10.9)	
≥65	7.4 (2.9–17.6)	21.3 (15.3–28.7)	17.4 (12.8–23.4)	
Vaccine history				0.763
Unvaccinated incomplete vaccination	14.3 (4.0–39.9)	35.1 (21.8–51.2)	29.4 (18.7–43.0)	
Complete vaccination	3.8 (1.1–13.0)	12.9 (8.3–19.4)	10.4 (6.8–15.5)	
Death				
Total	4.5 (1.6–12.5)	14.7 (10.2–20.7)	11.9 (8.4–16.6)	
Age (y)				1.000
<65	0	2.8 (4.9–14.2)	2.1 (0.4–10.9)	
≥65	5.6 (1.9–15.1)	17.7 (12.3–24.9)	14.4 (10.1–20.0)	
Vaccine history				0.301
Unvaccinated incomplete vaccination	14.3 (4.0–39.9)	27.0 (15.4–43.0)	23.5 (14.0–36.8)	
Complete vaccination	1.9 (0.3–10.1)	11.4 (7.2–17.8)	8.9 (5.6–13.7)	

Data are presented as attack rate (95% confidence interval). 95% confidence intervals were calculated using the Wilson score interval method. Group A comprised 2 day care centers for older adults and 2 nursing homes, while group B comprised 4 nursing hospitals.

[a]*p*-values were obtained by comparing the groups using the chi-square or Fisher exact tests. [b]The term "resident" refers to patients and older adults in day care centers for older adults, nursing homes, and nursing hospitals. [c]The severity rate was calculated as the percentage of the number of severe cases and the number of deaths among the confirmed cases, and the fatality rate was calculated as the percentage of deaths among confirmed cases during the study period.

https://doi.org/10.24171/j.phrp.2023.0188

had incomplete vaccinations. The fatality was lower in group A than in group B for both the total population and those aged ≥65 years. However, the fatality was higher in both groups in those who were not vaccinated or had incomplete vaccinations (Table 2).

Vaccination Effectiveness

Table 3 shows a comparison of VE in the 8 high-risk facilities. In group A, the VE for severity and death were 73.1% and 86.5%, respectively. In contrast, in group B, VE was 63.4% for severity and 57.7% for fatality. In total, the VE was 64.6% for severity (95% CI, 35.9%–80.4%) and 62.4% for death (95% CI, 26.4%–80.8%). As a result of the GLM, the OR for severe disease among vaccinated individuals was 0.27 (95% CI, 0.13–0.60); whereas for death, it was 0.31 (95% CI, 0.14–0.73) (Figure 3). When analyses were performed separately within the institutional groups, group A showed an OR of 0.24 (95% CI, 0.03–2.16) for severity and 0.12 (95% CI, 0.01–1.32) for death. However, these results were not statistically significant. Conversely, in group B, the OR for severity was 0.27 (95% CI, 0.12–0.64); whereas for death, it was 0.34 (95% CI, 0.14–0.87).

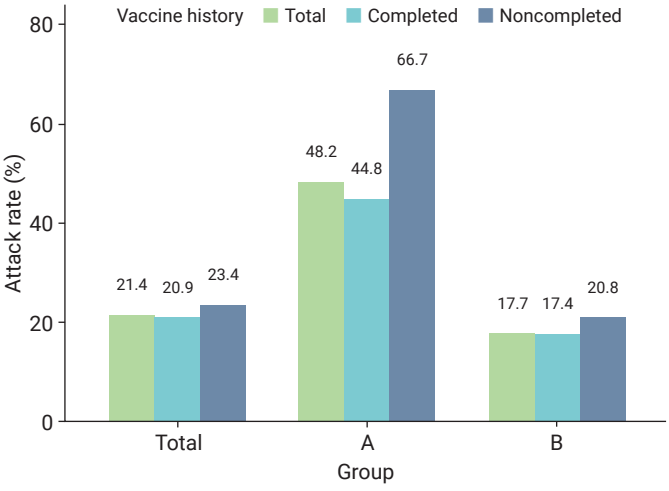

Figure 2. Comparison of attack rates by vaccine history. "Completed" in this figure refers to individuals who have received full vaccination, and "noncomplete" refers to individuals who have not been vaccinated at all or incompletely vaccinated. Group A included 2 older adult day care centers and 2 nursing homes, and group B included 4 nursing hospitals.

Table 3. Comparison of vaccine effectiveness[a] in 8 high-risk facilities

Variable	8 High-risk facilities		
	Group A	Group B	Total
Severity[b]	73.1 (−74.5 to 95.8)	63.4 (32.3 to 80.2)	64.6 (35.9 to 80.4)
Death[b]	86.5 (−37.9 to 98.7)	57.7 (14.7 to 79.0)	62.4 (26.4 to 80.8)

Data are presented as vaccine effectiveness (95% confidence interval). 95% confidence intervals were calculated using the Wilson score interval method. Group A comprised 2 day care centers for older adults and 2 nursing homes, while group B comprised 4 nursing hospitals.
[a][1−(complete vaccination %/unvaccinated or incomplete vaccination %)]×100. [b]The severity rate was calculated as the percentage of the number of severe cases and the number of deaths among the confirmed cases, and the fatality rate was calculated as the percentage of deaths among confirmed cases during the study period.

Discussion

Comparison of Confirmed Cases in Non-Medical and Medical Institutions

This study provides crucial epidemiological insights into the transmission dynamics of COVID-19 and the effectiveness of vaccination in high-risk facilities. A significant observation was the higher rate of confirmed cases in non-medical institutions (group A) than in medical institutions (group B). This disparity suggests potential structural or operational risk factors unique to group A, which is consistent with previous studies demonstrating that an increase in medical staff is associated with a decrease in infection risk [18,19]. Our study revealed that a considerable proportion of confirmed cases in both groups involved individuals aged ≥65 years, reflecting the increased COVID-19 susceptibility in older individuals. Moreover, residents (including patients and older adults) comprised the majority of patients with confirmed cases, suggesting an elevated risk due to

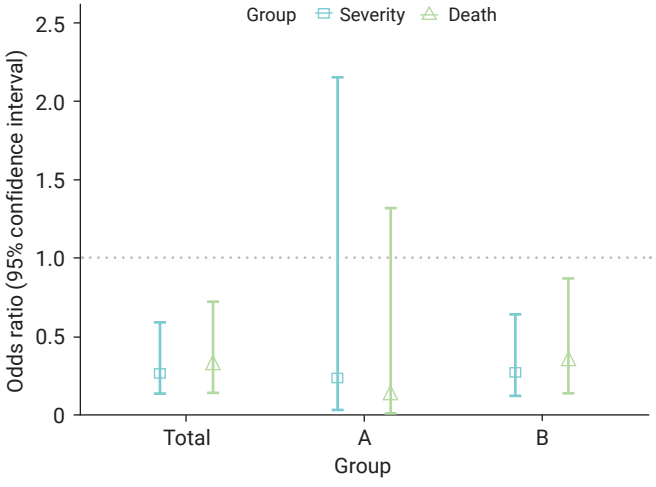

Figure 3. Odds ratio for severity and death among vaccinated patients by group. Group A included 2 older adult day care centers and 2 nursing homes, and group B included 4 nursing hospitals.

their living arrangements and potentially compromised immunity. This observation is consistent with that of a Spanish study that reported a higher infection rate among nursing home residents than among staff [20]. In terms of case severity, our findings indicate that baseline conditions in both healthcare and non-healthcare settings could potentially influence patient outcomes [21]. These findings highlight the necessity of considering these conditions when developing future infection control strategies for high-risk environments.

Comparison of AR, Severity, and Fatality according to Facility Type

We analyzed the ARs and severity of COVID-19 to provide crucial information for assessing the situation in high-risk facilities. We found that group A (non-medical institutions) had a higher overall AR than group B (medical institutions). This discrepancy was more pronounced when age and vaccination history were considered. For individuals aged ≥ 65 years, group A had an AR of 54.0%, which was significantly higher than that in group B (22.7%). Group A also had higher ARs among individuals who were unvaccinated, partially vaccinated, or fully vaccinated. In both groups, the AR was higher among unvaccinated and partially vaccinated individuals than among fully vaccinated individuals. Furthermore, the elevated severity rate and fatality among unvaccinated and partially vaccinated individuals underscore the importance of vaccination. Our findings are consistent with those of previous research on the Delta variant surge in India, confirming the effectiveness of vaccines as a protective measure against COVID-19 [22]. These findings suggest the need for strategies to improve vaccination rates in high-risk facilities.

Comparison of COVID-19 VE and Risk Level according to Facility Type

The severity and fatality rates in the complete vaccination group were 10.4% and 8.9%, respectively. Additionally, in the comparison of VE in high-risk facilities, significant disparities were observed between group A (day care centers and nursing homes for older adults) and group B (nursing hospitals) in terms of preventing severe disease and fatality. Group A demonstrated an effectiveness rate of 73.1% for preventing severe disease and 86.5% for preventing fatalities. In contrast, group B showed an effectiveness rate of 63.4% for preventing severe disease and 57.7% for preventing fatalities. These findings align with those of previous studies conducted in the United Kingdom [23] and further emphasize the strong protective effects of COVID-19 vaccination among older adults residing in day care centers and long-term care facilities. The differences in VE between groups A and B may have been influenced by various factors, including population characteristics, infection control measures, and healthcare resources specific to each setting [24]. Further GLM analysis further confirmed the consistency of the results with the initial VE assessment. However, it was not statistically significant for group A, which appears to be a result of widened CIs due to the limited sample size. However, it is noteworthy that significant OR results were reported in another study investigating COVID-19 outbreaks in nursing facilities located in Gwangju [25]. Considering these findings, the preventive effect of COVID-19 vaccination should be considered in future follow-up studies. This comprehensive analysis provides valuable insights into the efficacy of vaccination in high-risk facilities and emphasizes the importance of tailored strategies to maximize protection in these settings.

Limitation and Strengths

Our study has several limitations. First, our analysis primarily focused on older individuals in high-density settings and generalization of the results to the entire population may be limited. It is crucial to continuously monitor and study groups that can contribute to increased transmission and group infections, such as schools, crowded public spaces, and workplaces. This consideration extends beyond the scope of COVID-19 to encompass various infectious diseases. However, comparing the effectiveness of interventions and preventive measures between medical and non-medical institutions in high-risk facilities for older individuals is particularly valuable. This comparative analysis allowed a deeper understanding of the specific challenges and factors influencing the outcomes in each setting. By identifying similarities and differences between different settings, interventions and strategies can be tailored to effectively mitigate the risk of infection and optimize health outcomes in older populations. Second, we conducted a study based on information obtained during the Delta-predominant period. In Republic of Korea, the peak period of Delta predominance has passed. Even though the peak period of Delta predominance has passed, research related to COVID-19 vaccines continues globally. Our evaluation of VE in high-risk older adults is consistent with recent findings that emphasize the need for transparent information about the effectiveness and reliability of vaccination in older adults [26]. In addition, recent studies of vaccine hesitancy have highlighted the importance of consensus building using relevant evidence from healthcare professionals and scientists [27] and the potential for interventions [28]. This study is important to the extent that it holds

https://doi.org/10.24171/j.phrp.2023.0188

meaning in providing evidence related to VE in high-risk populations within a controlled setting, both nationally and internationally. Such evidence could be helpful in advancing health policy related to vaccination, and continuation of research targeting these high-risk groups is imperative.

Additionally, we conducted this study using information directly collected by central epidemiological investigators and local public health response personnel after the announcement of the COVID-19 vaccination program for older adults aged ≥65 years residing in medical and care facilities by the Korean government on March 23, 2021 [29]. In particular, we collected cluster information during the Delta-predominant period, which had a high fatality rate [30,31], to provide insights into how to manage the spread and prevalence of new COVID-19 variants. Furthermore, we analyzed the differences in the effectiveness of the preventive vaccine between the completely and incompletely vaccinated groups (including the unvaccinated group). We analyzed data from 8 medical and care facilities for older adults, a high-risk group in densely populated environments for which vaccination was prioritized. This evaluation of the effectiveness of public health response measures may be valuable in establishing response strategies in the event of future novel respiratory infections.

Implications for VE and Infection Control Policies

This study aimed to examine the effectiveness of preventive vaccines based on differences in communal facilities among high-risk COVID-19 management groups. This study confirmed that vaccination policies in medical facilities for older adults (day care centers, nursing homes, and nursing hospitals) could effectively reduce the risk of severe illness and death during the Delta-predominant period. Furthermore, as highlighted in this study, the appropriate establishment and use of vaccination in conjunction with infection control recommendations could be effective for the continuously emerging COVID-19 variants and new respiratory infections [32]. In addition, well-trained management personnel are recommended. Finally, future case studies based on field epidemiological investigations, such as this study, to analyze the effectiveness of infection control measures according to medical personnel standards in infection-prone facilities and obtain information from nursing hospitals during the COVID-19 outbreak can support the establishment of scientific evidence to formulate future infection control policies [33].

Conclusion

There were significant differences between the 2 institutional types. Our study demonstrated the effectiveness of COVID-19 vaccines in preventing severe disease and deaths in high-risk communal facilities. These findings highlight the importance of vaccination as a crucial strategy for managing respiratory infections including COVID-19. These results contribute to the existing literature and emphasize the need for further research to establish effective infection prevention measures. Developing unique response plans for each facility type and ensuring the presence of well-trained management personnel are critical. Through continual research and adjustments to strategies, we can optimize the protection of vulnerable populations and enhance public health outcomes.

Notes

Ethics Approval

This study was approved by the IRB of the KDCA (IRB No: KDCA 2021-12–02-PE-A). The Board waived the requirement for informed consent.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

These data sets are not publicly available. If you have any questions regarding this study, contact the corresponding author (ondalgl@korea.kr).

Authors' Contributions

Conceptualization: MJL, YJP; Data curation: DSK, SKP, JC, JJL, JMK, YMK; Formal analysis: MJL; Methodology: MJL, YJP; Project administration: JG, SEL; Visualization: MJL, MJH; Writing–original draft: MJL; Writing–review and editing: all authors. All authors read and approved the final manuscript.

Acknowledgements

The authors thank the public health response agents from each region for making this study possible.

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https://doi.org/10.24171/j.phrp.2023.0188

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0063
Osong Public Health Res Perspect 2023;14(5):427-432

Short Communication

Perceptions of older adults and generativity among older citizens in Japan: a descriptive cross-sectional study

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ABSTRACT

Objectives: As the population ages worldwide, including in Japan, there is a growing expectation for older adults to remain active participants in society. The act of sharing one's experiences and knowledge with younger generations through social engagement not only enriches the lives of older individuals, but also holds significant value for our society. In this study, we examined both positive and negative perceptions of older adults and investigated the correlation between these perceptions and generativity among older citizens. Additionally, we evaluated the impact of life satisfaction on these factors.

Methods: We conducted a survey of 100 older adults in Japan (mean age, 71.68 years) and utilized multiple regression analyses, using positive and negative perceptions of older adults, life satisfaction, and demographic factors as independent variables. The sub-categories of generativity—namely, generative action, concern, and accomplishment—were used as dependent variables.

Results: Participants who held a more positive perception of older adults demonstrated a higher level of generative actions and concerns. Additionally, participants who reported higher levels of life satisfaction also exhibited more generative actions, concerns, and accomplishments. Conversely, those who held a more negative perception of older adults were found to have higher levels of generative actions.

Conclusion: Enhancing positive perceptions of older adults among them can boost the sub-categories of generativity. This study, which was conducted from an exploratory perspective, has several limitations, including a potential sampling bias. A more comprehensive examination of the relationship between perceptions of older adults and generativity is anticipated in future research.

Keywords: Generation; Health status; Psychological well-being

Received: March 2, 2023
Revised: July 12, 2023
Accepted: August 9, 2023

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Introduction

Population aging is progressing worldwide, including in Japan, where individuals aged 65 and above comprise 29.1% of the total population [1]. The falling birthrate and aging population are anticipated to pose significant societal challenges. In light of these circumstances, it is crucial for older adults to actively participate in society to invigorate local communities and bolster the labor force [2]. The act of sharing one's experiences and knowledge with the younger generation through social participation not only enriches the lives of older citizens, but is also meaningful for our society.

In this study, we explored the concept of generativity, which pertains to the transmission of experiences and knowledge from older to younger generations. Erikson [3] defined generativity as the interest in nurturing and guiding the next generation, primarily through the act of parenting [4]. However, recent trends such as increased life expectancy and delayed marriages have led to a broader interpretation of generativity as a psychological and social issue encompassing adulthood and old age [5]. Indeed, research involving older adults has demonstrated that those with higher levels of generativity tend to experience greater life satisfaction [6,7] and are more likely to participate in local parenting support initiatives [4]. Generativity comprises the following elements: (1) generative action, which includes specific actions like sharing personal experiences; (2) generative concern, which signifies an interest in engaging with younger generations and in creative activities; and (3) generative accomplishment, which conveys a sense of societal contribution and involvement in passing experiences to younger generations [4]. By differentiating these components and identifying the psychological variables linked to them, we can effectively develop psychological interventions to enhance generativity among older individuals.

We also examined positive and negative perceptions of older adults. As the elderly population expands, the range of individual perceptions about older people is likely to broaden. In terms of the relationship between these perceptions and generativity, older adults who hold more positive views of themselves are often proud of their peer group (i.e., older adults), and are inclined to share their experiences and knowledge with younger generations. This is likely because older adults who maintain positive old-age stereotypes tend to have better mental health [8–10] and are more likely to engage actively in generative actions. On the other hand, those who hold negative views of older adults may be more reluctant to share their experiences with younger generations.

HIGHLIGHTS

- Passing on older adults' experiences to the younger generation is meaningful for our society.
- We conducted a survey of 100 older adults in Japan.
- Participants with more positive perceptions of older adults had more generativity.
- Participants with higher levels of life satisfaction had more generativity.
- A more detailed survey of the link between perceptions of older adults and generativity is expected.

The literature presents common stereotypes about old age as both negative, including descriptors like "slow" and "feeble," and positive, with terms such as "warm" and "gentle" [11,12]. These ambivalent stereotypes suggest that the positive and negative perceptions of the elderly are not mutually exclusive. In other words, a certain segment of the older population may view their social group in both a positive and negative light. With this in mind, we separately assessed the positive and negative perceptions of the elderly and explored the relationship between these perceptions and generativity.

A variable that must be accounted for when investigating this relationship is life satisfaction. Individuals with greater life satisfaction tend to have higher self-esteem [13,14] and fewer depressive tendencies [15,16], making them more likely to perceive the ingroup (i.e., older adults) in a positive light. Furthermore, there is a positive correlation between generativity and life satisfaction among older adults [6,7,17]. Consequently, we aimed to explore the relationship between positive and negative perceptions of older adults and generativity, after controlling for life satisfaction.

Materials and Methods

Participants

We performed a power analysis assuming a medium to large effect size ($f^2 = 0.20$, $N_{parameter} = 7$, $\alpha = 0.05$, $\beta_{power} = 0.80$), and the required sample size was $N = 80$. A total of 100 older Japanese, comprising 10 men and 90 women in urban areas, participated in this study (mean, 71.68 years; range, 65–86 years). They were enrolled in a health program implemented by a Japanese municipality in 2021 to train volunteers to read picture books to children. The ethics committee of the authors' institution approved this study.

https://doi.org/10.24171/j.phrp.2023.0063

Measurements

We utilized the 16-item measure developed by Toyoshima et al. [18] to evaluate both positive and negative perceptions of the elderly, with 8 items dedicated to each. Positive perceptions were gauged using descriptors such as "cheerful" and "active," while negative perceptions were measured with terms like "sickly" and "dependent." Participants were shown these descriptors and asked to rate the extent to which they believed each term applied to older adults. Their responses were recorded on a 5-point Likert scale, with 1, representing strong disagreement, to 5, representing strong agreement. The mean was used as the score (α = 0.76, 0.87, respectively), with higher scores indicating a more pronounced perception. While the correlations between this scale and existing ageism scales remain unknown, the items used in this study are believed to accurately represent stereotypes of old age in Japan [18,19].

We measured generativity using the revised Japanese version of the Generativity Scale [4], comprising 4 items related to generative action, 4 items related to generative concern, and 4 items related to generative accomplishment. Participants were asked to rate their responses on a 6-point Likert scale, with 0, indicating strong disagreement, to 5, indicating strong agreement. We calculated the mean score for each sub-category (α = 0.94, 0.85, and 0.87, respectively), with higher scores signifying greater generativity. A factor analysis revealed that the same factors identified in Murayama et al. [4] were extracted (refer to Open Science Framework [OSF], https://osf.io/unyh5/).

Life satisfaction was measured using the satisfaction with life scale [20]. This involved participant rating 5 items, one of which was "I am satisfied with my life," on a 7-point Likert scale. The scale ranged from 1, representing strong disagreement, to 7, indicating strong agreement. The average score was used (α = 0.83), with higher scores signifying greater life satisfaction. In terms of demographic variables, participants were queried about their educational background in terms of years, their age, and their gender.

Procedure and Analysis

Participants were briefed about the study and gave their consent to participate. They then responded to questions concerning their perceptions of older adults, generativity, life satisfaction, and demographic information. The statistical software R ver. 4.1.0 (The R Foundation) was utilized for the analysis. Multiple regression analyses were performed using the perceptions of older adults, life satisfaction, and demographic variables as independent variables, and the sub-categories of generativity (generative action, concern, and accomplishment) as dependent variables. The questionnaire items, data used in the analysis, R codes, and histograms for each indicator can be accessed on the OSF.

Results

Summary statistics for each indicator are presented in Table 1. Participants who held more positive perceptions of older adults demonstrated more generative actions, concerns, and accomplishments, respectively (r = 0.27, 95% confidence interval [CI], 0.08–0.44, $p = 0.006$; r = 0.24, 95% CI, 0.05–0.42, $p = 0.01$; r = 0.21, 95% CI, 0.02–0.39, $p = 0.04$). Additionally, participants who reported higher life satisfaction also showed more generative actions, concerns, and accomplishments, respectively (r = 0.38, 95% CI, 0.20–0.54, $p < 0.001$; r = 0.32, 95% CI, 0.13–0.49, $p = 0.001$; r = 0.46, 95% CI, 0.29–0.60, $p < 0.001$). The correlation coefficients between the variables for groups with higher and lower average years of education (M = 14.03) were included in the OSF. Consequently, correlations similar to those in the main analysis were found in both groups.

Multiple regression analyses were performed using

Table 1. Summary statistics for each indicator

No.	Mean ± SD	1	2	3	4	5	6	7
1	1.39 ± 0.98							
2	3.41 ± 0.83	0.55**						
3	1.99 ± 1.05	0.64**	0.46**					
4	3.24 ± 0.44	0.27**	0.24*	0.21*				
5	3.12 ± 0.55	−0.01	−0.05	−0.16	−0.35**			
6	4.32 ± 1.00	0.38**	0.32**	0.46**	0.24*	−0.35**		
7	14.03 ± 2.53	0.07	0.14	−0.01	0.05	0.04	0.06	
8	71.68 ± 5.15	−0.06	0.02	−0.14	0.06	−0.12	−0.07	−0.15

SD, standard deviation; 1, generative action; 2, generative concern; 3, generative accomplishment; 4, positive perception; 5, negative perception; 6, life satisfaction; 7, years of education; 8, age.
*$p < 0.05$, **$p < 0.01$.

positive and negative perceptions of the elderly, life satisfaction, and demographic variables as independent variables, with the sub-categories of generativity serving as dependent variables (Table 2). The results indicated that participants who held more positive perceptions of older adults demonstrated more generative actions and concerns (β = 0.25, 95% CI, 0.06–0.44, $p = 0.01$; β = 0.21, 95% CI, 0.004–0.41, $p = 0.046$). Conversely, participants with more negative perceptions of older adults showed more generative actions (β = 0.22, 95% CI, 0.02–0.43, $p = 0.03$). Participants who reported higher levels of life satisfaction exhibited more generative actions, concerns, and accomplishments (β = 0.38, 95% CI, 0.19–0.58, $p < 0.001$; β = 0.31, 95% CI, 0.11–0.51, $p = 0.003$; β = 0.43, 95% CI, 0.23–0.62, $p < 0.001$). A post-hoc power analysis was performed using the adjusted coefficient of determination (adjusted R^2) in multiple regression analyses ($N_{sample} = 100$, $N_{parameter} = 7$, α = 0.05), which yielded the following results $\beta_{power} = 0.95$ (generative action), $\beta_{power} = 0.75$ (generative concern), and $\beta_{power} = 0.95$ (generative accomplishment). While these are considered to be acceptable levels, the limitation caused by the small R^2 is discussed below.

Discussion

In this study, we surveyed older Japanese adults to explore the relationship between positive and negative perceptions of older adults and generativity, encompassing generative action, concern, and accomplishment [4]. The results suggested that, even when accounting for life satisfaction and demographic factors, participants who held more positive views of older adults were more likely to exhibit increased generative actions and concerns. Conversely, those with more negative perceptions of older adults demonstrated a higher level of generative actions.

Participants with more positive perceptions of older adults tended to exhibit better mental health [8–10] and a strong sense of pride in their association with the older adult social group, as well as in their personal accomplishments. These individuals are often more inclined to share their experiences and knowledge with younger generations and are more likely to take proactive steps to do so. Interestingly, while only life satisfaction influenced generative accomplishment, both life satisfaction and positive perceptions of older adults showed a positive correlation (r = 0.24) (Table 1). These results suggest that enhancing positive perceptions of older adults among them could potentially boost the sub-categories of generativity.

Meanwhile, participants with more negative perceptions of older adults demonstrated higher levels of generative behavior. This may be because those with negative views of older adults are more likely to use their own past and current behaviors as cautionary tales for younger generations. However, the correlation between negative perceptions of older adults and generativity was small (Table 1), so this finding should be interpreted with caution. Furthermore, given the moderate negative correlation between positive and negative perceptions of older adults (r = −0.35) [21], it may be possible to enhance positive perceptions of older adults without necessarily reducing negative ones. Among the ambivalent stereotypes of old age [11,12], focusing on increasing positive perceptions of older adults, rather than reducing negative ones, could be an effective strategy for boosting generativity among older adults.

Despite the above findings, our study has 4 major limitations. First, 90% of our participants were female. While we accounted for gender in our analysis, it remains uncertain whether the same results would be observed in an older male population. Previous studies have indicated that generativity tends to be higher among older males than older females [4,22], suggesting that the relationship between perceptions of older adults and generativity may differ by gender. It is worth noting that when we conducted the analysis using only data

Table 2. Results of multiple regression analyses for the sub-categories of generativity

	β (95% CI)			VIF
	Action	Concern	Accomplishment	
Positive perception	0.25 (0.06 to 0.44)*	0.21 (0.004 to 0.41)*	0.13 (−0.07 to 0.32)	1.16
Negative perception	0.22 (0.02 to 0.43)*	0.14 (−0.07 to 0.35)	0.03 (−0.17 to 0.23)	1.28
Life satisfaction	0.38 (0.19 to 0.58)**	0.31 (0.11 to 0.51)**	0.43 (0.23 to 0.62)**	1.19
Years of education	0.03 (−0.15 to 0.22)	0.13 (−0.06 to 0.32)	−0.06 (−0.24 to 0.12)	1.03
Age	−0.02 (−0.21 to 0.16)	0.06 (−0.13 to 0.26)	−0.13 (−0.31 to 0.06)	1.05
Sex (0 = male, 1 = female)	−0.15 (−0.33 to 0.03)	−0.11 (−0.30 to 0.08)	−0.07 (−0.25 to 0.12)	1.02
Adjusted R^2	0.19 (0.07 to 0.32)**	0.12 (0.01 to 0.23)**	0.19 (0.07 to 0.32)**	

β, standardized regression coefficients; CI, confidence interval; VIF, variance inflation factor.
*$p < 0.05$, **$p < 0.01$.

https://doi.org/10.24171/j.phrp.2023.0063

from women, the results were consistent with those in the main analysis (see OSF). Second, all participants in this study were Japanese. Therefore, the impact of cultural differences on the relationship between perceptions of older adults and generativity warrants further investigation in future research. Third, our participant pool was limited to those enrolled in a health program that trains volunteers to read picture books to children. Consequently, their health status and levels of generativity might be higher than those of the general older population. However, the average scores for the sub-categories of generativity in this study were not excessively high compared to the values in Murayama et al. [4], where participants were randomly selected older individuals aged 65 to 84 years from an urban area in Japan. Thus, it would be unjust to claim that our participants significantly deviate from the general older population. Finally, the coefficients of determination in our multiple regression analyses were relatively small. Prior research has identified agentic and communal goal attainment [23] and psychological well-being [17,24] as being strongly associated with generativity. Therefore, it is necessary to reevaluate our findings after controlling for these variables.

Conclusion

The findings of this study indicate that enhancing the positive perceptions of the ingroup (i.e., older adults) could potentially boost generativity. Concurrently, it has been documented that sharing their experiences and wisdom with younger generations can contribute to the preservation of older adults' physical and mental health. We anticipate a virtuous cycle where healthier older adults foster more positive perceptions of themselves, thereby increasing generativity. While a comprehensive interpretation of our data is challenging, it would be beneficial to further explore the interconnections among perceptions of older adults, generativity, and health status in older citizens. The heightened generativity in older adults could also promote intergenerational interaction, enabling younger generations to learn from the life experiences of their elders. We are eager to undertake empirical studies that concentrate on the generativity of older adults.

Notes

Ethics Approval

The ethics committee of Tokyo Metropolitan Institute for Geriatrics and Gerontology approved this study and performed in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from the participants.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

This work was supported by JSPS KAKENHI (22H01098).

Availability of Data

The data that support the findings of this study are openly available in the Open Science Framework (OSF) repository (https://osf.io/unyh5/).

Authors' Contributions

Conceptualization: YS, TT; Data curation: all authors; Formal analysis: YS; Funding acquisition: HS; Investigation: YS, TT; Methodology: YS; Project administration: HS; Supervision: HS; Writing–original draft: YS; Writing–review & editing: all authors. All authors read and approved the final manuscript.

Additional Contributions

The authors acknowledge the continued efforts in the management of survey by Senior Citizen Activities Promotion Section in Welfare Division of Hachioji City in Tokyo, Japan. The authors also thank members of the research team for social participation and healthy aging in Tokyo Metropolitan Institute for Geriatrics and Gerontology.

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https://doi.org/10.24171/j.phrp.2023.0063

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0182
Osong Public Health Res Perspect 2023;14(5):433-438

Brief Report

JYNNEOS vaccine safety monitoring in the Republic of Korea, 2022: a cross-sectional study

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ABSTRACT

Objectives: With the recent global mpox outbreak, the JYNNEOS vaccine (Modified Vaccinia Ankara-Bavarian Nordic) was developed as a third-generation smallpox vaccine and initially favored for mpox immunization. Vaccine-associated side effects contribute to vaccine hesitancy. Consequently, tracking adverse events post-immunization is crucial for safety management. This study used data from the national active vaccine safety surveillance conducted in Korea from August 25 to November 24, 2022 to detect potential safety signals and adverse events.

Methods: Data on health conditions following vaccination were gathered from web-based surveys and reported via active surveillance through the Immunization Registry Information System. This follow-up system functioned via a text message link, surveying adverse events and health conditions beginning on the second day post-vaccination. Information about specific adverse events, including both local and systemic reactions, was collected.

Results: The study included 86 healthcare workers who had received at least 1 dose of the JYNNEOS vaccine. Among the respondents, 79.1% reported experiencing at least 1 adverse event, with the majority being local reactions at the injection site. The incidence of adverse events was higher following the first dose (67.9%) than after the second dose (34.4%). The most frequently reported adverse event for both doses was mild pain at the injection site.

Conclusion: The study provides crucial information on the safety of the JYNNEOS vaccine, demonstrating that most adverse events were manageable and predominantly localized to the injection site. Nonetheless, additional research is needed on the safety of various vaccine administration techniques and the vaccine's effects on broader demographics.

Keywords: Adverse events; Mpox; Safety; Vaccination; Vaccines

Received: June 30, 2023
Revised: September 4, 2023
Accepted: September 8, 2023

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Introduction

The first case of mpox in Korea was confirmed on June 22, 2022. In response, the Korea Disease Control and Prevention Agency (KDCA) sought emergency use authorization for JYNNEOS, a third-generation smallpox vaccine derived from a live, attenuated orthopoxvirus, known as modified vaccine Ankara. By July 1, 2022, the Ministry of Food and Drug Safety (MFDS) had approved the emergency use of JYNNEOS, following a review by the Korea Orphan and Essential Drug Center. The vaccine was administered to adults aged ≥18 years as a 2-dose series (0.5 mL per dose) via subcutaneous injection, with doses given at least 28 days apart. The KDCA guidelines also specify that individuals who have previously been vaccinated against smallpox should receive only 1 dose [1]. Given the potential for exposure among laboratory and healthcare personnel due to their occupational activities, many countries recommend prioritizing these workers for vaccine access [2–4].

Accordingly, the KDCA's mpox vaccination strategy primarily targets individuals who have known or presumed exposure to mpox, or those who are at high risk for occupational exposure, such as healthcare workers, laboratory workers, and Epidemiological Investigation Officers [1,5]. This report presents an analysis of adverse event data collected from healthcare workers who received the JYNNEOS vaccination between August 25 and November 24, 2022.

HIGHLIGHTS

- The safety of the JYNNEOS vaccine in response to an mpox outbreak was investigated in Korea from August to November 2022.
- Post-vaccination health conditions were monitored utilizing web-based surveys and the Immunization Registry Information System.
- Among the 86 healthcare workers included in the study, 79.1% reported experiencing adverse events, primarily localized at the injection site, and a higher incidence was observed after the first dose.
- Most of these events were manageable, but further research on diverse populations and administration methods is needed.

Materials and Methods

Data Collection

Between August 25 and November 24, 2022, a total of 99 individuals, specifically those at occupational risk of exposure, voluntarily received at least 1 dose of the JYNNEOS vaccine at a healthcare center in Korea. For the analysis, we included 86 individuals who reported their health conditions after receiving at least 1 dose. The KDCA actively monitored the safety of the JYNNEOS vaccine. The day after their vaccination, individuals received text message notifications containing a health survey, which asked about potential adverse events for the 7 days following vaccination. This questionnaire was based on the Korean MFDS's guideline for evaluating the severity of adverse events in vaccine clinical trials, and was used to evaluate serious adverse events [6]. The survey assessed the severity of 12 symptoms and signs. In each survey, participants were asked if they were experiencing symptoms, and the severity of each symptom was classified as either mild, moderate (symptoms that do not interfere with daily life), or severe (symptoms that interfere with daily activities and necessitate emergency visits). During the surveillance period, all health conditions were recorded, even for those without symptoms.

Study Design

This study was carried out as a prospective, self-reported survey of adverse events over a period of 121 days, from August 2022 to November 2022. The data reported were analyzed according to age group (20–29, 30–39, 40–49, 50 years and above), dosage, severity of adverse events, and symptoms indicative of potential adverse events. Descriptive statistics were utilized to detail the adverse events experienced by the participants. All analyses were performed using SAS ver. 9.4 (SAS Institute).

IRB Approval

All activities described in this study were carried out under the supervision and approval of the public health authority. In line with government regulations, informed consent was duly obtained. The study, which involved text message-based surveillance, was granted an exemption from review by the Institutional Review Board of the KDCA (2022-08-09-PE-A).

Results

After applying the exclusion criteria detailed in Figure 1, we included a total of 86 individuals who reported their health conditions following at least 1 dose of the vaccine. Of these 86 respondents, 70 (81.4%) were fully vaccinated with a second dose, while 16 (18.6%) were partially vaccinated with just 1 dose due to the presence of a smallpox vaccination

https://doi.org/10.24171/j.phrp.2023.0182

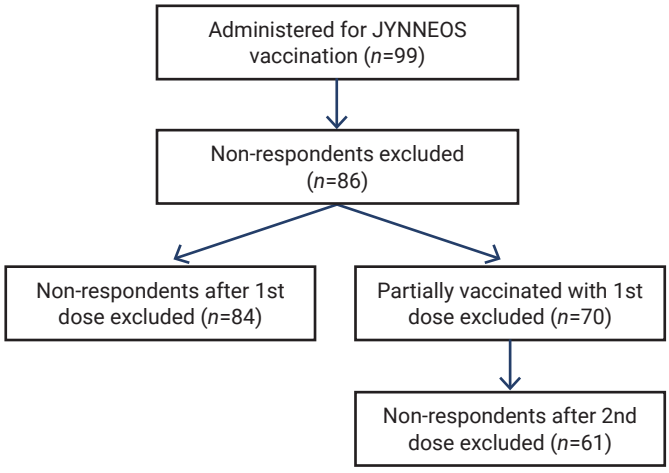

Figure 1. Flowchart illustrating the exclusion of sample subjects from the pool of vaccinated recipients.
Between August 25 and November 24, 2022, JYNNEOS vaccinations were administered to 99 individuals. Thirteen participants who did not report any consultations for any of the listed adverse events were excluded. Additionally, we had to exclude those with missing reports for each dose. After excluding non-respondents (*n*=2), the analysis of the first dose included 84 individuals. For the analysis of the second dose, we excluded those who had not received the second dose (*n*=16) and non-respondents (*n*=9), leaving 61 individuals included in the final analysis.

scar, which is a clear indicator of previous vaccination. All of the vaccine recipients were healthcare workers, including Epidemiological Investigation Officers. Among them, 51.2% were women, and 38.4% were aged between 30 and 39 years. A summary of the characteristics of the vaccine recipients is presented in Table 1.

Adverse Events by Signs and Symptoms

Of all the reports, 79.1% documented at least 1 adverse event (Table 1). The most commonly reported adverse events were local reactions, including pain, swelling, and erythema at the injection site. Systemic reactions such as pyrexia, fatigue, headache, and myalgia were reported infrequently. Regarding gastrointestinal disorders, there were no reports of nausea, but sporadic cases of diarrhea were noted, ranging from a minimum of 1 case (1.6%) to a maximum of 2 cases (2.4%). Skin disorders (excluding those at the injection site) were very rare, with a minimum of 1 case (1.6%) and a maximum of 7 cases (8.3%). These symptoms were mild and did not interfere with the individuals' daily activities. There were no reports of severe neurological disorders; however, mild symptoms, such as sweating and dizziness, were reported, ranging from a minimum of 1 case (1.6%) to a maximum of 2 cases (2.4%) (Table 2).

Table 1. Characteristics of JYNNEOS vaccine recipients with reports submitted to Immunization Registry Information System after vaccination (*n*=86), Republic of Korea, August 25 to November 24, 2022

Characteristic	No. of reports (%)[a]
Sex	
Male	42 (48.8)
Female	44 (51.2)
Age group (y)	
20–29	12 (14.0)
30–39	33 (38.4)
40–49	28 (32.6)
≥50	13 (15.1)
Dose in series	
First	86 (100.0)
Second[b]	70 (81.4)
Route of administration	
Subcutaneous	86 (100.0)

[a]The reported data included all recipients who had received at least 1 dose and submitted a report. [b]Individuals (*n*=16) who had been vaccinated in the past against smallpox were excluded.

Incidence of Adverse Events between First and Second Vaccine Doses

The incidence of adverse events following the first dose was 67.9%, and 34.4% after the second dose. The risk seems to be relatively lower after the administration of the second dose (Table 3). The most commonly reported adverse events for both the first and second doses were similar. The majority of these adverse events were local reactions, with the most common being pain, swelling, and erythema at the injection site. Following the first dose, the incidence of pain at the injection site was over 64.3%. Most of these were instances of mild pain that did not interfere with daily activities (*n*=51, 60.7%), and very few were reported as pain that interfered with daily activities (*n*=4, 4.8%). For the second dose, nearly half (49.2%) reported mild pain at the injection site that did not interfere with daily life. The incidence of swelling and erythema at the injection site after the first and second doses were relatively similar. However, the size of the affected areas with swelling and erythema appeared to be larger after the second dose. No serious adverse events were reported following either the first or second vaccinations. Table 2 presents the reporting rates for the first and second doses by symptom.

Discussion

We conducted the first study aimed at identifying adverse events in healthcare workers in Korea who had received a minimum of 1 dose of the JYNNEOS vaccine. The web-based survey was dispatched the day following their

Table 2. Reporting rates by local and systematic reactions. Republic of Korea, August 25 to November 24, 2022

Health event	First dose (*n* = 84)	Second dose (*n* = 61)
Local reaction (injection site)		
Pain		
None	29 (34.5)	21 (34.4)
Mild pain that does not interfere with daily life	51 (60.7)	30 (49.2)
Interference with activities of daily living	4 (4.8)	10 (16.4)
ED visit or hospitalization	0 (0)	0 (0)
Swelling		
None or with a diameter of 2.5 cm or less	62 (73.8)	32 (52.5)
With a diameter of 2.5–5 cm	17 (20.2)	19 (31.1)
With a diameter of 5.1–10 cm or difficulty moving arm	5 (6.0)	7 (11.5)
With a diameter of 5.1–10 cm or degree of interference with activities of daily	0 (0)	3 (4.9)
Erythema (redness)		
None or with a diameter of 2.5 cm or less	65 (77.4)	31 (50.8)
With a diameter of 2.5–5 cm	15 (17.9)	17 (27.9)
With a diameter of 5.1–10 cm	4 (4.8)	10 (16.4)
With a diameter of 10 cm or more	0 (0)	3 (4.9)
Systematic reaction		
Pyrexia		
None	79 (94.0)	55 (90.2)
Fever of 38.4 °C or less	5 (6.0)	5 (8.2)
Fever of 38.5–38.9 °C	0 (0)	1 (1.6)
Fever of 39.0–40.0 °C	0 (0)	0 (0)
Fever of 40.0 °C or less	0 (0)	0 (0)
Dyspnea		
None	84 (100.0)	61 (100.0)
Shortness of breathing not interfering with daily life	0 (0)	0 (0)
ED visit due to breathing difficulty	0 (0)	0 (0)
Fatigue		
None	62 (73.8)	41 (67.2)
Mild fatigue, but does not interfere with daily life	16 (19.0)	15 (24.6)
Interference with activities of daily living	6 (7.1)	5 (8.2)
ED visit or hospitalization	0 (0)	0 (0)
Headache		
None	72 (85.7)	52 (85.2)
Mild headaches that do not require medical attention	6 (7.1)	3 (4.9)
Managing pain with medicine; does not interfere with daily life	4 (4.8)	6 (9.8)
Pain is not manageable with medicine; interference with activities of daily life	2 (2.4)	0 (0)
ED visit or hospitalization	0 (0)	0 (0)
Myalgia		
None	65 (77.4)	47 (77.0)
Mild: does not interfere with daily life	15 (17.9)	11 (18.0)
Interference with activities of daily living	4 (4.8)	3 (4.9)
ED visit or hospitalization	0 (0)	0 (0)
Gastrointestinal disorders		
Nausea		
None	84 (100.0)	61 (100.0)
Mild nausea: does not interfere with daily life	0 (0)	0 (0)
Vomiting 1–2 times/d	0 (0)	0 (0)
Vomiting more than 3 times/d	0 (0)	0 (0)
ED visit or hospitalization	0 (0)	0 (0)
Diarrhea		
None	81 (96.4)	60 (98.4)
1–2 times/d	2 (2.4)	1 (1.6)
More than 6 times/day or received medical treatment	0 (0)	0 (0)
ED visit or hospitalization	0 (0)	0 (0)

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https://doi.org/10.24171/j.phrp.2023.0182

Table 2. Reporting rates by local and systematic reactions. Republic of Korea, August 25 to November 24, 2022

Health event	First dose (*n* = 84)	Second dose (*n* = 61)
Skin disorders (other than the injection site)		
Urticarial or erythema		
None	82 (97.6)	60 (98.4)
Mild: does not interfere with daily life	2 (2.4)	1 (1.6)
Interference with activities of daily living	0 (0)	0 (0)
ED visit or hospitalization	0 (0)	0 (0)
Swelling		
None	77 (91.7)	59 (96.7)
Mild: does not interfere with daily life	7 (8.3)	2 (3.3)
Interference with activities of daily living	0 (0)	0 (0)
ED visit or hospitalization	0 (0)	0 (0)
Neurologic disorders		
Syncope or seizure		
None	82 (97.6)	60 (98.4)
Sweating and dizziness	2 (2.4)	1 (1.6)
Loss of consciousness	0 (0)	0 (0)
Injury due to syncope	0 (0)	0 (0)
Seizure	0 (0)	0 (0)
Febrile seizure	0 (0)	0 (0)
Unconscious for a day or more after seizure	0 (0)	0 (0)
ED visit or hospitalization	0 (0)	0 (0)

Data are presented as *n* (%).
ED, emergency department.

Table 3. Reporting rate for adverse health events reported, Republic of Korea, August 25 to November 24, 2022

Seriousness	Total (*n* = 86)[a]	First dose (*n* = 84)[b]	Second dose (*n* = 61)[c]
No symptom	18 (20.9)	27 (32.1)	40 (65.6)
Non-serious	68 (79.1)	57 (67.9)	21 (34.4)
Serious	0 (0)	0 (0)	0 (0)

Non-serious events are when it does not meet any of the criteria for serious adverse events. Serious adverse events include symptoms that interfere with activities of daily living, and emergency visits.
[a]The total reported data includes the recipients among persons who had received at least 1 dose. [b]The reported data for the first dose includes the recipients among persons who had received the first dose, and submitted a report to the survey. [c]The reported data for the second dose includes the recipients among persons who had received the second dose, and submitted a report to the survey.

vaccination, and it required reporting for at least 1 day after each dose. The majority of adverse events were reactions at the injection site, and no new worrisome symptoms were identified. This aligns with the findings of previous studies [7,8].

This study offers timely insights into the safety of mpox vaccination. However, it should be noted that the findings detailed in this report are subject to several limitations, which are addressed below alongside recommendations for best practices in future research. First and foremost, only reactions at the subcutaneous injection site were observed. In a previous study, vaccine administration errors were reported more frequently following intradermal administration than subcutaneous administration of the JYNNEOS vaccine [7]. Given that the mpox vaccination was administered solely subcutaneously during the study period, data on adverse events following alternative routes of administration is limited. Further research is necessary to examine the effects of different injection techniques. This could contribute to a more comprehensive understanding of the safety of the method, particularly in comparison to intradermal injection.

Secondly, the study population was composed exclusively of healthy adults. Throughout the study period, mpox vaccinations were restricted and recommended solely for individuals with a high likelihood of exposure, such as those with occupational risks. The recipients of the vaccine were either healthcare workers, laboratory workers, or

epidemiological investigation officers. As a result, there was a lack of diversity among the research participants. Concerns about vaccine safety could also hinder vaccination efforts; further studies are suggested to ensure the safety of vaccination for vulnerable populations, such as those with HIV or within specific age groups [9].

The safety data from the KDCA for the JYNNEOS vaccine indicate that the subcutaneous administration of the JYNNEOS mpox vaccine is safe. It further recommends the use of the JYNNEOS vaccine in accordance with KDCA guidelines. The KDCA will persist in monitoring any adverse events to provide vaccination recommendations that prioritize safety.

Notes

Ethics Approval

All activities described in this study were reviewed and conducted with authorization from the public health authority. Informed consent was collected in accordance with government regulations, and the study with the text message-based surveillance was exempted from review by the Institutional Review Board of KDCA (2022-08-09-PE-A).

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

The data used in this study are protected under the Personal Information Protection Act.

Authors' Contributions

Conceptualization: JL, JP, HB, GYK; Data curation: JL, JP, HB; Formal analysis: JL, JP, HB; Investigation: JL, JP, HB; Methodology: all authors; Supervision: GYK; Validation: JL, JP, HB, GYK; Visualization: JL; Writing–original draft: JL; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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https://doi.org/10.24171/j.phrp.2023.0182

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Enacted January 1, 2010
Last revised April 1, 2022

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- Clinical Trials: PHRP accepts the ICMJE Recommendations for data sharing statement policies. Authors may refer to the editorial, "Data Sharing Statements for Clinical Trials: A Requirement of the International Committee of Medical Journal Editors," in the Journal of Korean Medical Science (https://dx.doi.org/10.3346/jkms.2017.32.7.1051).

Archiving Policy

The full text of PHRP has been archived in PubMed Central (https://www.ncbi.nlm.nih.gov/pmc/journals/2151/) from the first volume, 2010. According to the deposit policy (self-archiving policy) of Sherpa/Romeo (http://www.sherpa.ac.uk/), authors cannot archive pre-prints (i.e., pre-refereeing), but they can archive post-print (i.e., final drafts post-refereeing). Authors can archive the publisher's version/PDF. PHRP provides electronic backup and preservation of access to the journal content in the event the journal is no longer published by archiving the journal content in PubMed Central and the National Library of Korea.

Preprint Policy

A preprint can be defined as a version of a scholarly paper that precedes formal peer review and publication in a peer-reviewed scholarly journal. PHRP allows authors to submit the preprint to the journal. It is not treated as duplicate submission or duplicate publication. PHRP recommend authors to disclose it with DOI in the letter to the editor during the submission process. Otherwise, it may be screened from the plagiarism check program—Similarity Check (Crosscheck) or Copy Killer. Preprint submission will be processed through the same peer-review process with a usual submission. If the preprint is accepted for publication, authors are recommended to update the info at the preprint with a link to the published article in PHRP, including DOI at PHRP. It is strongly recommended that authors cite the article in PHRP instead of the preprint at their next submission to journals.

Peer Review Policy

All papers, including those invited by the editor, are subject to peer review. PHRP has adopted a double-blind peer review policy, where the author identities remain anonymous to the reviewers, and vice versa, and the identities of the reviewers and authors are visible to (decision-making) the editor throughout the peer review process. The Editorial Board selects reviewers based on expertise, publication history, and past reviews. During the peer review process, reviewers can interact directly or exchange information (e.g., via submission systems or email) with only an editor, which is known as "independent review." An initial decision will normally be made within 4–6 weeks after the reviewers agree to review a manuscript. No information about the review process or editorial decision process is published on the article page.

SUBMISSION & PEER REVIEW PROCESS

Online Submission

All manuscripts should be submitted online at https://mc04.manuscriptcentral.com/osongphrp (PHRP online submission system: ScholarOne). The entire process of manuscript submission, peer-review, and resubmission to PHRP is done through the online system.

Manuscripts submitted to PHRP will be preliminarily reviewed by the Editorial Office. Manuscripts not conforming to the instructions will be returned to the corresponding authors without being considered for publication. Submitted manuscripts are also screened for possible plagiarism or duplicate publication using Crossref Similarity Check. If a paper that might be regarded as duplicate or redundant had already been published in another journal or submitted for publication, the author should notify the fact in advance at the time of submission.

Any inquiry concerning manuscript submission should be directed to the editorial office at ophrp@korea.kr.

Peer Review Process

This journal operates a **double-blind** review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of 2 independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. The detailed review process is as follows.

- The Editorial Office of PHRP receives and reviews all submitted manuscripts, and all submitted manuscripts are considered confidential. The submitted manuscripts are initially screened for formatting. Once the manuscript is provisionally accepted, it is sent to the 2 most relevant referees for review.
- The referees are selected by the editor from the Editorial Board's database or the board members' recommendation. The referees are then requested to evaluate the manuscript based on originality, validity, presentation, and importance and interest, and, when considered necessary, statistics.
- Acceptance of a manuscript depends on the evaluation, critiques, and recommended decision made by the referees. A referee may recommend "accept," "minor revision," "major revision," and "reject." If there are conflicting decisions between referees, or between the author and referee(s),

the Editor-in-Chief has the full right to decide whether the manuscript will be published in the journal. Three repeated decisions of "major revisions" are equivalent to rejection, and rejected papers will not be considered further.

- The reviewed manuscript with comments, recommendations, and revisions is returned to the corresponding author. The corresponding author is to submit the revised manuscript accompanied by point-to-point replies to the comments given by the editor and how the revisions have been made. There should be a reasonable explanation for any noncompliance with the recommendations. In cases where references, tables, or figures are moved, added, or deleted during the revision process, renumbering must be done so that all references, tables, and figures are cited in numeric order. If the revised paper is not received within 2 months of decision, the manuscript is considered to have been withdrawn.
- When the final decision on the acceptance of the manuscript is made, the Editorial Office notifies the corresponding author. The peer-review process takes approximately 8–12 weeks.

MANUSCRIPT PREPARATION

General Requirements

- All manuscripts must be in grammatically correct English and should be created using MS Word. The manuscript must be double-spaced and written in an A4 page format. Do not leave a space between paragraphs. Only a single font (preferably Times New Roman) should be used in 11 point with margins of 2.5 cm.
- All pages should be paginated consecutively.
- All numbers should be written in Arabic numerals throughout the manuscript except for the first word of the sentence. Texts should be justified on both sides and not hyphenated and headings should be in bold letters, aligned in the center. If possible, avoid using abbreviated words at the beginning of sentences.
- Abbreviations: Where a term/definition is repeatedly referred to (i.e., 3 times in the text), it is written in full when it first appears, followed by the subsequent abbreviation in parentheses (even if it was previously defined in the abstract); thereafter, the abbreviation is used.
- Gene nomenclature: Current standard international nomenclature for genes should be adhered to. Genes should be typed in italic font and include the accession number. For human genes, use the genetic notation and symbols approved by the HUGO Gene Nomenclature Committee (http://www.genenames.org/) or refer to PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez).
- Units: Système International (SI) units must be used, with the exception of blood pressure values, which are to be reported in mmHg. Please use the metric system for expressions of length, area, mass, and volume. There should be a space between the numerals and the unit symbol. When indicating time, the 24-hour system is to be used.
- Math formulae: Present simple formulae in the line of normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by "exp." Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Reporting Guidelines for Specific Study Designs

For specific study designs, such as randomized control studies, studies of diagnostic accuracy, meta-analyses, observational studies, and non-randomized studies, authors are encouraged to consult the reporting guidelines relevant to their specific research design. A good source of reporting guidelines is the EQUATOR Network (https://www.equator-network.org/) and NLM (https://www.nlm.nih.gov/services/research_report_guide.html).

Manuscript Types

PHPR publishes editorials, original articles, review articles, guidelines, data profiles (including cohort profiles), special articles, short communications, viewpoints, editorials, commentaries, and correspondence, and book reviews.

- **Original articles** are papers containing results of basic and clinical investigations, which are sufficiently well documented to be acceptable to critical readers. These articles should be written in the following format: title page; abstract and keywords; main body (introduction, materials and methods, results, discussion, conclusion [if any]); references; and tables and figure legends. Manuscript limitations are 5,000 words, excluding the abstract, references, and tables and figure legends.
- **Review articles** provide concise reviews of subjects important to medical researchers, and can be written by an invited medical expert. These have the same format as original articles, but the details may be more flexible depending on the content. Manuscript limitations are 6,500 words from introduction to conclusion, 100 references, 10 figures and 10 tables. The abstract should

not exceed 200 words, and must be written as one unstructured paragraph.

- **Guidelines** are similar to original articles, but provide evidence-based recommendations expected to impact clinical research and practice. This category can include consensus-based statements of reporting standards or clinical practice guidelines.
- **Data Profiles (including Cohort Profiles)** present large data sets from specific populations that could be analyzed in epidemiological studies. Data Profiles should be structured with the following headings in the main text: Introduction, Collection, Data Resource Use, Strengths and Weaknesses, and Access. Cohort Profiles present up-to-date information about large population-based cohorts for which long-term data collection is planned. Data Profiles should be structured with the following headings in the main text: Introduction, Study Participants, Measurements, Key Findings, Strengths and Weaknesses, and Access. The main text of Data and Cohort Profiles is limited to 4,000 words, with an unstructured abstract of up to 200 words, a maximum of 7 tables and figures, and no more than 40 references.
- **Special Articles** deal with topics or issues that are relevant to public health, but without following a traditional study format. For example, articles in this category may address scientific methodology, wide-ranging ethical and social issues, scientific methodology, or other scholarly topics. Reports from consensus committees and working groups can be published as Special Articles. This category has a main text limit of 3,500 words, with an unstructured abstract of no more than 200 words, a maximum of 7 tables and figures, and no more than 40 references.
- **Brief reports** deal with issues of importance to biomedical researchers. The maximum length of the manuscript should be 2,000 words, including tables and figures.
- **Short communications** follow the general rules of the original article. The maximum length of the manuscript should be 3,000 words, including tables and figures.
- **Viewpoints** may deal with almost any topic deemed to be important in the fields of public health, ethics, health law, prevention, or health policy, and are not typically written in response to a specific article. Viewpoints should have a clear focus and present material in a well-organized and scholarly manner, but should not contain novel research findings or previously unpublished data. Although we welcome unsolicited viewpoint contributions, we request that authors contact the Editorial Office (ophrp@korea.kr) prior to submission to confirm that the proposed topic is suitable for the journal. The main text of Viewpoints is limited to 3,000 words, with an unstructured abstract of up to 150 words, a maximum of 4 tables and figures, and no more than 30 references.
- **Editorials** provide invited perspective on an area of PHRP, dealing with very active fields of research, current interests, fresh insights, and debates. An abstract is not required and a brief unstructured text should be prepared. Although editorials are normally invited or written by an editor, unsolicited editorials may be submitted. Manuscript limitations are 1,000 words and 20 references.
- **Commentaries** are brief articles with a narrow focus. The journal commissions most commentaries, but unsolicited commentaries will also be considered. Commentaries may undergo peer review. The length of commentaries should be limited to 1,000 words, 10 references, and 1 figure or small table.
- **Correspondence** is a comment from readers regarding a published article with a reply from the authors of the article. Manuscript limitations are 500 words, 2 tables/figures, and 5 references.
- **Book reviews** may be published. Please dispatch a book to the editorial office if you think the book is essential to public health personnel.

Title Page

Title page should include (1) the title of the article (less than 50 words); (2) name of the authors (first name, middle initial, last name in capitals) and institutional affiliation including the name of department(s) and institution(s) of each author; (3) name, full address (including the postal code) of the institutional affiliation, telephone and e-mail address of the corresponding author; (4) a running title of 50 characters or less including blank spaces; and (5) notes (disclaimers). Notes include ethics approval and consent to participate, conflict of interest, funding, availability of data, authors' contributions, additional contributions, and ORCID of all authors. All contributors who do not meet the criteria for authorship as defined above should be listed in an additional contribution section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Authors should disclose whether they had any writing assistance and identify the entity that paid for this assistance.

Abstract and Keywords

An abstract and 3–6 relevant keywords (in alphabetical order)

are required. Abstracts should be no more than 250 words in length. Abstracts should be structured, with the following section headings: Objectives, Methods, Results, Conclusion. For selecting keywords, refer to the MeSH browser (http://www.ncbi.nlm.nih.gov/mesh).

Highlights

All papers must include 3–5 short sentences presenting short summary or findings in the next of title page. The highlight section should be no more than 100 words, including spaces.

Main Body

- **Introduction** should provide concise yet sufficient background information about the study to provide the readers with a better understanding of the study, avoiding a detailed literature survey or a summary of the results.
- **Materials and methods** should contain detailed procedures of the study or experiment including investigation period, methods of subject selection, and information on subjects such as age, sex or gender, and other significant features, in order to enable the experiment to be repeated. A procedure that has been already published or standardized should be described only briefly using literature citations. Clinical trials or experiments involving laboratory animals or pathogens must elaborate on the animal care and use and experimental protocols, in addition to mentioning approval from the relevant committees. The sources of special equipment and chemicals must be stated with the name of the manufacturer. All statistical procedures used in the study and criteria for determining significance levels must be described. Ensure correct use of the terms "sex" (when reporting biological factors) and "gender" (identity, psychosocial or cultural factors). Unless inappropriate, report the sex and/or gender of study participants, the sex of animals or cells, and describe the methods used to determine sex or gender. If the study involved an exclusive population (only one sex, for example), authors should justify why, except in obvious cases (e.g., prostate cancer). Authors should define how they determined race or ethnicity, and justify its relevance. Institutional Review Board approval and informed consent procedures can be described as follows: The study protocol was approved by the Institutional Review Board of OOO (IRB No: OO-OO-OO). Informed consent was confirmed (or waived) by the IRB.
- **Results** should be presented in logical sequence. Only the most important observations should be emphasized or summarized, and the main or the most important findings should be mentioned first. Tables and figures must be numbered in the order they are cited in the text, kept to a minimum, and should not be repeated. Supplementary materials and other details can be separately presented in an appendix. The authors should state the statistical method used to analyze the results (statistical significance of differences) with the probability values given in parentheses.
- **Discussion** should contain an interpretation and explanation of the results and important aspects of the study, followed by the conclusions drawn from them. Information already mentioned in the Introduction or Results sections should not be repeated and the main conclusions of the study may be presented in the discussion.
- **Conclusion** (if any) must be linked with the purpose of the study stated in the abstract, and clearly supported by the data produced in the study. New hypotheses may be stated when warranted, but must be clearly labeled.

References

Authors are responsible for the accuracy and completeness of their references and for correct text citations.

- References are presented with [ ] following a surname in the main text, such as Kim [1] and Kim et al. [2]. When a reference is cited within the content, it is shown as [3] or [4,5] at the end. References should be searchable online.
- The last names and initials of all the authors (up to 3) should be included. For articles with more than 3 authors, list the first 3 authors only followed by "et al."
- References cited in tables or figure legends should be included in sequence at the point where the table or figure is first mentioned in the main text.
- Do not cite abstracts unless they are the only available reference to an important concept.
- Uncompleted work or work that has not yet been accepted for publication (i.e., an "unpublished observation" or "personal communication" should not be cited as a reference). In the references list, references should be limited to those cited in the text and listed in the order in which they appear in the text. The journals should be abbreviated according to the style used in the list of journals indexed in the NLM Journal Catalog (http://www.ncbi.nlm.nih.gov/nlmcatalog/journals).
- Use of DOI is highly encouraged. Note that missing data will be highlighted at the proof stage for the author to correct.

- Other types of references not described below should follow the ICMJE Recommendations (https://www.nlm.nih.gov/bsd/uniform_requirements.html).

Please refer to the following examples.

- **Journal articles**
  1. Park AK, Kim IH, Kim J, et al. Genomic surveillance of SARS-CoV-2: distribution of clades in the Republic of Korea in 2020. Osong Public Health Res Perspect 2021; 12:37-43.
  2. Hyun J, Lee JH, Park Y, et al. Interim epidemiological and clinical characteristic of COVID-19 28 cases in South Korea. Public Health Wkly Rep 2020;13:464-74. Korean.
  3. Gultekin V, Allmer J. Novel perspectives for SARS-CoV-2 genome browsing. J Integr Bioinform 2021 Mar 15 [Epub]. https://doi.org/10.1515/jib-2021-0001.
- **Books**
  1. Riffenburgh RH, Gillen DL. Statistics in medicine. 4th ed. Academic Press; 2020.
  2. Miller DD. Minerals. In: Damodaran S, Parkin KL, editors. Fennema's food chemistry. 5th ed. CRC Press; 2017. p. 627-80.
  3. Ministry of Employment and Labor. Statistics on occupational injuries and illnesses, 2008. Ministry of Employment and Labor; 2009.
- **Websites**
  1. World Health Organization (WHO). COVID-19 vaccines [Internet]. WHO; 2021 [cited 2021 Mar 15]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines.
- **Conference papers**
  1. Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, IE. Springer; 2002. p. 182-91.
- **Dissertation**
  1. Park HY. The role of the thrombomodulin gene in the development of myocardial infarction [dissertation]. Yonsei University; 2000.

Tables and Figures

Tables should be simple, self-explanatory, and supplemental, and should not duplicate the text or figures. Each table must be on a separate page, not exceeding 1 page when printed, and have a concise and informative title. The tables should be numbered with Arabic numerals in consecutive order. Each column should be appropriately headed with units in parentheses if numerical measures are given. All units of measurements and concentrations must be indicated. Footnotes are followed by the source notes, other general notes, abbreviation, notes on specific parts of the table ([a], [b], [c], [d]...), and notes on level of probability (*, **, *** for *p*).

Figures should be numbered with Arabic numerals consecutively in figure legends. The figures must not be interfered and must be clearly seen. The legend for each light microscopic image should include name of the stain and magnification. Electron microscopic images should contain an internal scale marker. All figures may be altered in size by the editor. The legends should briefly describe the data shown, explain abbreviations or reference points, and identify all units, mathematical expressions, abscissas, ordinates, and symbols.

Figures that are drawn or photographed professionally should be sent as JPG or PPT files. However, if an article receives approval for publication, files must be submitted as .tiff or .pdf. Each figure must have a caption explaining the figure. The preferred size of the images is 8×8 cm but 16.5 cm in width×8 cm in length is also acceptable. It is authors' full responsibility to submit images of sufficient quality for accurate reproduction and to approve the final color galley proof. All images must be correctly exposed, sharply focused, and prepared in files of 500 dpi or more.

When tables and figures are mentioned together in the text, they should be presented in parentheses as follows: (Table 1; Figure 1), (Tables 1, 2; Figures 1–3).

Appendix and Supplemental Data

If any materials are not enough to be included in the main text such as questionnaires, they can be listed in the Appendix. Any supplementary materials that help the understanding of readers or contain too great an amount of data to be included in the main text may be placed as supplementary data. Not only a recording of the abstract, text, audio or video files, but also data files should be added here.

FINAL PREPARATION FOR PUBLICATION

Final Version

After the paper has been accepted for publication, the author(s) should submit the final version of the manuscript. The names and affiliations of the authors should be double-checked, and if the originally submitted image files were of poor resolution, higher-resolution image files should be submitted at this

time. Symbols (e.g., circles, triangles, squares), letters (e.g., words, abbreviations), and numbers should be large enough to be legible on reduction to the journal's column widths. All symbols must be defined in the figure caption. If references, tables, or figures are moved, added, or deleted during the revision process, renumber them to reflect such changes so that all tables, references, and figures are cited in numeric order.

Manuscript Corrections

Before publication, the manuscript editor will correct the manuscript such that it meets the standard publication format. The author (s) must respond within 48 hours when the manuscript editor contacts the corresponding author for revisions. If the response is delayed, the manuscript's publication may be postponed to the next issue.

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The author(s) will receive the final version of the manuscript as a PDF file. Upon receipt, the author(s) must notify the editorial office of any errors found in the file within 48 hours. Any errors found after this time are the responsibility of the author(s) and will have to be corrected as an erratum.

Errata and Corrigenda

To correct errors in published articles, the corresponding author should contact the journal's editorial office with a detailed description of the proposed correction. Corrections that profoundly affect the interpretation or conclusions of the article will be reviewed by the editors. Corrections will be published as corrigenda (corrections of the author's errors) or errata (corrections of the publisher's errors) in a later issue of the journal.

NOTICE: These recently revised instructions for authors will be applied beginning with the February 2023 issue.

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Author's checklist

General Requirements

- ▫ The corresponding author (or the representative author of the co-corresponding authors) is the submitter of this manuscript.
- ▫ All manuscripts should be written in English.
- ▫ The main document with manuscript text and tables should be prepared in an MS Word (docx) or RTF file format.
- ▫ Manuscripts should be double-spaced in A4-size pages.
- ▫ Manuscripts should include line numbers.
- ▫ All pages should be numbered consecutively, starting with the abstract.

Title Page

- ▫ The title page and the rest of the manuscript text are prepared separately in two files (not combined together).
- ▫ The title page is arranged in the following order: article title, authors' full name(s), affiliation(s), and corresponding author's information, running title (less than 50 characters), notes.
- ▫ The notes section including (1) ethics approval and consent to participate, (2) conflicts of interest, (3) funding, (4) availability of data, (5) author contributions, (6) additional contributions, and ORCID is in title page, not in the manuscript.

Abstract

- ▫ The abstract does not exceed 250 words (Objectives, Methods, Results, Conclusion) for original articles and 200 words for reviews. Up to 3–6 keywords are listed at the bottom of the abstract.

Main Text

- ▫ The manuscript is organized according to following sequence: Title page, Abstract and keywords, Main text, References, Tables, and Figure legends.

Tables and Figures

- ▫ All tables and figures are numbered in the order of their appearance in a main text.
- ▫ Tables are included at the end of the manuscript as editable text and not as images.
- ▫ Figures are as separate files, in jpg, ppt, tiff, or pdf format.

References

- ▫ References are listed in proper format.
- ▫ All references listed in the reference section are cited in the text and vice versa.

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Copyright Transfer and Conflict of Interest Disclosure Form

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Everyone who is listed as an author in this article should have made a substantial, direct, intellectual contribution to the work and should take public responsibility for it.
This paper contains works that have not previously published or not under consideration for publication in other journals.

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All authors are responsible for recognizing any conflict of interest that could bias their work in the acknowledgments, disclosing all financial support and any other personal connections.

Please check the appropriate box below:

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