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Editorial

As the incidence of Omicron increases, so will the number of deaths

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Although the vaccination rate against coronavirus disease 2019 (COVID-19) is very high in the Republic of Korea, it is thought that the peak of the Omicron surge has yet to arrive despite the ever-increasing number of cases, which has recently exceeded 100,000 daily. Among the various viewpoints that have been presented regarding this issue, Horton [1], Editor-in-Chief of The Lancet, criticized the European Union World Health Organization (WHO) Regional Director Dr. Kluge, who said that the end game was now in sight. Horton addressed the issue that the data marshaled in favor of the purported impending end of the epidemic only came from some Western European countries, and he seemed to be of the view that the situation in Eastern Europe was different and could become a source of the epidemic again, given the low vaccine uptake rate in Africa. However, in this context, the head of the WHO, Tedros Adhanom Ghebreyesus visited the Republic of South Africa, the first beneficiary of the hub-and-spoke vaccination strategy, and said that 70% of vaccinations would be done by June–July, and that the situation would improve [2].

Since Omicron is different from other variants in terms of its phylogenetic evolution, the possibility of an epidemic involving other variants is predicted this winter. In other words, it remains unclear whether Omicron will be eradicated, or whether it will occur annually or continue to mutate and become a larger epidemic. Nonetheless, in general terms, the risk of disease occurrence—on a local, global, or endemic scale—or eradication is mediated through disease outbreaks. Endemic diseases are highly likely to occur every year, but the socioeconomic damage is lower, and a massive outbreak will result in remnants of active cases in some areas and the possibility of re-spreading in winter [3].

At the same time, the United Kingdom (UK) and European countries are pushing for a step-by-step recovery of daily life by lifting masks, relaxing social distancing, and reducing border control measures in response to the decreasing number of critically ill patients and fatalities after 3 doses of the COVID-19 vaccine. The Republic of Korea has been cautious about policy changes, but the 3-dose vaccination rate for adults over the age of 18 is 59.4%, which is close to the rate of 66% in the UK (February 17) and higher than that in the United States (43.2% as of February 22). Therefore, it was judged that even though the spread of COVID-19 was receding gradually, the likelihood of a rapid increase in the number of patients was low. Furthermore, the number of critically ill patients and deaths was low. This reasoning supported the choice to ease the public health measures step by step and to switch to home treatment according to very rapid increase in mild cases, but there is also an argument that concerns are warranted about

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relaxing stringent anti-COVID-19 measures because that relaxation will take place quickly. However, the government announced that if Omicron's virulence is estimated as the fatality rate for each week of the epidemic, rather than the cumulative fatality rate, the fatality rate of Omicron is lower than the original estimate; therefore, it would be better to relax social distancing and release the restrictions on freedom and economic activity. These claims were made at the same time. Let us take a closer look at the rationale for these arguments.

The rationale for prudent implementation is as follows:

First, although the fatality rate of Omicron is low, considering the active immunity caused by vaccination and the natural immunity caused by infections with the delta variant (as the scale of delta infections has been very large), it has been estimated that if the Omicron epidemic continues in our country, the overall scale of deaths will be similar and the burden of disease will increase [4,5]. To exemplify this reasoning using concrete data, according to a press release on February 21, when comparing the delta- and Omicron-infected patients from the third week of January to the third week of February, the severity rate decreased from 1.4% to 0.38%, and the fatality rate decreased from 0.7% to 0.18%. These rates both decreased to a quarter of the initial level, but the total number of patients increased by 15.6 times, so it is possible that the number of critically ill patients or the number of deaths might quadruple.

Second, Omicron countermeasures were prepared with a focus on respiratory specialist clinics, which were in charge of the transition to at-home treatment. However, the expansion of primary care clinics in accordance with the increase in the number of patients was insufficient, and there were reports of deaths during at-home care due to inadequacies in the transport system, medication system, and patient management system, we need more time to preparedness and need to reduce volume of positive cases by public health measures, such as trace-test-isolation-quarantine supported by Information and Communications Technology.

Lastly, although there have been clear changes in perceptions of this disease over the past 2 years, as exemplified by a marked decrease in the severity of infection and fear scores compared to 2020, it is nonetheless noteworthy that 40.2% of residents responded that they would not be able to receive treatment for COVID-19 due to a lack of treatment facilities such as hospital beds. As the number of confirmed cases increases, the number of respondents who were concerned about the increase in severity and death took second place, accounting for 33.2% of the total, and 19.1% of the respondents also said that it would be difficult to receive timely treatment for other diseases due to the spread of COVID-19. It is necessary to consider this gap in perceptions between healthcare providers and the public [6].

Turning to the opposing point of view, if the virulence of delta and Omicron is estimated as the fatality rate for each variant's cohort, rather than the cumulative fatality rate, it is predicted that the fatality rate of Omicron will be lower based on recent epidemiological trends. The number of patients hospitalized due to the Omicron variant is also small, so there is no shortage of beds, and the death rate is almost the same as that of influenza, so the public does not need to be anxious. Thus, there is no possibility of a major problem, even if measures against COVID-19 transmission are actually lifted.

The general public finds it difficult to accept the government's explanation. In other words, the excessively conservative prediction of the number of patients is causing distrust. Furthermore, the number of patients with BA.2 (stealth Omicron), a sub-lineage of Omicron, is increasing in some countries. This variant has a faster rate of transmission than the original Omicron variant, a higher immune evasion rate, resistance to existing treatments, and already accounts for 4.9% of infections in the Republic of Korea [4]. Therefore, while monitoring this situation, it is necessary to take a cautious approach, while avoiding anxiety, until the end of the pandemic. Prevention policies to reduce the number of patients— such as prompt screening and voluntary isolation at home, wearing a mask, preventing mass outbreaks, and promptly vaccinating those who are not vaccinated—should not be hastily chosen as policies to favor or disadvantage the upcoming election. In any case, the basic policy direction to protect the lives of the people must be faithfully observed. In addition, as long as cases continue to be imported from the epicenters of variants, international cooperation will inevitably become more important to prevent the spread of this disease.

Therefore, in order to make policy decisions accurately, there is a need for a system that can provide accurate knowledge of the facts. Instead of simple theoretical modeling or simulation, it should be possible to know the situation in the field as it unfolds. The government is responsible for the real-time identification of inpatients, critically ill patients, and fatalities. It is also necessary to establish a system that can quickly synthesize the results of various epidemiological data and interventions in real time to make decisions. Considering that the end game of Omicron is a period of preparation for a larger epidemic, rather than the end of COVID-19, improvements should be made to supplement the shortcomings of the countermeasures that have been taken to date.

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Review Article

Points to consider for COVID-19 vaccine quality control and national lot release in Republic of Korea: focus on a viral vector platform

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ABSTRACT

Due to the global public health crisis caused by the coronavirus disease 2019 (COVID-19) pandemic, the importance of vaccine development has increased. In particular, a rapid supply of vaccines and prompt deployment of vaccination programs are essential to prevent and overcome the spread of COVID-19. As a part of the vaccine regulations, national lot release is regulated by the responsible authorities, and this process involves the assessment of the lot before a vaccine is marketed. A lot can be released for use when both summary protocol (SP) review and quality control testing are complete. Accelerated lot release is required to distribute COVID-19 vaccines in a timely manner. In order to expedite the process by simultaneously undertaking the verification of quality assessment and application for approval, it is necessary to prepare the test methods before marketing authorization. With the prolonged pandemic and controversies regarding the effectiveness of the COVID-19 vaccine against new variants, public interest for the development of a new vaccine are increasing. Domestic developers have raised the need to establish standard guidance on the requirements for developing COVID-19 vaccine. This paper presents considerations for quality control in the manufacturing process, test items, and SP content of viral vector vaccines.

Keywords: COVID-19 vaccines; National lot release; Quality control; Regulatory science; Summary protocol; Viral vector platform

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Introduction

A common issue currently facing countries throughout the world is how to return to pre-

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coronavirus disease 2019 (COVID-19) daily life when the COVID-19 pandemic ends. COVID-19 is a respiratory syndrome caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which first emerged in Wuhan, Hubei Province, China in December 2019. In the absence of specific therapeutics or vaccines to prevent this new infectious disease, it has spread all over the world. Accordingly, the World Health Organization (WHO) declared the COVID-19 outbreak as a public health emergency of international concern on January 30, 2020. The WHO then made the assessment that COVID-19 could be characterized as a pandemic. At that time, about 120,000 people in 110 countries were infected with the virus [1,2].

In the exceptional situation of a pandemic, the pharmaceutical industry, regulatory authorities, governments, and international organizations have coordinated collaborative strategies, and many national regulatory authorities have eased regulations to accelerate the development of COVID-19 vaccines. In order to facilitate access to COVID-19 vaccines, regulatory authorities have executed innovative and agile regulatory measures such as conditional marketing authorizations or emergency use authorizations. These measures are intended to ensure that patients could be supplied with COVID-19 therapeutics or vaccines as quickly as possible. These new regulatory applications involve expedited regulatory reviews for COVID-19 vaccines without compromising their safety, effectiveness, and quality [3–8]. As a result, the vaccine development process, which normally takes about 10 years, has been shortened to within about 1 year, from development to review and approval, and then to vaccination. Tables 1 and 2 show the authorization and approval status of COVID-19 vaccines by stringent regulatory authorities. In the Republic of Korea, the AstraZeneca vaccine, a viral vector platform vaccine was first authorized on February 10, 2021, followed by the Pfizer-BioNTech vaccine, Janssen vaccine, and Moderna vaccine, which have been approved and used [9].

In the Republic of Korea, it is stipulated that vaccines shall obtain lot release approval from the Ministry of Food and Drug Safety (MFDS) after undergoing the examination and verification of the data on manufacturing and quality management in accordance with the Pharmaceutical Affairs Act, Article 53. For biologics such as vaccines, reviews have been implemented to further confirm the quality of each lot of the product before its release onto the market. A national lot release system has been implemented to review the summary protocol (SP) for its production and quality control tests. The SP refers to a summarized document regarding the manufacturing process and test results of a product, ranging from raw materials to the final product (Figure 1). When the MFDS receives the application for a national

Table 1. Authorization and approval status of COVID-19 vaccines (overseas: ICH founding & standing regulatory members)

Platform	Viral vector vaccines						mRNA vaccines			
	ChAdOx1-SARS-CoV-2 vaccine		Ad26.COV2.S vaccine							
Developer	AstraZeneca/Oxford		Janssen				Pfizer-BioNTech		Moderna	
Product information	Name	Approval/date	Name	Approval/date			Name	Approval/date	Name	Approval/date
Authority										
United States, FDA	-	-	Janssen COVID-19 vaccine	EUA/February 27, 2021			Comirnaty (COVID-19 vaccine, mRNA)	MA/August 23, 2021 (EUA/December 11, 2020)	Moderna COVID-19 vaccine	EUA/December 18, 2020
EC, EMA	Vaxzevria (previously AstraZeneca COVID-19 vaccine)	CMA/January 29, 2021	COVID-19 vaccine Janssen	CMA/March 11, 2021			Comirnaty	CMA/December 21, 2020	Spikevax (previously Moderna COVID-19 vaccine)	CMA/January 6, 2021
Japan, MHLW/ PMDA	Vaxzevria	SAFE/May 21, 2021	-	-			Comirnaty	SAFE/February 14, 2021	COVID-19 Moderna	SAFE/May 21, 2021
Canada, HC	Vaxzevira COVID-19 vaccine	ISAD IO/February 26, 2021	Janssen (Johnson & Johnson) COVID-19 vaccine	ISAD IO/March 5, 2021			Comirnaty COVID-19 vaccine	MA/September 16, 2021 (ISAD IO/ December 9, 2020)	Spikevax COVID-19 vaccine	MA/September 16, 2021 (ISAD IO/ December 23, 2020)
Switzerland, Swissmedic	-	-	Janssen COVID-19 vaccine	TPA/March 22, 2021			Comirnaty	TPA/December 19, 2020	Spikevax	TPA/January 12, 2021

Available from regulatory authority websites as of October 2021 (sources: FDA, https://www.fda.gov/emergency-preparedness-and-response/counterterrorism-and-emerging-threats/coronavirus-disease-2019-covid-19; EMA, https://www.ema.europa.eu/en; PMDA, https://www.pmda.go.jp/english/about-pmda/0002.html; Health Canada, https://www.canada.ca/en/health-canada/services/drugs-health-products/covid19-industry/drugs-vaccines-treatments/vaccines.html; Swissmedic, https://www.swissmedic.ch/swissmedic/en/home.html).

COVID-19, coronavirus disease 2019; ICH, International Council for Harmonization; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; FDA, Food and Drug Administration; MA, marketing authorization; EUA, emergency use authorization; EC, European Commission; CMA, conditional marketing authorization; MHLW, Ministry of Health, Labour and Welfare; PMDA, Pharmaceuticals and Medical Devices Agency; SAFE, special approval for emergency; HC, Health Canada; ISAD IO, interim order pathway for COVID-19-related drugs and vaccines; TPA, temporary authorization; -, unauthorized or unapproved.

Table 2. Authorization and approval status of COVID-19 vaccines (domestic)

No.	License holder	Product	Approval date (manufacture/import)	Platform
1	AstraZeneca Korea Co. Ltd.	Vaxzevria injection COVID-19 vaccine (SARS-CoV-2 viral vector vaccine)	February 10, 2021 (manufacture) May 21, 2021(import)	Viral vector vaccine
2	Pfizer Pharmaceuticals Korea Ltd.	Comirnaty injection (tozinameran) (SARS-CoV-2 mRNA vaccine)	March 5, 2021 (import)	mRNA vaccine
3	Janssen Korea Ltd.	COVID-19 vaccine Janssen injection (SARS-CoV-2 viral vector vaccine)	April 7, 2021 (import)	Viral vector vaccine
4	Green Cross Corp.	Moderna Spikevax injection (SARS-CoV-2 mRNA vaccine)	May 21, 2021 (import)	mRNA vaccine

COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
Available from Ministry of Food and Drug Safety website (source: https://nedrug.mfds.go.kr).

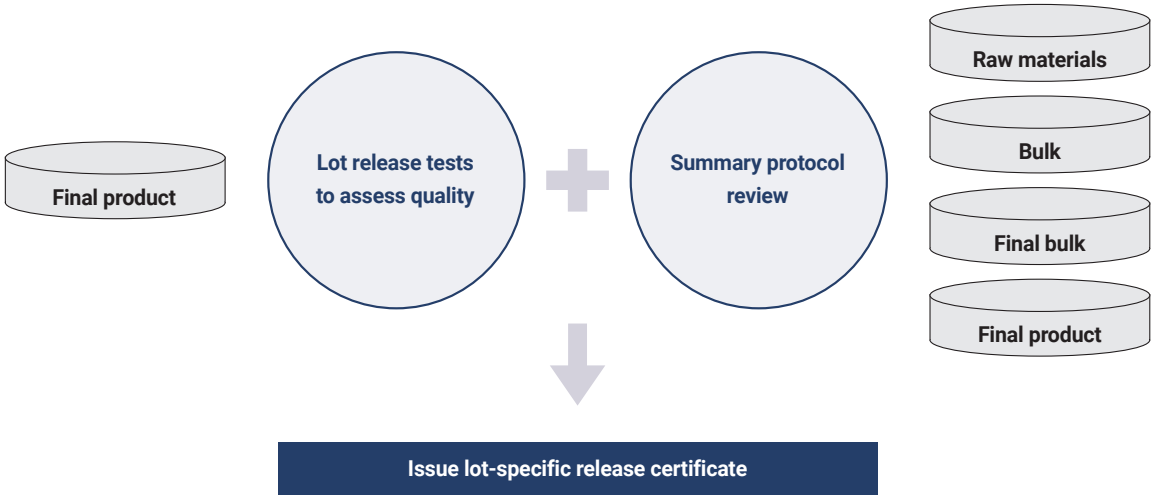

Figure 1. National lot release system in the Republic of Korea.

lot release, the applicant is notified of the result after lot release testing and a review of the data concerning the product's manufacturing and quality control. If the criteria are satisfied, a certificate of approval for the national lot release shall be issued [10]. Normally, the applicant is notified of the specifications and test methods of products for lot release, and then the subsequent lot release process is carried out after marketing authorization. However, as COVID-19 vaccines were designated as crisis-response medical products (specifically, as preventive vaccines for a pandemic infectious disease), they are subject to expedited national lot release. The MFDS has prioritized COVID-19 vaccines over other lot releases, and rapidly verified the quality of the vaccines [11]. The MFDS has committed to the improvement of the national quality control system for biologics and established a comprehensive lot release system. The National Institute of Food and Drug Safety Evaluation (NIFDS), an affiliated organization of the MFDS, is responsible for this task.

To push ahead with the lot release further, simultaneous applications for marketing authorization and lot release have been conducted. The MFDS reviewed the safety and quality verification methods prior to the drug approval process of COVID-19 vaccines, and established the test methods before the application for lot release. Necessary equipment and instruments were also prepared. MFDS expedited the lot release process, which generally takes 2 or 3 months, and approved the lot release within 20 days. By the third quarter of 2021, the MFDS has approved the national lot release of 67.64 million doses (for domestic use) of COVID-19 vaccines. As of November 2021, 87.05 million doses—an amount with which more than 44 million people can be fully vaccinated—were approved for lot release. Thus, it became feasible to swiftly provide the necessary quantity of vaccines in a timely manner amid the spread of COVID-19. The status of domestic COVID-19 vaccine lot release and product information is available from the MFDS website (https://www.mfds.go.kr/vaccine_covid19.jsp) and

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the integrated drug information system (https://nedrug.mfds.go.kr), respectively.

Vaccine manufacturing technology has been continuously developed as a solution to prevent infectious diseases. A vaccine platform refers to a technology for vaccine development by changing specific antigens or genetic information. A single vaccine platform commonly addresses a specific pathogen. However, the COVID-19 pandemic yielded a competitive situation, wherein various platforms were used to develop new vaccines with the goal of rapidly obtaining an effective vaccine. Currently, COVID-19 vaccine platform modalities include inactivated virus vaccines, attenuated virus vaccines, recombinant viral vector vaccines, recombinant subunit vaccines, DNA vaccines, and RNA vaccines [12,13]. As of December 3, 2021, there are 135 candidates in the clinical phase of development, of which vaccines using protein subunits accounted for 34.8% (*n*=47), RNA vaccines accounted for 15.6% (*n*=21), and viral vectors (non-replicating) accounted for 14.8% (*n*=20) (Table 3) [14].

Since the manufacturing technology and quality control processes required for each platform are novel and unique, the establishment of test methods is time-consuming. Furthermore, each vaccine platform has advantages and drawbacks, including differences in production, efficacy, safety profile, and immune response. Therefore, it is essential to recognize the characteristics of every vaccine candidate and the specific quality considerations for each platform [12]. If new technology platform development guidelines are provided for use in quality control and testing, the vaccine development timeline could be significantly shortened. Accordingly, the NIFDS published 2 national lot release guidelines for new technology platforms for COVID-19 vaccines (viral vector vaccines and mRNA vaccines) by referring to the approval and review data of COVID-19 vaccines by the MFDS, WHO, and European Medicines Quality Committee (EDQM) guidelines [15,16].

Table 3. Global COVID-19 vaccine candidates in clinical development (*n*=135)

Platform	*n* (%)
Protein subunit	47 (34.8)
Viral vector (non-replicating)	20 (14.8)
DNA	15 (11.1)
Inactivated virus	18 (13.3)
RNA	21 (15.6)
Viral vector (replicating)	2 (1.5)
Virus-like particle	6 (4.4)
Virus-like particle+antigen presenting cell	2 (1.5)
Live-attenuated virus	2 (1.5)
Viral vector (non-replicating)+antigen presenting cell	1 (0.7)
Bacterial antigen-spore expression vector	1 (0.7)

COVID-19, coronavirus disease 2019.
Data as of December 3, 2021 (source: https://www.who.int).

Viral vector vaccines are defined as live viruses that are genetically engineered to express 1 or more heterologous antigens [17]. Recombinant viral vector vaccines are generated by cloning the gene for an antigen from the pathogen into an avirulent host such as an adenovirus. Viral vector vaccines enter cells and produce the vaccine antigen, stimulating an immune response. Developing a recombinant viral vector vaccine that mimics a pathogen (e.g., SARS-CoV-2), which is the cause of the COVID-19 pandemic, but is not virulent, is known to be a safe option [12,13]. Several recombinant viral vectors derived from other viruses have emerged as gene delivery systems [18]. In particular, adenoviruses were initially characterized and have been used as gene delivery vectors since the early stages of gene therapy. Adenoviral vectors have been explored as vaccine agents for infectious diseases. Thus, as logical vaccine candidates, adenoviral vector vaccines are expected to be useful as a response to the COVID-19 pandemic as they are known to induce a potent and balanced immune response [19]. Two viral vector vaccines are currently commercialized in Republic of Korea. These vaccines contain adenovirus engineered to carry a SARS-CoV-2 gene, using a chimpanzee-derived adenovirus (AstraZeneca vaccine) or a human-derived adenovirus (Janssen vaccine) vector as a delivery system.

In this paper, we describe considerations for the management of COVID-19 viral vector vaccines under the regulatory system in the Republic of Korea, with a particular focus on specific lot release tests and information to be included in the SP. This paper is intended to provide an internationally harmonized guideline by comparing and reviewing the EDQM document for recombinant viral vector vaccines [17], the WHO and Official Control Authority Batch Release (OCABR) guidelines [20–23], and the United States Pharmacopoeia (USP) COVID-19 vaccine quality assessment toolkits [24].

Considerations for Quality Control in Each Stage of the Production of Viral Vector Vaccines

The quality control tests that can be performed at each stage of manufacturing a viral vector-based COVID-19 vaccine are as follows. This document is expected to help COVID-19 vaccine developers prepare an appropriate analytical strategy during vaccine development. Additional tests may be considered on a case-by-case basis, depending on the characteristics of the vector backbone and the

specific manufacturing process established.

Cell Substrates for Viral Vector Propagation

The vector can be propagated in human diploid cell lines, continuous cell lines, chick-embryo cells, or in the amniotic membrane of chick embryos derived from specific pathogen-free chickens [17]. More specific test information on human diploid cells and immortal cell lines used as cell substrates for the propagation of recombinant viral vectors can be obtained from the MFDS guidelines [25,26] and European Pharmacopoeia Chapter 5.2.3 [27]. The cell substrates need to be used with a cell banking system.

The most widely used method of viral vector development involves homologous recombination [28]. Certain replication-defective viral vectors may require testing for replication-competent viruses. Replication-competent viral vectors may incur significant issues with quality control when there is a wide homology region between the viral genome and the genome of the complementation cell. This expression could reduce the replication capacity by minimizing the homology between both genomes. It is recommended to use cells without homology between the vector and sequence for production.

Viral Vector Seed Lot

Recombinant viral vector production generally uses a seed lot system (master virus strain and working virus strain). The number of passages is controlled based on evidence regarding the maximum number of passages, and production cannot exceed the maximum passage level. Historical records, including information on the strain origin of the viral vector used for vaccine production, and in particular, subsequent manipulation of deleted or modified regions, should be confirmed. Genetic insert and flanking control regions should be described in detail, including the nucleotide sequences. The origin of genetic inserts into the vectors and the engineering methods should be documented. A relevant characterization could be carried out based on the nature of the viral vector and the results of pre-clinical studies.

Propagation and Harvest

It is preferable to produce vectors without the use of antibiotics. Penicillin or streptomycin should not be used at any stage of manufacture if insufficient data support doing so. It is necessary to provide information on control cell culture. A single harvest refers to the biological material prepared from a single production run.

Bulk

A bulk refers to the one containing active ingredient prior to formulation. The purification process could be applied to a pool of single harvests. The purification process should be validated for removal of impurities. The following test items may be considered for controlling the bulk: identification, vector concentration, infectious vector titer, ratio of vector particle concentration to infectious vector titer, ratio of infectious vector titer to total protein concentration, transgene expression, host-cell protein, host-cell DNA, vector aggregation, residual reagents, residual antibiotics, the absence of replication-competent viral vectors, microbial control (microbial limit or sterility), and endotoxin.

Final Bulk

The final bulk may consist of 1 or more bulks. In preparing the final bulk, any excipients such as stabilizers are added to the product. The following tests could be considered for control of the final bulk: microbial control (microbial limit or sterility) and preservative.

Final Product

Control tests for the final product are shown in Table 4. If a test for bovine serum albumin or ovalbumin (if applicable) has been carried out on the bulk or the final bulk, they may be omitted on the final product.

Guidance on Quality Control for Viral Vector Vaccines

Test Items and Methods

Regulatory authorities in the countries such as the United States, Europe, and Japan are supporting the development of COVID-19 vaccines by providing guidance, quality standards, and training materials to vaccine developers and manufacturers [20–24]. In the context of the COVID-19 pandemic, the EDQM published the first analytical strategy options on the quality control of COVID-19 recombinant viral vector vaccines in November 2020. In this document, the mandatory tests at each stage of viral vector vaccine manufacturing (from raw materials to the final product) are presented [20]. The USP provided a toolkit for the quality evaluation items and test methods for each COVID-19 vaccine platform (mRNA vaccine, inactivated vaccine, and viral vector vaccine) in July 2021 [24]. Japan registered a monograph of test items and standards for domestically authorized COVID-19 vaccines (Pfizer-BioNTech, Moderna, and AstraZeneca) in the Minimum Requirements for Biological Products (MRBP) [22]. The NIFDS published a Guideline on National Lot Release for COVID-19 Viral Vector Vaccines [15], and SARS-CoV-2 viral vector vaccines fee for national lot release was added to the Regulation on

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Table 4. Comparison of quality control tests for final products

Category		United States (USP)	Europe (EDQM)	Korea (MFDS)	Japan (NIID)[a]
Item	Attribute	Possible test methods			
Identity	Sequence confirmation	DNA extraction & sequencing (USP 1125, 1126) restriction analysis (USP 1129, 1126)	Immunochemical methods (EP 2.7.1), NAT (EP 2.6.21), LC (EP 2.2.29)	Immunochemical methods. NAT, LC	Suitable methods
	Vector detection	qPCR, ddPCR, RT-PCR (USP 1125, 1126, 1127), ELISA (USP 1103)			
Purity	Vector aggregates	Light-scattering (USP 1430.2, 1430.3, 1430.5, 1430.6, 1430.7), SEC-MALS (USP 621, 1430.1)	Light-scattering	Light-scattering	-
Potency	Infectious vector titer	Plaque assays (USP 111, 1235, 1237), CCID50, cell-based qPCR (USP 1032, 1033, 1034)	Plaque assays or CCID50 assay by immunostaining, qPCR, flowcytometry/FACS, fluorescent focus assay	Plaque assays or CCID50 assay by immunostaining, qPCR, flowcytometry, fluorescent focus assay	Immunostaining
	Transgene expression	Western blot (USP 1104), ELISA (USP 1103), LC-MS (USP 621, 736, 1736), RP-HPLC (USP 621)	Immunochemical assay (EP 2.7.1), biochemical assay, flow cytometry (EP 2.7.24)	Immunochemical assay, biochemical assay, flow cytometry	-
Quantity	Virus particle (vector concentration)	Light-scattering & DLS (USP 1430.2, 1430.6), CZE (USP 1053), qPCR (USP 1125, 1126, 1127)	qPCR (EP 2.6.21)	qPCR	LC
Appearance	Compendial test	USP 1, 790	EP 2.7.1, 2.6.21, 2.2.29	Visual observation	Criteria specified
pH	Compendial test	USP 791	EP 2.2.3	KP general test	JP general test
Container content for injections (includes extractable volume)	Compendial test	USP 697	EP 2.9.17	KP general test	-
Sterility	Compendial test	USP 71	EP 2.6.1	KP general test	JP general test
Endotoxin	Compendial test	USP 85	EP 2.6.14	KP general test	JP general test
Osmolality	Compendial test	USP 785	EP 2.2.35	KP general test	-

USP, United States Pharmacopoeia; EDQM, European Medicines Quality Committee; MFDS, Ministry of Food and Drug Safety; NIID, National Institute of Infectious Diseases; EP, European Pharmacopoeia; NAT, nucleic acid test; LC, liquid chromatography; qPCR, quantitative polymerase chain reaction; ddPCR, droplet digital PCR; RT-PCR, reverse transcription PCR; ELISA, enzyme-linked immunosorbent assay; SEC, size exclusion chromatography; MALS, multi-angle light-scattering; CCID50, cell culture infectious dose 50%; FACS, fluorescence-activated cell sorting; MS, mass spectrometry; DLS, dynamic light-scattering; CZE, capillary zone electrophoresis; RP, reverse phase; HPLC, high-performance liquid chromatography; KP, The Korean Pharmacopoeia; JP, Japanese Pharmacopoeia; -, not applicable.

[a]Chimpanzee adenoviral vector vaccine only.

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Fees for Pharmaceutical Approval, etc. on October 21, 2021 [29]. In addition, in order to ensure the transparency and clarify of the lot release system, revisions of the Regulation for Designation, Approval Process, and Method of Pharmaceuticals for National Lot Release, which describes the amount of samples and test items for newly authorized COVID-19 vaccines, are in progress, and the proposed amendment will be announced within this year.

The test items, objectives, and possible methods in Table 4 are necessary information to be considered in the quality control of the final product, but they are not set criteria for lot release. Generally, test specifications and methods subject to lot release should be in compliance with the MRBP (NFDS Notification). However, the Minister of the MFDS may adjust them partially considering the characteristics of the product according to the approved Specification and Analytical Procedures.

National Lot Release Tests

Specifications and test methods include product-specific tests such as identification, purity, potency, and tests for preservatives and stabilizers used in manufacturing. Further, sterility and endotoxin tests are conducted to confirm product safety. In addition, in accordance with the MRBP (MFDS Notification), the details of the necessary criteria such as the nature, condition, and quality of vaccines could be determined. Table 5 demonstrates the recommended test items and methods for viral vector vaccines. The test items performed for the national lot release of viral vector vaccines (AstraZeneca and Janssen vaccines) authorized in Korea are as follows. The lot release test items of AstraZeneca vaccine include appearance, pH, sterility, endotoxin, identity, potency (infectivity), virus particle concentration, purity (ratio of DNA to protein, ratio of virus particle to infectious virus), and container content for injections. The test items for Janssen vaccine lot release include appearance, pH, sterility, endotoxin, identity (virus identity, virus protein identity), potency (transgene expression, infectious titer, and ratio of virus particle to infectious virus), concentration, purity, and container content for injections. The test items for both vaccines are included in the revised content of the Regulation for Designation, Approval Process, and Method of Pharmaceuticals for National Lot Release.

Table 6 shows the test items for COVID-19 viral vector vaccines tested by the National Regulatory Authority and National Control Laboratory. The test items performed by the European Network of Official Medicines Control Laboratories are specified in the OCABR guideline [21], and the Australian government provides guidance on the assessment items for COVID-19 vaccine batch release through the Therapeutic Goods Administration website [23].

Table 5. National lot release test items and methods in the Republic of Korea

Test item		Test methods
General	Appearance	Confirm color, shape formulation, etc. by visual observation
	Container content for injections	Compendial test such as KP general test 65
	pH	Compendial test such as KP general test 76
Product- specific	Quantity	Vector concentration (virus particles) such as qPCR, liquid chromatography, etc.
	Identity	Identification of vector including genetic insert such as liquid chromatography, qPCR and gel electrophoresis system, etc.
	Potency	Determination of transgene expression by qPCR, ELISA, immunostaining, etc.
	Purity	Determination of vector aggregation such as liquid chromatography, light-scattering tests
Safety	Sterility	Compendial test such as KP general test 12
	Endotoxin	Compendial test such as KP general test 40

KP, The Korean Pharmacopoeia; qPCR, quantitative polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay.

Table 6. Viral vector vaccine laboratory test items performed by the NRA/NCL

NRA/NCL	EMA (OMCL)	Australia (TGA)	Korea (MFDS)
Test items	Appearance, potency, identity (potency may serve as an identity test)	Composition and strength, purity and integrity, endotoxin	Appearance, pH, quantity, identity, potency, purity, sterility, endotoxin, container content for injection

NRA, National Regulatory Authority; NCL, National Control Laboratory; EMA, European Medicines Agency; OMCL, European Network of Official Medicines Control Laboratories; TGA, Therapeutic Goods Administration; MFDS, Ministry of Food and Drug Safety.

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SP Review

The manufacturer's SP is the summarized information taken from the manufacturing and testing results according to Good Manufacturing Practice requirements to ensure that the lot meets the specifications of the marketing authorization. The SP contains data on all appropriate production steps and controls, and it is certified and signed by the person in charge of the manufacturing company [30].

As shown in Table 7, information on the production and storage at each stage of the manufacturing process, including information on the passage history of cell strains/virus strains used as raw materials, as well as the test methods, specifications, and results should be provided in the SP. For reference, an SP template is included with the Guideline on National Lot Release for COVID-19 Viral Vector Vaccines [15]. The manufacturer or importer will be notified of the SP form. However, it is thus possible that SP for a specific product may differ in detail from the provided model.

Conclusion

The still ongoing COVID-19 pandemic demands a global response due to the occurrence and spread of variants with novel mutations. Around the world, people are looking for explicit scientific evidence and useful guidance to deal with this unprecedented pandemic. The currently predominating Delta variant lowers the effectiveness of vaccination, leading

Table 7. Content of the summary protocol for viral vector vaccines

Category	Description
1. General	a. Information about the product that has been applied for lot release
	1) Name of applicant, name of representative, and address
	2) Name of the preparation pursuant to the MRBP (MFDS Notification)
	3) Product name, lot number, manufacturing date, expiry date, period of usage, packaging unit, storage
	4) Amount that has been applied for national lot release
	b. Summary on manufacturing information
	1) Lot number of the raw materials (master cell strains, working cell strains/virus strains), material (master cell line/virus strain, production cell line/virus strain), manufacturing date, etc.
	2) Bulk, lot number of the final bulk, manufacturing date and manufacturer
	3) Lot number of final product, manufacturing date, manufacturer, total amount of production, and bottling date
2. Raw materials	With regard to the raw materials, testing items, methods, results, detailed information related to the manufacturing, etc. must be included. For cell strains and virus strains, information on the passage history, number of passages, etc. must be included
3. Bulk	a. Information about half-finished products (intermediates) that are produced during the manufacturing of bulk, name, lot number, manufacturing date, manufacturer, storage conditions (temperature and container), period of storage, amount of production, etc.
	b. Information about bulk, lot number, manufacturing date, manufacturer, storage conditions (temperature and container), period of storage, amount of production, major production methods, etc.
	c. Information about quality control, information related to the testing items, methods, specifications, period, results, etc. of a test performed according to the quality control manual and approval items of a bulk and materials used for producing the bulk
4. Final bulk	a. Information about the material used for producing the final bulk, corresponding material's ingredient name, lot number, added amount, etc.
	b. Information about the final bulk, lot number, manufacturing date, manufacturer, storage conditions (temperature and container), period of storage, amount of production, etc.
	c. Information about quality control, testing items, methods, specifications, period, results, etc. of a test conducted according to the quality control manual and approval items of a final bulk
5. Final product	a. Information about the drug product, lot number, manufacturer, specification of a container, filling amount, bottling date, manufacturing date, total amount of production, storage, expiration date (shelf life), etc.
	b. Information about quality control, testing items, methods, specifications, period, results, etc. of a test conducted according to the quality control manual and approval items of a final product
	c. For imported drug products, information that is recorded additionally by an importer, lot number, manufacturer, manufacturing date, date of customs clearance, imported amount, storage method, expiry date (shelf life) in addition to the testing items, methods, specifications, period, results, etc. of a test conducted by the importing party should be included.
	d. If attached reconstitution solution is included, regarding information about manufacturer, lot number, manufacturing date, expiration date, specifications, and test results

MRBP, Minimum Requirements for Biological Products; MFDS, Ministry of Food and Drug Safety.

to the 4th wave of the epidemic [31]. The Delta variant is eightfold less sensitive to ChAdOx1 vaccine-elicited antibodies than the wild-type Wuhan-1. It was also confirmed that the neutralizing antibodies from those who had recovered or been vaccinated showed decreased effectiveness against the Delta variant [32]. Although the current vaccines could reduce the hospitalization and mortality rate, it is also necessary to develop a next-generation vaccine to cope with breakthrough infections. Vaccination is recognized as the best game-changer that mitigates the current pandemic [33], considering its superior effectiveness and capability as a safe public health intervention to prevent infections. Therefore, the rapid production and supply of safe and effective vaccines to protect people and mitigate the economic and social impacts of infectious diseases should be prioritized [34,35].

In the middle of the 20th century, vaccine production technology made substantial advances as vaccines were produced by virus propagation in cell culture. Through these technological innovations, vaccine development platforms have been diversified [12]. Historically, 4 classic platforms (inactivated virus, live-attenuated virus, protein subunit, and virus-like particle) constituted most vaccine products [13]. However, in response to COVID-19, developers are looking for more up-to-date technologies in order to avoid the safety and efficacy concerns associated with traditional platforms targeting SARS-CoV-2 [12]. The currently commercialized COVID-19 vaccines were developed quickly, even though they were based on recent technologies. The reasons for the success of vaccine development with a rapid timeline are as follows: SARS-CoV-2 is closely related to SARS-CoV, and through the accumulated data on SARS-CoV and Middle East respiratory syndrome coronavirus, a spike protein has been identified as the antigenic target for coronavirus vaccines [36,37]. This enabled the more rapid design and development of SARS-CoV-2 vaccine candidates after the emergence of this new virus, as well as sharing the SARS-CoV-2 gene sequence, diversifying vaccine development strategies, supporting development costs, and shortening the period from vaccine approval to distribution by expediting the regulatory response system to COVID-19. In other words, the rapid development and deployment of COVID-19 vaccines resulted from organically harmonized efforts and collaboration to manage this public health crisis.

One of the hurdles regarding COVID-19 vaccine development is how to decide upon the most appropriate platform to target SARS-CoV-2. It has not been determined which kind of platform would be the most effective against the novel coronavirus. Therefore, all vaccine platforms are being explored as COVID-19 vaccine candidates. Inactivated, attenuated, and recombinant vaccines using pathogens or antigenic proteins have been successful for targeting other infectious diseases. Novel platforms such as viral vector vaccines or nucleic acid vaccines have not been sufficiently used to fully establish their safety and efficacy in humans, but these platforms have been used worldwide as major vaccines for COVID-19. There are 135 candidates in the clinical development stage, of which viral vector vaccines and nucleic acid vaccines account for about 43.0% (36 nucleic acid platforms and 22 viral vector platforms) as of December 3, 2021 (Table 3).

Unlike chemically synthesized pharmaceuticals, vaccines are manufactured from biological sources. They should be produced consistently and show comparability from lot to lot, demonstrating clinical efficacy, immunogenicity, and safety. Furthermore, since vaccines are biological products with a complex nature and inherent potential for variability, it is difficult to produce vaccines with consistency, safety, and effectiveness even under similar manufacturing conditions. Therefore, an independent review of manufacturing and quality control data from each vaccine lot is essential to assure the consistent quality of manufactured lots [30]. Reliable and authorized guidance needs to provide support through regulatory requirements for quality management. Since the outbreak of the COVID-19 pandemic, regulatory agencies such as the WHO, Europe, United States, and Japan have provided high-quality documents in order to support COVID-19 vaccine developers currently working on candidate vaccines based on recent technologies, such as nucleic acid vaccines and recombinant viral vector vaccines. The Republic of Korea has published lot release guidelines on COVID-19 vaccine platforms (viral vector vaccines, mRNA vaccines) to harmonize the global regulatory requirements for lot release and to accelerate vaccine development [15,16].

This paper is intended to serve as guidance for regulatory systems dealing with the lot release. Furthermore, this paper also provides general considerations for quality control and tests applicable to vaccine development and quality evaluation using the viral vector platform. Lot release test items and methods provided are prepared based on drug approval and review cases, and national lot release cases for domestically approved replication-defective adenoviral vector vaccines. This information will help vaccine developers to minimize industry trials and expedite commercialization by providing predictable regulatory requirements for the same platform vaccine. However, it should be noted that quality control items and considerations may be different depending on the replication-competent viral vector and/or the nature of the vector backbone as the vaccine delivery system. The quality

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tests provided herein have been drawn up using available knowledge to date, and the content may be updated as needed to change the regulatory system and/or to reflect further experience gained with new products. Emerging infectious disease disasters such as the COVID-19 pandemic are expected to occur periodically. In order to accelerate vaccine development for novel infectious diseases such as COVID-19, technical support for globally harmonized quality requirements is urgently needed.

Notes

Ethics Approval
Not applicable.

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

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Authors' Contributions
Conceptualization: JHJ, JTH; Data curation: NL, ShK, SC, MY, JS, EL, SS, JHK; Writing-original draft: JHJ, NL, HJO; Writing-review & editing: JTH, HJO.

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Review Article

Worldwide prevalence of fungal coinfections among COVID-19 patients: a comprehensive systematic review and meta-analysis

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ABSTRACT

Microbial coinfections can increase the morbidity and mortality rates of viral respiratory diseases. Therefore, this study aimed to determine the pooled prevalence of fungal coinfections in coronavirus disease 2019 (COVID-19) patients. Web of Science, Medline, Scopus, and Embase were searched without language restrictions to identify the related research on COVID-19 patients with fungal coinfections from December 1, 2019, to December 30, 2020. A random-effects model was used for analysis. The sample size included 2,246 patients from 8 studies. The pooled prevalence of fungal coinfections was 12.60%. The frequency of fungal subtype coinfections was 3.71% for *Aspergillus*, 2.39% for *Candida*, and 0.39% for other. The World Health Organization's Regional Office for Europe and Regional Office for Southeast Asia had the highest (23.28%) and lowest (4.53%) estimated prevalence of fungal coinfection, respectively. Our findings showed a high prevalence of fungal coinfections in COVID-19 cases, which is a likely contributor to mortality in COVID-19 patients. Early identification of fungal pathogens in the laboratory for COVID-19 patients can lead to timely treatment and prevention of further damage by this hidden infection.

Keywords: Coinfection; Coronavirus; COVID-19; Fungi; Meta-analysis

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-COV-2), a novel coronavirus, is the causative agent of the recent coronavirus disease 2019 (COVID-19) pandemic [1,2], and it is mainly transmitted by respiratory droplets. The conventional method for diagnosing COVID-19 is a reverse transcription-polymerase chain reaction test [3], but other methods with similar nanotechnology-based strategies can be used for the detection of RNA viruses [4].

Microbial coinfections can increase the mortality rate of COVID-19 patients and lead to prolonged hospitalizations [5]. In particular, fungal coinfections pose a significant threat to COVID-19 patients [6]. Studies have indicated that the detection of fungal coinfections in patients infected with COVID-19 can be difficult and creates a challenging situation when deciding on the proper treatment for these patients; furthermore, fungal coinfections can remain undetectable even after death, thereby skewing estimates of mortality rates [7]. The clinical and radiological features of viral respiratory diseases, such as COVID-19, are similar to those of respiratory diseases caused by fungal pathogens, creating another challenge in diagnosing fungal coinfections [8]. The limited sensitivity of fungal pathogen detection tests is a real problem in identifying fungal pathogens, as exemplified by reports that approximately 50% of *Candida* infections are not detectable by blood culture [9]. Various studies have noted that coinfections with fungi such as *Aspergillus* and *Candida* can increase mortality rates, especially in critically ill patients [10] such as those hospitalized in critical care wards with COVID-19. Therefore, the prevalence of fungal coinfections in COVID-19 patients should be routinely monitored [10]. In the context of monitoring different types of coinfections among COVID-19 patients, this study aims to identify the prevalence of fungal coinfection. Our goal is to provide evidence that will be valuable for identifying coinfections in COVID-19 patients and help clinicians to provide suitable treatments, especially in the early stages of the disease.

Materials and Methods

This study was registered in the International Prospective Register of Systemic Reviews (CRD42021240030, https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=240030) [11]. All required steps of the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) protocol were conducted (Suppl. 1). We applied the keywords: COVID-19, coronavirus, SARS-CoV-2 infection, SARS-CoV-2, polymicrobial infection, fungal AND coinfections, fungal AND secondary infections, and mixed infections for all included studies.

Literature Search

An in-depth search without language restrictions was performed to find related articles in Web of Science, Medline, Scopus, and Embase. Other databases such as MedRxiv and Social Science Research Network (SSRN) were also reviewed to enhance the accuracy of the search and to obtain any other associated articles. The search period was December 1, 2019, to December 30, 2020. We used Medical Subject Headings (MeSH) keywords for searching the databases. The patient, intervention, comparison, outcome (PICO) framework was applied in this article, as described below for Medline, following the suggestion made by Salvador-Olivan et al. [12]: population/patient: COVID-19 patients; intervention/exposure: none; comparison: none; outcome: fungal coinfection.

The search strategy (Box 1) and keywords used for database searches (Box 2) are available in Suppl. 2.

In addition, we used Google Scholar to explore the gray literature, had a mycologist identify important research, and manually searched reference lists to find related articles. All extracted articles were exported to Endnote ver. X6 and duplicate articles were removed. The screening process was conducted in 3 phases. Articles were first reviewed by title, then by abstract, and finally the full texts were reviewed. To gain access to articles with data that were not publicly available, emails were sent to the corresponding authors. Articles with no response from the corresponding author were excluded. Two independent raters reviewed the 3 phases independently. A third evaluator resolved any inter-rater discrepancies. Study selection processes were conducted based on blinding and task separation. The kappa coefficient for the inter-rater agreement was 94%.

Inclusion and Exclusion Criteria

Epidemiological research assessing the prevalence of fungal coinfections among COVID-19 cases was recorded without restriction. We excluded case-control studies, clinical trials, and review studies. We also excluded case reports and case series studies with sample sizes less than 10.

Data Extraction

The author's name, type of study, year of the study, country, sample size, subjects' age and sex, the number of subjects, and fungal species were recorded from the eligible articles. Patients confirmed to have COVID-19 and fungal coinfection were included in the study.

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Variable Definition

We classified the fungal coinfections based on transmission mode and clinical features. The World Health Organization (WHO) regional classification, which included the Regional Office for Africa, the Regional Office for the Americas, the Regional Office for the Eastern Mediterranean, the Regional Office for Europe, the Regional Office for Southeast Asia, and the Regional Office for the Western Pacific, was used to categorize the countries.

Quality Assessment

The quality of the studies was evaluated using the Newcastle-Ottawa Scale [13]. Two researchers independently assessed the articles and, for each study, an overall score was determined. The studies were classified as very good, good, satisfactory, and unsatisfactory [14].

Statistical Analysis

We performed the statistical analysis with Stata ver. 14.0 (StataCorp., College Station, TX, USA). We also extracted the number of COVID-19 infection cases, the prevalence of COVID-19 with fungal coinfection, and the fungal species. The Cochran Q test was used to identify heterogeneity as quantified by the I^2 index, with $I^2 > 0.7$ indicating high heterogeneity. "Metaprop" commands were applied in a forest plot to estimate the pooled prevalence with a 95% confidence interval (CI). A random-effects model was used to estimate the pooled prevalence [15–20]. Meta-regression analysis was used to evaluate the effect of the WHO region, sample size, and age on heterogeneity. The "metabias" command was used to evaluate publication bias. We adjusted the prevalence rate with the "metatrim" command, using the trim-and-fill method if there was publication bias. Statistical significance was indicated by a $p < 0.05$.

Results

Medline, Scopus, Web of Science, and Embase searches resulted in 1,010 studies, and 41 articles were obtained from other sources. After removing duplicate papers, 510 studies remained. During the first round of screening, 301 studies were excluded, 114 studies were excluded in the second round, and 87 studies were excluded in the third round, leaving 8 studies [21–28] with a sample size of 2,246 cases. Figure 1 shows the selection process flow chart. The different features of the studies are illustrated in Table 1 and more details about coinfections by fungal type are available in Table S1 [21–28]. The European area had the highest number of studies on this subject ($n = 4$) and Southeast Asia provided the lowest number of studies ($n = 1$). All studies were published in 2020. Four case series, 1 cohort study, 1 letter to the editor, and 1 cross-sectional study were included in our research.

Pooled Prevalence of Fungal Coinfections in COVID-19 Patients

Table 1 shows the prevalence of fungal coinfections in the included studies [21–28]. Figure 2 illustrates the prevalence of fungal coinfections in a forest plot [21–28]. Hughes et al. [24] reported the lowest prevalence of fungal coinfections (prevalence, 0.36%; 95% CI, 0.07–1.05) in the United Kingdom and Intra et al. [25], in a study conducted in Italy, reported the highest prevalence of fungal coinfections (prevalence, 31.15%; 95% CI, 19.90–44.29). Figure 2 shows the estimated pooled prevalence of fungal coinfections (prevalence, 12.60%; 95% CI, 7.84–17.36; I^2, 96.1%; $p < 0.001$). For every 1,000 people infected with COVID-19, 78 to 173 experienced fungal coinfections.

Pooled Prevalence of Fungal Coinfections Based on Different Subgroups

Figure 3 provides prevalence data on fungal coinfections according in detail. The most frequent genus among fungal coinfections was *Aspergillus* (pooled prevalence, 3.71%; 95% CI, 0.54–9.11), with other taxa being the least frequent (pooled prevalence, 0.39%; 95% CI, 0.01–2.15). The pooled prevalence of *Candida* was 2.39% (95% CI, 0.92–4.42). The highest and lowest pooled prevalence of fungal coinfections occurred in the Regional Office for Europe and Regional Office for Southeast Asia regions, with 23.28% (95% CI, 1.67–58.41) and 4.53% (95% CI, 3.01–6.52), respectively. The pooled prevalence for the Regional Office for the Western Pacific is presented in Figure 3.

Heterogeneity and Meta-Regression

Significant heterogeneity was found among the studies, as shown in Table 2 ($p < 0.001$). The I^2 index for total fungal coinfections and different species was 85%. Meta-regression showed that the WHO region size (coefficient, −0.0118; $p = 0.924$), sample size (coefficient, −0.001; $p = 0.132$), age (coefficient, −0.0171; $p = 0.110$) and quality score (coefficient, 0.001; $p = 0.297$) had no significant effect on the heterogeneity of the studies (Figure 4).

Publication Bias

The Egger test showed no significant publication bias in the present meta-analysis.

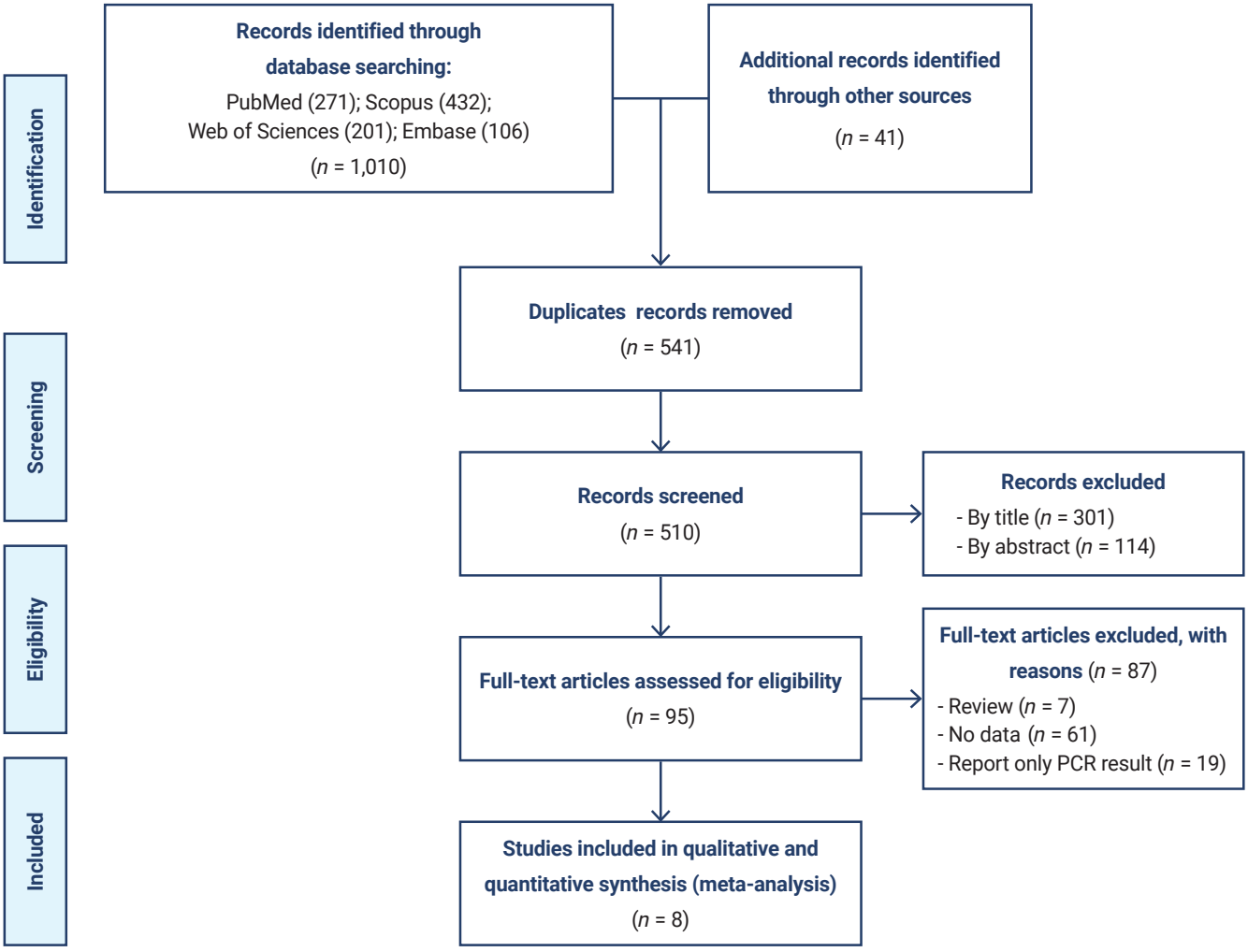

Figure 1. Study flowchart based on the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guideline.
PCR, polymerase chain reaction.

Table 1. Characteristics of studies included in the meta-analysis

Study	Country	Design	Publication year	Mean age (y)	Sample size	Fungal coinfection prevalence (%) (95% CI)
Zhu et al. [21]	China	Retrospective case series	2020	-	257	23.35 (18.31–29.00)
Agrifoglio et al. [22]	Spain	Letter to the editor	2020	58.7	139	10.79 (6.17–17.17)
Chowdhary et al. [23]	India	Cross-sectional	2020	-	596	2.52 (1.41–4.11)
Hughes et al. [24]	United Kingdom	Retrospective case series	2020	69.5	836	0.36 (0.07–1.05)
Intra et al. [25]	Italy	Retrospective case series	2020	-	61	31.15 (19.90–44.29)
Segrelles-Calvo et al. [26]	Spain	Cohort	2021	59.6	215	22.79 (17.36–28.99)
Wang et al. [27]	China	Retrospective case series	2020	73	104	7.69 (3.38–14.60)
Zhang et al. [28]	China	Retrospective cohort	2020	64.76	38	15.79 (6.02–31.25)

CI, confidence interval; -, not reported.

Discussion

The fungal microorganisms in hospitals are neglected factors contributing to the overall health risks of hospitalized patients [29].

Opportunistic infections can often be seen in patients

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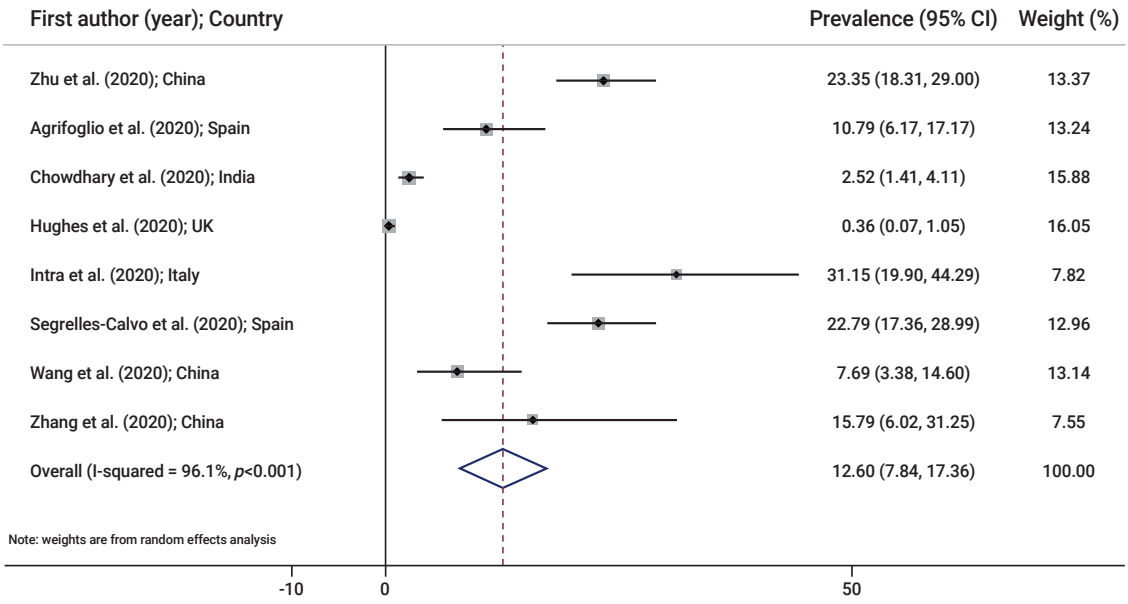

Figure 2. Forest plot of fungal coinfections among coronavirus disease 2019 cases. Each study is denoted in terms of author, year, and region. Each line sector's midpoint shows the estimated prevalence, the length of the line segment shows the 95% confidence interval (CI), and the diamond shows the pooled estimate.

infected with respiratory viruses [30]. Microbial agents including *Acinetobacter, Klebsiella, Enterobacter, Aspergillus*, and *Candida* are common causative agents of secondary infections in COVID-19 patients [5]. Invasive fungi play an important role among the different types of microbial pathogens causing coinfections in COVID-19 patients and are associated with increased mortality rates [31]. Invasive fungal infections, including *Candida* and *Aspergillus* infections, are common among immunocompromised patients in critical condition [32]. Respiratory viruses and fungal pathogens have similar clinical features, which may create difficulties for clinicians in their differential diagnosis, especially at the early onset of the disease [33]. For example, diagnosing a blood infection with *Candida* is challenging, even today, because of the low number of pathogens in the infected tissue. An estimated 50% of candidiasis cases cannot be detected by blood culture [34]. This problem is critical because it can interfere with determination of the correct drug to prescribe for patients in the initial stages of the infection [35]. The early detection of COVID-19-associated pulmonary aspergillosis (CAPA) has become problematic. A study in France reported negative serology and culture tests for *Aspergillus* coinfection in COVID-19 patients during the initial stage of the viral infection, and the CAPA was only confirmed after the patients' deaths [36]. This report shows the importance of early detection of fungal coinfections and the impact of a late diagnosis on increasing the mortality rate. Fungemia has been reported as a complication of SARS-CoV-2 infection [37]. Another risk factor for fungal coinfection could be probiotic consumption, especially in patients admitted to the intensive care unit (ICU). Two patients admitted to the ICU presented with blood infections from *Saccharomyces cerevisiae* after receiving a probiotic supplement. Data suggested that damage to the intestinal mucosal barrier due to the COVID-19 infection created an opportunity for the fungi to relocate from the probiotics to the bloodstream and cause a blood infection. The study suggests avoiding consumption of prophylactic probiotics by COVID-19 patients in the ICU [38]. Central venous catheterization, frequent use of antibiotics, and steroid therapy are risk factors for fungal coinfection [39]. Different types of antifungal treatment have been used to treat COVID-19 patients infected with fungal pathogens. Antifungal drugs including amphotericin B, micafungin, and fluconazole are some of the main drugs prescribed to treat fungal coinfections [5].

Among *Candida* species, *Candida albicans* is the organism most frequently found in critically ill COVID-19 patients. *Candida auris* is the second most common species causing invasive candidiasis [39,40]. Resistance to antifungal therapy is another problem reported in the studies of fungal infection. Multiple studies reported differing percentages of antifungal resistance in *C. auris*. One study indicated that 67% of the patients who died from an invasive *C. auris* coinfection had received micafungin, yet manifested persistent candidemia [23]. *Aspergillus* is another frequent

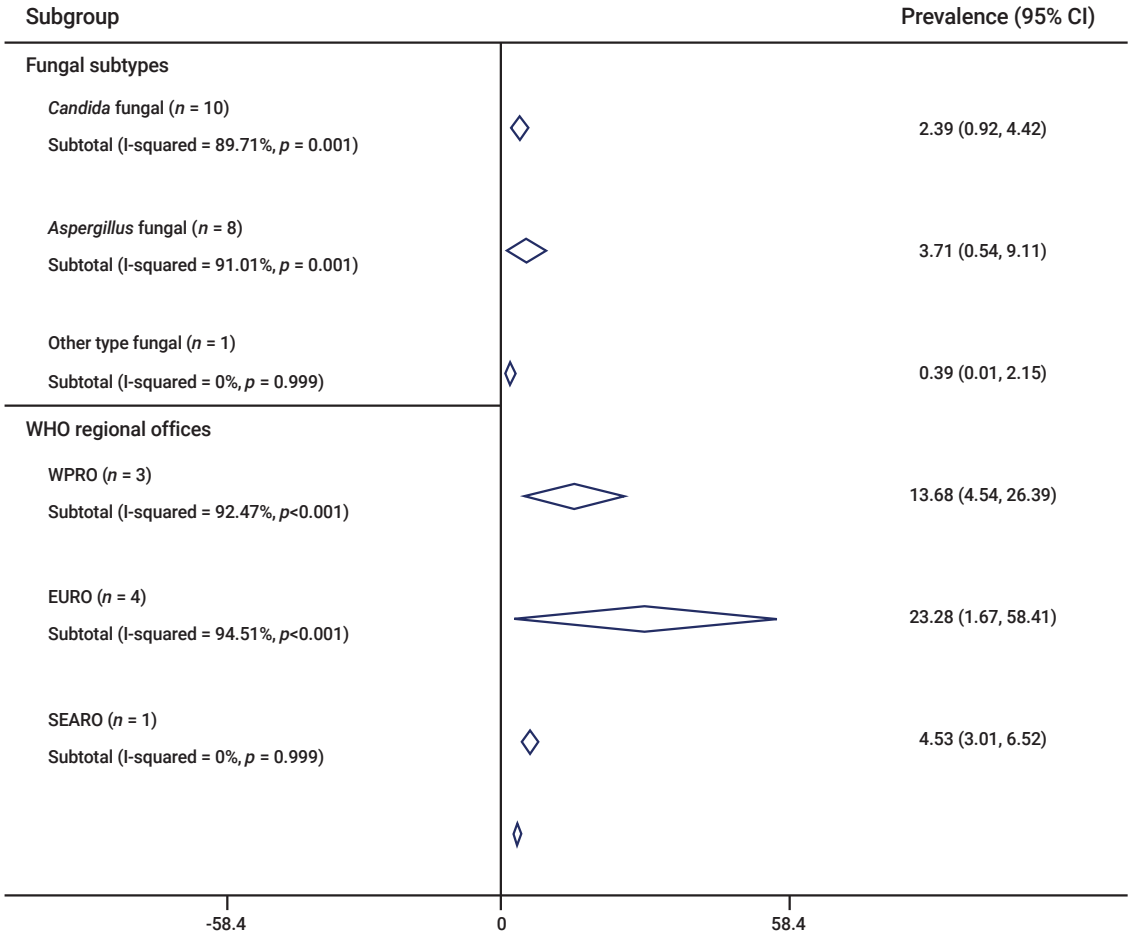

Figure 3. Pooled prevalence with 95% confidence intervals (CIs) and heterogeneity indices of coronavirus disease 2019 fungal coinfection cases based on fungal taxa and geographic area. The diamond shows the pooled prevalence and the diamond length indicates the 95% CI.
WHO, World Health Organization; WPRO, Regional Office for the Western Pacific; EURO, Regional Office for the European Region; SEARO, Regional Office for Southeast Asia.

Table 2. Meta-regression results for the identification of heterogeneity determinants in the studies

Variable	Coefficient	95% CI	*p*-value
Age (y)	−0.0171	−0.0414 to 0.007	0.110
WHO region (score)	−0.0118	−0.304 to 0.280	0.924
Sample size (*n*)	−0.001	−0.001 to 0.001	0.132
Quality score	0.001	−0.002 to 0.002	0.297

CI, confidence interval; WHO, World Health Organization.
Coding of WHO regions: Regional Office for the Western Pacific (WPRO), Regional Office for Europe (EURO), Regional Office for Southeast Asia (SEARO).

opportunistic fungal genus. A study conducted in England showed invasive *Aspergillus* infection to be an underestimated danger in COVID-19 patients. Our study showed that COVID-19 patients had a 12.6% coinfection rate with *Aspergillus* [41]. A recent study showed that patients infected with the influenza virus who presented with severe acute respiratory distress syndrome (ARDS), rapidly developed invasive pulmonary aspergillosis (IPA). This finding is concerning because almost 40% of COVID-19 cases develop ARDS and are therefore susceptible to IPA [42]. A study conducted in China reported a 23.3% rate of *Aspergillus* coinfection among COVID-19 patients [21].

Other studies indicated lower rates of *Aspergillus* coinfection among SARS-CoV-2 cases, ranging from 3.2% to 5%. It is crucial to note that none of these articles mentioned the specific diagnostic procedures necessary to confirm the presence of fungal pathogens in patients. This may have been because of low sensitivity or the limited availability of gold standard tests during the studies [42,43]. CAPA has been widely reported in the European region, up to 35% [44]. The

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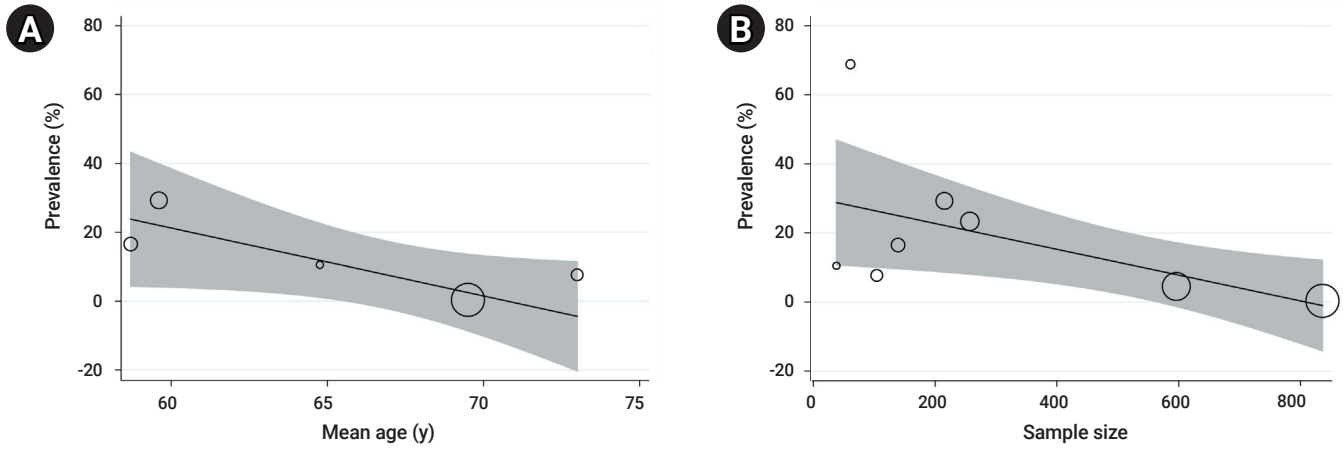

Figure 4. The relationship between age (A) and sample size (B) with the prevalence of fungal coinfections using meta-regression. The circle's size indicates the study's precision. There is no significant association of the prevalence of fungal coinfections with age or sample size.

overall mortality rate among reported case series with confirmed CAPA ranged from 44.5% to 66.7% [42]. A meta-analysis performed on fungal coinfection studies reported the overall proportion of fungal coinfections in COVID-19 patients was 0.12%, the overall proportion of *Aspergillus* was 0.06%, and the overall pooled mortality rate was 0.17%. This study also showed a significant difference when comparing ICU cases with mixed hospitalized populations (0.36 vs. 0.06) [45]. Another study reported a 4% fungal coinfection rate among COVID-19 cases and an 8% rate of fungal superinfections [46]. Our results showed more consistency, with a uniformly high prevalence of fungal coinfections among COVID-19 patients. We found that *Aspergillus* was the most frequent fungal taxon in COVID-19 cases and candidiasis the second most frequent fungal coinfection. We also found that the Regional Office for Europe reported the highest coinfection rate (23.28%) and that the Regional Office for Southeast Asia reported the lowest coinfection rate (4.53%). As previously mentioned, the low rate of reported coinfections in Southeast Asia (especially China) could be because there was a lack of detection equipment or the specific or gold standard tests were not performed to detect fungal coinfections. Our results also support the practical use of antifungal treatment, especially in the early stages of COVID-19 infection, to reduce the mortality rate for critically ill patients. It should be noted that the results of some studies were based on small sample populations and therefore do not provide robust evidence from which to draw conclusions.

Strengths and Limitations

We faced some limitations while performing our analysis. First, we could not conduct a sex-specific estimation because of limited data in the primary research. Secondly, we estimated the pooled prevalence based on geographical data provided by the WHO. Therefore, our estimations in the spatial analysis of the different geographical areas [47–51] were not robust because of inconsistent study numbers. It should be noted that many of the studies suffered from significant sources of bias, but we assessed the quality of the included studies and considered the effect of the quality score as a source of heterogeneity. The meta-regression analysis showed that the quality score did not affect the pooled frequency estimates. Finally, in many instances, the analysis was based on very few studies. Therefore, the evidence supporting the results is low, and the findings must be interpreted with caution. Nonetheless, strength of this study is that it performed a comprehensive search and meta-analysis to determine the pooled prevalence of different fungal subtypes. Another limitation in our study is the significant heterogeneity and difficulty in interpreting results due to different sets of data, including various regimens, drug doses, durations, center settings, and population samples. The fact that many of the reviewed studies suffered from significant sources of bias should also be taken into consideration.

Conclusion

Our results showed that 12.60% of COVID-19 patients were infected with fungal pathogens. We also found that *Aspergillus* and *Candida* were the most frequent fungal genera among the patients. Due to the difficulties in detecting fungal coinfections, particularly in the initial stages of COVID-19 infection, we support the routine use of antifungal treatments in COVID-19 patients.

Supplementary Material

Table S1. Details of fungal coinfections in included studies; Suppl. 1. PRISMA 2020 checklist; Suppl. 2. Search strategy for Medline (MeSH, Medical Subject Headings). Supplementary data are available at https://doi.org/10.24171/j.phrp.2021.0293.

Notes

Ethics Approval
Not applicable.

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

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None.

Availability of Data
All data extracted and analyzed during this study are included in this published article. For other data, these may be available through the corresponding author upon reasonable request.

Authors' Contributions
Study Design and Idea: SS; Conceptualization: JM, NJ, MZ, RS; Data curation: SAR; MZ, SF; Formal analysis: RP, PM, RS; Funding acquisition: all authors; Investigation: IP, SAR, SF; Methodology: RP, PM; Project administration: RS, SS, RP; Resources: JM, NJ, SF; Software: RP, PM; Supervision: IP, MV, SS, RP; Validation: JM, NJ, MV; Visualization: MV; Writing–original draft: all authors; Writing–review & editing: all authors.

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Review Article

Yersinia pestis antibiotic resistance: a systematic review

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ABSTRACT

Yersinia pestis, the cause of plague and a potential biological weapon, has always been a threatening pathogen. Some strains of *Y. pestis* have varying degrees of antibiotic resistance. Thus, this systematic review was conducted to alert clinicians to this pathogen's potential antimicrobial resistance. A review of the literature was conducted for experimental reports and systematic reviews on the topics of plague, *Y. pestis*, and antibiotic resistance. From 1995 to 2021, 7 *Y. pestis* isolates with 4 antibiotic resistance mechanisms were reported. In *Y. pestis* 17/95, 16/95, and 2180H, resistance was mediated by transferable plasmids. Each plasmid contained resistance genes encoded within specific transposons. Strain 17/95 presented multiple drug resistance, since plasmid 1202 contained 10 resistance determinants. Strains 16/95 and 2180H showed single antibiotic resistance because both additional plasmids in these strains carried only 1 antimicrobial determinant. Strains 12/87, S19960127, 56/13, and 59/13 exhibited streptomycin resistance due to an *rpsl* gene mutation, a novel mechanism that was discovered recently. *Y. pestis* can acquire antibiotic resistance in nature not only via conjugative transfer of antimicrobial-resistant plasmids from other bacteria, but also by gene point mutations. Global surveillance should be strengthened to identify antibiotic-resistant *Y. pestis* strains by whole-genome sequencing and drug susceptibility testing.

Keywords: Drug resistance; Plasmids; *rpsl* gene mutation; *Yersinia pestis*

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Introduction

Yersinia pestis, an obligate parasite and Gram-negative bacterium, is the causative agent of plague and has killed an estimated 200 million people in 3 major pandemics in the 14th, 17th, and 19th centuries. The first pandemic, known as the Black Death, killed more than 30% of Europe's population. Alexandre Yersin, a bacteriologist, discovered it during a plague investigation in Hong Kong in 1894 [1]. Plague is now rare, and it is generally considered as a disease of the past. However, it still appears in many areas, including Asia, Africa, and

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America, in the 21st century, and it remains a threat to public health [2]. Every year, at least 2,000 cases of plague are documented. *Y. pestis* can grow within a wide range of temperatures, and its optimal pH for growth is 7.2 to 7.6 [3]. It dies rapidly when exposed to ultraviolet light, intensive desiccation, temperatures exceeding 40°C [4].

It is generally accepted that *Y. pestis* evolved from *Yersinia pseudotuberculosis* 1,500–2,000 years ago [5]. However, *Y. pestis* was recently found in the remains of a hunter-gatherer from 5,000 years ago via ancient DNA analysis, suggesting that *Y. pestis* emerged about 7,000 years ago, which is substantially older than considered before [6]. This early strain isolated from ancient human remains seems to be less virulent and less contagious than later strains of *Y. pestis* [6]. *Y. pestis* strain CO92 was the first to be sequenced [7], and other strains have been sequenced subsequently [8–11]. Specific characteristics of the genome and molecular mechanisms of *Y. pestis* contribute to its infection of fleas, virulence, and subsistence against the host immune response [12].

According to biologists, many wild animal species are highly susceptible to *Y. pestis*. It lives in small rodents that are mostly found in rural and semi-rural areas of Africa, Asia, and the United States. The effects of plague on these populations are unknown, but certain characteristics make rodent species more vulnerable to the disease. However, due to a series of gene mutations, gene acquisition and loss, the molecular mechanisms and lifestyle of *Y. pestis* have changed dramatically [5]. *Y. pestis* contains a particular group of virulence factors that enable colonization in the flea and allow growth and subsistence in mammal macrophages [13]. For instance, it evolved to lose major genes for better adaptation to the flea vector. Its virulence determinants for hosts are mainly contained on 3 well-characterized plasmids (pYV/pCD1, pPla/pPCP1 and pFra/pMT), as well as in the pathogen's chromosome [12]. Specific virulence proteins are encoded, such as Yersinia outer membrane proteins (Yops) and the protease Pla, which cause host cell death and escape from phagocytosis by macrophages through multiple sophisticated strategies [14]. In addition to the well-known virulence genes, novel virulence factors have been detected in recent years. With the introduction of genome-wide fitness profiling and mutant screening methods, more genes were identified as being involved in bacterial intracellular survival in the early stage of bubonic plague, as well as in the infection process [15,16]. Gene sequencing not only revealed the genetic information of *Y. pestis*, but also phylogeographic information on its evolution. An analysis of 345 core genome sequences from *Y. pestis* based on the National Center for Biotechnology Information (NCBI) international database and genome sequencing revealed 3,315 single-nucleotide polymorphisms (SNPs). Combining the results of phylogenetic trees and strain foci, the evolution of *Y. pestis* mainly occurred in Asia, including China, Mongolia, and Middle East [11].

Materials and Methods

Literature Search

The systematic review follows the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Figure 1). A literature search was performed in Medline, Embase, and Web of Science from their inception to December 2021. Firstly, articles were identified using the key terms "*Yersinia pestis*" or "Plague." These words were then combined in a second search with the subject headings "Antibiotic Resistance," "Antimicrobial Resistance," or "Drug Resistance" to identify references related to *Y. pestis* antibiotic resistance.

In total, 1,475 potentially related publications were identified through the database search. Twenty-four other articles were identified via a manual review of article references. As a result, 1,499 articles were screened based on the title, abstract, and content. All publications that contained content on the life cycle/transmission of *Y. pestis* and its antibiotic resistance were included, along with those that dealt with the clinical manifestations and diagnosis, latest treatment, and prevention of plague. Finally, 68 full-text articles were included in this systematic review. The flow chart of the literature search is shown in Figure 1.

Results

Epidemiology

As an obligate parasite, the life cycle of *Y. pestis* consists of rodents, transmission from fleas to animal reservoirs, and transmission between mammal hosts. Fleas feed on a rodent infected with *Y. pestis*, which survive within the flea gut. The moving vector then delivers the bacteria intradermally through a bite into a new mammalian host. Lagomorphs, artiodactyls, carnivores, hyracoids, insects, marsupials, and primates can be infected [4]. The movement of *Y. pestis* through different types of mammals promotes the distribution of plague between areas [17]. Humans can be infected with *Y. pestis* by flea bites, contact with infected animals, and intake of undercooked contaminated meat. Therefore, people surrounded by animals are more likely to be infected. Moreover, plague can be transmitted between people by respiratory droplets from the coughs of infected

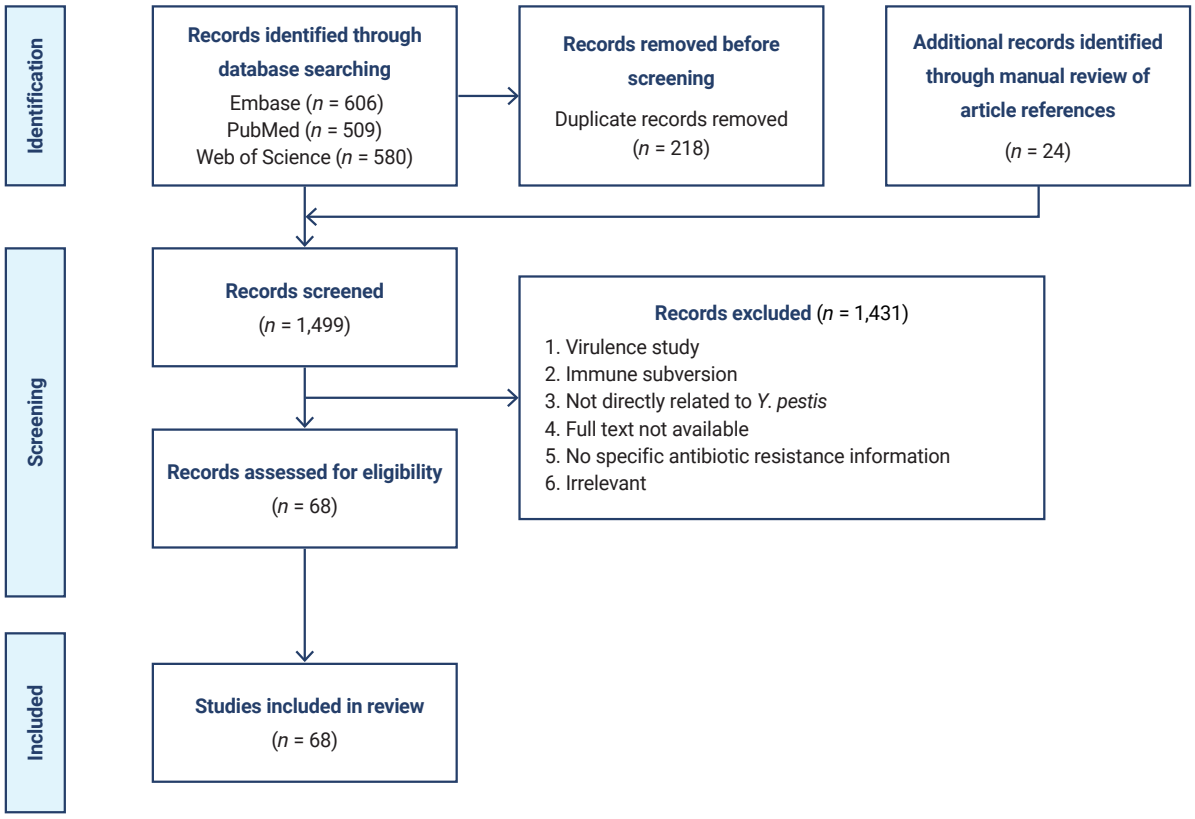

Figure 1. Systematic review flow chart of the literature search. Boxes on the left: steps of the literature search, including identification, screening, and included. Boxes in the middle: n refers to the number of articles remaining after each step.

patients [18].

When *Y. pestis* enters *in vivo*, the infection begins (Figure 2). Firstly, it can enter neutrophils and macrophages actively or passively. It has generally been thought that *Y. pestis* bacteria are killed in neutrophils, while they can live on and proliferate within macrophages [19]. Nevertheless, it has been proven that *Y. pestis* can also survive and duplicate before the type III secretion system (T3SS) is upregulated at the body temperature of fleas [20]. After that, infected neutrophils would be ingested by macrophages in a cleaning process known as efferocytosis [12]. During the efferocytosis period, a cytokine named 1L-1RA is secreted, which blocks the inflammatory response and upregulates the T3SS [21]. This may partly explain why *Y. pestis* adapts to the extracellular lifestyle with antiphagocytic abilities even after escaping from macrophages. Thus, it is difficult for phagocytes to ingest the bacteria in the late stage of the infection. As the bacteria arrive at the target organs via lymphatic system, virulence factors such as Yops translocate into the host cells through T3SS, which both activates the signal-transducing pathway and promotes contact between Yops and host cells [13].

Detection and Diagnosis of Plague

A rapid diagnosis is required to ensure that plague-suspected patients receive timely medical administration to reduce the risk of mortality. Several biological diagnostic tests have been developed to identify *Y. pestis*. Isolation of this pathogen from clinical specimens remains the gold standard for plague diagnosis according to World Health Organization recommendations [12]. The use of selective cefsulodin-irgasan-novobiocin medium under an appropriate culture temperature are essential to isolate the bacteria [22]. Nonetheless, this method is quite time-consuming (about 3 to 5 days) and labor-intensive [12]. Therefore, automatic detection methods have been developed for faster diagnostic results. The F1 antigen has been widely adopted as the target for discriminating *Y. pestis*, since it is the typical immunogenic protein of the pathogen [23–25]. The F1 dipstick assay and enzyme-linked immunosorbent assay have been developed to identify the F1 antigen, and these methods have been proven practical with trained staff at local points of care [12]. However, these methods that depend on an immunological reaction have some limitations, including low sensitivity and delayed

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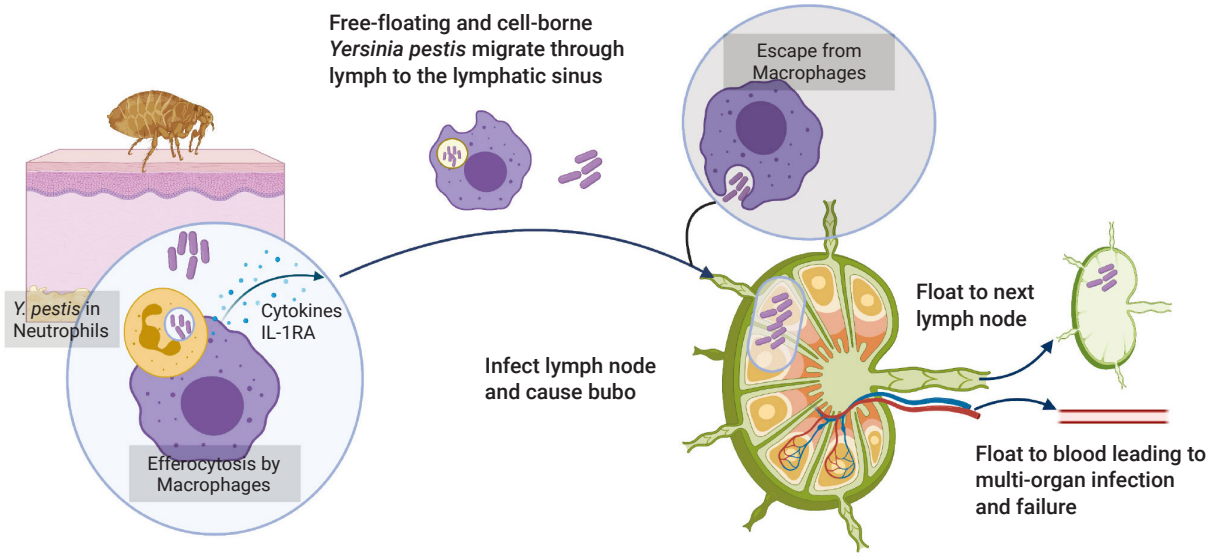

Figure 2. Life cycle of *Yersinia pestis in vivo*.

detection [26,27]. The detection results may be negative if samples are collected in the early stage of infection when massive multiplication has not occurred inside the host [27]. Other detection methods, such as phage lysis and mass spectrometry, are sometimes misleading [12,28].

Molecular genetic tests have been developed to reduce delays in detection and increase the sensitivity of diagnostics. Conventional polymerase chain reaction (PCR) targeting the *caf1, pla, yopM*, and *inv* gens can offer results within 3 to 4 hours [12]. Nonetheless, PCR-based diagnostic methods need to be performed in a laboratory with level II biosafety, technical expertise, and specialized instruments [29,30]. Thus, portable real-time quantitative PCR thermocyclers have been invented to enable medical staff to perform tests in the field without special equipment [18]. The recombinase polymerase assay and loop mediated isothermal amplification (LAMP) assay have been developed to make the tests easier and more efficient than conventional PCR [26]. A LAMP assay pair on *caf1* and *3a* has been described, but it was only tested in artificially spiked human blood samples [26]. Another LAMP assay targeting the *caf1* gene was developed for detection of *Y. pestis* in plague biological samples. The sensitivity and specificity of this test were quite reliable compared with the gold standard [31]. In fact, the diagnosis of *Y. pestis* is generally confirmed by a combination of several detection methods. Culture-based identification offers provisional results, while molecular biology tests or antigens detection tests provide more precise results [27].

Clinical Manifestations

There are numerous specific clinical manifestations of plague depending on the route of transmission [32]. Three major types of plague are generally reported: bubonic, septicemic, and pneumonic [33]. As the most frequent form, bubonic plague is characterized by swollen regional lymph nodes and usually occurs after infected flea bites. In some cases, patients may suffer red and hot skin, headache, fever (up to 39°C), chills, malaise, and severe pain of the lymph nodes near the flea-bite region [18]. Nausea, emesis, and dizziness are less-common manifestations. Without treatment in time, *Y. pestis* can spread to the lungs and other organs, leading to more severe clinical symptoms [4]. Septicemic plague occurs when the blood is infected. This type may arise primarily and secondarily to bubonic plague, generally accompanied by systemic symptoms, including sudden chills, high fever, multiple organ failure, nausea, and diarrhea. Disseminated intravascular coagulation, purpura and acral cyanosis, necrosis, and hemorrhage in the skin and serosal surfaces may present later.

The most virulent and dangerous type is pneumonic plague, which results in nearly 100% mortality and is easily transmissible via airborne droplets. The incubation period is quite short, usually 1 to 3 days, after large-scale exposure to *Y. pestis*. This form manifests with the abrupt onset of chills, fever, weakness, headache, chest discomfort, severe coughing, and sometimes bloody or watery mucus [34].

Other forms of plague are rarely reported. Pharyngeal plague sometimes occurs when people eat uncooked meat contaminated by *Y. pestis* [4]. This form of plague may present with distinctive clinical symptoms, including acute pharyngitis and tonsillitis, accompanied by enlargement of the cervical lymph nodes [35]. Gastrointestinal plague is

transmitted in the same way, and it manifests as vomiting, abdominal pain, diarrhea, tenesmus, and mucous stool accompanied with systemic symptoms [36]. Cutaneous plague is also described in rare cases. The infection is transmitted through flea bites, with the rapid formation of herpes and pustules [18].

Treatment

Although plague, especially the pneumonic form, can cause high and rapid mortality, the disease can be treated with early antimicrobial treatment [37]. Based on a systematic review of 762 published clinical cases from 1937 to 2019, aminoglycosides, tetracyclines, fluoroquinolones, and sulfonamides proved to be effective against plague. However, the case fatality rate of patients treated with sulfonamides was slightly higher, which is likely due to the time period, since sulfonamides were generally used from 1937 to 1949. Fluoroquinolones have been exclusively administered since the 1980s, when they were developed [38]. According to the latest recommendations on antimicrobial treatment for plague from the Centers for Disease Control and Prevention (CDC), aminoglycosides and fluoroquinolones are the mainstays of antimicrobial treatment for plague, while tetracyclines, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMX) could also be options for treatment depending on the age and pregnancy status of the patient [37]. Nevertheless, a study of plague in Vietnam revealed that patients treated with TMP-SMX had a longer duration of fever and some developed complications [38]. Furthermore, the World Health Organization does not recommend chloramphenicol as plague therapy considering its severe adverse effects, including reversible bone marrow suppression, aplastic anemia, and "gray baby" syndrome. Tetracyclines have few major adverse events, but they are contraindicated for pregnant women and infants [39]. Table 1 displays the first choices of treatment protocols for plague from the CDC [37]. Apart from doxycycline (for bubonic and pharyngeal plague), the other antibiotics in the table can be used to treat all forms of plague.

Prevention

Evidence shows that pneumonic and septicemic plague spread more easily from person to person because bloody sputum and aerosols mixed with *Y. pestis* are infectious [40]. Preventive measures comprise physical isolation, oral prophylactic antibiotics, and vaccines.

Patients are recommended to stay physically isolated for the initial 48 hours during antibiotic treatment until they improve clinically. Face masks, gloves, and eye protectors are standard precautions against respiratory droplets [41].

Close contacts (<2 m) of patients infected with pneumonic plague and individuals exposed to airborne droplets are at high risk of infection [34]. Therefore, they are suggested to receive orally administered ciprofloxacin, levofloxacin,

Table 1. Current recommended treatment protocol from the CDC

Antibiotic	Dose		Duration (d)	Route of administration
	Adult	Child		
Ciprofloxacin	400 mg/8 h	10 mg/kg per 8 or 12 h (maximum 400 mg/dose)	7–10	IV
	750 mg/12 h	15 mg/kg per 8 or 12 h (maximum 500 mg/dose per 8 h or 750 mg/dose per 12 h)		PO
Levofloxacin	750 mg/24 h	Weight < 50 kg: 8 mg/kg per 12 h (maximum 250 mg/dose)	7–10	IV or PO
		Weight ≥ 50 kg: 500–750 mg per 24 h		
Moxifloxacin	400 mg/24 h	Alternative option	7–10	IV or PO
Doxycycline (for bubonic and pharyngeal plague)	200 mg loading does, then 100 mg/12 h	Weight < 45 kg: 4.4 mg/kg loading dose, then 2.2 mg/kg every 12 h (maximum 100 mg/dose)	7–10	IV or PO
		Weight ≥ 45 kg: same as adult dose		
Gentamicin	5 mg/kg every 24 h	4.5–7.5 mg/kg every 24 h	7	IV or IM
Streptomycin	1 g/12 h	15 mg/kg every 12 h (maximum 1 g/dose)	7	IV or IM

Based on [37].
Pediatric regimens are for children aged ≥1 month to ≤17 years.
CDC, Centers for Disease Control and Prevention; IV, intravenous; PO, by mouth; IM, intramuscular.

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doxycycline, and moxifloxacin as the first options for 7 days according to the CDC recommendations [37]. However, in a mass casualty setting, doxycycline, ciprofloxacin, and tetracycline are suggested as the preferred choice for postexposure prophylaxis based on the suggestions of Working Group on Civilian Biodefense. In contrast, gentamicin or streptomycin are recommended when a modest number of patients need treatment [42].

Vaccination is another precautionary method against the plague. A live attenuated vaccine was invented and applied in 1931 in Madagascar and remained in use in Russia and China [43]. In addition, a killed whole-cell vaccine was licensed and used by the United States Army. However, it was not continued since studies proved that this type of vaccine was only effective against bubonic plague without the ability to prevent pneumonic plague [34]. Most advanced research in recent years focused on molecular vaccines against pneumonic plague. The F1 and V antigens of *Y. pestis* were combined, since some strains do not contain the F1 antigen [44]. Animal trials showed that 2 doses of the F1/V vaccine could completely protect mice from pneumonic and bubonic plague. Nevertheless, no tests of the F1-V were conducted in humans [12]. Additionally, DNA vaccines have been developed as part of a prime-boost strategy by attenuating *Y. pestis* strains with genetic engineering, but none have progressed to the clinical stage [4]. An oral vaccine with live attenuated *Y. pseudotuberculosis* strains, designated as Yptb5, was recently developed. Although this mutant can provide complete protection against a pulmonary challenge with 5.5×10^3 colony-forming units (CFU) of *Y. pestis*, while partial protection (50% survival) against $100LD_{50}$ of *Y. pestis* [45].

Antibiotic Resistance

Antibiotic resistance or antimicrobial resistance (AMR) refers to the ability of microbes (including bacteria, fungi, and parasites) to resist antibiotics, making antimicrobial drugs ineffective [46]. Since antimicrobials are the primary options for plague treatment and prophylaxis, antibiotic resistance in *Y. pestis* increases the risks posed by this disease and makes it more challenging to control. Understanding the mechanism of AMR in *Y. pestis* is the key to solving this problem.

Y. pestis strains with antibiotic resistance

To date, 5 *Y. pestis* strains with unique AMR and multidrug resistance (MDR) were isolated and identified in Madagascar with complete genome sequencing (Table 2) [47]. In addition, 1 streptomycin-resistant strain of the bacterium was found, and the genome was recently sequenced in the Tibetan region of China [48].

Y. pestis strains 12/87, 56/13, 59/13, and S19960127 exhibited high streptomycin resistance due to a novel mechanism, namely a ribosomal protein S12 gene (*rpsl*) mutation. For isolates 56/13, 59/13, and 12/87, the minimum inhibitory concentrations (MICs) were over 1,024 μg/mL, sharing 1 SNP (position 215,373 in the CO92 reference genome). However, strain 12/87 was highly distinct from strains 56/13 and 59/13 based on a phylogenetic analysis. However, as the first strain discovered exhibited the novel mechanism of antibiotic resistance, S19960127 displayed quite high resistance to streptomycin (MIC = 4,096 μg/mL). It shared the same SNP (215373) as those found in Madagascar [47,48].

Plasmid-mediated high-level resistance to antibiotics was identified in strains 17/95, 16/95, and IP2180H (Table 2). Strain 17/95 presented resistance to at least 8 antimicrobial agents to different degrees, including streptomycin (MIC > 2,048 μg/mL), ampicillin (MIC = 2,048 μg/mL), kanamycin (MIC = 2,048 μg/mL), spectinomycin (MIC = 2,048 μg/mL), sulfonamides (MIC = 1,024 μg/mL), tetracycline (MIC = 1,024 μg/mL), minocycline (MIC = 512 μg/mL) and chloramphenicol (MIC = 128 μg/mL). However, it was susceptible to TMP [49]. *Y. pestis* 16/95 was only resistant to streptomycin (MIC = 1,024 μg/mL) and remained susceptible to others therapeutics or prophylactics against plague. Strain IP2180H exhibited high-level resistance to doxycycline (MIC > 16 μg/mL), while it was susceptible to other antibiotics such as ampicillin, kanamycin, moxifloxacin, levofloxacin, and imipenem [50].

Apart from the above strains, some reported AMR/MDR

Table 2. Strains of *Yersinia pestis* with antimicrobial resistance

Type of strains	Location of isolation	Year	Host of isolation	Resistance phenotype	Mechanism
12/87	Andapa in Madagascar	1987	Human	Streptomycin	*rpsl* gene mutation
17/95 (IP275)	Ambalavao in Madagascar	1995	Human	Multiple drug resistance	Plasmid IP1202
16/95	Ampitana in Madagascar	1995	Human	Streptomycin	Plasmid IP1203
S19960127	Tibet in China	1996	Human	Streptomycin	*rpsl* gene mutation
IP2180H	Antananarivo in Madagascar	1998	Rat	Doxycycline	Plasmid pIP2180H
56/13, 59/13	Faratsiho in Madagascar	2013	Humans	Streptomycin	*rpsl* gene mutation

strains, however, did not provide a genetic basis and transferability of resistance [51,52]. For instance, an MDR *Y. pestis* strain, MNG 3122, was isolated in Mongolia from a marmot. However, its antibiotic susceptibility profile was different from that of *Y. pestis* 17/95. It presented drug resistance to gentamicin, tetracycline, doxycycline, TMP-SMX, and chloramphenicol [52]. In addition, some AMR strains were discovered during 1996 to 1998 in Madagascar, including ampicillin-resistant and chloramphenicol-resistant isolates from a clinic in Mahajanga District. A tetracycline-resistant isolate from a rat and ampicillin-resistant isolate from a flea were discovered in Antananarivo District [51].

Recent studies on the detection of the strains of *Y. pestis* with AMR have been conducted (Table 3) [53–55]. PCR and susceptibility tests were primarily used. In the PCR method, various AMR-associated genes were targeted to determine the strains with resistance based on the resistance phenotype of *Y. pestis* of interest in a particular study. The results were all negative, as listed in Table 3 [53–55].

Table 3. Detection of antibiotic resistance in *Yersinia pestis* in different regions

Year of detection	Location/year of isolation	No. of isolates	Purpose	Method	Results
2006	China/1943–2005	271	Detect anti-streptomycin *Y. pestis*	PCR (*strA-strB* genes)	No targeted genes were detected
2010	North America/1943–2002	713	Detect MDR *Y. pestis*	PCR (IncA/C plasmid backbone)	No targeted genes were detected
2011	North and South America, Asia, Africa/1940–2007	392	Detect resistance against gentamicin, streptomycin, tetracycline, doxycycline, ciprofloxacin, levofloxacin, chloramphenicol and trimethoprim-sulfamethoxazole	Suspensibility test based on CLSI guidelines	Highly susceptible to tested antibiotics
2018	Qinghai province in China/1950–2016	282	Detect anti-streptomycin, anti-β-lactam, and anti-sulfonamide *Y. pestis*	PCR (*strAB* genes, *tem*/shv/ctx-m genes, *sul1*/*sul2*/*sul3* genes were targeted) & Kirby-Bauer disc agar diffusion method test based on NCCLS guidelines	No targeted genes. All strains sensitive to tested antibiotics

Based on [53–55].
PCR, polymerase chain reaction; MDR, multidrug resistance; CLSI, Clinical and Laboratory Standards Institute; NCCLS, National Committee for Clinical Laboratory Standards.

Transferability of antibiotic resistance in *Y. pestis*

The horizontal transfer of DNA between unrelated organisms is a significant source of variation that leads to new strains of bacterial pathogens. As discussed above, 3 isolates (17/95, 16/95, and IP2180H) presented antibiotic resistance attributed to conjugative plasmids with high rates of transferability, which could be spread to endemic and foreign plague populations, as well as other bacteria, in the same way [4]. These plasmids can be transferred horizontally along with other transferable elements, including transposons and integrons, leading to increased phenotypic diversity [56].

As a member of the A/C incompatibility group of plasmids, the antibiotic plasmid pIP1202 of *Y. pestis* 17/95 can transfer to *Y. pestis* 6/69cN and *Escherichia coli* K802N at frequencies of 1.5×10^{-2} and 1×10^{-2} per donor CFU, respectively. Meanwhile, pIP1202 can retransfer from *E. coli* to *Y. pestis* and *E. coli* at frequencies of 1.1×10^{-4} and 5.7×10^{-5}, respectively. The lack of stability of pIP1202 in *E. coli* can be attributed to the lower frequency of its transfer from *E. coli* to other strains [49].

As for the self-transferable plasmid pIP1203 in *Y. pestis* 16/95, high-frequency transfers were observed from 16/95 to *Y. pestis* 6/69cN and *Y. pseudotuberculosis* IP32790cN (3×10^{-1} and 1×10^{0} per donor CFU, respectively). Moreover, retransfer of the plasmid pIP1202 occurred from *Y. pestis* 6/69cN to *Y. pestis* 6/69cNR and *Y. pseudotuberculosis* IP32790cNR at frequencies of 2×10^{-1} and 5×10^{-1} per donor CFU, respectively. Plasmid pIP1203 was found to belong to the IncP group [57].

The mobile plasmid pIP2180H (a member of the IncH1group)

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can transfer from *Y. pestis* IP2180H to *Y. pestis* CO92 (1.5×10^{-1} per donor CFU), *Y. pseudotuberculosis* (5×10^{-2} per donor CFU) and *E. coli* (2×10^{-1} per donor CFU) at quite high transfer frequencies [50].

For strains carrying mutated *rpsl* genes, a study documented that these AMR *Y. pestis* isolates can be transmitted among individuals via respiratory droplets during a pneumonic plague outbreak [47]. Since bubonic plague can cause secondary pneumonic plague without appropriate treatment, an immediate surveillance system seems quite important to detect and identify patients infected with AMR strains.

Mechanisms of antibiotic resistance in *Y. pestis*

Plasmid-mediated antibiotic resistance

Plasmid IP1202 (182913bp) in *Y. pestis* 17/95 confers the ability to produce a series of proteins that inactivate antimicrobial agents. Its resistance mechanisms have constantly been revealed at the molecular level, as presented in Table 4 [42,49]. As this isolate remained susceptible to TMP, synergism did not occur between sulfonamides and TMP for plague treatment [42]. Some of the antibiotic resistance genes, including *aadA, sul1, tetRA,* and bla_{TEM-1}, were located in the Tn21 transposon [58], which has been commonly found as the carrier of diverse resistance genes [59].

Streptomycin resistance in *Y. pestis* 16/95 was due to the plasmid pIP1203 (40 kbp), in which the *strA* (801 bp) and *strB* (834 bp) genes encoded aminoglycoside 3"-O-phosphotransferase [aph(3")-*I*] and a 6-O-phosphotransferase [*aph(6)-I*] [57]. These 2 determinants were part of the Tn5393 transposon, which is generally found in phytopathogenic bacteria, such as *Snodgrassella alvi, Salmonella enterica serovar Typhimurium*, and *Aeromonas* spp. [60–62]. The inverted terminal repeat (IR), which is frequently found at the same position in the *str* genes in various DNAs, was identified downstream from the *strB* genes. Additionally, a partial sequence of the *tnpR* gene of Tn5393 was located upstream from *strA*. The sequences IR-*tnpR-strA-strB*-IR were inserted in the R751backbone [42].

Y. pestis IP2180H exhibited single resistance to doxycycline and susceptibility to levofloxacin, moxifloxacin, kanamycin, ampicillin, and imipenem. An additional 171 kbp plasmid was discovered in the strain, designated pIP2180H. Its antibiotic resistance was conferred by the production of tetracycline efflux protein encoded by *tetB* genes located in the Tn10 transposon in the plasmid. Tn10 also carried *tetR* and *tetC*, which coded for regulators. It was also highly homological to the pB71 plasmid, a multidrug-resistant plasmid in a *Salmonella enterica* strain. However, the pIP2180H plasmid did not present resistance to the same antimicrobial agents as the *S. enterica* strain did [50].

Gene mutation-associated streptomycin resistance

As a first-line treatment option against plague, streptomycin can bind to the aminoacyl-tRNA recognition site of 16S rRNA (rrs), impairing translational proofreading and leading to the blockade of protein synthesis and subsequent cell death of sensitive bacteria [63]. Ribosomal protein S12 (rpsl) increases the stabilization of the high-order pseudoknot structure formed by rrs; therefore, amino acid substitutions in the *rpsl* protein influence the conserved structure of rrs and induce the failure of interactions between streptomycin and rrs, conferring high-level streptomycin resistance [48,64].

Based on whole-genome sequencing and alignment analysis with the CO92 strain, 4 isolates (56/13, 59/13, 12/87 and S19960127) were found to have an *rpsl* gene mutation at 128 bp, resulting in the amino acid substitution of Lys to Arg at site 43 (K43R) in the *rpsl* protein [48]. Subsequently, as mentioned before, this mutation disrupted streptomycin binding to rrs by altering its tertiary structure.

Table 4. Antibiotic resistance determinants of the pIP1202 plasmid

Resistance phenotype	Mechanism	Resistance gene
β-lactams		
Ampicillin	A TEM-1 penicillinase	bla_{TEM-1}
Tetracycline	efflux	$tetRA_{classD}$
Phenicols		
Chloramphenicol	Type I chloramphenicol acetyltransferase	*catI*
Sulfonamides	Dihydropteroate synthase	*sul1*, *sul2*
Aminoglycosides		*aphA, aadA, strAB*
Kanamycin	Type I 3'-aminoglycoside phosphotransferase	
Streptomycin	3”-9-aminoglycoside adenylyl transferase	
Spectinomycin		

Based on [42,49].

Mutations associated with streptomycin resistance have been consistently identified in the *rpsl, rrs*, and *gidB* genes in *Mycobacterium tuberculosis* (MTB) since 1993 in diverse geographic localities [65]. In contrast, this mechanism of AMR in *Y. pestis* was never reported until 2021, while *Y. pestis* strains containing mutated *rpsl* genes have existed since 1987 according to recent studies [47]. It is probable that detecting strains with a single method (Table 3) [53–55], such as PCR for target genes in plasmids, would reduce the accuracy of streptomycin susceptibility monitoring since it may have missed gene-mutated isolates.

The reasons for the *rpsl* gene mutation were not further investigated through *in vivo* or *in vitro* tests. However, a study about streptomycin resistance in MTB isolates in Nepal assumed that the inappropriate practice of streptomycin monotherapy for MTB in the region was an important factor [63]. As a first-line treatment option for plague, streptomycin is easier to access in developing countries, leading to its wide application in clinical settings, which probably caused the drug-induced gene mutation.

Two strains (17/95, S19960127) have complete genome data in the GenBank, while strains 16/95 and IP2180H only had sufficient gene annotations of specific plasmids. Information on the genes associated with AMR is listed in Table 5.

Other possible mechanisms of drug resistance

Bacterial membrane vesicles (BMVs) have recently been investigated to clarify their connection with antibiotic resistance in pathogenic and non-pathogenic bacteria. They are released by bacteria themselves. A study showed that BMVs could transform bacteria with genes encoding enzymes, resulting in their dissemination and increased AMR. Eddy et al. [66] found that the BMVs of *Y. pestis* contain a penicillin-binding protein activator that regulates peptidoglycan synthesis. However, detailed the mechanism of BMVs against antibiotics was not investigated in that study.

Discussion

Based on the mechanism of AMR/MDR in strains 17/95, 16/95, and IP2180H, it can be inferred that the transposons cause the transmission of antibiotic resistance genes in bacteria. Ten resistance determinants were identified in pIP1202, mostly encoded within the Tn21 transposon, resulting in MDR. In contrast, all the antibiotic resistance-associated genes in pIP1203 and pIP2180H were characterized in Tn5393 and Tn10, respectively. The 3 transposons identified in *Y. pestis* are typical carriers of AMR genes, which have been widely discovered in various bacteria [59]. Tn10 is a composite transposon, while Tn5393 and Tn21 are non-composite transpositions. The former type, carrying drug resistance genes, poses the most serious challenge for treatment against infectious diseases since these transposons allow bacteria to survive toxic compounds created by humans [67]. However, non-composite transposons play a very important role in the genetic evolution of bacteria and the spread of AMR due to transpositions in the transposons, which can cause the increase and decrease of bacterial virulence [59]. As a result, actions should be taken to prevent the transmission of these mobile elements among bacteria.

To date, there was not sufficient evidence confirming the reason for the *rpsl* gene mutation in *Y. pestis* with streptomycin resistance. However, cases in Madagascar that occurred in 2013 excluded streptomycin application-induced gene mutation, since sputum samples from patients surviving from plague were collected before the administration of streptomycin [47,48]. As for the cases in 1996 in Qinghai-Tibet plateau, 4 people were involved in the plague outbreak and the S19960127 strain was isolated from the necropsy organ samples from 1 patient who received streptomycin treatment for 8 days [48]. It seems that further research should be conducted *in vivo* or *in vitro* to clarify the origin of streptomycin resistance in *Y. pestis*. As discussed above, the frequent use of streptomycin in plague treatment may have played an important role in the emergence of this mutation. Since drugs mediate gene mutations, vaccines and antibiotics targeting novel gene points (such as DNA adenine methyltransferase) may be an inevitable choice for defeating the bacteria [68].

It should be noticed that no plague patients in Tibet who were treated with unified administration (intramuscular streptomycin, oral TMP/SMX, oral tetracycline) survived, while all cases given a similar treatment recovered in Madagascar in 2013 [47,48]. The significant difference in the treatment effects between the 2 outbreaks, on one hand, corresponded to the streptomycin suspensibility tests, in which the S19960127 strain exhibited higher streptomycin resistance than 59/13 and 56/13. On the other hand, more advanced medical conditions probably contributed to the higher cure rate of plague in the more recent outbreak. Last, it should be noted that the 3 patients infected with pneumonic plague in Tibet died quite soon after treatment (3 to 8 days) due to inappropriate medication. Not only does this fact illustrate the high mortality of pneumonic plague, but it also underscores the importance of rapid detection of antibiotic resistance of strains isolated from people in the early stage of infection. Drug susceptibility monitoring of *Y. pestis* isolates should not only include whole-genome sequencing to identify the well-known resistance genes, but

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Table 5. Genome information of AMR genes in AMR strains

Strain	Name of the genome	Accession	Size (Mb)	GC%	Protein	Start	Stop	Locus	Locus tag	Protein product	Length	Protein name
17/95 (IP275)	Plasmid pIP1202	CP000603.1	0.18	52.9	212	32222	33037	*aphA*	YpIP275_ pIP1202_0052	ABO42185.1	271	Aminoglycoside 3\'-phosphotransferase (plasmid)
						34715	35518	*strA*	YpIP275_ pIP1202_0055	ABO42054.1	267	Streptomycin resistance protein A (plasmid)
						35518	36354	*strB*	YpIP275_ pIP1202_0056	ABO42239.1	278	Streptomycin resistance protein B (plasmid)
						40824	41483	*cat*	YpIP275_ pIP1202_0063	ABO42042.1	219	Chloramphenicol acetyltransferase (plasmid)
						48941	49756	*sul2*	YpIP275_ pIP1202_0073	ABO42075.1	271	Dihydropteroate synthase (plasmid)
						141580	142440	bla_{TEM-1}	YpIP275_ pIP1202_0175	ABO42128.1	286	Beta-lactamase (plasmid)
						147544	148728	*tetA*	YpIP275_ pIP1202_0182	ABO42139.1	394	Tetracycline resistance protein TetA, class D (plasmid)
						148824	149480	*tetR*	YpIP275_ pIP1202_0183	ABO42071.1	218	Tetracycline repressor protein TetR, class D (plasmid)
						152529	153368	*sul1*	YpIP275_ pIP1202_0188	ABO42234.1	279	Dihydropteroate synthase (plasmid)
						153873	154883	*aadA*	YpIP275_ pIP1202_0190	ABO42050.1	336	Aminoglycoside adenylyltransferase (plasmid)
S19960127	Chromosome	NZ_CP045640.1	4.55	47.5	3849	4461357	4461731	*rpsL*	S96127_RS21075	WP_200911418.1	124	30S ribosomal protein S12
16/95	Plasmid pIP1203	HG326189	0.047	64.5	50	612	1415	*strA*	-	SPF31211.1	267	Aminoglycoside 3'-phosphotransferase
						1646	2251	*strB*	-	SPF31212.1	201	Aminoglycoside 3'-phosphotransferase
IP2180H	Plasmid pIP2180H	ERS1681497	0.17	45.4	203	82627	83250	*tetR(B)*	P2180_RS00535	WP_000088605.1	207	Tetracycline resistance transcriptional repressor
						83332	84537	*tet(B)*	P2180_RS00540	WP_001089068.1	401	Tetracycline efflux MFS transporter
						84650	85244	*tetC*	P2180_RS00545	WP_000428546.1	197	Tetracycline resistance-associated transcriptional repressor

AMR, antimicrobial resistance; MFS, major facilitator superfamily.

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also drug suspensibility testing based on globally accepted guidelines. The whole-genome sequencing technology should aim to offer immediate and accurate resistance information on the genetic level so that AMR/MDR strains could be identified in time, helping clinicians to initiate appropriate treatment strategies in a timely manner. Drug suspensibility testing should be conducted to discover *Y. pestis* strains with unknown drug resistance genes to avoid missing AMR/MDR isolates.

Conclusion

Y. pestis can acquire antibiotic resistance in nature not only via conjugative transfer of AMR plasmids from other bacteria, but also by gene point mutations. The health care system should enhance the surveillance of *Y. pestis* by taking measures to control transposon transmission and detect antibiotic-resistant strains with whole-genome sequencing and drug suspensibility testing. Discovering drugs targeting novel gene points is also of urgent importance considering drug-induced gene mutations.

Notes

Ethics Approval
Not applicable.

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

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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.

Additional Contributions
Management and Science University (Selangor, Malaysia) provided statistical support.

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Original Article

Associations of pre-existing cardiovascular morbidity with severity and the fatality rate in COVID-19 patients: a systematic review and meta-analysis

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ABSTRACT

Objectives: The aim of this study was to evaluate the association of pre-existing cardiovascular comorbidities, including hypertension and coronary heart disease, with coronavirus disease 2019 (COVID-19) severity and mortality.

Methods: PubMed, ScienceDirect, and Scopus were searched between January 1, 2020, and July 18, 2020, to identify eligible studies. Random-effect models were used to estimate the pooled event rates of pre-existing cardiovascular disease comorbidities and odds ratio (OR) with 95% confidence intervals (95% CIs) of disease severity and mortality associated with the exposures of interest.

Results: A total of 34 studies involving 19,156 patients with COVID-19 infection met the inclusion criteria. The prevalence of pre-existing cardiovascular disease in the included studies was 14.0%. Pre-existing cardiovascular disease in COVID-19 patients was associated with severe outcomes (OR, 4.1; 95% CI, 2.9 to 5.7) and mortality (OR, 6.1; 95% CI, 2.9 to 12.7). Hypertension and coronary heart disease increased the risk of severe outcomes by 2.6 times (OR, 2.6; 95% CI, 1.9 to 3.6) and 2.5 times (OR, 2.5; 95% CI, 1.7 to 3.8), respectively. No significant publication bias was indicated.

Conclusion: COVID-19 patients with pre-existing cardiovascular comorbidities have a higher risk of severe outcomes and mortality. Awareness of pre-existing cardiovascular comorbidity is important for the early management of COVID-19.

Keywords: Coronary disease; COVID-19; Hypertension

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Introduction

The ongoing coronavirus disease 2019 (COVID-19) pandemic poses a significant public health threat to all nations worldwide [1,2]. As of August 23, 2021, COVID-19 has infected approximately 212,763,099 people, including roughly 4,447,912 patients who have died. Regrettably, these numbers have kept increasing worldwide, indicating that the peak is far from over and the global community remains on edge as the number of infected patients continues to escalate.

Several studies from different countries have reported that pre-existing cardiovascular comorbidities are prevalent among COVID-19 patients [3–6]. Understanding the association of cardiovascular comorbidities with the severity and outcomes of COVID-19 may highlight a cohort of patients who require more intensive monitoring during the early phase of infection [7,8]. Epidemiological studies have reported different mortality rates for COVID-19 patients with cardiac manifestations and pre-existing cardiovascular diseases, particularly hypertension and coronary artery disease [8].

Several studies have investigated the association between pre-existing cardiac disease and COVID-19 severity and fatality, and the pooled effects have been estimated in a number of meta-analyses. However, previous reviews varied in how COVID-19 severity was defined; did not report the country of the studies, and reported substantial heterogeneity. Therefore, the present meta-analysis was performed with the following aims: (1) to estimate the overall prevalence rate of pre-existing cardiovascular disease and cardiac manifestations in COVID-19 patients, and (2) to evaluate the association of pre-existing hypertension and coronary heart disease with the severity of COVID-19 and the mortality rate in COVID-19 patients using a random-effect model that incorporates heterogeneity.

Materials and Methods

Data Search

Three databases (PubMed, Science Direct, and Scopus) were searched between January 1, 2020, and July 18, 2020. The following combined keywords were used for searching the databases: cardiovascular and COVID-19; cardiovascular and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2); cardiovascular, and SARS-CoV-2; cardiovascular, hypertension, and COVID-19. Furthermore, the lists of references of all relevant studies were also manually checked to identify further studies. The protocol for this meta-analysis is registered at PROSPERO CRD42020191768. The meta-analysis was reported following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement [9].

Study Selection

The study selection was limited to articles in English and studies on adult humans. Case reports, review articles, and editorials were excluded from this analysis. Studies were selected if they provided adequate details on pre-existing cardiovascular disease comorbidities, particularly in patients with positive diagnoses for COVID-19 and hypertension. Studies that did not provide enough details on the number of cases with severe or fatal outcomes were excluded.

Data Abstraction

For studies that met the inclusion criteria, the following data were extracted from each study using a standardized form: the surname of the first author; the design of the study; ratios of clinical characteristics of interest; sample size, country, data relevant to cardiovascular disease comorbidities factor; and pertinent data for arrhythmia and acute cardiac injury as outcomes, and the number of cases with severe and non-severe outcomes, and the number of survivors and non-survivors. As reported in the included studies, severe disease was identified if patients needed to be admitted to the intensive care unit, needed vital life support, or required mechanical ventilation. Non-survivors were defined as cases of death. Two investigators (FA and MA) extracted the relevant data.

Quality Assessment

We used the Joanna Briggs Institute (JBI) critical appraisal checklist for case series to assess the risk of bias [10]. The JBI includes 10 items dealing with confounding, selection, and information bias to assess the internal validity of the case series. The answers for each of the 10 items in the JBI checklist could be "yes," "no," "unclear," or "not applicable." A detailed description of how to use the JBI tool is provided by Munn et al. in 2020 [10]. It is advised that the results of the quality assessment of the included studies should not be shortened and reported as a score [10]. The quality assessment of the included studies in this meta-analysis was carried out by SA.

Quantitative Data Synthesis and Analysis

Data analysis was carried out using Comprehensive Meta-Analysis V2 (Biostat, Englewood, NJ, USA). A p-value of <0.05 was considered statistically significant. Random-effect models were used to estimate the pooled event rates of pre-existing cardiovascular disease comorbidities as

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well as the odds ratio (OR) with 95% confidence intervals (95% CIs) of disease severity and mortality associated with the exposures of interest. A random-effect model was used to incorporate heterogeneity among studies [11]. Heterogeneity in any analysis was tested by using the I^2 statistic ($p < 0.1$), which estimates the percentage of variation in study results that is explained by between-study heterogeneity rather than sampling error. Usually, an I^2 value >50% indicates considerable heterogeneity [11]. Funnel plots and Egger test were used to assess the presence of publication bias.

Results

Search Results and Study Characteristics

A total of 1,601 articles were identified from the 3 databases examined and other sources. After excluding duplicated or overlapping articles and removing reviews and editorials, 169 articles met the primary search criteria. For the quantitative part of our study, 34 studies that reported the event rate of pre-existing cardiovascular disease, arrhythmia, or acute cardiac injury as disease complications were included in the meta-analysis (Figure 1). Most studies

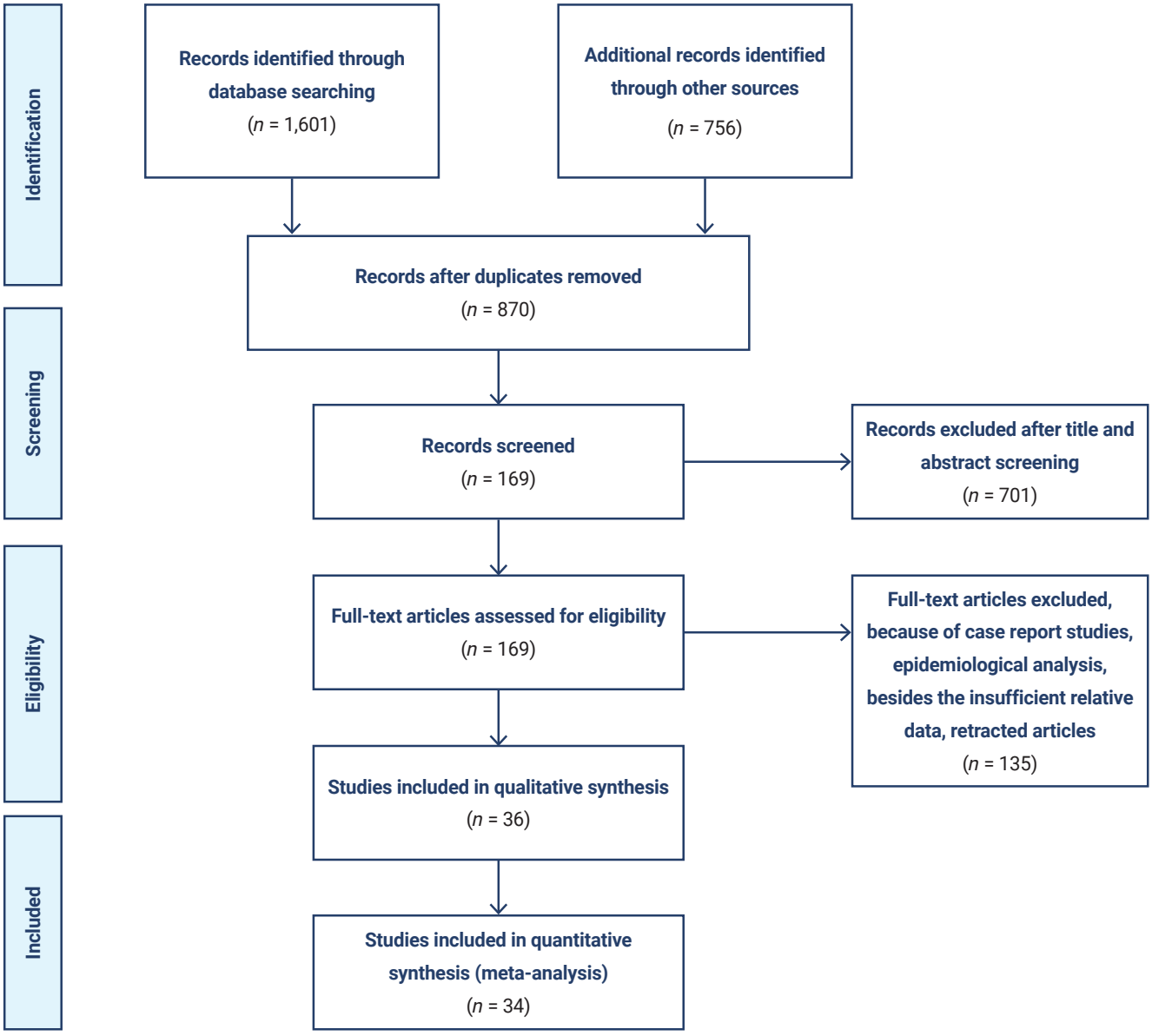

Figure 1. Flow chart of the literature search and study selection.

were conducted in China (n=21) and the United States of America (n=8), while 4 studies were conducted in Italy and 1 study reported results from different parts of the world. The setting for most of the included studies was the hospital (Table 1) [3–5,12–42].

Quantitative Analysis

The proportions of cardiovascular disease comorbidities and cardiac manifestations in COVID-19 patients

Relevant data regarding the event rate of pre-existing cardiovascular diseases, including hypertension and coronary heart disease, in 19,156 patients with COVID-19 were collected from 34 studies (Table 1) [3–5,12–42]. The pooled prevalence of pre-existing cardiovascular diseases or coronary heart disease among the included studies was 14% (95% CI, 11% to 18%), as is shown in Figure 2.

Pre-existing cardiovascular disease, hypertension, and coronary heart disease and the risk of severity outcomes and mortality in COVID-19

Table 2 summarizes the results of the current analysis. COVID-19 patients with pre-existing cardiovascular comorbidities were 4 times more likely to have severe outcomes (OR, 4.1; 95% CI, 2.9 to 5.7) (Figure 3) or not survive the disease (OR, 6.1; 95% CI; 2.9 to 12.7) (Figure 4), compared to patients with no pre-existing cardiovascular or coronary heart diseases. Severe disease was defined as patients needing to be admitted to the intensive care unit, needing vital life support, or requiring mechanical ventilation. Hypertension as a comorbid factor was associated with 2.6 times higher risk for severe outcomes (OR, 2.6; 95% CI, 1.9 to 3.6) and a 3 times higher fatality rate (OR, 3.2; 95% CI, 2.0 to 5.0) (Figures 5 and 6). However, coronary heart disease was associated with a 2.5 times higher risk for severe outcomes (OR, 2.5; 95% CI, 1.7 to 3.8) (Figure 7).

Quality of the Included Studies

Table S1 shows the quality assessment of the studies on cardiovascular disease as a comorbidity in COVID-19 patients using JBI's tool [3–5,12–42]. Most of the studies did not define participants' eligibility criteria. Moreover, most studies were unclear regarding whether they included consecutive participants and whether the inclusion was complete. The majority of the studies diagnosed COVID-19 and the outcomes of interest using valid and reliable methods. All included studies in this analysis reported the demographic and the clinical characteristics, as well as the outcomes of the participants. However, most of the multi-center studies did not present the demographic and the

Table 1. Number of patients with CVD comorbidities among coronavirus disease 2019 patients

Study	Country	Condition	Setting	Comorbidities	Sample size (n)	Events (n)	Non-events (n)	Severe cases ratio	Non-severe cases ratio	Non-survivors	Survivors
Wang et al. [3]	China	CVD	Zhongnan Hospital of Wuhan, University in Wuhan, China	HP, CVD, DM, CLD, CRVD, COPD, CKD, Ca, HIV	138	20	115	9/36	11/102		
Goyal et al. [14]	USA	CAD	An 862-bed quaternary referral center and an affiliated 180-bed nonteaching community hospital in Manhattan	DM, obesity, HP, COPD, asthma, CAD	393	54	339	25/130	29/263		
Zhang et al. [15]	China	CVD	Zhongnan Hospital of Wuhan University, Wuhan, China	HP, CVD, DM, CLD, CRVD, COPD, CKD, Ca, immunosuppression	221	22	199	13/55	9/166		
Hu et al. [16]	USA	CVD	Tianyou Hospital, Wuhan University of Science and Technology, China	HP, CVD, DM, CLD, CRVD, COPD, CKD, Ca, cirrhosis	323	34	289	30/172	4/151		
Guo et al. [17]	China	CHD	The Seventh Hospital of Wuhan City, China	HP, CHD, DM, COPD, CKD, Ca, cardiomyopathy	187	29	158				
Zhang et al. [18]	China	CVD	The Seventh Hospital of Wuhan City, China	HP, DM, CHD, HL, CG, CVD, CKD, CRVD, COPD, arrhythmia cholelithiasis, fatty liver, thyroid diseases	140	15	125	10/58	5/82		

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Table 1. Continued

Study	Country	Condition	Setting	Comorbidities	Sample size (*n*)	Events (*n*)	Non-events (*n*)	Severe cases ratio	Non-severe cases ratio	Non-survivors	Survivors
Du et al. [19]	China	CHD	Hannan Hospital and Wuhan Union Hospital of Wuhan City, China	HP, DM, CHD, CRVD, CLD, COPD, CKD, Ca	85	10	75				
Rosenberg et al. [20]	USA	CVDs	25 hospitals in the New York City, metropolitan region	Obesity, cancer, CKD, COPD, DM, HP, CAD, CHD, dementia	1,438	468	1,000				
Lei et al. [21]	China	CVDs	Renmin Hospital, Zhongnan Hospital, Tongji Hospital, and Central Hospital in Wuhan	HP, Ca, DM, CVD, CRVD, COPD, CKD	34	7	27	6/15	1/19		
Mercuro et al. [22]	USA	CVDs	An academic tertiary care center in Boston, Massachusetts	HP, CHF, DM, CAD, AF, COPD, asthma	90	19	71				
Saleh et al. [23]	USA	CVDs	14 hospitals of the New York State Northwell Health system	HP, HL, DM, AF, CAD, COPD, CKD, CHF	201	52	149				
Inciardi et al. [24]	Italy	Cardiac disease	Civil Hospitals of Brescia, Lombardy, Italy	HP, HL, DM, HF, AF, CAD, COPD, CKD, Ca	99	53	46				
Bhatla et al. [25]	USA	CVDs	The Hospital of the University of Pennsylvania	CHD, HF, HP, AF, DM, COPD, CLD, CKD	700	203	497			48/79	155/621
Sala et al. [26]	Italy	CAD	Seven COVID units at a third-level hub center, San Raffaele Hospital, Italy	CAD, COPD, HP, DM, Obesity, AF	132	9	123				
Enzmann et al. [27]	USA	CVD	Three hospitals in the Dakotas	Asthma, CHF, CVD, DM, RD, CKD, cirrhosis, Ca, immunosuppression	150	93	57				
Guan et al. [4]	China	CHD	552 hospitals in 30 provinces, autonomous regions, and municipalities in mainland China	COPD, DM, HP, CHD, CRVD, HBV, CKD, immunosuppression	1,099	27	1,072	10/173	17/926		
Qin et al. [28]	China	CVD	Tongji Hospital	COPD, HP, CVD, CLD, DM, tuberculosis, Ca, CKD	452	27	425	24/286	3/166		
Huang et al. [29]	China	CVD	2 hospitals in the Hubei provinces, China	HP, DM, CHD, Ca	223	13	210	9/98	4/125		
Huang et al. [30]	China	CVD	Designated hospital in Wuhan	DM, HP, CVD, COPD, Ca, CLD	41	6	35	3/13	3/28		
Wan et al. [31]	China	CVD	Chongqing University Three Gorges Hospital,	DM, CVD, HP, COPD, Ca, CLD	135	7	128	6/40	1/95		
Shi et al. [32]	China	CVD	Renmin Hospital of Wuhan University	HP, DM, CAD, CRVD, CHF, CKD, COPD, Ca, HBV	416	61	355	36/82	25/334		
Zhou et al. [33]	China	CVD	Jinyintan Hospital and Wuhan Pulmonary Hospital	HP, DM, CHD, COPD, Ca, CKD	191	59	132			41/54	18/137
Lagi et al. [34]	Italy	CHD	University Hospital, Florence, Italy	DM, COPD, CHD, HP, HBV, CRVD, CKD	84	12	72	5/16	7/68		
Wang et al. [35]	China	CVD	Zhongnan Hospital of Wuhan University in Wuhan and Xishui Hospital, Hubei Province, China	HP, CVD, DM, CLD, CVD, COPD, CKD	107	13	94			7/19	6/88

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Table 1. Continued

Study	Country	Condition	Setting	Comorbidities	Sample size (*n*)	Events (*n*)	Non-events (*n*)	Severe cases ratio	Non-severe cases ratio	Non-survivors	Survivors
Chen et al. [36]	China	CVD	Wuhan Tongji Hospital	HP, DM, CVD, CHF, COPD, Ca, HBV, HIV, CRVD, CKD, CG, metabolic arthritis, autoimmune disease	274	23	251			16/19	7/36
Yang et al. [37]	China	Chronic cardiac disease	Wuhan Jin Yin-Tan Hospital	CVD, COPD, CRVD, DM, Ca, Dementia	52	5	47			3/32	2/20
Chen et al. [12]	China	CVD	Wuhan Jinyintan Hospital	CVD, CRVD, Ca	99	40	59				
Jin et al. [38]	China	CVD	The Health Commission of Zhejiang province in designated hospitals	HP, DM, CLD, Ca, CKD, CHD COPD, immunosuppression	651	5	646				
Richardson et al. [13]	USA	CVD	12 hospitals in New York City, Long Island, and Westchester County, New York	Ca, CVD, HP, CAD, CHF, COPD asthma	5,700	966	4,734				
Yan et al. [39]	China	CVD	Tongji Hospital, Wuhan, China	HP, CVD, CRVD, CKD, COPD, CLD	193	31	162			27/108	4/85
Guan et al. [40]	China	Cardiovascular disease	575 hospitals in 31 provinces/ autonomous regions/provincial municipalities across mainland China	HP, DM, COPD, Ca, CVD	1,590	59	1,531	20/59	234/1,531		
Gold et al. [41]	USA	CVD	Seven hospitals in metropolitan Atlanta	DM, CVD, CHF, Arrhythmia, COPD, asthma, Obesity, HP, CKD, Ca	305	78	227				
Grasselli et al. [5]	Italy	CVD	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan	HP, CVD, HL, DM, Ca, COPD, CKD, CLD	1,591	223	1,368				
Wang et al. [42]	China	CVD	Tongji Hospital	HP, DM, CVD, COPD	344	40	304			22/133	18/211
Wang et al. [3]	China	HP	Zhongnan Hospital of Wuhan University in Wuhan, China	HP, CVD, DM, CLD, CRVD, COPD, CKD, Ca, HIV	138	43	95	21/36	21/102		
Goyal et al. [14]	USA	HP	An 862-bed quaternary referral center and an affiliated 180-bed nonteaching community hospital in Manhattan	DM, Obesity, HP, COPD, Asthma, CAD	393	197	196	70/130	127/263		
Zhang et al. [15]	China	HP	Zhongnan Hospital of Wuhan University, Wuhan, China	HP, CVD, DM, CLD, CRVD, COPD, CKD, Ca, immunosuppression	221	55	166	26/55	28/166		
Hu et al. [16]	USA	HP	Tianyou Hospital, Wuhan University of Science and Technology, China	HP, CVD, DM, CLD, CRVD, COPD, CKD, Ca, cirrhosis	323	105	218	66/172	39/151		
Guo et al. [17]	China	HP	The Seventh Hospital of Wuhan City, China	HP, CHD, DM, COPD, CKD, Ca, cardiomyopathy	187	61	126				
Zhang et al. [18]	China	HP	The Seventh Hospital of Wuhan City, China	HP, DM, CHD, HL, CG, CVD, CKD, CRVD, COPD, arrhythmia cholelithiasis, fatty liver, thyroid diseases	140	42	98	22/58	20/82		

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Table 1. Continued

Study	Country	Condition	Setting	Comorbidities	Sample size (*n*)	Events (*n*)	Non-events (*n*)	Severe cases ratio	Non-severe cases ratio	Non-survivors	Survivors
Du et al. [19]	China	HP	Hannan Hospital and Wuhan Union Hospital of Wuhan City, China	HP, DM, CHD, CRVD, CLD, COPD, CKD, Ca	85	32	53				
Rosenberg et al. [20]	USA	HP	25 hospitals in the New York City, metropolitan region	Obesity, cancer, CKD, COPD, DM, HP, CAD, CHD, dementia	1,438	816	622				
Lei et al. [21]	China	HP	Renmin Hospital, Zhongnan Hospital, Tongji Hospital, and Central Hospital in Wuhan	HP, Ca, DM, CVD,CRVD,COPD, CKD	34	13	21	9/15	4/19		
Mercuro et al. [22]	USA	HP	An academic tertiary care center in Boston, Massachusetts	HP, CHF, DM, CAD, AF, COPD, asthma	90	48	42				
Saleh et al. [23]	USA	HP	14 hospitals of the New York State Northwell Health system	HP, HL, DM, AF, CAD, COPD, CKD, CHF	201	121	80				
Inciardi et al. [24]	Italy	HP	Civil Hospitals of Brescia, Lombardy, Italy	HP, HL, DM, HF, AF, CAD, COPD, CKD, Ca	99	63	36				
Bhatla et al. [25]	USA	HP	The Hospital of the University of Pennsylvania	CHD, HF, HP, AF, DM, COPD, CLD, CKD	700	347	353	62/79	285/621		
Sala et al. [26]	Italy	HP	Seven COVID units at a third-level hub center, San Raffaele Hospital, Italy	CAD, COPD, HP, DM, obesity, AF	132	60	72				
Guan et al. [4]	China	HP	552 hospitals in 30 provinces, autonomous regions, and municipalities in mainland China	COPD, DM, HP, CHD, CRVD, HBV, CKD, immunosuppression	1,099	165	934	41/173	124/926		
Qin et al. [28]	China	HP	Tongji Hospital	COPD, HP, CVD, CLD, DM, tuberculosis, Ca, CKD	452	135	317	105/286	30/166		
Huang et al. [29]	China	HP	2 hospitals in the Hubei provinces, China	HP, DM, CHD, Ca	223	40	180	38/98	12/125		
Huang et al. [30]	China	HP	Designated hospital in Wuhan	DM, HP, CVD, COPD, Ca, CLD	41	6	35	2/13	4/28		
Wan et al. [31]	China	HP	Chongqing University Three Gorges Hospital	DM, CVD, HP, COPD, Ca, CLD	135	13	122	4/40	9/95		
Shi et al. [32]	China	HP	Renmin Hospital of Wuhan University	HP, DM, CAD, CRVD, CHF, CKD, COPD, Ca, HBV	416	127	289	49/82	78/334		
Zhou et al. [33]	China	HP	Jinyintan Hospital and Wuhan Pulmonary Hospital	HP, DM, CHD, COPD, Ca, CKD	191	58	133			26/54	32/137
Lagi et al. [34]	Italy	HP	University Hospital, Florence, Italy	DM, COPD, CHD, HP, HBV, CRVD, CKD	84	31	53	5/16	26/68		
Wang et al. [35]	China	HP	Zhongnan Hospital of Wuhan University in Wuhan and Xishui Hospital, Hubei Province, China	HP, CVD, DM, CLD, CVD, COPD, CKD	107	26	81			10/19	16/88
Richardson et al. [13]	USA	HP	12 hospitals in New York City, Long Island, and Westchester County, New York	Ca, CVD, HP, CAD, CHF, COPD, asthma	5,700	3026	2674				

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Table 1. Continued

Study	Country	Condition	Setting	Comorbidities	Sample size (*n*)	Events (*n*)	Non-events (*n*)	Severe cases ratio	Non-severe cases ratio	Non-survivors	Survivors
Yan et al. [39]	China	HP	Tongji Hospital, Wuhan, China	HP, CVD, CRVD, CKD, COPD, CLD	193	73	120			57/108	16/85
Guan et al. [40]	China	HP	575 hospitals in 31 provinces/ autonomous regions/provincial municipalities across mainland China	HP, DM, COPD, Ca, CVD	1,590	269	1,321				
Gold et al. [41]	USA	HP	Seven hospitals in metropolitan Atlanta	DM, CVD, CHF, Arrhythmia, COPD, asthma, Obesity, HP, CKD, Ca	305	206	99				
Grasselli et al. [5]	ITALY	Hypertension	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan	HP, CVD, HL, DM, Ca, COPD, CKD, CLD	1,591	509	1,082				
Wang et al. [42]	China	Hypertension	Tongji hospital	HP, DM, CVD, COPD	344	141	203			69/133	72/211

CVD, cardiovascular disease; HP, hypertension; DM, diabetes mellitus; CLD, chronic liver disease; CRVD, cerebrovascular disease; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; Ca, cancer; HIV, human immunodeficiency virus; CAD, coronary artery disease; CHD, coronary heart disease; HL, hyperlipidemia; CG, chronic gastritis; CHF, congestive heart failure; AF, atrial fibrillation; HF, heart failure; RD, rheumatologic disease; HBV, hepatitis B virus infection.

clinical characteristics by site or clinic.

Assessment of Publication Bias

Publication bias was evaluated visually using a funnel plot. As shown in Figure 8 on the event rate of pre-existing cardiovascular comorbidity, a visual symmetry indicates the absence of publication bias. The Egger test also revealed no significant publication bias (Egger test, $p = 0.09$).

Discussion

In the present meta-analysis, we examined 36 independent studies reporting clinical data on 19,156 patients with COVID-19 worldwide. The studies included in this meta-analysis include the latest research available on COVID-19 from January to July 2020. Our pooled analyses indicated that pre-existing cardiovascular diseases, in particular hypertension and coronary heart disease, are prevalent among patients with COVID-19. Our pooled analyses also clearly showed that the presence of pre-existing cardiovascular disease, including hypertension and coronary heart disease, is associated with COVID-19 severity and/or fatality. This association can be confounded by older age, patients with poor outcomes may be older and have more cardiovascular events [43]. In this analysis meta-regression (data are not shown) using the method of moments of the effect of age, reported as mean or median, on association of pre-existing cardiovascular disease with COVID-19 outcomes revealed that age was significantly associated only with estimated OR for severity in patients with pre-existing cardiovascular disease.

In comparison, another meta-analysis of 6 published studies from China including 1,527 patients with COVID-19 that reported a 16.4% prevalence of cardio-cerebrovascular disease [44]. Another analysis of 7 Chinese studies showed that the prevalence of cardiovascular disease and that of hypertension were 21% and 8%, respectively [45]. Our meta-analysis on data from different countries reported a 14% prevalence of cardiovascular disease.

Pre-existing cardiovascular disease was associated with a 4-fold and 6-fold greater risk of disease severity and fatality, respectively. A previous study that analysed data of COVID-19 patients until March 20, 2020 found that cardiovascular disease increased the odds of combined critical/fatal cases of COVID-19 by 5 times [46] and in particular, hypertension was found to increase the odds of combined critical and fatal cases by 2.7 times. The main difference between our analysis and that by Zheng et al. [46] is that we analysed data separately for COVID-19 severity and mortality, while Zheng et al. [46] combined

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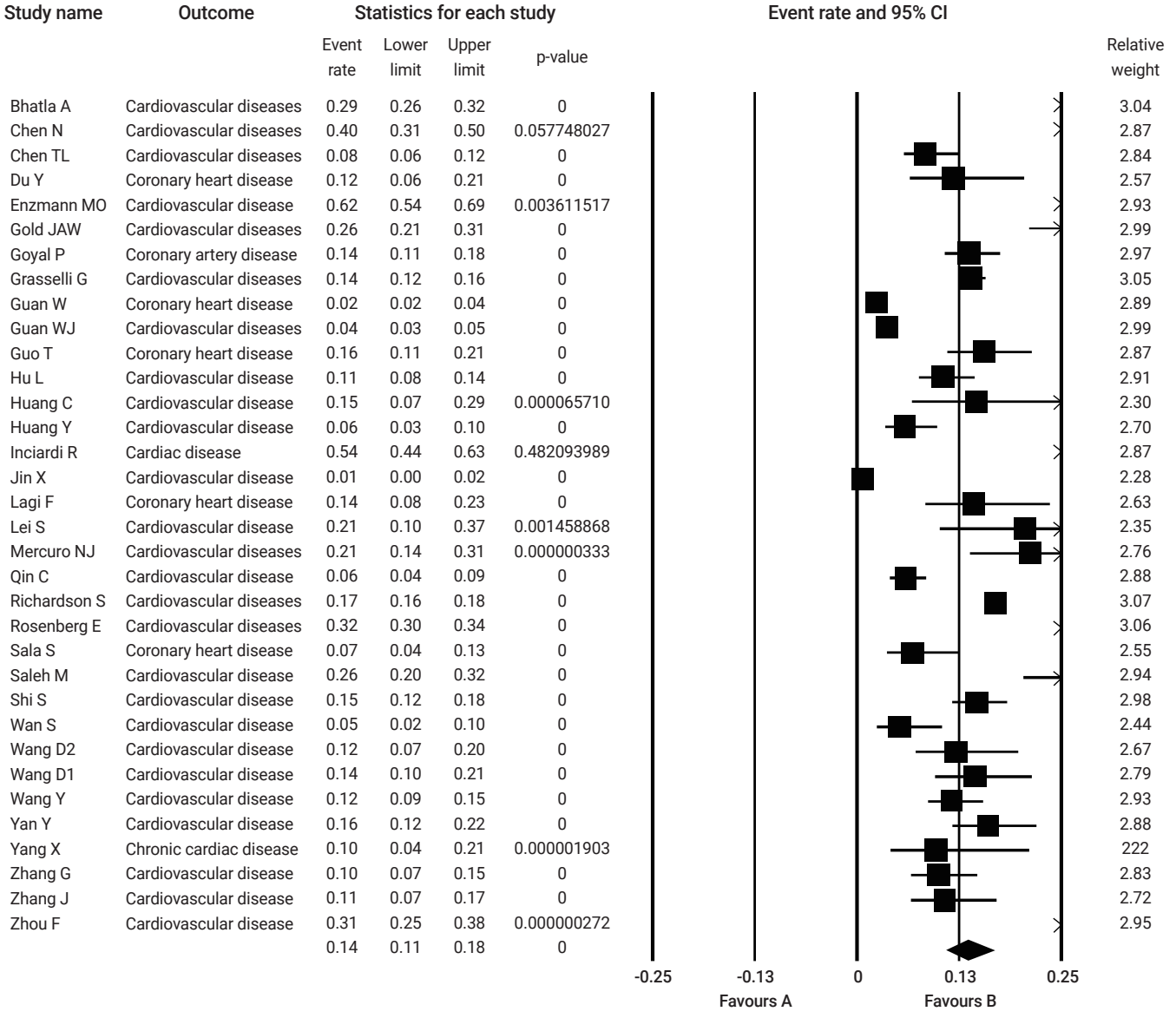

Figure 2. Pooled event rate of pre-existing cardiovascular disease in patients with coronavirus disease 2019. CI, confidence interval.

Table 2. Effect of cardiovascular comorbidities on severity and mortality outcomes associated with coronavirus disease 2019

Comorbidity	Severity		Mortality	
	No. of studies	OR (95% CI)	No. of studies	OR (95% CI)
Pre-existing cardiovascular diseases	14	4.1 (2.9 to 5.7)	7	6.1 (2.9 to 12.7)
Hypertension	14	2.6 (1.9 to 3.6)	4	3.2 (2.0 to 5.0)
Coronary heart disease	4	2.5 (1.7 to 3.8)	-	-

OR, odds ratio; CI, confidence interval; -, no data available to run analysis.

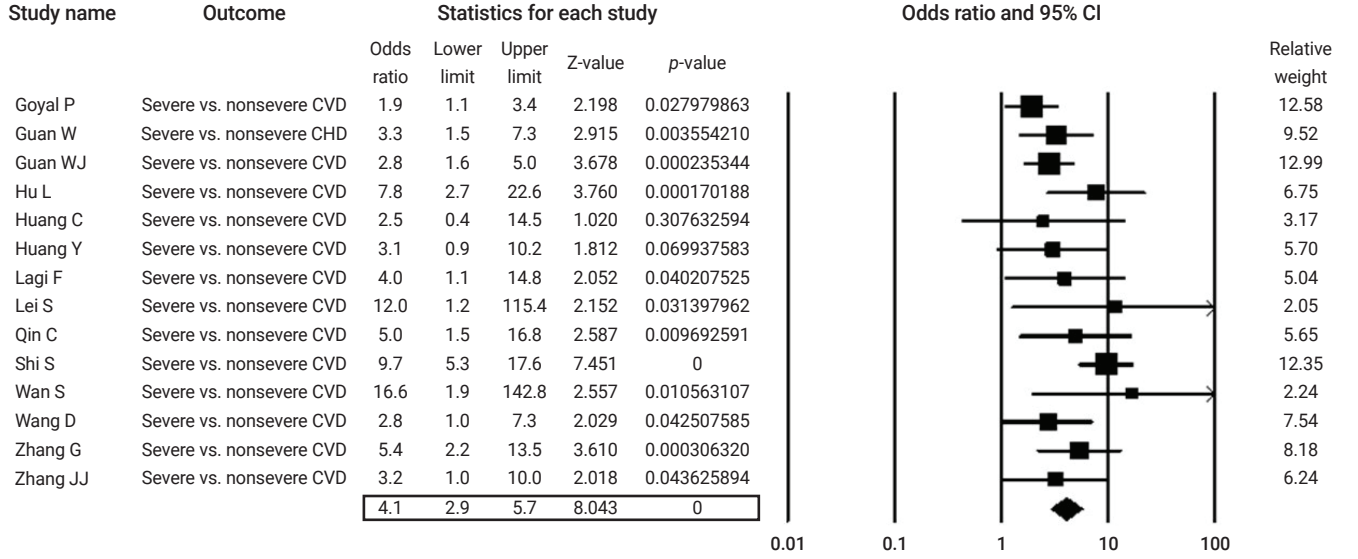

Figure 3. Forest plot of the odds ratios of pre-existing cardiovascular disease (CVD) in severe cases compared to non-severe cases of coronavirus disease 2019.
CI, confidence interval; CHD, coronary heart disease.

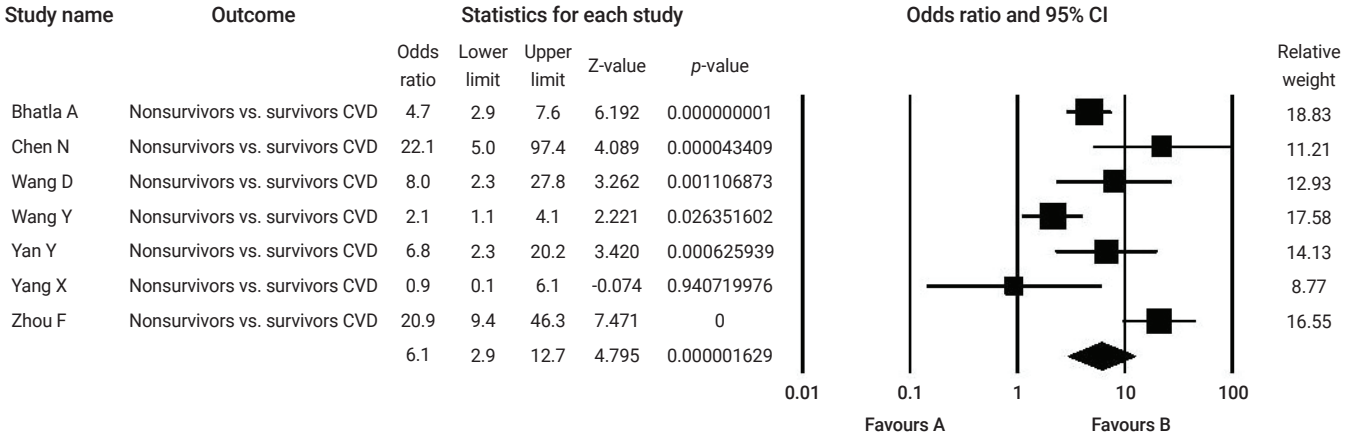

Figure 4. Forest plot of the odds ratios of pre-existing cardiovascular disease (CVD) in non-survivor compared to survivor coronavirus disease 2019 patients. CI, confidence interval.

data on COVID-19 critical conditions and mortality. Another previous meta-analysis [44] that included only studies from China reported that comorbid hypertension increased COVID-19 severity by 2-fold, suggesting the prognostic impact of this comorbidity. Our results clearly confirm previous findings and add to them. Li et al. [44] were not able to provide data on cardiovascular comorbidities and death from COVID-19 as data collection was incomplete, and most of the included studies in their analysis did not analyse comorbidities in death cases. Another analysis by Luo et al. [47] included a larger number of studies and found that hypertension was associated with 2.5 times higher odds of mortality; however, considerable heterogeneity was also reported. In this analysis, the relationship between hypertension comorbidity and COVID-19-induced death was pooled using data from China and other countries using a random-effect model to account for heterogeneity. Hypertension was associated with a 3-fold increased fatality rate. The American Heart Association and the American College of Cardiology define hypertension as systolic blood pressure (BP) ≥130 or diastolic BP ≥80 mmHg, and hypertension is a primary risk factor associated with atherosclerotic cardiovascular disease [48]. In line with our analysis, several studies identified high rates of hypertension among severely symptomatic COVID-19 patients [5,12,13]. Roughly half of United States patients

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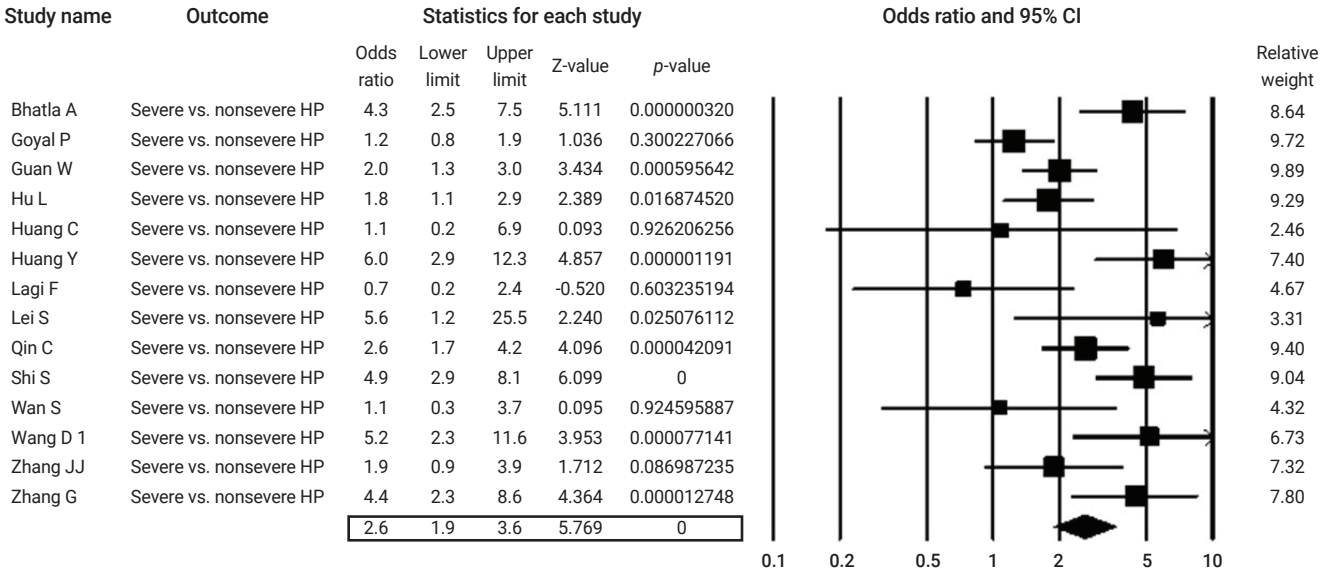

Figure 5. Forest plot of the odds ratios of pre-existing hypertension (HP) in severe compared to non-severe coronavirus disease 2019 cases. CI, confidence interval.

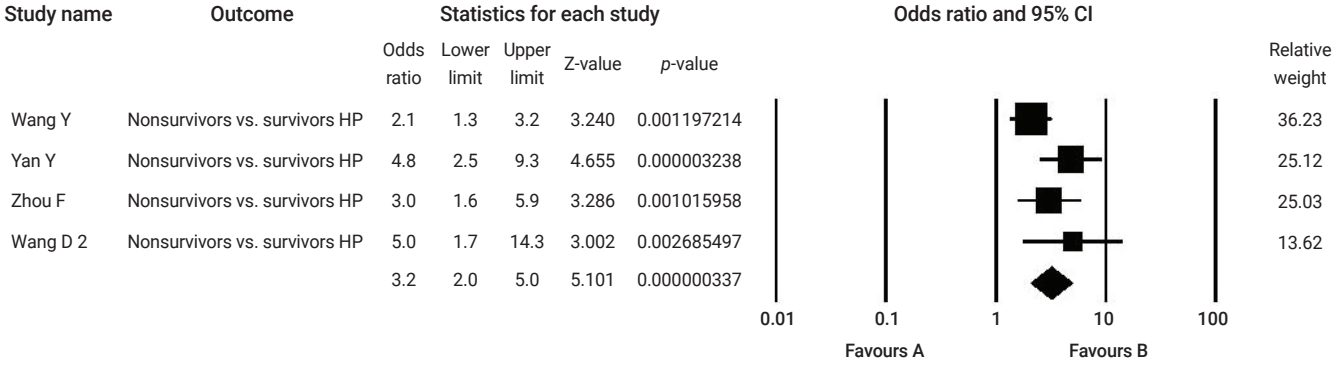

Figure 6. Forest plot of the odds ratios of pre-existing hypertension (HP) non-survivor compared to survivor coronavirus disease 2019 patients. CI, confidence interval.

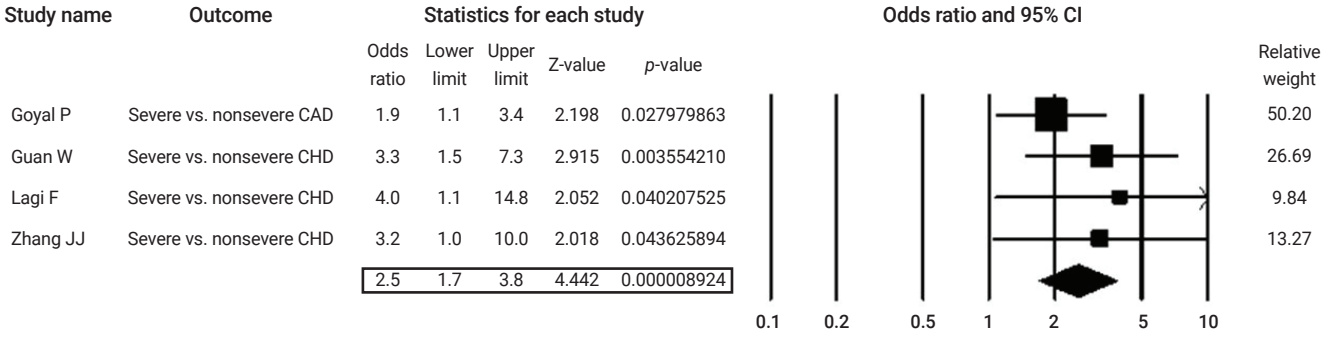

Figure 7. Forest plot of the odds ratios of pre-existing coronary heart disease (CHD) in severe cases compared to non-severe coronavirus disease 2019 cases.
CAD, coronary artery disease; CI, confidence interval.

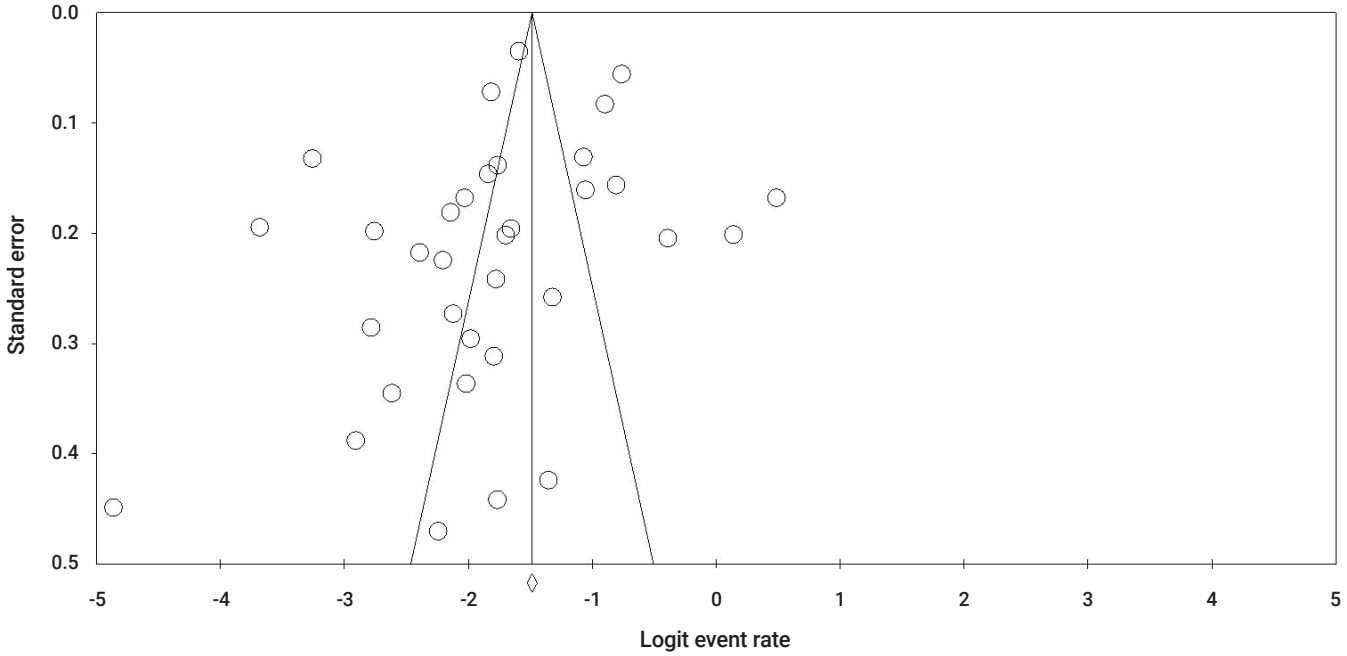

Figure 8. Funnel plot for publication bias based on cardiovascular comorbidity.

with hypertension are prescribed angiotensin-converting enzyme (ACE) inhibitors, aldosterone receptor blockers, and aldosterone antagonists, collectively called renin-angiotensin-aldosterone system (RAAS) inhibitors [49]. The modulator of the RAAS is the ACE2 receptor, which is used by SARS-CoV-2 to bind via its spike (S) protein to allow entry into attached cells. The activation of the RAAS is suggested as a mechanism for severe lung injury, especially in COVID-19 patients [50]. Inhibition of the protective signaling pathways in cardiac myocytes may result in secondary the downregulation of ACE2 expression within the myocardium. Finally, COVID-19 infection induces profound changes in coagulation pathways that create a hypercoagulable state and risk of microvascular thrombosis [51].

A strength of our pooled analysis is that it included more studies than some of the previous ones, and thus a larger sample size from different countries compared to the previous meta-analyses. Hence, our pooled analysis is the most inclusive and up-to-date analysis. The mechanism by which pre-existing cardiovascular disease increases the risk of COVID-19 adverse outcomes is also thought to be through the way that drugs for this disease work [52]. However, studies did not report data on the type of medications prescribed for each comorbidity, and hence we were not able to perform subgroup analyses by medication type. Such analyses are needed in further research. Another strength of this analysis is that visual symmetry in the funnel plot indicates the absence of publication bias. A limitation of this analysis is that most studies did not report the eligibility criteria and whether participants were recruited consecutively. Therefore, selection bias is a likely concern in the included studies. Other biases in the included studies are less likely since all studies sufficiently addressed other points in the JBI tool. Another limitation of this analysis is the possible effect of confounding factors including age, sex, and presence of other comorbidities that contribute to heterogeneity of the included studies. However, we used a random-effect model that addresses heterogeneity.

Conclusion

In summary, the present evidence showed that pre-existing cardiovascular disease in general, as well as hypertension and coronary heart disease, are highly associated with the severity and the mortality rate of COVID-19. Awareness of pre-existing cardiovascular comorbidities is important for the early management of COVID-19.

Supplementary Material

Table S1. Quality assessment of the studies on cardiovascular disease as a comorbidity in coronavirus disease 2019 patients using the Joanna Briggs Institute's tool. Supplementary data are available at https://doi.org/10.24171/j.phrp.2021.0186.

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Notes

Ethics Approval
Not applicable.

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

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None.

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

Authors' Contributions
Conceptualization: FA; Data curation: FA, SA, MA; Formal analysis: SA; Investigation: FA; Methodology: FA, SA; Project administration: FA; Software: SA; Supervision: FA; Validation: all authors; Visualization: FA, SA; Writing–original draft: FA, SA; Writing–review & editing: all authors.

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Original Article

Generalized anxiety and sleep quality among health care professionals during the COVID-19 pandemic: a cross-sectional study from a tertiary healthcare institution in Eastern India

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ABSTRACT

Objectives: With the emergence of the coronavirus disease 2019 (COVID-19) pandemic, healthcare professionals (HCPs) have experienced high levels of stress and anxiety because of the high risk of infection for themselves and their families. This has led to acute sleep problems for HCP. This study was designed to assess the anxiety and sleep quality of HCPs during the COVID-19 pandemic.

Methods: This cross-sectional study analyzed 370 HCPs employed at All India Institute of Medical Sciences Patna over 3 months, using the standard Generalized Anxiety Disorder 7-item scale (GAD-7) for suspected GAD and the Pittsburgh Sleep Quality Index for sleep quality. Results were tabulated and multivariable binomial logistic regression analysis was done to determine the predictors of poor sleep. Significance was attributed to $p < 0.05$.

Results: Of the 370 HCPs screened, 52 (14.1%; 95% confidence interval [CI], 10.8%–18.1%) were found to have GAD and 195 (52.7%; 95% CI, 47.5%–57.9%) were found to be poor sleepers. The presence of any addictive habit (adjusted odds ratio [AOR], 1.833; 95% CI, 1.12–2.8), unprotected contact with COVID-19 cases (AOR, 1.902; 95% CI, 1.1–3.3), and the presence of GAD (AOR, 5.57; 95% CI, 2.5–12.4) were found to be predictors of poor sleep quality among HCPs.

Conclusion: A significant proportion of HCPs were found to have suspected GAD and were poor sleepers. This highlights the need for measures to confront this problem.

Keywords: Anxiety; Anxiety disorders; COVID-19; Sleep disorders; Sleep quality

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Introduction

India is one of the countries most affected by the coronavirus disease 2019 (COVID-19) pandemic. On November 1, 2020, India reported 8,223,000 confirmed cases of COVID-19, rising

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to 10,600,000 by the end of January 2021, with a death toll reaching 153,000 (110 deaths per million population) [1,2]. Not only has COVID-19 caused substantial social and economic disruptions, but it also remains a serious challenge for the health care system and other public services. From day 1 of the COVID-19 pandemic, frontline workers have been working day and night to help mitigate the impact of the pandemic and control its spread. Frontline workers include healthcare personnel, personnel involved in maintaining law and order, and workers involved in the supply and distribution of essential goods [3].

Health care professionals (HCPs) need a special mention because of their direct exposure through involvement in the assessment, quarantine, isolation, and treatment of confirmed COVID-19 cases. They are at constant risk of contracting the virus from their patients and fear the possibility of transmitting infections to their family members and being unable to safeguard their families from this pandemic. This stress may lead to acute sleep problems, including poor sleep quality and short sleep duration. Because of the unpredictable nature of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) transmission and the novel nature of COVID-19, mitigation, prevention and control, and management strategies are ever-evolving. The absence of clear-cut strategies during the initial stages of the COVID-19 pandemic, together with the high risk of exposure, has added to the stress levels of HCPs [4].

Infectious disease outbreaks, such as the current COVID-19 pandemic, are associated with major psychological distress and have significant impacts on mental illness [5–7]. HCPs often experience sleep problems, anxiety, depression, and stress when facing major public health threats [8,9]. Career anxiety, job insecurity, decreased job satisfaction, and increased organizational and professional turnover have been positively associated with the COVID-19 fears of HCPs [10]. Reports in the literature state that 31% to 50% of physicians have poor sleep quality [11–13]. HCPs are under stress in general due to the nature of their job, dealing with sickness, suffering, and death. They often have irregular work schedules and frequent long shifts, leading to burnout, which may adversely impact their sleep [14–17]. A systematic review from Africa revealed that 9.5% to 73.3% of HCPs suffered from anxiety disorders and 12.5% to 71.9% dealt with depression [18].

Few studies have concentrated on the sleep quality of HCPs during pandemic events, in particular the outbreak of COVID-19 in India [17,19]. This study was conducted during the first wave of the COVID-19 pandemic in India when resources were scarce. All India Institute of Medical Sciences (AIIMS) Patna is a dedicated COVID-19 facility in the eastern state of Bihar, India, with a 500-bed capacity earmarked for COVID-19 patients during the pandemic. The HCPs, including all categories of healthcare workers, have made nonstop efforts to provide the best possible healthcare to the COVID-19 patients and to help control the pandemic in Bihar. In this context, the current study was designed to assess the anxiety levels and sleep quality of HCPs working at AIIMS Patna, India during the COVID-19 pandemic. In addition, we identified the factors associated with poor sleep quality among HCPs.

Materials and Methods

Study Design and Duration

This was a hospital-based, cross-sectional study that was carried out for a duration of 3 months (November 2020 to January 2021).

Study Setting

This study was conducted at AIIMS Patna, an Institute of National Importance under the Ministry of Health and Family Welfare, Government of India. AIIMS Patna is engaged in providing comprehensive healthcare to the people of Bihar. This institute was declared a dedicated COVID-19 hospital during the pandemic with a 500-bed capacity for serving the people of Bihar and neighboring states.

Study Population

The study's population included all HCPs involved in COVID-19 management, primarily doctors (medical faculty, senior residents, junior residents, interns) and nursing officers. HCPs who were on psychiatric medications at the time of the study (self-reported) were excluded.

Sample Size and Sampling Technique

With a 43.9% prevalence of poor sleep quality among HCPs during the COVID-19 pandemic [20], the minimum sample size was calculated to be 218 at 15% relative precision and 95% confidence interval (CI). The final sample size was calculated to be 242 after considering a 10% non-response rate. Nonetheless, we intended to include all eligible employees at our institution in the study.

The list of all healthcare employees of AIIMS Patna and their contact numbers were obtained from the administrative section. After all staff nurses and doctors involved in COVID-19 management in any capacity were identified and their contact details (WhatsApp number/email) were collected, the study tool was shared and they

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were asked to participate voluntarily. A total of 370 HCPs participated in the study.

Study Tool and Technique

Information was collected using a pre-designed, standard questionnaire on Google Forms that was sent to the eligible participants via WhatsApp and email. Digital consent was incorporated into the Google Forms link, and participants could proceed only after giving consent.

The questionnaire had 3 sections and all questions were in English. Section A included the sociodemographic details section B included questions to screen for anxiety using the Generalized Anxiety Disorder 7-item scale (GAD-7) [21], and section C included questions about subjective sleep quality using the Pittsburgh Sleep Quality Index (PSQI) [22]. The GAD-7 scale includes 7 questions and each question is scored 0–3, with 0 indicating "not at all sure" and 3 indicating "nearly every day." The overall score for the GAD-7 scale ranges from 0 to 21. Scores of 5, 10, and 15 are used as the cut-off points for mild, moderate, and severe anxiety, respectively. Participants with a threshold score of ≥10 are considered to have generalized anxiety disorder, and further evaluation is recommended. This cut-off was found to be 85% sensitive and 82% specific in screening for generalized anxiety disorder, with excellent internal consistency (Cronbach alpha, 0.92) and good test-retest reliability (interclass correlation coefficient, 0.83) [21].

The PSQI includes questions related to usual sleep habits during the past month divided into 7 categories: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medications, and daytime dysfunction. Overall sleep quality was rated as very good, fairly good, fairly bad, and very bad. Scores were given to each category (0–3) and the global score ranged from 0 to 21. High scores indicate increased sleeping disturbance. Participants with scores ≥5 are considered poor sleepers. The cut-off of ≥5 had a diagnostic sensitivity of 89.6% and specificity of 86.5% for distinguishing poor sleepers and an overall reliability coefficient (Cronbach alpha) of 0.83 [22].

Statistical Analysis

The collected information was entered in MS Excel and the statistical analysis was done using IBM SPSS ver. 22.0 (IBM Corp., Armonk, NY, USA) Descriptive analyzes were conducted to describe the demographic characteristics and COVID-19-related data of HCPs who treated COVID-19 patients. Categorical variables such as sex, presence of generalized anxiety disorder, and poor sleeping were expressed as proportions and percentages. Continuous variables such as GAD-7 scores and PSQI scores were expressed as the median and interquartile range (IQR). The median difference in the GAD-7 scores among various groups was assessed using the Mann-Whitney U-test/Kruskal-Wallis test. A multivariable binomial logistic regression analysis was done to determine the predictors of suspected GAD and poor sleep quality among the HCP. Statistical significance was attributed to $p < 0.05$

Ethical Considerations

This study was approved by the Institute Ethics Committee, AIIMS, Patna (AIIMS/Pat/IEC/2020/697). The principles of ethics were adhered to throughout the study and thereafter.

Results

General Characteristics of Study Participants

The mean age of the HCPs was 28.8 ± 5.2 years. Among the 370 HCP, 246 (66.5%) were male, 247 (66.8%) lived off-campus in their own homes, 187 (50.5%) were nursing staff, and 168 (45.4%) had at least 1 addictive habit (of those, 25 [14.9%] used tobacco). A total of 22 participants (5.9%) had a history of psychiatric disorders but were not under any antipsychotic medication/care at the time of the study. We found that 77 (20.8%) had been in contact with a COVID-19 patient without any protection (Table 1).

Among the 370 HCPs, 196 (53.0%) had no symptoms related to COVID-19 for 1 month before the survey. Of the 174 HCPs (47.0%) who had symptoms, 103 (59.2%) had a cough, 100 (57.5%) had fever, 91 (52.3%) had body aches, 55 (31.6%) had a loss of appetite, and 33 (19.0%) had a loss of smell and/or taste sensation. A total of 71 participants (19.2%) reported testing positive for COVID-19 prior to or during the study period (Table 1).

Generalized Anxiety Disorder among HCPs (GAD-7)

The GAD-7 scores of the participants are provided in Table 1. The median (IQR) global GAD-7 score was 2.5 (0–6). HCPs with a history of psychiatric illness had a median (IQR) score of 5 (0–10.5). There were statistically significant differences in the median (IQR) scores when comparing HCPs with high-risk persons in their family to those without (3 [1–7] vs. 2 [0–5], $p < 0.001$) and when comparing those who tested positive for COVID-19 to those who tested negative (3 [1–8] vs. 2 [0–5], $p < 0.02$) (Table 1).

Among all participants, 246 (66.5%) had minimal/no anxiety, 72 (19.5%) had mild anxiety, 37 (10.0%) had moderate anxiety, and 15 (4.1%) had severe anxiety. We found that 12 interns (34.3%), 30 junior residents (32.6%), 7 senior residents (20.0%), 8 faculty (38.1%), and 52 nursing staff (27.8%) had mild to moderate anxiety (Figure 1). Overall,

Table 1. Sociodemographic details and distribution of the global GAD-7 and PSQI scores of HCPs ($n = 370$)

Variable	*n* (%)	GAD-7 scores	Global PSQI scores
		Median (IQR)	
Age (y), mean ± SD	28.8 ± 5.2		
Sex			
Male	246 (66.5)	2 (0–5)	5 (3–7)
Female	124 (33.5)	3 (0–6.5)	5 (3–7)
Residence			
Dormitory	123 (33.2)	3 (1–7)	5 (3–7)
Own home	247 (66.8)	2 (0–6)	5 (3–7)
Designation			
Interns	35 (9.5)	3 (0–7)	4 (3–6)
Junior resident	92 (24.9)	3 (1–7)	5 (3–7)
Senior resident	35 (9.5)	2 (0–5)	5 (2–7)
Medical faculty	21 (5.7)	5 (0.5–12.5)	6 (2.5–11)
Nursing staff	187 (50.5)	2 (0–5)	5 (3–7)
Addictive habits[a)]			
No	202 (54.6)	2 (0–5)	4 (3–6)
Yes	168 (45.4)	3 (0–7)	5 (3–7.5)
Tobacco ($n = 168$)[a,b)]	25 (14.9)	4 (0.5–12)	6 (5–9)
Beverages ($n = 168$)[a)]	152 (90.5)	3 (0–7)	5 (3–8)
Other habits ($n = 168$)	13 (7.7)	2 (0–6)	4 (2–6)
History of psychiatric illness			
No	348 (94.1)	2 (0–6)	5 (3–7)
Yes	22 (5.9)	5 (0–10.5)	5 (4–8.5)
High-risk family member[a,b)]			
No	204 (55.1)	2 (0–5)	4 (3–6)
Yes	166 (44.9)	3 (1–7)	5 (3–8)
Unprotected contact with COVID-19 positive patient[a,b)]			
No	227 (61.4)	2 (0–5)	4 (2–6)
Yes	77 (20.8)	4 (1–9)	6 (3.5–9)
Don't know	66 (17.8)	3 (0–7)	5 (3–8)
Tested positive for Covid-19[a,b)]			
No	258 (69.7)	2 (0–5)	4 (3–6)
Yes	71 (19.2)	3 (1–8)	5 (3–9)
Was not tested	41 (11.1)	3 (0–6.5)	5 (3.5–7)

GAD-7, Generalized Anxiety Disorder 7-item scale; PSQI, Pittsburgh Sleep Quality Index; HCP, healthcare professional; IQR, interquartile range; SD, standard deviation; COVID-19, coronavirus disease 2019.

[a)]Statistically significant ($p < 0.05$) for median (IQR) global PSQI scores using the Mann-Whitney U-test for presence of any addictive habit, tobacco consumption, and the presence of a high-risk group in the family and using the Kruskal-Wallis test for unprotected contact with a COVID-19 positive patient and testing positive for COVID-19. [b)]Statistically significant ($p < 0.05$) for median (IQR) global GAD-7 scores using the Mann-Whitney U-test for tobacco consumption and the presence of high-risk group in the family and using the Kruskal-Wallis test for unprotected contact with a COVID-19 positive patient and testing positive for COVID-19.

a total of 52 (14.1%; 95% CI, 10.8–18.1) out of 370 HCPs were screened and found to have suspected generalized anxiety disorder.

Sleep Quality among HCPs (PSQI)

The PSQI scores of participants are presented in Table 1. The median (IQR) global PSQI score was 5 (3–7). There was a statistically significant difference in the median scores when comparing HCPs who: (1) had high-risk persons in their family (median [IQR] PSQI score: 5 [3–8]) to those who did not (median [IQR] PSQI score: 4 (3–6); $p < 0.001$); (2) those who had unprotected contact with COVID-19 cases (median [IQR] PSQI score: 6 [3.5–9]) to those who did not (median [IQR] PSQI score: 4 [2–6]; $p < 0.001$); and (3) those who tested positive for COVID-19 (median [IQR] PSQI score: 5 [3–9]) to those who tested negative (median [IQR] PSQI score: 4 [3–6];

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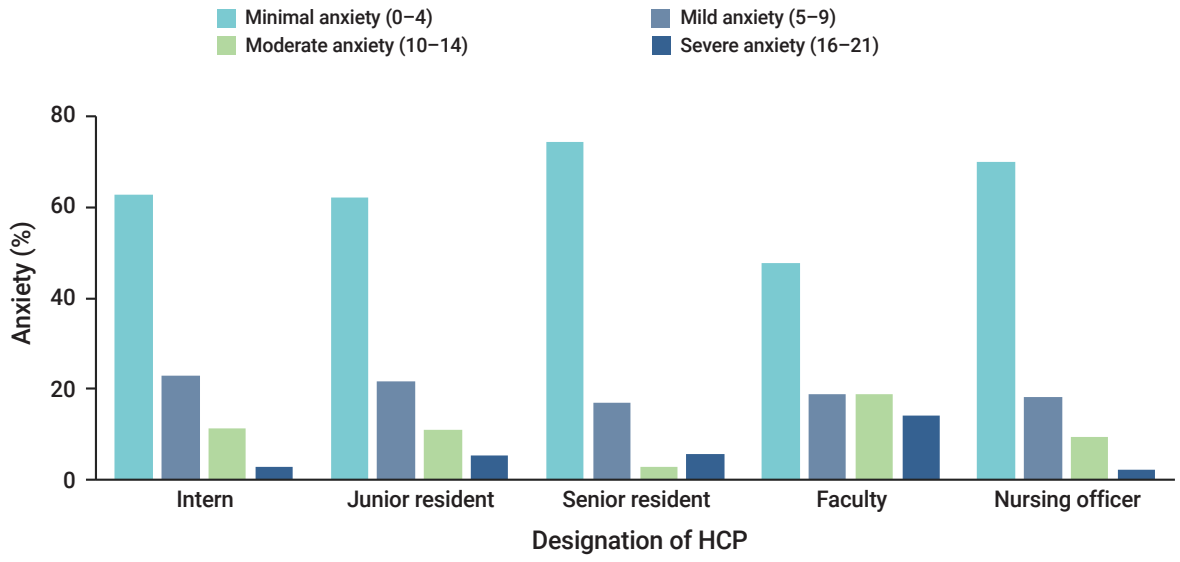

Figure 1. Distribution of the severity levels of generalized anxiety disorder among different healthcare professionals (HCPs).

$p = 0.02$) (Table 1).

The median (IQR) PSQI scores of HCPs (maximum score of 3) were as follows: sleep latency, 1 (1–2); sleep duration, 1 (0–2); sleep disturbances, 1 (0–1); daytime dysfunction, 1 (1–2); and 0 out of a maximum score of 3 for subjective sleep quality, habitual sleep efficiency, and use of sleep medicines.

Among the HCPs, 296 (80.0%) had altered sleep latency, 263 (71.1%) had altered sleep duration, 236 (63.8%) had impaired habitual sleep efficiency, and 289 (78.1%) had daytime dysfunction (Figure 2). Overall, more than half of the HCPs ($n = 195$, 52.7%; 95% CI, 47.5%–57.9%) were found to be poor sleepers.

Predictors of Suspected Generalized Anxiety Disorder

Using univariate analysis, the presence of high-risk persons in the family was found to be a significant predictor of suspected GAD (unadjusted odds ratio [OR], 2.42; 95% CI, 1.3–4.4). However, other variables including the presence of addictive habits, a history of psychiatric illness, unprotected contact with COVID-19 cases, and having tested positive for COVID-19 were also considered in multivariable logistic regression.

The presence of high-risk persons (elders, children, pregnant/lactating mothers, those with chronic diseases) in the family was found to be an independent predictor of suspected generalized anxiety disorder (adjusted OR [AOR], 2.22; 95% CI, 1.2–4.1), with a predictive accuracy of 85.9% (Table 2).

Predictors of Poor Sleep Quality

In the univariate analysis, the presence of addictive habits (crude OR, 1.982; 95% CI, 1.3–3.007), unprotected contact with COVID-19 cases (crude OR, 2.182; 95% CI, 1.2–3.7), and suspected generalized anxiety disorder (crude OR, 6.083; 95% CI, 2.7–13.3) were found to be significant predictors of poor sleep quality. These variables, along with the presence of high-risk persons in the family, were considered in multivariable logistic regression.

The presence of an addictive habit (AOR, 1.833; 95% CI, 1.1–2.8; $p = 0.006$), history of unprotected contact with a COVID-19 case (AOR, 1.902; 95% CI, 1.1–3.3; $p = 0.02$), and suspected generalized anxiety disorder (AOR, 5.57; 95% CI, 2.5–12.4; $p < 0.001$) were found to be independent predictors of poor sleep among HCPs, with a predictive accuracy of 62.4% (Table 3).

Discussion

Sleep quality and anxiety levels are important concerns for HCPs since these issues can impact their performance and result in poor patient outcomes [23,24]. Sleep problems are, in turn, associated with psychological distress [13]. During the COVID-19 pandemic, HCPs have been overburdened with work and the risk of contracting SARS-CoV-2 remains high. An Indian Council of Medical Research serosurvey conducted during December 2020 and January 2021 reported that almost one-fourth (25%) of healthcare workers showed evidence of SARS-COV-2 infection [25]. It is important to determine the proportion of HCPs with GAD and poor

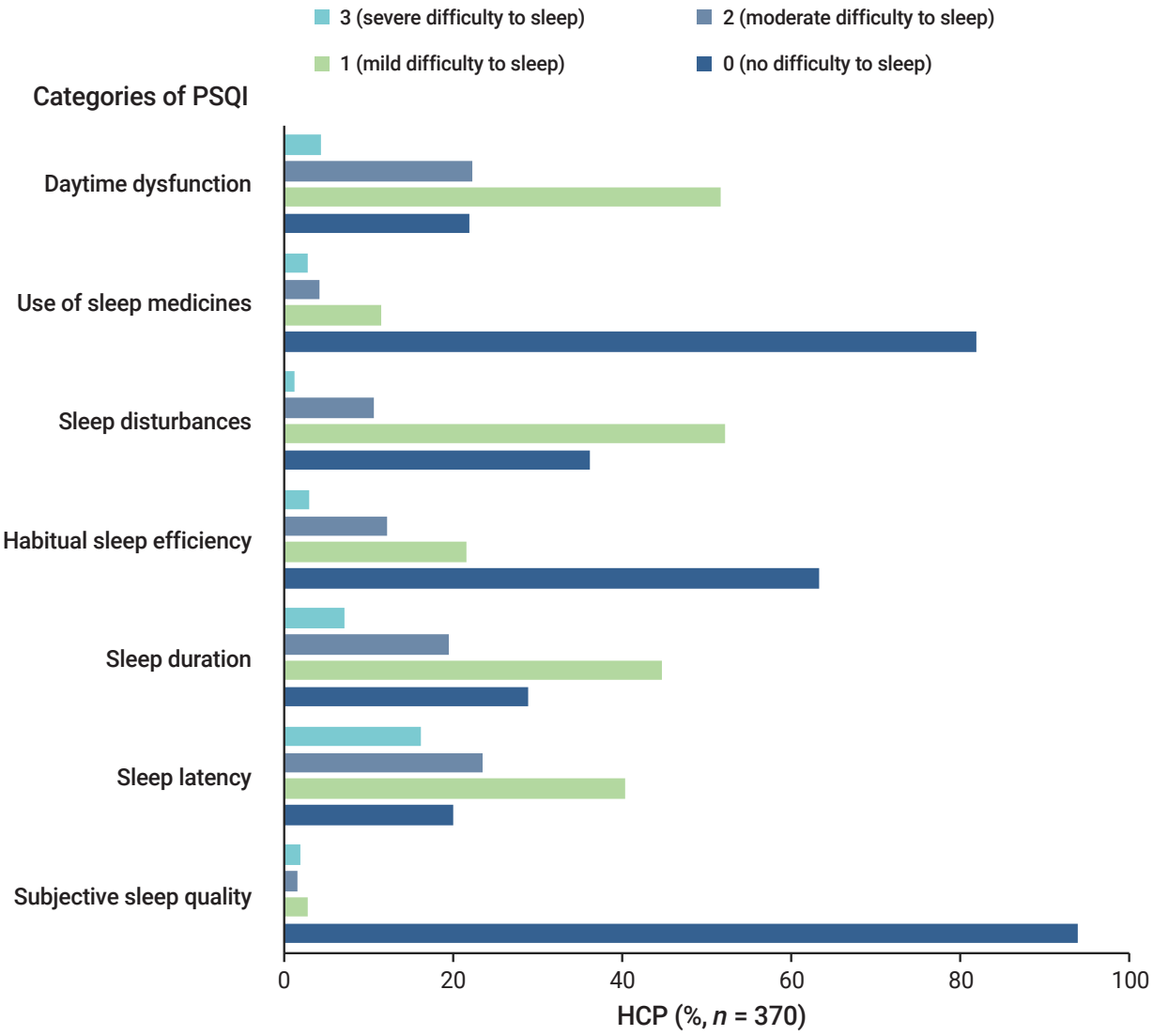

Figure 2. Distribution of varying grades of sleep difficulty among healthcare professionals (HCPs), across various categories of the Pittsburgh Sleep Quality Index (PSQI).

sleep in order to plan for future emergencies. Our cross-sectional survey screened 370 HCPs working in a COVID-19 dedicated hospital and found the prevalence and predictors of suspected generalized anxiety disorder and poor sleep among HCP.

In our study, one-fifth of HCPs were exposed to COVID-19 without protection, whereas a study in China [26] reported only 2.7% exposure among HCPs. This may be due to the limited resources in our hospital, including personnel and beds, which led to higher exposure to SARS-COV-2 among HCPs.

The results of screening in our study showed that 14.1% of HCPs had suspected generalized anxiety disorder. Similar findings (e.g., a prevalence of anxiety among HCPs of 11.5%) have been reported in other studies [27,28]. However, a study from Turkey reported that 39.4% of HCPs had anxiety [29]. In our study, 29.5% of HCPs had mild to moderate anxiety and 14.1% had severe anxiety, which is similar to a study from Saudi Arabia, which showed that more than 25% of HCPs had mild to moderate anxiety and nearly 10% had severe anxiety [30]. A study from southern Ethiopia reported a higher prevalence of mild to moderate anxiety among HCPs (35.6%) [31]. The reported prevalence of anxiety among the Indian public is 28% [32]. Our study showed that the prevalence of mild to moderate anxiety in nursing staff was 27.8%, while Meo et al. [30] reported almost double the anxiety (51.7%) in the same group. Magnavita et al. [33], in a 1-year prospective study in Italy,

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Table 2. Predictors of suspected GAD among HCPs ($n = 370$)

Variable	Severity of anxiety		Crude OR (95% CI)	AOR (95% CI)
	Not suspected GAD (%)	Suspected GAD (%)		
Age (y), mean ± SD	28.75 ± 5.1	29.21 ± 5.6	1.016 (0.96–1.07)	
Sex				
Male ($n = 246$)	212 (86.2)	34 (13.8)	1	
Female ($n = 124$)	106 (85.5)	18 (14.5)	1.059 (0.5–1.96)	
Residence				
Dormitory ($n = 123$)	104 (84.6)	19 (15.4)	1	
Own home ($n = 247$)	214 (86.6)	33 (13.4)	0.84 (0.4–1.55)	
Designation				
Intern ($n = 35$)	30 (85.7)	5 (14.3)	1	
Residents and medical faculty ($n = 148$)	123 (83.1)	25 (16.9)	1.22 (0.43–3.45)	
Nursing staff ($n = 187$)	165 (88.2)	22 (11.8)	0.8 (0.2–2.3)	
Addictive habits[a]				
No ($n = 202$)	178 (88.1)	24 (11.9)	1	1
Yes ($n = 168$)	140 (83.3)	28 (16.7)	1.483 (0.8–2.7)	1.272 (0.6–2.338)
History of psychiatric illness[a]				
No ($n = 348$)	302 (86.8)	46 (13.2)	1	1
Yes ($n = 22$)	16 (72.7)	6 (27.3)	2.46 (0.9–6.6)	1.85 (0.6–5.2)
High-risk family member[a]				
No ($n = 204$)	185 (90.7)	19 (9.3)	1	1
Yes ($n = 166$)	133 (80.1)	33 (19.9)	2.42 (1.3–4.4)[b]	2.22 (1.2–4.1)[b]
Unprotected contact with COVID-19 patient[a]				
No ($n = 293$)	257 (87.7)	36 (12.3)	1	1
Yes ($n = 77$)	61 (79.2)	16 (20.8)	1.872 (0.9–3.5)	1.75 (0.8–3.4)
Tested positive for COVID-19[a]				
No ($n = 299$)	260 (87.0)	39 (13.0)	1	1
Yes ($n = 71$)	58 (81.7)	13 (18.3)	1.494 (0.7–2.98)	1.257 (0.6–2.5)

GAD, generalized anxiety disorder; HCP, healthcare professional; OR, odds ratio; CI, confidence interval; AOR, adjusted OR; SD, standard deviation; COVID-19, coronavirus disease 2019.
[a]Used for adjustment of the final model; Nagelkerke $R^2 = 0.068$ (6.8%). [b]$p < 0.05$ indicates statistical significance.

reported that anxiety among HCPs was 25% and that even positive attitudes of doctors regarding the COVID-19 vaccine and procedural justice did not improve the mental health of HCPs.

The presence of high-risk family members was found to be an independent risk factor for anxiety among HCPs due to fear of unknowingly infecting their family members with SARS-CoV-2 and this was confirmed by another study on a large group of HCPs [34]. A study by Teshome et al. [31] in 2020 showed that factors such as contact with confirmed/suspected COVID-19 cases, lack of access to COVID-19 media updates, the absence of confidence in one's ability to cope with the stresses of COVID-19, and other COVID-related worries increased the rate of anxiety disorder among HCPs. A systematic review from Africa showed that the availability of personal protective equipment and information regarding preventive measures reduced anxiety among HCPs, while substance use, history of chronic illness, low resilience, and low social support increased the odds of anxiety [18]. Another study from Iran predicted that less work experience, high levels of exposure to COVID-19 patients, having a history of mental illness, and underlying medical conditions increased the odds of higher anxiety among HCPs [35].

In our study, almost half of the HCPs (52.7%) were found to be poor sleepers during this pandemic, which is in line with a study from China and a multicenter study from India, China, and Nepal [26,36]. The prevalence of sleep disorders during the COVID-19 pandemic was reported to range from 18.5% to 61% [37–39]. However, a study from

Table 3. Predictors of poor quality of sleep among HCPs (*n* = 370)

Variable	Quality of sleep		Crude OR (95% CI)	AOR (95% CI)
	Good sleepers (*n* = 175, %)	Poor sleepers (*n* = 195, %)		
Age (y) (mean ± SD)	28.7 ± 5.4	28.9 ± 4.9	1.008 (0.9–1.04)	
Sex				
Male (*n* = 246)	116 (47.2)	130 (52.8)		
Female (*n* = 124)	59 (47.6)	65 (52.4)	0.983 (0.6–1.6)	
Residence				
Dormitory (*n* = 123)	57 (46.3)	66 (53.7)	1.059 (0.7–1.6)	
Own home (*n* = 247)	118 (47.8)	129 (52.2)		
Designation[a)]				
Intern (*n* = 35)	20 (57.1)	15 (42.9)		1
Residents and medical faculty (*n* = 148)	63 (42.6)	85 (57.4)	1.799 (0.8–3.8)	1.527 (0.7–3.4)
Nursing staff (*n* = 187)	92 (49.2)	95 (50.8)	1.377 (0.6–2.8)	1.67 (0.8–3.6)
Addictive habits[a)]				
No (*n* = 202)	111 (55.0)	91 (45.0)		1
Yes (*n* = 168)	64 (38.1)	104 (61.9)	1.982 (1.3–3.007)[b)]	1.833 (1.1–2.8) (*p* = 0.006)[b)]
History of psychiatric illness				
No (*n* = 348)	166 (47.7)	182 (52.3)	1	
Yes (*n* = 22)	9 (40.9)	13 (59.1)	1.317 (0.5–3.2)	
High-risk family member[a)]				
No (*n* = 204)	104 (51.0)	100 (49.0)	1	1
Yes (*n* = 166)	71 (42.8)	95 (57.2)	1.392 (0.9–2.1)	1.159 (0.75–1.8)
Unprotected contact with COVID-19 patient[a)]				
No (*n* = 293)	257 (87.7)	36 (12.3)	1	1
Yes (*n* = 77)	25 (32.5)	52 (67.5)	2.182 (1.2–3.7)[b)]	1.902 (1.1–3.3) (*p* = 0.02)[b)]
Tested positive for COVID-19				
No (*n* = 299)	145 (48.5)	154 (51.5)	1	
Yes (*n* = 71)	30 (42.3)	41 (57.7)	1.287 (0.7–2.1)	
Suspected GAD[a)]				
No (*n* = 52)	167 (52.5)	151 (47.5)	1	
Yes (*n* = 318)	8 (15.4)	44 (84.6)	6.083 (2.7–13.3)[b)]	5.57 (2.5–12.4) (*p* < 0.001)[b)]

HCP, healthcare professional; OR, odds ratio; CI, confidence interval; AOR, adjusted OR; SD, standard deviation; COVID-19, coronavirus disease 2019; GAD, generalized anxiety disorder.
[a)]Used for multivariable regression model ($p < 0.2$); Nagelkerke $R^2 = 0.149$ (14.9%). [b)]$p < 0.05$ indicates statistical significance.

Turkey reported that up to 92% of surveyed HCPs were poor sleepers [40]. One in every 2 HCPs was reported to have poor sleep quality in a systematic review [11]. Nearly three-quarters of frontline workers in Bahrain were reported to be poor sleepers [16].

The mean global PSQI score in our study was found to be 5.18 ± 3.16, which was lower than that found in a study in Riyadh, Saudi Arabia (8.9 ± 3.8) [30]. In our study, HCPs scored a median 1 out of 3 for each category of the PSQI (sleep latency, sleep duration, sleep disturbances, and daytime dysfunction), while a study by Jahrami et al. [16] showed mean PSQI scores of 1.6, 1, 1.3, and 1.1 out of 3 in these same categories, respectively.

Our study found that the presence of any addictive habits, unprotected contact with COVID-19 patients, and a positive screen for suspected generalized anxiety disorder were significant independent predictors of poor sleep and increased the odds of poor sleep quality among HCPs. A study from Turkey showed that the female sex, being a physician, and depression predicted poor sleep quality [29]. Another study from Taiwan reported that symptoms of anxiety, fear of COVID-19, and stress predicted poor sleep quality and insomnia [41]. In our study, the unadjusted odds of poor sleep for the nursing staff was 1.377, while a study by Zhou et al. [37] showed 3 times higher odds of poor sleep among nursing staff. Zhou et al. [37] also showed that older age and working in the emergency department were predictors of poor sleep quality among HCPs. In our study, resident

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doctors and faculty showed higher odds of poor sleep than others (interns and nursing staff). Factors influencing this could be working in an unfamiliar and chaotic work environment, a heavy clinical workload, burnout, and constant fear of infection for themselves and their family members. These biopsychosocial factors affect sleep quality [42]. It has been reported that sleep is the moderating factor in the relationship between occupational stress and anxiety [43].

Our study showed that HCPs with suspected generalized anxiety disorder had 5.7 times higher odds of poor sleep, which is in line with a study from Serbia done among HCPs treating COVID-19 patients [44].

All of these findings confirm the negative impact of the COVID-19 pandemic on the psychological well-being of HCPs in terms of severe GAD and poor sleep. The findings from our study will add to the limited but growing pool of literature on the mental health of HCPs during the COVID-19 pandemic.

Conclusion

A significant proportion of HCPs had suspected generalized anxiety disorder and were poor sleepers. The presence of a high-risk family member was the only significant independent predictor of suspected generalized anxiety disorder and the presence of addictive habits, unprotected contact with COVID-19 cases, and the presence of suspected generalized anxiety disorder were independent predictors of poor sleep quality among HCPs. This study provides further evidence concerning the negative impact of the COVID-19 pandemic on the mental health of HCPs. HCPs should be given optimum days off and the administration should provide the human resources necessary at times such as this pandemic to optimize mental well-being. HCPs also need ongoing institutional support regarding their roles and responsibilities, the risks involved, and resources available for self-protection. Emphasis should also be placed on the early detection of sleep problems among HCPs to help improve their ability to tackle future pandemics.

Strength and Limitations

To plan for the future, it is important to understand the anxiety, apprehension, workload, and sleep quality of HCPs, especially during an unprecedented situation like the COVID-19 pandemic, in order to provide an optimal level of healthcare without putting strain on the healthcare delivery workforce. This study is one of only a few to highlight the anxiety levels and sleep quality among HCPs in eastern India.

Our study was not without limitations. This was a study conducted at a single center with limited personnel, which is a situation similar to other parts of the country, considering the constraints on personnel in the healthcare sector. A causal relationship could not be established due to the cross-sectional design of the study. The self-reporting tool used for the collection of information may have led to some reporting bias, although the data collection was anonymous to ensure the privacy and confidentiality of information and to minimize reporting bias. This study did not differentiate pandemic-related anxiety and sleep problems from other causes of anxiety and sleep problems. The anxiety and sleep disorder results were probable and diagnostic using the screening questionnaires. However, the results clearly indicated that anxiety and sleep problems do exist among HCPs in the situation of the COVID-19 pandemic, and this problem requires attention and intervention.

Notes

Ethics Approval

This study was approved by the Institute Ethics Committee, AIIMS Patna (AIIMS/Pat/IEC/2020/697). We adhered to the principles of ethics throughout the study and thereafter and this study was conducted in accordance with the principles of the Declaration of Helsinki. An online survey was used and only those who gave consent to participate in the study were included.

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: BNN, SP; Data curation: RR, MV, PKS; Data analysis: RR; Methodology: BNN, RR; Supervision: BNN, SP; Writing-original draft: RR, PKS; Writing-review & editing: all authors.

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Original Article

Predictors of health-related quality of life in Koreans with cardiovascular disease

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ABSTRACT

Objectives: This study aimed to identify the predictors of health-related quality of life (HRQoL) in Korean adults with cardiovascular disease (CVD).

Methods: This was a cross-sectional study with a stratified multistage probability sampling design. Data from the 2016 to 2019 Korea National Health and Nutrition Examination Survey (n=32,379) were used. Among the participants aged 19 years or older (n=25,995), 1,081 patients with CVD were extracted after excluding those with missing data and those who had cancer. The participants' HRQoL was measured using the three-level EuroQoL Group's five-dimension questionnaire (EQ-5D) scale. Data were analyzed using the t-test, one-way analysis of variance, and general linear regression for complex samples.

Results: The most potent predictors of HRQoL in Korean adults with CVD were limited activity (β=−0.103, p<0.001), poor perceived health (β=−0.089, p<0.001), depression (β=−0.065, p<0.01), low household income (β=−0.033, p<0.05), unemployment (β=−0.023, p<0.05), and older age (β=−0.002, p<0.01), which explained 37.2% of the variance.

Conclusion: Comprehensive interventions that address both physical and mental factors and social systems that provide financial help need to be implemented to improve the HRQoL of Korean adults with CVD.

Keywords: Cardiovascular diseases; Myocardial ischemia; Quality of life; Stroke

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Introduction

Cardiovascular disease (CVD), which generally refers to ischemic heart disease and stroke, is a major cause of mortality and morbidity. According to the World Health Organization (WHO), ischemic heart disease and cerebrovascular disease are the 2 leading causes of death worldwide, accounting for 16% and 11% of all deaths, respectively [1]. As of 2019 in Korea, heart disease was the second leading cause of death, and cerebrovascular disease was the fourth leading cause of death [2]. In total, CVD accounted for 18.8% of deaths in the Korean population in 2019 [2]. It is the largest single disease in terms of the cause of death in Koreans, and the

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diseases that cause CVD-stroke and diabetes mellitus-are ranked first and second in the list of diseases that incur the greatest cost of care [3]. In response, the Korean government announced a comprehensive plan to address the disease in 2007 and has implemented CVD prevention and management projects in various communities.

Health-related quality of life (HRQoL) is an important parameter for evaluating public health policy and is a strong predictor of mortality and morbidity [4,5]. Research on HRQoL is also important because it assesses patients' perspectives about their health and could also be used to assess healthcare systems. HRQoL measurement enables the evaluation of CVD prevention and management projects in various communities [6]. Quality of life (QoL) is a multidimensional concept that refers to subjective physical, mental, social, and financial well-being in relation to one's purpose and expectations with regard to one's own life [7].

Patients with recurrent ischemic heart disease have a mortality rate that is twice as high as that of their counterparts [8]. Stroke not only has a high mortality rate, but also results in permanent functional disability in 15% to 30% of survivors [9] and causes both physical and mental problems [10]. The condition therefore affects patients' activities of daily living [10], which highlights the importance of continuing disease management and prevention. CVD-inducing diseases such as hypertension, diabetes mellitus, hyperlipidemia, and obesity are related to individuals' health-related lifestyle [11,12]. Lifestyle habits that facilitate the prevention and management of CVD include abstinence from cigarettes and alcohol, reduced salt intake, appropriate exercise, body weight control, and stress reduction [12]. Therefore, individuals with CVD must engage in long-term and regular exercise and lifestyle management to enjoy a healthy life. For this reason, people with CVD are more likely to experience low QoL than the general population.

A previous study reported that the QoL of individuals with CVD was improved by engaging in regular physical activity, which contributed to improving heart disease, hypertension, obesity, depression, and immune functions [13]. Additionally, the QoL of older adults with CVD was improved through walking and CVD prevention programs [7,14]. Studies on individuals with CVD have primarily focused on health behaviors, which are individual factors. Since QoL is a multidimensional concept, research on QoL in people with CVD must include multidimensional factors, including mental, social, and financial domains. Therefore, more multidimensional support and efforts are required rather than emphasizing only individual efforts. In addition, CVD is a representative chronic disease of old age. As Korea rapidly progresses toward becoming an aging society, there is increasing interest in, and demand for, health interventions to promote improvements in QoL. Although factors related to QoL in patients with CVD (e.g., physical activity, depression, and obesity) have been identified, the existing research is very limited. Therefore, a multidimensional investigation of the predictors of QoL in individuals with CVD is required. This will contribute to improving QoL in individuals with CVD

To understand the QoL of individuals with CVD—a significant cause of death in the Korean population—a comprehensive and multidimensional approach to examining QoL predictors is needed. This study aimed to identify the predictors of QoL in Korean adults with CVD using data from the nationally representative Korea National Health and Nutrition Examination Survey (KNHANES). Ultimately, this investigation aimed to present foundational data for developing interventions to improve QoL in patients with CVD.

Materials and Methods

Data Source and Participants

This study conducted a secondary analysis of the 2016–2019 KNHANES data, which were originally collected by the Korea Disease Control and Prevention Agency (KDCA) of the Ministry of Health and Welfare.

The KNHANES is a legally grounded survey mandated by Article 16 of the National Health Promotion Act and approved by the KDCA's Institutional Review Board (IRB No. 2018-01-03-P-A, 2018-01-03-C-A). Data were collected through a health examination, health interview, and nutrition survey by trained investigators via face-to-face interviews. Participants provided written informed consent before completing the KNHANES survey. For this study, an agreement to adhere to the requirements for the use of statistical data was submitted through the KNHANES website to receive approval for the use of the KNHANES raw data. Upon receiving approval, the data were downloaded from the website.

Among the participants of the KNHANES VII (2016–2018) and VIII (2019), 1,081 adults aged 19 years or older who had been diagnosed with CVD (i.e., stroke, myocardial infarction, or angina) by a physician, did not have cancer, and had no missing values in terms of QoL, general factors, health-related factors, and disease-related factors were included in the analysis, as shown in Figure 1.

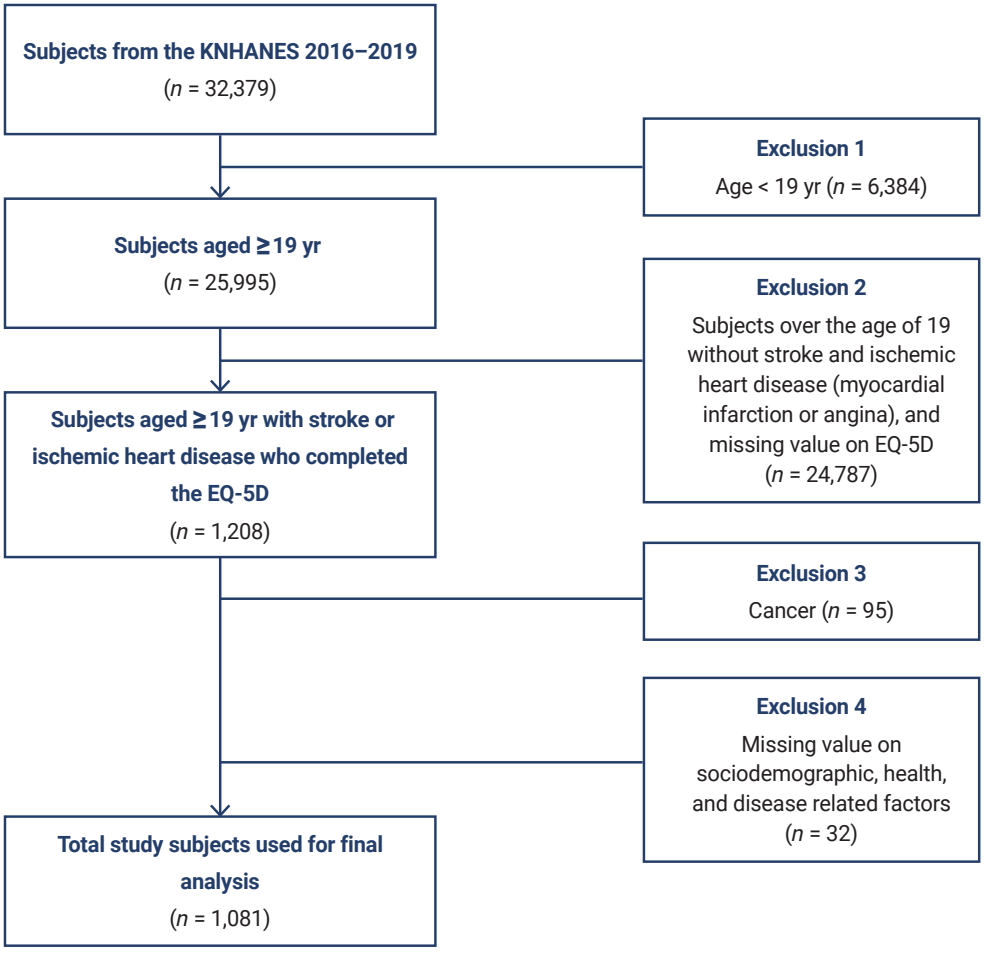

Figure 1. Flow chart of the study sample.
KNHANES, Korea National Health and Nutrition Examination Survey; EQ-5D, three-level EuroQoL Group's five-dimension questionnaire.

Measurements and instruments

Sociodemographic characteristics

The queried sociodemographic characteristics included age, sex, marital status, education level, household income, and employment status. The mean age of patients with CVD was 65.81 years, and the raw data for marital status (married or unmarried (including those who are single, divorced or widowed)) and education level (elementary or below, ≤6 years; middle school, ≤9 years; high school, ≤12 years, or college or beyond, ≥13 years) were used as obtained. Household income was calculated by dividing the monthly household income by household size; the values were classified into quartiles (low, mid-low, mid-high, and high), and the corresponding raw data were used. The employment data were reclassified for analysis in this study to simply reflect whether the participants were employed or unemployed.

Health-related factors

Health-related factors included smoking, drinking, obesity, activity restriction, physical activity, perceived health, perceived stress, and depression. The participants' current smoking status was classified as "yes" or "no," and drinking was used as shown in the raw data (defined as monthly drinking frequency: ≥1 drink/month in the past year, never drank alcohol, or <1 drink/month in the past year). Obesity was reclassified to reflect whether the participants were obese (body mass index [BMI], ≥25 kg/m^2) or non-obese.

With respect to the participants' physical characteristics, the raw data classified activity restriction as "yes" or "no" to indicate whether their activities of daily living or social activity were limited due to their condition, and the data were used as obtained. Using the WHO Global Physical Activity Questionnaire, the raw data classified physical activity into low or moderate-high based on the practice (moderate-high) or non-practice (low) of moderate physical activity for 2 hours and 30 minutes per week, vigorous physical activity for 1 hour and 15 minutes per week, or combined moderate and vigorous physical activity for the corresponding durations per week; the data were used as provided.

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With regard to the participants' psychological characteristics, their perceived health was reclassified into good, moderate, and poor, and their perceived stress was used as provided in the raw data (high or low). In the raw data, depression was determined based on whether an individual had been diagnosed with it by a physician, and the data were used as obtained.

Disease-related factors

In this study, CVD was defined as ischemic heart disease (myocardial infarction or angina) and stroke. Participants who reported that they had been diagnosed with myocardial infarction, angina, or stroke by a physician in the raw data were considered as individuals with CVD. The presence of diabetes, hypertension, and dyslipidemia was also determined based on a "yes" response in the raw data to the item that queried whether they had been diagnosed with these conditions by a physician.

Health-related QoL

QoL was measured based on the 5 domains of the three-level EuroQoL Group's five-dimension questionnaire (EQ-5D) approved by the EuroQol Group: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each item was rated on a 3-point Likert scale (1=no problem, 2=moderate problem, and 3=serious problem). The KDCA weighted the EQ-5D index for the Korean population, where a score closer to 1 indicates a better QoL. The EQ-5D index score range from 1 (no problems were reported for all 5 EQ-5D domains) to −0.171 (severe problems were reported in all 5 EQ-5D domains), where negative scores are considered worse than death, 0 represents death, and 1 indicates perfect health [15,16].

Statistical Analysis

The collected data were analyzed using IBM SPSS for Windows ver. 21.0 (IBM Corp., Armonk, NY, USA). The KNHANES data were collected using stratified 2-stage sampling, thus, a complex sample design using weights for all data analyzes was used. Prior to the analysis, the data were confirmed to have no multicollinearity based on a tolerance of above 0.1, and a variance inflation factor of less than 10 (0.998–1.298). The participants' general characteristics and health-related factors were analyzed using the frequency of the complex sample, weighted percentages, and descriptive statistics. The differences in QoL based on the participants' general characteristics, health-related factors, and disease-related factors were analyzed using the t-test and one-way analysis of variance for complex samples. The predictors of HRQoL in individuals with CVD were identified using a 3-step hierarchical regression analysis. In step 1, significant demographic factors (age, sex, education level, household income, and employment) that were similar to those in previous studies were entered. In step 2, significant physical factors (drinking, obesity, and limited activity) and significant mental factors (perceived health, perceived stress, and depression) were added. In step 3, the significant causative diseases of CVD (diabetes and hypertension) were added.

Results

Participants' General Characteristics

Table 1 presents the general characteristics of the participants. A total of 1,081 participants were included, and their mean age was 65.81±0.45 years. In total, 59.2% of the participants were male and 40.8% were female. The majority (96.2%) of the participants were married. With regard to their education level, 40.5% of the sample had completed elementary school or lower and 25.6% had completed high school. The most common household income status was low (34.5%), followed by mid-low (26.6%), mid-high (22.9%), and high (15.9%). There were more unemployed participants (57.3%) than employed participants (42.7%).

Most of the participants were non-smokers (81.4%). In total, 45.7% of the participants drank at least once a month, whereas 54.3% did not consume alcohol. A total of 32.3% of the participants were obese (BMI ≥25 kg/m^2), whereas 67.7% were not (BMI <25 kg/m^2). Furthermore, 76.5% of the participants' physical activities were not limited by their condition. The participants reported that they engaged in either low (68.4%) or moderate to high (31.6%) physical activity. Almost half (44.5%) of the participants perceived themselves as having poor health, whereas 41.5% and 14.1% of them perceived themselves as having moderate and good health, respectively. In total, 26.1% of the participants reported a high level of perceived stress, whereas the rest (73.9%) reported a low level of stress. A total of 9.6% of the participants were diagnosed with depression. The prevalence of CVD-inducing diseases, namely hypertension, dyslipidemia, and diabetes mellitus, was 61.1%, 42.0%, and 28.3%, respectively.

HRQoL Differences according to the Characteristics of Patients with CVD

As shown in Table 2, the HRQoL of patients with CVD significantly differed based on their age, sex, education level, household income level, employment, drinking, obesity, activity restriction, physical activity, perceived stress and perceived health, and whether they had depression,

Table 1. General characteristics of individuals with cardiovascular disease (n = 1,081)

Characteristic	n or mean ± SE	Weighted %
Sociodemographic characteristics		
Age (y)	65.81 ± 0.45	
19–49	53	8.3
50–64	292	34.7
65–74	409	30.4
≥ 75	327	26.6
Sex		
Male	609	59.2
Female	472	40.8
Marital status		
Married	1048	96.2
Unmarried	33	3.8
Education level		
College or more	140	15.6
High school	253	25.6
Middle school	190	18.3
Elementary school or less	498	40.5
Household income		
High	144	15.9
Middle-high	221	22.9
Low-middle	290	26.6
Low	426	34.5
Employment		
Yes	424	42.7
No	657	57.3
Health-related factors		
Current smoking		
Yes	184	18.6
No	897	81.4
Monthly drinking		
Yes (≥ 1/mo)	456	45.7
No	625	54.3
Obesity		
Yes (≥ 25 kg/m^2)	356	32.3
No (< 25 kg/m^2)	725	67.7
Limited activity		
Yes	271	23.5
No	810	76.5
Physical activity		
Moderate to high	340	31.6
Low	741	68.4
Perceived health status		
Good	140	14.1
Moderate	453	41.5
Poor	488	44.5
Perceived stress		
High	268	26.1
Low	813	73.9
Depression		
Yes	105	9.6
No	976	90.4
Disease-related factors		
Stroke		
Yes	498	47.0
No	583	53.0
Ischemic heart disease (myocardial infarction or angina)		
Yes	645	58.4
No	436	41.6
Comorbidity (hypertension)		
Yes	680	61.1
No	401	38.9
Comorbidity (diabetes mellitus)		
Yes	304	28.3
No	777	71.7
Comorbidity (dyslipidemia)		
Yes	461	42.0
No	620	58.0

SE, standard error.

diabetes mellitus, and hypertension. The QoL of male was higher than that of female (p < 0.001). Additionally, the QoL of the employed participants was higher than that of the unemployed participants. The QoL of the participants with a higher education level and household income level was also higher (p < 0.001).

In terms of health-related factors, the QoL was higher among alcohol users (p < 0.001), individuals without physical activity limitations (p < 0.001), obesity (p = 0.046), and those who engaged in moderate to vigorous physical activity (p < 0.001) than among their counterparts. Furthermore, the QoL of those with better perceived health, low perceived stress, and no depression was higher (p < 0.001). The QoL of those who also had hypertension (p = 0.004) and those with diabetes mellitus (p = 0.002) was lower. The QoL of patients with CVD did not significantly differ based on their marital status, smoking status, and the presence of dyslipidemia.

Predictors of the HRQoL of Patients with CVD

Table 3 shows the results of the general linear regression for complex samples that was conducted to identify the predictors of QoL in patients with CVD.

In model 1, multiple sociodemographic factors (female sex, education level, low household income, and unemployment) were identified as significant negative predictors. Of these, unemployment was the most potent predictor. These predictors accounted for 16.1% of the variance in the QoL of patients with CVD.

Health-related factors were included in model 2. Older age, low household income, unemployment, limited activity, poor perceived health, and depression were identified as significant negative predictors of QoL. Of these, limited activity and poor perceived health were identified as the most potent predictors. These predictors accounted for 37.0% of the variance in the QoL of patients with CVD.

Diseases that increased the risk of CVD were included in model 3. Older age, low household income, unemployment, limited activity, poor perceived health, and depression were identified as significant negative predictors of QoL. The causative diseases of CVD (hypertension and diabetes mellitus) did not predict QoL. Limited activity and perceived health were identified as the most potent predictors. These predictors accounted for 37.2% of the variance in the QoL of patients with CVD.

Discussion

This study aimed to present foundational data for developing interventions to improve QoL in Korean adults with CVD by identifying predictors of their QoL using data from the

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Table 2. Differences in HRQoL based on the characteristics of individuals with cardiovascular disease (n = 1,081)

Characteristic	Mean ± SE	p
Sociodemographic characteristics		
Age (y)	0.87 ± 0.00	< 0.001
19–49	0.93 ± 0.02	
50–64	0.89 ± 0.01	
65–74	0.88 ± 0.01	
≥ 75	0.81 ± 0.01	
Sex		< 0.001
Male	0.90 ± 0.07	
Female	0.83 ± 0.01	
Marital status		0.624
Married	0.87 ± 0.01	
Unmarried	0.86 ± 0.03	
Education level		< 0.001
College or more	0.95 ± 0.01	
High school	0.89 ± 0.01	
Middle school	0.87 ± 0.01	
Elementary school or less	0.83 ± 0.01	
Household income		< 0.001
High	0.93 ± 0.01	
Middle-high	0.91 ± 0.01	
Low-middle	0.88 ± 0.01	
Low	0.80 ± 0.01	
Employment		< 0.001
Yes	0.93 ± 0.01	
No	0.82 ± 0.01	
Health-related factors		
Current smoking		0.165
Yes	0.89 ± 0.01	
No	0.87 ± 0.01	
Monthly drinking		< 0.001
Yes (≥ 1/mo)	0.91 ± 0.01	
No	0.84 ± 0.01	
Obesity		0.046
Yes (≥ 25 kg/m^2)	0.89 ± 0.01	
No (< 25 kg/m^2)	0.86 ± 0.01	
Limited activity		< 0.001
Yes	0.74 ± 0.01	
No	0.91 ± 0.01	
Physical activity		< 0.001
Moderate to high	0.91 ± 0.01	
Low	0.85 ± 0.01	
Perceived health status		< 0.001
Good	0.95 ± 0.01	
Moderate	0.93 ± 0.01	
Poor	0.79 ± 0.01	
Perceived stress		< 0.001
High	0.82 ± 0.01	
Low	0.89 ± 0.01	
Depression		< 0.001
Yes	0.73 ± 0.03	
No	0.88 ± 0.01	
Disease-related factors		
Comorbidity (hypertension)		0.004
Yes	0.86 ± 0.01	
No	0.89 ± 0.01	
Comorbidity (diabetes mellitus)		0.002
Yes	0.84 ± 0.01	
No	0.88 ± 0.01	
Comorbidity (dyslipidemia)		0.238
Yes	0.86 ± 0.01	
No	0.88 ± 0.01	
HRQoL (EQ-5D)	0.87 ± 0.01	

HRQoL, health-related quality of life; SE, standard error; EQ-5D, three-level EuroQoL Group's five-dimension questionnaire.

KNHANES (2016–2019).

In this study, the most potent predictor of HRQoL in individuals with CVD was limitation of physical activity. Limited activity refers to the restriction of one's activities of daily living or social activities due to health problems or physical or mental disabilities. A low level of mobility has been reported as a predictor of QoL in stroke survivors [17]. A stroke could potentially induce severe disability, and Korean adults aged 50 years or older who had experienced stroke had a low HRQoL [18]. Our results also corroborated the high positive correlation between stroke patients' activities of daily living and QoL [10]. Approximately 46% of stroke survivors require assistance with their activities of daily living, and 30% of them are incapable of independent living [19]. Patients who are dependent on others or require assistance with activities of daily living have been shown to have a markedly lower QoL than others [20]. Limited physical activity has also been identified as a predictor of the HRQoL of individuals with diabetes mellitus [21] and older adults with osteoarthritis [22]. Therefore, limitation of physical activity is an important predictor of HRQoL. The management of physical mobility can improve QoL and extend the lifespan [23]. Until now, CVD management has mainly focused on disease prevention. Of course, interventions that prevent the deterioration of motor function/disabilities of individuals with CVD should be continued. At the same time, however, interventions should be strengthened to enable people with CVD to perform their daily activities well. A program for strengthening physical function should be prepared in the current community-centered CVD prevention project. Social assistance and related systems should be further strengthened so that there is no inconvenience when moving due to physical activity limitations. Reducing discomfort caused by limitation of physical activity will improve the QoL of people with CVD. Thus, in addition to health-related interventions, better support for individuals with limited physical activity during their activities of daily living and systems that facilitate their movement are needed.

In this study, the second predictor of HRQoL of individuals with CVD was perceived health. We can predict that in individuals with CVD, poor perceived health will be associated with lower HRQoL. Individuals with other chronic diseases also had lower HRQoL when they perceived their health as poor [21,22]. Perceived health has been found to be more important than other clinical indicators as a strong predictor of risk for mortality [24]. CVD is a chronic disease that requires lifestyle modifications through regular exercise and lifestyle management. Therefore, individuals with CVD must monitor the status of their health on an

Table 3. General linear regression analysis of the HRQoL of individuals with CVD (n = 1,081)

Variable	Model 1		Model 2		Model 3	
	β	SE	β	SE	β	SE
Sociodemographic factors						
Age (y)	0.000	0.001	−0.002**	0.001	−0.002**	0.001
Sex						
Female (ref.: male)	−0.035**	0.013	−0.013	0.012	−0.014	0.012
Education level (ref.: college of more)						
High school	−0.031**	0.014	−0.009	0.013	−0.008	0.013
Middle school	−0.038**	0.015	−0.009	0.012	−0.009	0.013
Elementary school or less	−0.048**	0.017	−0.024	0.014	−0.023	0.014
Household income (ref.: high)						
Middle-high	0.010	0.014	0.003	0.013	0.004	0.013
Low-middle	−0.005	0.015	−0.003	0.013	−0.002	0.013
Low	−0.059**	0.018	−0.034*	0.016	−0.033*	0.015
Employment						
No (ref: yes)	−0.069***	0.012	−0.024*	0.010	−0.023*	0.010
Health-related factors						
Monthly drinking						
No (ref: yes)			−0.009	0.010	−0.009	0.010
Obesity (ref.: yes, ≥ 25 kg/m^2)						
No (< 25 kg/m^2)			−0.012	0.009	−0.016	0.008
Limited activity						
Yes (ref.: no)			−0.102***	0.014	−0.103***	0.014
Physical activity						
No (ref.: yes)			−0.016	0.008	−0.016	0.008
Perceived health status (ref.: good)						
Moderate			0.008	0.011	0.008	0.011
Poor			−0.091***	0.012	−0.089***	0.012
Perceived stress						
High (ref.: low)			−0.010	0.011	−0.010	0.012
Depression						
Yes (ref: no)			−0.065**	0.022	−0.065**	0.003
Disease-related factors (ref.: no)						
Comorbidity (diabetes mellitus)					−0.010	0.023
Comorbidity (hypertension)					−0.001	0.010
Comorbidity (diabetes mellitus and hypertension)					−0.016	0.015
R^2	0.161***		0.370***		0.372***	

HRQoL, health-related quality of life; CVD, cardiovascular disease; SE, standard error.
*p < 0.05, **p < 0.01, ***p < 0.001.

ongoing basis. A positive perception of one's health can bring about lifestyle changes. Mental interventions that instill a positive outlook toward one's health should thus be implemented alongside interventions that target individuals' physical function. Instilling a positive attitude among such individuals with respect to their health through a combination of physical and mental interventions could contribute to improving their HRQoL.

Another predictor of HRQoL in individuals with CVD was depression, a psychological factor. Korean adults aged 50 years or older with depression had a lower HRQoL than their counterparts without depression [25]. The incidence of depression was higher among patients with ischemic heart disease than among those without ischemic heart disease [26]. The 2018 European Society of Cardiology guidelines stated that the prevalence of depression among patients with coronary artery disease ranges from 15% to 30%, which is higher than that among the general population (10%) [27]. Another study reported that approximately 40% of stroke patients had depression [19]. Depression is negatively associated with QoL [28], and post-stroke depression is negatively correlated with QoL and activities

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of daily living [10]. Depression has also been reported to be associated with physical and mental factors in individuals with chronic diseases [27,29]. Therefore, interventions to prevent and alleviate depression to improve HRQoL in CVD patients should also be implemented. In Korea, various community-led CVD prevention and management projects are underway. However, these projects have mainly focused on disease-related knowledge education. An intervention to screen depression in individuals with CVD should also be included. It is also necessary to prepare a system to manage individuals with confirmed depression. Identifying and preventing depression, which is a predictor of HRQoL in individuals with CVD, in advance will improve their QoL.

Age, household income, and employment status were also predictors of HRQoL in individuals with CVD. Individuals with CVD who had a low household income and were unemployed had a low HRQoL. In a previous study, Korean adults aged 50 to 69 years who had a low household income and were unemployed displayed a low HRQoL [25]. The mean age of our participants was 65.81 years, suggesting that Koreans with CVD are primarily older adults. The prevalence of various diseases and the burden of their costs of care increase as they age. Older people are unable to address these changes because most of them are unemployed and economically inactive. This situation negatively impacts their QoL. In particular, CVD is the disease that consumes the most medical expenses, so this patient population would inevitably have a high financial burden. In particular, the main predictors of HRQoL in individuals with CVD were perceived health and depression, age, household income, and employment, in addition to physical factors. In other words, the HRQoL of individuals with CVD constitutes interactions among physical, psychological, and economic factors. In this context, strengthened social welfare programs that can alleviate psychological and economic problems should be implemented to help improve the QoL of individuals with CVD.

The limitations of this study are as follows. The measure of HRQoL used in this study included mobility and anxiety/depression. Therefore, these factors may have influenced the predictors of QoL in individuals with CVD. To address this limitation, we suggest further studies on QoL in individuals with CVD in the future. Another limitation is the cross-sectional design of this study. Therefore, there is a limit to elucidating the causal relationships of variables that were identified as predictors of QoL.

Conclusion

This study aimed to identify the predictors of HRQoL in individuals with CVD using data from the KNHANES. The results of this study indicated that the negative predictors of HRQoL among Korean adults with CVD were older age, low household income, unemployment, limited activity, poor perceived health, and depression. The most potent predictor was limited activity, followed by perceived health and depression. Thus, the current community-led CVD prevention projects should implement interventions that target both physical and mental aspects in order to simultaneously instill healthier routines and positive perceptions about one's health. Furthermore, the financial hardship experienced by patients should not be simply deemed an individual problem. Instead, it should be addressed by society through bolstered social welfare and support systems in order to improve the QoL of this patient population.

The main limitation of this study is attributed to the fact that its results are focused only on the Korean population. Thus, the generalizability of the study's findings is limited. Future studies that focus on different ethnic and global populations will enable the development of support policies specifically tailored to the populations of different countries, thereby improving the QoL of cardiovascular patients.

Notes

Ethics Approval

The KNHANES is a legally grounded survey mandated by Article 16 of the National Health Promotion Act and was approved by the KDCA's Institutional Review Board (IRB No. 2018-01-03-P-A, 2018-01-03-C-A).

Conflicts of Interest

The author has no conflicts of interest to declare.

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Availability of Data

All data analyzed in this study are included in this article. For other data, these may be available through the author upon reasonable request

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Original Article

Neck circumference and incidence of cerebrovascular disease over 12 years among Korean adults

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ABSTRACT

Objectives: Neck circumference is associated with a distinctive fat storage process that confers additional metabolic risk. Hence, this study aimed to investigate the correlation between baseline neck circumference and the incidence of cerebrovascular disease using a prospective community-based sample of Korean adults over 12 years of follow-up, after controlling for selected covariates.

Methods: Participants with non-cerebrovascular disease were divided into 4 groups (Q1–Q4) based on their baseline neck circumference. Cox proportional hazards analysis was used to calculate hazard ratios and 95% confidence intervals (CIs) to evaluate the relationship between neck circumference and cerebrovascular disease incidence over a 12-year period.

Results: Among this study's 3,662 participants, 128 (3.50%) developed cerebrovascular disease. The incidence of cerebrovascular disease increased from 2.2% in Q1 to 4.3% in Q2, 2.5% in Q3, and 5.0% in Q4. When compared to Q1, the relative risks of cerebrovascular disease development were 0.57 (95% CI, 0.25–1.31), 0.86 (95% CI, 0.38–1.96), and 0.79 (95% CI, 0.30–2.07) in man and 1.86 (95% CI, 0.66–5.20), 3.50 (95% CI, 1.25–9.86), and 4.71 (95% CI, 1.50–14.77) in woman in Q2, Q3, and Q4, respectively, after adjusting for most risk factors related to cerebrovascular disease.

Conclusion: The relationship between neck circumference and cerebrovascular disease was stronger in woman than in man, indicating potential differences between the sexes. These results are meaningful for evaluating and surveilling neck circumference as a promising tool for identifying subgroups of vulnerable and at-risk populations.

Keywords: Cerebrovascular disorders; Korea; Neck; Public health practice

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Introduction

Cerebrovascular disease, a disease of the blood vessels that supply the brain [1], is the fourth most common cause of death in Korea after cancer, heart disease, and pneumonia, and in

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2019, the number of deaths from cerebrovascular disease in Korea reached 42 per 100,000 people [2]. Although the number of deaths due to cerebrovascular disease has been decreasing substantially, the incidence and prevalence of cerebrovascular disease have not been considerably reduced owing to population aging and the lack of new diagnostic techniques [3]. Depending on the specific part of the brain that is damaged, cerebrovascular disease can be accompanied by various symptoms, including motor disorders, sensory disorders, speech disorders, and emotional disorders [4]. Therefore, cerebrovascular disease is a serious national health problem that causes substantial economic losses for the nation, as well as the burden of individual medical costs, thereby necessitating early prevention and diagnosis of this chronic disease.

Except for congenital cerebrovascular diseases, such as genetic abnormalities, most acquired cerebrovascular diseases can be prevented by controlling risk factors [5] such as hypertension, smoking, obesity, and diabetes [6]. Since the incidence of cerebrovascular disease increases with age [5], controlling these modifiable risk factors may reduce the incidence of cerebrovascular disease. Therefore, a screening tool that can detect individuals at high risk of developing cerebrovascular disease at an early stage is required; however, currently, most cerebrovascular diseases are only diagnosed at the onset of symptoms [7].

Neck circumference (NC) is an easy, reliable, and widely affordable anthropometric indicator. NC can be measured with a measuring tape just below the laryngeal prominence of the neck in an upright position [8]; therefore, it can be measured easily and quickly. NC is related to several factors, including age, sex, body mass index (BMI), weight, waist circumference, and systolic blood pressure [9–12]. These indicators also correspond to the risk factors for cerebrovascular diseases [13]. Growing evidence suggests that NC is a novel indicator of upper-body subcutaneous fat distribution [14]. In particular, upper-body fat distribution with increased visceral fat has been shown to be a better predictor of metabolic complications of obesity than the degree of overweight [15]. Furthermore, as an indicator of subcutaneous adipose tissue distribution in the upper-body [16], NC has been found to be associated with cardiometabolic risk factors in populations with diverse ethnic backgrounds [17–19]. In the Framingham Heart Study, participants with a large NC had more cardiometabolic risk factors than those with a small NC, even after adjusting for BMI [20]. Additionally, NC is regarded as an indicator of upper-body obesity and is positively correlated with changes in blood pressure and other components of metabolic syndrome [21].

Ultrasound, computed tomography, and magnetic resonance imaging are the gold standard imaging modalities for determining obesity, body fat distribution, and further diagnosis. However, these methods are frequently costly and impractical in large-scale or long-term investigations; thus, reliable, simple, and easy-to-implement measurements of obesity and body fat distribution are required [12]. Compared to standard measures, NC may be a useful and convincing alternative measure for obesity, a better indicator of metabolic risk [19], and may also be effective in screening health risks in the general public, particularly for large samples where non-invasive, low-cost, and simple-to-implement measures are needed.

Previous studies in Korea have shown significant relationships between NC and obesity, diabetes, cardiovascular disease, and obstructive sleep apnea [22–25]. However, to the best of our knowledge, the potential association between NC and the incidence of cerebrovascular disease has not been previously investigated in middle-aged and older Korean individuals. Hence, this study aimed to determine the association of baseline NC with the incidence of cerebrovascular disease using a prospective community-based sample of Korean adults over a 12-year follow-up period, after controlling for selected covariates.

Materials and Methods

Study Population

The present study was performed using data from the Korean Genome and Epidemiology Study (KoGES), a prospective community-based cohort study. The study cohort consisted of 40- to 69-year-old residents of Ansan or Ansung City, near the capital city of Seoul. Assessments were conducted biennially between 2003 and 2015. Initially, 8,603 participants were included in the baseline survey. NC was measured in Ansan as an anthropometric index related to respiratory diseases. Participants with missing data or cerebrovascular disease at baseline were excluded. Thus, a total of 3,662 participants were included in the final analysis (Figure 1). All participants volunteered for the study, and informed consent was obtained from each patient.

Definition of Cerebrovascular Disease

The diagnosis of cerebrovascular disease in this study was defined as a history of stroke (including cerebral infarction and cerebral hemorrhage), which was established by an answer of "yes" to the question, "Have you ever been diagnosed with cerebrovascular disease by a doctor in a hospital in the past?"

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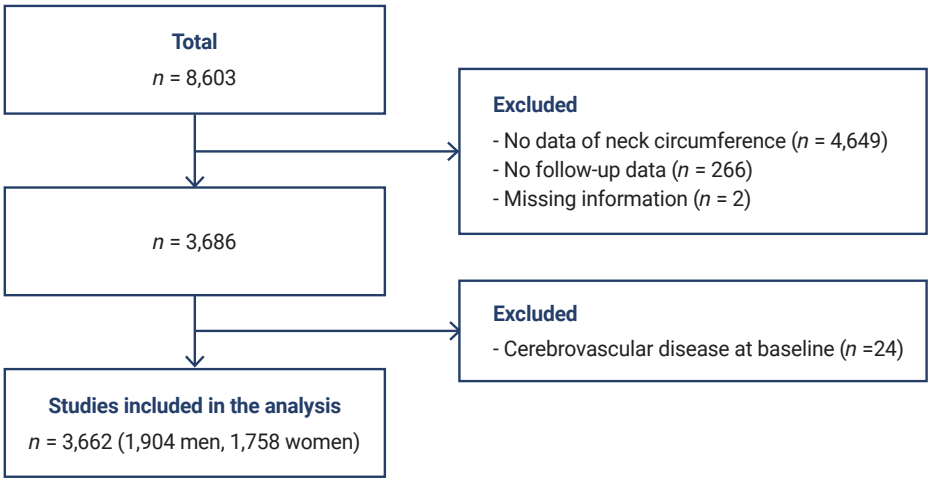

Figure 1. Flow diagram of participant inclusion and exclusion.

Measures

Participants in the KoGES were examined biannually by trained interviewers using a questionnaire that included participants' demographic factors (age, marital status, and employment), health behaviors (smoking, drinking, and regular exercise), and comorbidities (hypertension, diabetes, and hyperlipidemia). Anthropometric parameters (height, body weight, and NC) were measured using standard methods.

NC (cm) was measured using non-stretchable plastic tape to the nearest 1 mm from the level just below the laryngeal prominence perpendicular to the long axis of the neck with the head positioned in the Frankfurt horizontal plane.

The variables included as covariates in this study were demographic factors, health behaviors, comorbidities, and BMI. Age was categorized into 3 groups: 40 to 49, 50 to 59, and ≥60 years. Marital status was classified as never-married and married (with the presence or absence of a spouse), and employment was classified as a dichotomous variable (yes/no). Smoking and alcohol consumption status were each classified into 3 categories: non-, former, and current. Exercise habits were divided into 2 categories: none or irregular (≤1 session/wk) and regular (≥2 sessions/wk). One session of exercise was defined as exercising for at least 30 minutes. Comorbidities were defined as the presence or absence of hypertension, diabetes, and hyperlipidemia. BMI was calculated as body weight (kg) divided by height squared (m^2).

Statistical Analysis

The participants' baseline characteristics were analyzed using the mean and standard deviation for continuous variables and frequency and percentage for categorical variables. Differences among groups were analyzed using an analysis of variance with the Scheffé *post-hoc* analysis method for continuous variables and the chi-square test for categorical variables. The cumulative rates of the incidence of cerebrovascular disease were estimated using Kaplan-Meier survival curves, and equality was compared using log-rank tests. Cox proportional hazards analyzes were conducted to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between NC and cerebrovascular disease incidence over 12 years. Multivariate Cox proportional hazards regression analysis was performed to identify the association between NC and cerebrovascular disease incidence after controlling for covariates (demographic factors, health behaviors, comorbidities, and BMI) at baseline. The analyses were performed using IBM SPSS ver. 25.0 (IBM Corp., Armonk, NY, USA). For all tests, statistical significance was set at $p < 0.05$.

Ethics Statement

The Institutional Review Board of Pukyong National University approved the study protocol (IRB No. 1041386-201907-HR-29-01). The study protocol was also approved by the Ethics Committee of KoGES at the Korean National Institute of Health, and the study was performed in accordance with approved guidelines.

Results

Of the total study population of 3,662 adults aged ≥40 years, 52.0% were man (*n*=1,904). The mean NC was 37.7±2.1 cm, 32.9±1.8 cm, and 35.4±3.1 cm in man, woman, and the total study population, respectively. Participants' mean age at study entry was 50.5±7.5 years for the total

study population; and 50.1±7.3 and 50.9±7.8 years for man and woman, respectively. The average proportion of the population with spouses and the rate of employment was higher for man. Man also had a higher proportion of current smokers and drinkers. Woman was less likely than man to exercise regularly. The total population's average BMI was 24.6±2.9 kg/m^2, which was similar in man and woman. Woman had a higher proportion of participants with hyperlipidemia and hypertension, but that of diabetes was lower (Table 1).

A thicker NC was found in woman who were older, married, and unemployed, while in man, current drinkers were found to have a thicker NC than those who did not drink. The presence of chronic disease (hypertension, diabetes, and hyperlipidemia) was associated with a thicker NC in both man and woman (Table 2).

Over the follow-up period (0–12 years), 128 adults developed cerebrovascular disease, including 69 men and 59 women. The incidence was significantly higher in man (3.5/1,000 person-years) than in woman (3.2/1,000 person-years). Table 3 demonstrates the incidence of cerebrovascular disease rates over an average of 10.6±2.9 years in man and woman, stratified by quartiles of NC. As expected, a thicker NC was correlated with a higher incidence of cerebrovascular disease in the total study population. No significant differences in incidence were found among man, but there were significant differences between the thinnest and thickest NC groups in woman.

Table 4 provides the HRs, controlled for covariates, of cerebrovascular disease incidence in association with NC according to sex. The HRs of the incidence of cerebrovascular disease in the thickest quartile group of NC (man, ≥39.1 cm; woman, ≥34.0 cm) were consistently higher than those in the thinnest NC group (man, <36.2 cm; woman, <31.5 cm). Compared to the lowest quartile (i.e., the reference group), the fully multivariate-adjusted HRs of the incidence of cerebrovascular disease in the thickest NC group were 0.79 (95% CI, 0.30–2.07) for man and 4.71 (95% CI, 1.50–14.77) for woman. Regarding the sex-related differences between NC and risk of cerebrovascular disease occurrence, man showed no statistically significant differences between the quartiles; however, in woman, the higher quartiles (Q3, ≥32.8 and <34.0 cm; Q4, ≥34.0 cm) had a higher incidence of cerebrovascular disease than the reference

Table 1. Baseline characteristics of the study population

Characteristic	Man (*n*=1,904)	Woman (*n*=1,758)	Total (*n*=3,662)
Neck circumference (cm)	37.7±2.1	32.9±1.8	35.4±3.1
Age (y)	50.1±7.3	50.9±7.8	50.5±7.5
Age groups (y)			
40–49	1,125 (59.1)	969 (55.1)	2,094 (57.2)
50–59	509 (26.7)	491 (27.9)	1,000 (27.3)
≥60	270 (14.2)	298 (17.0)	568 (15.5)
Marital status			
Never married	10 (0.5)	12 (0.7)	22 (0.6)
Married (spouse present)	1,848 (97.1)	1,543 (87.8)	3,391 (92.6)
Married (spouse absent)	46 (2.4)	203 (11.5)	249 (6.8)
Employment	1,684 (88.4)	584 (33.2)	2,268 (61.9)
Smoking			
Non-smoker	415 (21.8)	1,695 (96.4)	2,110 (57.6)
Former smoker	749 (39.3)	21 (1.2)	770 (21.0)
Current smoker	740 (38.9)	42 (2.4)	782 (21.4)
Drinking			
Non-drinker	314 (16.5)	1,114 (63.4)	1,428 (39.0)
Former drinker	149 (7.8)	37 (2.1)	186 (5.1)
Current drinker	1,441 (75.7)	607 (34.5)	2,048 (55.9)
Regular exercise	1,088 (57.1)	979 (55.7)	2,067 (56.4)
Body mass index (kg/m^2)	24.5±2.7	24.6±3.1	24.6±2.9
Hypertension	243 (12.8)	233 (13.3)	476 (13.0)
Diabetes	171 (9.0)	91 (5.2)	262 (7.2)
Hyperlipidemia	23 (1.2)	41 (2.3)	64 (1.7)

Data are presented as mean±standard deviation or *n* (%).

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Table 2. Differences in baseline characteristics of the study population by quartiles of neck circumference

Neck circumference (quartile)	Man (*n* = 1,904, 52.0%)					Woman (*n* = 1,758, 48.0%)					Total (*n* = 3,662, 100%)				
	Q1 (*n* = 451)	Q2 (*n* = 493)	Q3 (*n* = 477)	Q4 (*n* = 483)	*p*	Q1 (*n* = 428)	Q2 (*n* = 448)	Q3 (*n* = 448)	Q4 (*n* = 434)	*p*	Q1 (*n* = 909)	Q2 (*n* = 906)	Q3 (*n* = 923)	Q4 (*n* = 924)	*p*
Age (y)	50.6 ± 7.8	50.2 ± 7.3	49.9 ± 7.4	49.8 ± 6.6	0.331	49.3 ± 6.9	50.3 ± 7.6	51.7 ± 7.9	52.2 ± 8.3	< 0.001	49.8 ± 7.3	51.5 ± 7.9	50.6 ± 7.7	49.9 ± 7.0	< 0.001
Age groups (y)					0.113					< 0.001					< 0.001
40–49	257 (57.0)	285 (57.8)	295 (61.8)	288 (59.6)		272 (63.6)	260 (58.0)	228 (50.9)	209 (48.2)		551 (60.6)	468 (51.7)	518 (56.1)	557 (60.3)	
50–59	119 (26.4)	139 (28.2)	110 (23.1)	141 (29.2)		106 (24.8)	124 (27.7)	137 (30.6)	124 (28.6)		238 (26.2)	266 (29.4)	252 (27.3)	244 (26.4)	
≥ 60	75 (16.6)	69 (14.0)	72 (15.1)	54 (11.2)		50 (11.7)	64 (14.3)	83 (18.5)	101 (23.3)		120 (13.2)	172 (19.0)	153 (16.6)	123 (13.3)	
Marital status					0.486[a]					0.001[a]					< 0.001
Never married	3 (0.7)	5 (1.0)	1 (0.2)	1 (0.2)		4 (0.9)	3 (0.7)	2 (0.4)	3 (0.7)		7 (0.8)	5 (0.6)	8 (0.9)	2 (0.2)	
Married (spouse present)	434 (96.2)	474 (96.1)	467 (97.9)	473 (97.9)		392 (91.6)	392 (87.5)	395 (88.2)	364 (83.9)		813 (89.4)	807 (89.1)	868 (94.0)	903 (97.7)	
Married (spouse absent)	14 (3.1)	14 (2.8)	9 (1.9)	9 (1.9)		32 (7.5)	53 (11.8)	51 (11.4)	67 (15.4)		89 (9.8)	94 (10.4)	47 (5.1)	19 (2.1)	
Employment	392 (86.9)	430 (87.2)	432 (90.6)	430 (89.0)	0.260	164 (38.3)	157 (35.0)	145 (32.4)	118 (27.2)	0.005	340 (37.4)	394 (43.5)	713 (77.2)	821 (88.9)	< 0.001
Smoking					0.086					0.374					< 0.001
Non-smoker	119 (26.4)	99 (20.1)	103 (21.6)	94 (19.5)		410 (95.8)	428 (95.5)	436 (97.3)	421 (97.0)		861 (94.7)	729 (80.5)	324 (35.1)	196 (21.2)	
Former smoker	153 (33.9)	206 (41.8)	190 (39.8)	200 (41.4)		8 (1.9)	4 (0.9)	5 (1.1)	4 (0.9)		17 (1.9)	81 (8.9)	302 (32.7)	370 (40.0)	
Current smoker	179 (39.7)	188 (38.1)	184 (38.6)	189 (39.1)		10 (2.3)	16 (3.6)	7 (1.6)	9 (2.1)		31 (3.4)	96 (10.6)	297 (32.2)	358 (38.7)	
Drinking					0.050					0.503					< 0.001
Non-drinker	91 (20.2)	73 (14.8)	72 (15.1)	78 (16.1)		260 (60.7)	281 (62.7)	281 (62.7)	292 (67.3)		560 (61.6)	487 (53.8)	229 (24.8)	152 (16.5)	
Former drinker	45 (10.0)	35 (7.1)	30 (6.3)	39 (8.1)		9 (2.1)	11 (2.5)	11 (2.5)	6 (1.4)		22 (2.4)	34 (3.8)	63 (6.8)	67 (7.3)	
Current drinker	315 (69.8)	385 (78.1)	375 (78.6)	366 (75.8)		159 (37.1)	156 (34.8)	156 (34.8)	136 (31.3)		327 (36.0)	385 (42.5)	631 (68.4)	705 (76.2)	
Regular exercise	245 (54.3)	276 (56.0)	286 (60.0)	281 (58.2)	0.322	229 (53.5)	254 (56.7)	253 (56.5)	243 (56.0)	0.768	507 (55.8)	514 (56.7)	502 (54.4)	544 (58.9)	0.261
BMI (kg/m^2)	21.7 ± 1.9	23.7 ± 1.6	25.3 ± 1.6	27.2 ± 2.1	< 0.001	21.9 ± 1.9	23.6 ± 1.9	25.5 ± 2.1	27.6 ± 2.9	< 0.001	22.8 ± 2.2	24.8 ± 2.9	24.4 ± 2.8	26.5 ± 2.2	< 0.001
Hypertension	32 (7.1)	43 (8.7)	73 (15.3)	95 (19.7)	< 0.001	26 (6.1)	45 (10.0)	59 (13.2)	103 (23.7)	< 0.001	74 (8.1)	118 (13.0)	122 (13.2)	162 (17.5)	< 0.001
Diabetes	24 (5.3)	32 (6.5)	45 (9.4)	70 (14.5)	< 0.001	7 (1.6)	12 (2.7)	20 (4.5)	52 (12.0)	< 0.001	20 (2.2)	58 (6.4)	75 (8.1)	109 (11.8)	< 0.001
Hyperlipidemia	2 (0.4)	7 (1.4)	11 (2.3)	3 (0.6)	0.035	7 (1.6)	7 (1.6)	8 (1.8)	19 (4.4)	0.014	14 (1.5)	21 (2.3)	15 (1.6)	14 (1.5)	0.510

Data are presented as mean ± standard deviation or *n* (%).

Q, quartile; Q1, lowest; Q2, low-middle; Q3, middle-high; Q4, highest; BMI, body mass index.

[a]Fisher exact test.

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Table 3. Cerebrovascular disease incidence by quartiles of neck circumference by sex

Neck circumference	Man			Woman			Total		
	Total	Incidence (%)	Incidence rate per 1,000 p-y	Total	Incidence (%)	Incidence rate per 1,000 p-y	Total	Incidence (%)	Incidence rate per 1,000 p-y
Q1	451	14 (3.1)	3.0	428	6 (1.4)	1.3	909	20 (2.2)	2.0
Q2	493	11 (2.2)	2.1	448	11 (2.5)	2.3	906	39 (4.3)	4.0
Q3	477	20 (4.2)	4.1	448	21 (4.7)	4.4	923	23 (2.5)	2.4
Q4	483	24 (5.0)	4.8	434	21 (4.8)	4.6	924	46 (5.0)	4.8
Total	1,904	69 (3.6)	3.5	1,758	59 (3.4)	3.2	3,662	128 (3.5)	3.3

p-y, person-years; Q, quartile; Q1, lowest; Q2, low-middle; Q3, middle-high; Q4, highest.

Table 4. HRs of cerebrovascular disease incidence in relation to neck circumference over the 12-year study follow-up

Neck circumference	Man		Woman		Total	
	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
Q1	1		1		1	
Q2	0.57 (0.25–1.31)	0.183	1.86 (0.66–5.20)	0.239	1.40 (0.78–2.52)	0.260
Q3	0.86 (0.38–1.96)	0.723	3.50 (1.25–9.86)	0.018	0.72 (0.34–1.52)	0.385
Q4	0.79 (0.30–2.07)	0.636	4.71 (1.50–14.77)	0.008	1.36 (0.58–3.18)	0.475

Model were adjusted for demographic factors (age, marital status, employment), health behaviors (smoking, drinking, regular exercise), body mass index, and comorbidities (hypertension, diabetes, hyperlipidemia).
HR, hazard ratio; CI, confidence interval; Q, quartile; Q1, lowest; Q2, low-middle; Q3, middle-high; Q4, highest.

group (Q1, <31.6 cm). The significance of the relationship between NC and the incidence of cerebrovascular disease was confirmed with additional multivariate-adjusted Cox regression models. The Kaplan-Meier survival curves presented in Figures 2 and 3 show the overall survival rate of woman with a thinner NC; in man, Q2 of NC showed a better overall survival rate.

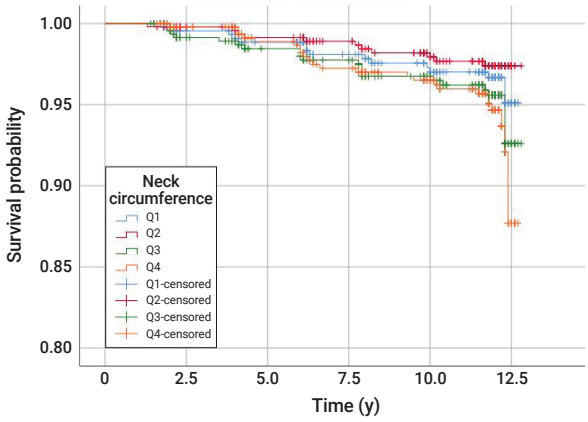

Figure 2. Kaplan-Meier survival curves stratified by neck circumference quartiles for man.

Discussion

In this prospective survey study using community-based cohort data, a thicker NC at study entry (i.e., baseline) was significantly associated with a higher incidence of cerebrovascular disease over the 12 years of follow-up in Korean woman aged over ≥40 years. Furthermore, the significance of this relationship between NC and cerebrovascular disease incidence was stronger in woman than in man, even after adjusting for the selected covariates.

Previous studies have shown a significant negative correlation between NC and incident stroke in various adult populations [26,27]. In a similar manner, the powerful association found in our study was independent of age, socioeconomic factors, health behaviors, and comorbidities. While other studies concentrated on older adults (mean age of 65 years) [27], our study population was younger, with an average age of 50 years (range, 40–71 years). Taken together, these results present the anticipatory value of NC for future incident cerebrovascular disease among middle-aged and elderly adults.

This study demonstrated that there were differences in the association between NC and cerebrovascular disease incidence with respect to sex. The mean values of NC were significantly different between man and woman (37.7 cm and 32.9 cm, respectively). When comparing the incidence rate of cerebrovascular diseases per 1,000 person-years, man and woman had similar incidence rates (3.5 and 3.2, respectively), but in man, the incidence did not increase along with the NC quartile. These results were judged to reflect the self-reports from the study participants without

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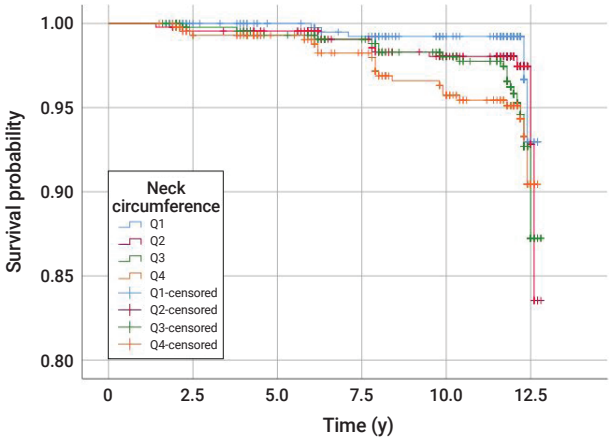

Figure 3. Kaplan-Meier survival curves stratified by neck circumference quartiles for woman.

determining whether cerebrovascular disease occurred through a standardized diagnostic process; therefore, it is thought that disease incidence was underestimated. Thus, additional studies are needed to accurately evaluate the occurrence of cerebrovascular disease over time.

Only a few studies have examined the sex-specific relationship between relevant factors; however, there have been mixed findings [28,29]. Marrugat et al. [28] estimated the incidence of cerebrovascular disease in the Spanish population in 2002 and found that the cumulative incidence of cerebrovascular disease per 100,000 people was 218 for man and 127 for woman. In contrast, Ben-Noun and Laor [29] reported a significant association between NC and BMI, waist circumference, waist-to-hip ratio, total cholesterol, low-density lipoprotein-cholesterol, triglycerides, glucose, uric acid, and blood pressure, which were found to be more relevant for woman than for man. These factors are also positively associated with the onset of cerebrovascular disease. Therefore, it can be predicted that a higher NC in woman may increase their risk of developing cerebrovascular diseases. By using population-based data, the present study provides new information on the association between NC and cerebrovascular disease in the Korean population.

Similar to cerebrovascular disease, the incidence of cardiovascular disease has also been reported to increase rapidly after the age of 40, then proportionally with age, and it also varies according to sex [13,27]. Woman in particular, experience a variety of menopausal symptoms such as reduced levels of female sex hormones, increased weight, increased stress, and decreased physical activity after the age of 45 [30]. In addition, as age increases, the prevalence of various chronic diseases, including hypertension, diabetes, and hyperlipidemia also increases; this leads to an increase in the incidence of cardiovascular disease and mortality from coronary artery disease in older individuals [1]. As a result, man is more likely than woman to develop cardiovascular disease before they reach the age of 60, but woman is more likely to develop cardiovascular disease in their 60s or older, and woman is 2 times more likely to develop cardiovascular disease than man in their 80s or older [31]. Middle-aged woman is known to have an increased quantity of internal fat due to menopause [32], which leads to a concurrent increase in NC. As such, NC should be carefully monitored in this population of woman.

Among various anthropometric measures, NC is an indicator of the distribution of subcutaneous tissue in the upper body, and it can be measured quickly and relatively inexpensively [23]. In addition, because no special equipment is required, it is easy and feasible to educate people visiting public health centers or primary medical centers on how to measure NC. The increasing evidence supporting the relationship between NC and disease prediction suggests that simply assessing and monitoring NC has great potential for disease prevention in community-based settings. Moreover, it is important that adults, children, and adolescents are educated about NC and its related long-term health effects, as this may motivate them to modify their health behaviors, such as nutrition and physical activity. NC measurements can serve as a practical screening assessment tool for the early detection of individuals vulnerable to disease in community-based health promotion initiatives.

The limitations of this study include the use of self-reported data, particularly regarding health behaviors and comorbidities. However, since these data were collected from face-to-face interviews by trained interviewers, the validity of the data was improved. Additionally, because of the nature of nationwide population-based survey studies, the measurements of the data were rather simple. Nevertheless, the relationships of the baseline variables with NC and cerebrovascular disease incidence shown in this study are consistent with those of a previous study that used other standardized instruments [8]. Furthermore, we were not able to address other covariates, such as disease severity. Due to the lack of specific data on the diagnosis of diseases in this set of survey data, attention should be paid to the broad interpretation of the association between NC and the risk of developing cerebrovascular diseases. Finally, since this study identified the association between NC and cerebrovascular disease incidence by adjusting for the age of adult woman, we suggest further studies should investigate NC and the incidence of cerebrovascular disease according to menopausal status in middle-aged woman. The advantages of this study are its prospective study

design with a relatively long follow-up period and the use of population-based interview data with population rates over the follow-up period. To the best of our knowledge, this is the first study to explore the longitudinal and independent relationship between NC and the incidence of cerebrovascular disease in middle-aged and older Korean adults, controlling for various covariates.

Conclusion

The results of this study showed that Korean adult woman with a thicker NC had a higher risk of developing cerebrovascular disease. This relationship was not influenced by age, socioeconomic status, selected health behaviors, or comorbidities. Therefore, the results we presented here may be meaningful for evaluating and surveilling NC as a promising tool for identifying subgroups of vulnerable and at-risk populations. To develop customized interventions to reduce and maintain upper-body fat in Korean adults, further research using a longitudinal assessment of NC and follow-up research to track sex-specific differences is warranted.

Notes

Ethics Approval

The Institutional Review Board of Pukyong National University approved the study protocol (IRB No. 1041386-201907-HR-29-01). The study protocol was also approved by the Ethics Committee of KoGES at the Korean National Institute of Health, and the study was performed in accordance with the approved guidelines.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Availability of Data

All data were obtained from the Korean Genome and Epidemiology Study (KoGES) of the National Research Institute of Health (NIH).

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Corrigendum

Correction to "Prevalence and Associated Factors of Hypertension Subtypes Among the Adult Population in Nepal: Evidence from Demographic and Health Survey Data" [Osong Public Health Res Perspect 2019;10(6):327–36]

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In the article entitled "Prevalence and Associated Factors of Hypertension Subtypes Among the Adult Population in Nepal: Evidence from Demographic and Health Survey Data" [1], the name of the second author, Animesh Talukdar, was incorrect.
The corrected name is as follows: **Animesh Talukder**.

Reference

1. Gupta RD, Talukdar A, Haider SS, Haider MR. Prevalence and associated factors of hypertension subtypes among the adult population in Nepal: evidence from demographic and health survey data. Osong Public Health Res Perspect 2019;10:327–36.

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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**

4. Riffenburgh RH, Gillen DL. Statistics in medicine. 4th ed. London: Academic Press; 2020.
5. Miller DD. Minerals. In: Damodaran S, Parkin KL, editors. Fennema's food chemistry. 5th ed. Boca Raton, FL: CRC Press; 2017. p. 627-80.
6. Ministry of Employment and Labor. Statistics on occupational injuries and illnesses, 2008. Gwacheon, KR: Ministry of Employment and Labor; 2009.

- **Websites**

7. World Health Organization (WHO). COVID-19 vaccines [Internet]. Geneva: WHO; 2021 [cited 2021 Mar 15]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines.

- **Conference papers**

8. 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. Berlin: Springer; 2002. p.182-91.

- **Dissertation**

9. Park HY. The role of the thrombomodulin gene in the development of myocardial infarction [dissertation]. Seoul: Yonsei University; 2000.

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