OSONG PUBLIC HEALTH
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Vol. 15, No. 3, June 2024

Pages 187-264

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OSONG PUBLIC HEALTH
AND RESEARCH PERSPECTIVES

Vol. 15, No. 3, June 2024

pISSN 2210-9099
eISSN 2233-6052

Vol. 15, No. 3, June 2024

Korea Disease Control and
Prevention Agency

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

https://ophrp.org

pISSN 2210-9099
eISSN 2233-6052

Vol. 15, No. 3, June 2024

Published on 30 June 2024

Aims & Scope

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

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Publisher: The Korea Disease Control and Prevention Agency

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

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♾ This paper meets the requirements of KS X ISO 9706, ISO 9706-1994 & ANSI/NISO Z39.48-1992 (Permanence of Paper).

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

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pISSN 2210-9099 eISSN 2233-6052

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2024.0181
Osong Public Health Res Perspect 2024;15(3):187-188

Editorial

Strengthening the health system, including innovative budget mobilization, is an urgent issue for the Expanded Programme on Immunization

Jong-Koo Lee

National Academy of Medicine of Korea, Seoul, Republic of Korea

This year marks the 50th anniversary of the introduction of the World Health Organization (WHO) EPI, originally known as the Expanded Programme on Immunization. On May 23, 1974, during the 27th World Health Assembly (WHA) in Geneva, Switzerland, WHO member states advocated for the development of immunization and surveillance programs targeting diphtheria, pertussis, tetanus, measles, poliomyelitis, tuberculosis, and smallpox. At that time, despite the availability of vaccines, these 7 disease continued to be major causes of mortality among children.

At a special event of the WHA on May 28 this year, WHO Director-General Dr. Tedros highlighted that immunizations have enabled 40% of children to celebrate their first birthday who would not otherwise been able to. He noted that vaccines have prevented approximately 154 million deaths, with the most significant impacts coming from vaccines against measles, tetanus, pertussis, and tuberculosis. This statement was part of a study examining the contribution of vaccination to improved survival and health over 50 years of the EPI [1]. Dr. Young-mee Ji, Commissioner of the Korea Disease Control and Prevention Agency (KDCA), emphasized in this event that the EPI has significantly contributed not only to immunization but also to Universal Health Coverage in many developing countries. She highlighted the critical role of WHO's regional and country offices in this success, stating that the roles of country offices should be further expanded. While personally participating in measles vaccination campaigns (2007, 2010, 2014) in Philippine, Dr. Ji underscored that the EPI has made substantial contributions to health system strengthening.

Reflecting on the history of the EPI, 1974 stands out as a pivotal year in public health. At that time, despite the availability of vaccines, many children remained unvaccinated. Former WHO Director General Dr. Halfan Mahler urged member states to intensify efforts at all levels to address this issue, which led to the establishment of the EPI. Now, 50 years later, the initiative is known as the Essential Programme on Immunization. It recommends 13 vaccines globally and up to 17 additional vaccines in specific countries [2]. According to the WHO, global immunization efforts have saved at least 154 million lives over the past 50 years.

Despite these successes, the recent coronavirus disease pandemic has widened immunity gaps and increased the number of zero-dose children, leading to a rise in diseases such as measles [3]. Reports from the 33rd WHO Western Pacific Region Technical Advisory Group

Received: June 24, 2024
Accepted: June 25, 2024

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on Immunization meeting in Manila last week highlighted ongoing Big Catch-Up campaigns in response to the increased cases of measles and pertussis.

To combat these challenges, new support organizations such as Gavi have been established to procure vaccines, and innovative vaccine delivery platforms, including patches and nasal sprays, are under development to enhance accessibility. However, innovative funding mechanisms are essential to ensure that vaccines are distributed to all children and that issues of equity in vaccination coverage are tackled. Strengthening health systems, such as governance, financing, information management, and the quality of human resources is crucial for improving vaccination rates and reducing the number of children left unvaccinated.

In the Republic of Korea, immunization programs play a crucial role in controlling infectious diseases. Following a decline in vaccination rates due to adverse events related to Japanese encephalitis vaccines in 1994, an Advisory Committee on Immunization Programs was established. This committee focused on compensation for adverse events, implementing guidelines for safe vaccination, and providing training for frontline health workers. Despite these measures, a significant measles outbreak occurred in 2000–2001, attributed to the low uptake of the second dose of the measles vaccine. This led to further strengthening of the health systems. Improvements were made in reporting diseases and outbreak investigations, including monitoring adverse vaccine reactions. Additionally, improvements were made to the registration of vaccination and the school entrance examination program. In 2002, child vaccination programs were transferred to the Communicable Disease Control division of the Korea National Institute of Health. After the severe acute respiratory syndrome outbreak, the Immunization Management Division was established in 2003 within the newly formed Korea Centers for Disease Control and Prevention.

In 2004, the terminology for routine and temporary vaccinations was updated to "national essential vaccinations," and measures were implemented to alleviate the financial burden of vaccinations administered at private medical institutions. An electronic reporting system was also introduced to manage vaccination records, issue certificates, and provide recall and reminder services for vaccinations. By 2014, all essential vaccinations offered at medical institutions became free, representing a major advancement in public health. The funding for these vaccination programs was supported by an increase in tobacco taxes, leading to a substantial growth in the budget for health promotion accounts, which included vaccination programs. This budget expanded from 105.2 billion Korean won (KRW) in 2013 to 357.6 billion KRW in 2023 [4]. Considering the central government's subsidy of 1/2 to 1/3 of the cost, more than double the budget is being invested.

As we look forward to the next 50 years, it is essential to continue improving health systems and securing funding to improve access to both essential and new vaccinations. The Korea National Institute of Infectious Diseases is dedicated to developing innovative vaccine platforms that aim to boost global health, especially for children in low- and middle-income countries. The efforts of the KDCA, under the leadership of Commissioner Dr. JM Jee, are commendable and play a significant role in the global fight against infectious diseases.

Notes

Ethics Approval
Not applicable.

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

Funding
None.

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https://doi.org/10.24171/j.phrp.2024.0181

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0148
Osong Public Health Res Perspect 2024;15(3):189-200

Review Article

Effectiveness of virtual reality-supported exercise therapy in improving upper extremity function and activities of daily living among patients after stroke: a systematic review of randomized control trials

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ABSTRACT

Objectives: This systematic review describes the effectiveness of virtual reality (VR)-supported exercise therapy on upper limb motor function and activities of daily living after stroke.
Methods: Studies published through January 24, 2022, were identified using CINAHL, Cochrane Library, Embase, Medline, and Web of Science. Randomized control trials comparing VR treatment with conventional therapy (CT) for upper extremity rehabilitation after stroke were included. Methodological quality was assessed using the Cochrane risk-of-bias tool.
Results: Of 9 included studies, 5 concluded that the VR group outperformed control participants, 1 indicated the superiority of VR-supported exercises alone over CT, and 3 found VR comparable to CT in promoting upper limb motor function. Five studies analyzed independence in daily living, with 4 reporting no significant difference between VR and CT groups. No strong evidence indicated long-term benefits of VR-assisted exercise. All included studies demonstrated low risk of bias concerning random sequence generation, allocation concealment, outcome assessment blinding, incomplete outcome data, and selective reporting bias. However, a high risk of bias was observed regarding participant blinding due to the nature of the intervention.
Conclusion: Most studies suggested that VR, used alongside CT, can improve motor function following stroke. However, the evidence was insufficient to conclude that VR outperforms conventional approaches.

Keywords: Rehabilitation; Review; Stroke; Upper extremity; Virtual reality

Received: June 2, 2023
Revised: January 19, 2024
Accepted: February 6, 2024

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Introduction

Stroke is the second leading cause of death worldwide, with an annual mortality figure of approximately 5.5 million deaths [1]. Additionally, up to 50% of survivors experience a permanent

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disability [2]. Stroke is characterized by neurological deficits and long-term disability resulting from a focal injury to the central nervous system, such as cerebral infarction, intracerebral hemorrhage, or subarachnoid hemorrhage [3].

Changes related to impaired upper arm function include difficulty performing tasks, abnormal postural adjustments, an inability to control grasping, and functional disability. These challenges make it difficult for individuals to participate in activities of daily living (ADLs) such as eating, dressing, and grooming [4,5]. Research indicates that 40% of stroke survivors experience reduced function in the affected arm, and approximately 70% of patients report diminished arm function [6]. Separately, over 80% of patients have been found to exhibit impaired upper limb motor function after stroke, with 66% continuing to have a non-functional upper limb long-term [7,8]. Upper limb impairments are often more pronounced than those of the lower limb [9] and include functional limitations and uncoordinated motion [10]. Conventional rehabilitation techniques such as physiotherapy, neurodevelopmental therapy, and proprioceptive neuromuscular facilitation are effective to similar degrees in improving motor function. However, these methods can be resource-intensive and costly, often requiring specialized facilities that are not widely available. Virtual reality (VR) has emerged as a novel rehabilitation strategy and an enjoyable alternative to enhance motor recovery after stroke [11,12]. VR is an emerging technology that provides an interactive simulation, creating an environment that closely mimics reality for users. VR has been described as "the use of interactive simulations created with computer hardware and software to present users with opportunities to engage in environments that appear and feel similar to real-world objects and events" [13]. VR is based on several principles, including goal-oriented tasks, adjustable task difficulty, task repetition, feedback, and the promotion of increased user motivation and enjoyment [14]. VR systems can be categorized as immersive or non-immersive. Non-immersive VR systems allow users to interact with the virtual environment displayed on a computer screen while maintaining a sense of the real world. Immersive VR systems, which include large screen projections, head-mounted displays, or Cave Automatic Virtual Environment (Balance Near Automatic Virtual Environment) systems, block out all real-world perception, immersing the user solely in computer-generated imagery [15].

Adults with persistent hemiparesis have recently been shown to benefit from VR therapy, with reasonably good patient compliance [16]. Engaging in physical exercises through VR programs can help patients with neurological disorders enhance their motor learning and control, as well as their functional capacities [17]. Research suggests that training in fine hand-motion rehabilitation can be more effective when it incorporates immersive VR technology [18]. Several studies have indicated that VR technology produces better outcomes than conventional therapies in improving upper extremity function [19–24] and ADL performance [22–24] after stroke. However, a systematic review conducted by Laver et al. [25] reported no appreciable difference between VR-based training and conventional treatment in the recovery of upper limb motor function. Due to the variability of findings regarding VR intervention, a systematic review is necessary to thoroughly explore the effects of VR rehabilitation on upper arm function. The aim of this review was to evaluate the benefits of VR in the motor recovery of upper limb function and in promoting independence in ADLs, focusing on studies conducted a maximum of 2 years after stroke.

HIGHLIGHTS

- Healthcare providers can integrate virtual reality (VR)-supported exercises into rehabilitation programs for survivors of stroke.
- The use of VR as a supplement to conventional therapy shows promise as an adjuvant for this patient population.

Materials and Methods

Eligibility Criteria

We conducted this systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (Supplementary Material 1). We incorporated studies that met the following inclusion criteria: (1) randomized controlled trials (RCTs) that compared VR with conventional interventions in upper limb rehabilitation, with no more than 2 years elapsed since the stroke, and that met predefined PICOTS criteria (Table 1); (2) the intervention group received VR-supported exercise therapy either alone or in combination with conventional therapy, while the control group received conventional therapy, recreational therapy, or standard care alone; (3) the study participants were at least 18 years old with any type of stroke (hemorrhagic or ischemic); and (4) the article was accessible and written in English. We excluded studies that compared 2 types of VR treatment, those involving robotics-supported therapy or VR treatment administered through telerehabilitation, and those conducted in home settings. Studies that included

https://doi.org/10.24171/j.phrp.2023.0148

Table 1. PICOTS criteria for included studies

	Criteria
Population	Patients with a maximum of 2 years elapsed since stroke
Intervention	Virtual reality treatment (immersive or non-immersive)
Comparators	Conventional techniques/recreational activity/ standard treatment
Outcome	Upper limb function and activity
Time	Published in the last 10 years
Study design	Randomized controlled trial

participants with cognitive impairments and those that did not focus exclusively on upper limb function were also excluded.

Search Strategy

This review was conducted between October 2022 and March 2023. All reviewers independently performed a literature search across 5 databases: CINAHL, Cochrane Library, Embase, Medline, and Web of Science. The keywords used for the literature search were "stroke," "cerebrovascular accident," "virtual reality," "virtual environment," "motor deficits," "neurorehabilitation," and "upper limb function." These keywords were used either individually or in combination, employing the Boolean operators "OR" and "AND". No restrictions were imposed on the year of publication, and the most recent study publication date was January 24, 2022.

Outcome Measures

The primary outcome was upper limb function and activity, while the secondary outcomes were independence in daily living and the long-term benefits of VR therapy. We conducted a narrative synthesis of data from the identified studies, which encompassed participant characteristics, attributes of the interventional and control groups, upper limb function and activity, independence in ADLs, and the effects of VR therapy during the follow-up period.

Data Collection and Processing

The screening procedure was performed independently by 2 reviewers to identify relevant studies based on predetermined eligibility criteria. Initially, the titles of potential studies were screened, followed by an examination of the abstracts and full texts of the articles for inclusion in the review. Studies were assessed against the inclusion and exclusion criteria before being selected. Data from the articles were extracted and analyzed independently by the first 2 reviewers, with their findings subsequently verified by a third reviewer. Each reviewer examined the data individually to lead to a consensus while minimizing bias. After extracting data from all included articles, a table was created. This table included the author, publication year, country of origin, number of participants, type of VR used, nature and duration of the intervention, outcome variables and scales used, and results for each study (Table 2) [11,26–33].

Assessment of Study Risk of Bias

The risk of bias in the selected documents was assessed using the Cochrane risk-of-bias tool [34]. The evaluation covered several areas: allocation concealment and random sequence generation (both related to selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), completeness of outcome data (attrition bias), and selective data reporting (reporting bias) (Table 3) [11,26–33]. The level of bias was categorized into 3 grades: low, high, and unclear risk of bias (Figure 1). The reviewers independently assessed the biases in the included studies and, in the event of any discrepancies, consulted a third reviewer for resolution.

Data Synthesis

We conducted a narrative synthesis of data from the selected studies, encompassing participant characteristics, descriptions of the intervention and control groups, details of the VR interventions, and comparisons between VR and conventional therapy. This synthesis also included assessments of upper limb function and activity, independence in ADLs, and the impact of VR therapy over the course of follow-up.

Results

A total of 589 relevant studies were identified. Of these, 412 were duplicates, 30 did not meet the eligibility criteria, and 112 were excluded after screening titles and abstracts. Ultimately, 35 studies were assessed for eligibility, and 9 RCTs were included in the systematic review (Figure 2).

Study Characteristics

The studies included in this review were conducted in 7 countries: 2 studies in the Republic of Korea [26,27], 2 in China [28,29], 1 in Canada, 1 in Norway [30], 1 in Saudi Arabia [31], 1 in the United Kingdom [32], and 1 in Turkey [33]. The total sample size across the included articles was 558 participants, with 282 in the experimental groups and 276 in the control groups. The time since stroke onset ranged from 9 days to 24 months. Six studies [11,26,28–30,33] recruited participants within 6 months after stroke, while 3 trials [27,31,32] included participants for whom more than 6 months had elapsed. In 1 study [32], the intervention and control groups were subdivided into patients with ischemic and hemorrhagic stroke, whereas the other studies did not

Table 2. Characteristics of included studies

No.	Study	Year	Country	Time elapsed since stroke	Participant (*n*)	Type of VR	Intervention	Duration of intervention	Outcome variables and scales	Results
1	Saposnik et al. [11]	2016	Canada	Within 3 mo of enrollment	121 (VR: 59, control: 62)	Non-immersive	Experimental group: VR treatment Control group: recreational activity (playing cards, bingo, etc.)	10 Sessions×60 min/session over 2 wk	UE-motor function: WMFT, BBT, FIM, SIS, Barthel index, dynamometer	No significant difference was found between the VR and recreational therapy group on FM, FIM, SIS, Barthel index, or grip strength in the post-intervention and follow-up period. Regarding ADL, similar improvement was observed between the groups.
2	Brunner et al. [30]	2017	Norway	Within 3 mo of stroke	120 (VR: 62, control: 58)	Non-immersive	Experimental group: VR training+standard rehabilitation Control group: conventional training+standard rehabilitation	16 Sessions×60 min/session over 4 wk in both groups	UE-motor function: ARAT Dexterity assessment: BBT Independence in ADL: FIM	No significant difference was noticed between the 2 groups ($p=0.714$) in motor function improvement or ADL ($p=0.777$). Regarding ADL, both groups showed similar improvement, representing a nonsignificant difference ($p=0.777$).
3	Kiper et al. [32]	2018	United Kingdom	12 mo	136 (VR: 68, control: 68)	Non-immersive	Experimental group: 1 h of CR and 1 h of RFVE Control group: r2 h of CR	2 h session/d×5 d/wk×4 wk	UE-motor function: FMA-UE ADL: FIM	RFVE therapy combined with CR treatment promotes better upper limb outcomes on the FMA-UE scale ($p<0.001$), NIHSS ($p\leq0.014$), and ESAS ($p\leq0.022$) compared to CR. Regarding ADL, a significant difference was also found on the FIM ($p<0.001$) scale.
4	Chen et al. [29]	2022	China	1–3 mo	36 (VR: 18, control: 18)	Non-immersive	Experimental group: VR training Control group: conventional therapy	60 min×5 sessions/wk×2 wk	UE-motor function: ARAT, FMA-UE	The improvement in ARAT and FMA-UE in the VR group was significantly higher than in the control group.

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https://doi.org/10.24171/j.phrp.2023.0148

Table 2. Continued

No.	Study	Year	Country	Time elapsed since stroke	Participant (*n*)	Type of VR	Intervention	Duration of intervention	Outcome variables and scales	Results
5	Ikbali Afsar et al. [33]	2018	Turkey	1–6 mo	35 (VR: 19, control: 16)	Non-immersive	Experimental group: conventional therapy+VR therapy (Xbox Kinect game system) Control group: conventional rehabilitation only	CT: 60 min session/d×5 d/wk×4 wk VR therapy: 30 min/d	UE-motor function: FMA-UE, Gross manual dexterity: BBT, ADL: FIM	A significant difference was found between groups in both BBT score and FMA-UE ($p<0.05$). Regarding ADL, no significant difference was observed ($p=0.677$).
6	Lee et al. [27]	2016	Republic of Korea	Diagnosis of stroke at least 6 mo prior	18 (VRBT group: 10; bilateral training group: 8)	Non-immersive	Experimental group: bilateral upper extremity exercises in a VR environment in addition to CT Control group: bilateral upper extremity exercises while watching irrelevant videos in addition to CT Control group: bilateral upper extremity exercises while watching irrelevant videos in addition to CT	VR-based bilateral training: 30 min session/d×3 d/wk×6 wk Conventional therapy: 30 min session/d×3 d/wk×6 wk	UE-motor function: JHFT, BBT, GPT Muscle strength: DMMT	The VRBT group exhibited significant improvement in upper extremity function and muscle strength ($p<0.05$).
7	Wang et al. [28]	2017	China	4 wk–6 mo	26 (VR: 13, control: 13)	Immersive	Experimental group: Leap Motion VR+conventional treatment Control group: conventional therapy (stretches, strength, balance, gait training)	Experimental group: Leap Motion VR: once a day (45 min), 5 d/wk for 4 wk+CT: once a day (45 min), 5 d/wk for 4 wk Control group: twice a day each 45 min, 5 d/wk for 4 wk	UE-motor function: WMFT	A significant difference was found between the control and experimental groups in WMFT-Time ($p<0.01$).

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https://doi.org/10.24171/j.phrp.2023.0148

Table 2. Continued

No.	Study	Year	Country	Time elapsed since stroke	Participant (*n*)	Type of VR	Intervention	Duration of intervention	Outcome variables and scales	Results
8	Kwon et al. [26]	2012	Republic of Korea	Within 3 mo of the stroke	26 (VR: 13, control: 13)	Immersive	Experimental group: VR therapy in addition to CT Control group: CT only	VR therapy: 30 min/d×5 d/wk×4 wk Conventional therapy: 70 min/d×5 d/wk×4 wk	UE-motor function: FMA, MFT Independence in ADL: K-MBI	The VR group showed improvement in both FMA and MFT scores. In the CT group, only the FMA score improved. K-MBI (ADL performance) improved in both groups, with no significant differences observed in upper extremity function or ADL performance ($p > 0.05$).
9	El-Kafy et al. [31]	2021	Saudi Arabia	6–24 mo	40 (VR: 20, control: 20)	Non-immersive	Experimental group: 1 h CT+1 h VR therapy Control group: 2 h CT	2 h session×3 sessions/wk×3 mo	UE-motor function: WMFT, ARAT	The experimental group displayed greater improvement in all measured scales (ARAT, WMFT, and WMFT-Time) compared to the control group.

VR, virtual reality; UE, upper extremity; WMFT, Wolf Motor Function Test; BBT, Box and Block Test; FIM, Functional Independence Measure; SIS, Stroke Impact Scale; ARAT, Action Research Arm Test; ADL, activities of daily living; CR, conventional rehabilitation; RFVE, reinforced feedback in a virtual environment; FMA-UE, Fugl-Meyer Assessment-Upper Extremity; NIHSS, National Institutes of Health Stroke Scale; ESAS, Edmonton Symptom Assessment System; CT, conventional therapy; JHFT, Jebsen–Taylor Hand Function Test; GPT, Grooved Pegboard Test; DMMT, Digital Manual Muscle Test; VRBT, virtual reality-based bilateral upper extremity training; MFT, Manual Function Test; K-MBI, Korean version of the modified Barthel index.

Table 3. Risk of bias assessment

Study	Year	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting bias	Other, ideally specified
Saposnik et al. [11]	2016	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Brunner et al. [30]	2017	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk
Kiper et al. [32]	2018	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Chen et al. [29]	2022	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Ikbali Afsar et al. [33]	2018	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Lee et al. [27]	2016	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Wang et al. [28]	2017	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk
Kwon et al. [26]	2012	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
El-Kafy et al. [31]	2021	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk

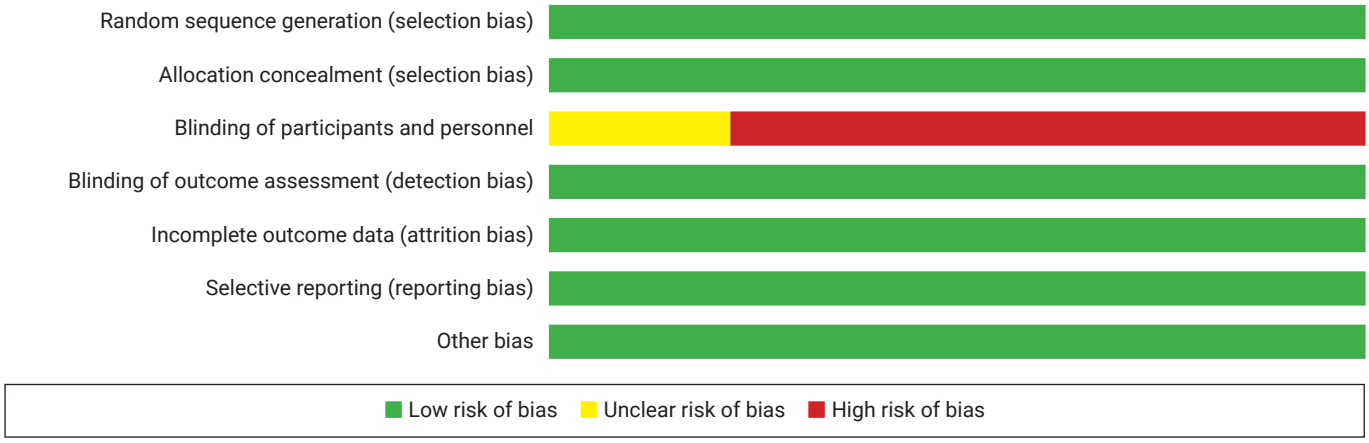

Figure 1. Risk of bias graph.

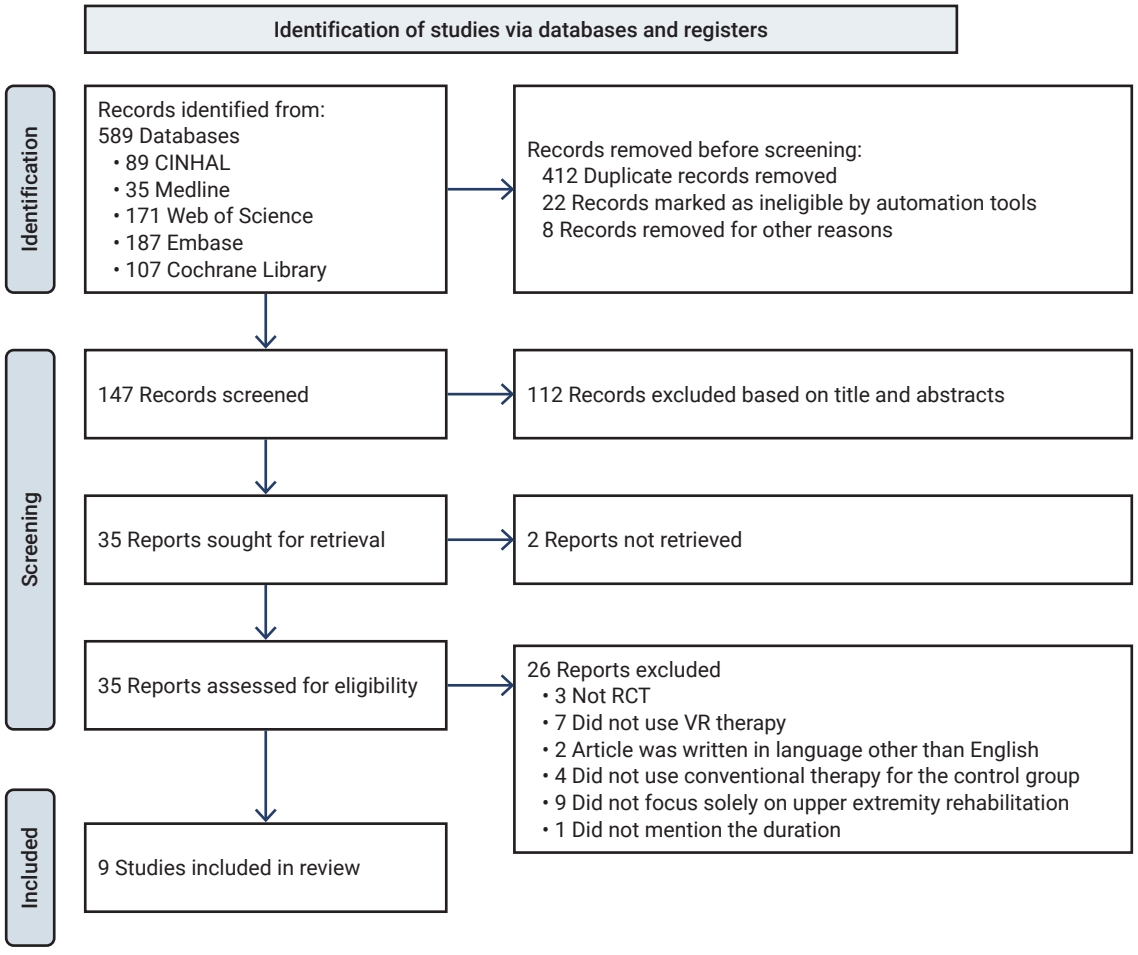

Figure 2. PRISMA flowchart for the selection of articles for integrative review.
PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RCT, randomized controlled trial; VR, virtual reality.

report specific details about the type of stroke. The frequency of training varied from 3 to 5 sessions per week, and the number of intervention sessions ranged from 10 to 36. The duration of each session varied from 30 to 120 minutes. In 1 study [30], the experimental and control groups were further segmented based on the severity of hand paresis, specifically into mild to moderate hand paresis and severe hand paresis categories. Few studies investigated whether the benefits of VR treatment were sustained in the long term after the therapy concluded. One study [30] reassessed outcomes 3 months post-intervention, while another study [11] conducted an assessment 1 month after the intervention ended.

Risk of Bias and Study Quality

The risk of bias for the included studies is detailed in Table 3 and Figure 3 [11,26–33]. All 9 studies employed random sequence generation, allocation concealment, and blinding of outcome assessors. None of the studies exhibited selective reporting or attrition bias, the latter of which pertains to participant dropout. In 7 studies [11,26,27,29,31–33], the participants and personnel were not blinded to the allocated intervention. For the remaining 2 studies [28,30], the risk of performance bias was unclear. Therefore, a high risk of performance bias was found overall, since blinding of participants was not feasible due to the nature of the intervention. Apart from performance bias, all included studies were assessed as having a low risk of bias in all other domains. The outcome assessors were adequately blinded by withholding knowledge of the allocated interventions. Based on the quality assessment of the included studies, the total bias score was determined to be 1 out of 7, indicating a low risk of bias.

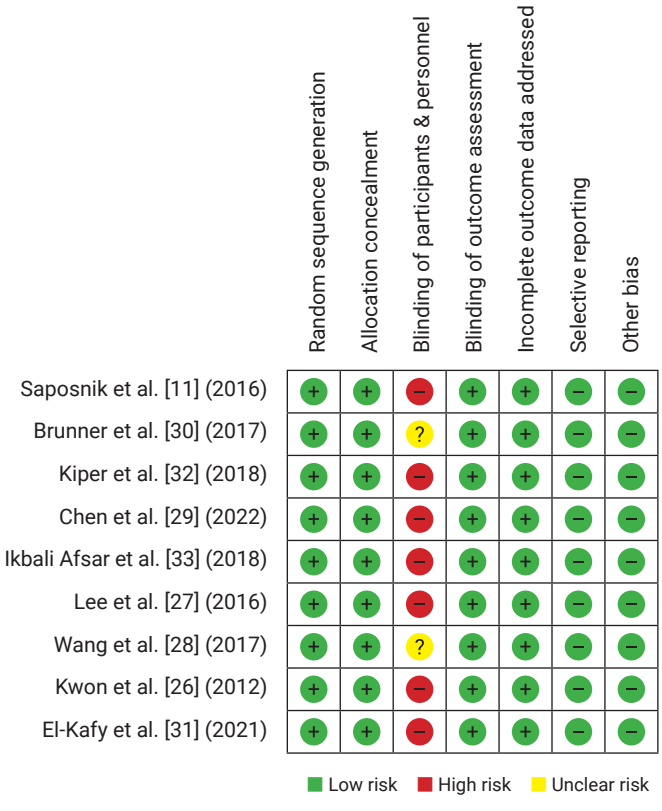

Figure 3. Risk of bias summary for the included trials.

Intervention Group Characteristics

In 7 studies [26–28,31–33], the intervention group received a combination of conventional treatment and VR therapy, while in 2 studies [29,30] the intervention group was treated exclusively with VR. The VR-based interventions employed in these studies utilized systems that provide feedback within a virtual environment, including You Grabber [30], Armeo Spring [31], VR-based bilateral upper extremity training (VRBT) [27], A2, YiKang, reinforced feedback in a virtual environment (RFVE) [32], a Leap Motion-based VR system [28], and the Interactive Rehabilitation and Exercise? system [26]. Other studies involved the intervention group using commercially available gaming systems for VR-based therapy, such as the Wii (Nintendo) [11] and the Xbox Kinect [33], within a VR environment.

Control Group Characteristics

In 5 studies [11,29–32], interventions were administered to both groups with matching frequency (number of sessions per week), session duration, and treatment duration. However, in the remaining 4 studies [26–28,33], the duration of therapy for the control group did not match that of the intervention group.

Measurement Tools

A variety of scales were used in the 9 studies to assess improvements in upper limb motor function, arm function, and ADLs. The scales employed included the Fugl-Meyer Assessment-Upper Extremity (FMA-UE) [26,29,32,33], the Box and Block Test (BBT) [11,27,30,33], the Functional Independence Measure (FIM) [11,30,32,33], the Barthel index [11], the Korean version of the modified Barthel index [26], the Action Research Arm Test (ARAT) [29–31], the Wolf Motor Function Test (WMFT) [11,28,31], hand grip strength [31], the Grooved Pegboard Test [27], the Jebsen–Taylor Hand Function Test [27], the Brunnstrom stage scale [33], the Stroke Impact Scale (SIS) [11], the Edmonton Symptom Assessment System (ESAS) [32], the Manual Function Test [26], and a dynamometer [11].

https://doi.org/10.24171/j.phrp.2023.0148

Comparison of VR Therapy versus Conventional Therapy/Standard Care/Recreational Therapy

The results of this comparison are detailed in the following sections.

Upper limb function and activity

Upper limb function and activity were assessed in all studies. Of the 9 articles, 5 [27,28,31–33] concluded that the stroke survivors receiving VR treatment experienced better outcomes than those administered conventional treatment. One study [29] reported that VR-supported exercises alone were superior to conventional therapy, while 3 studies [11,26,30] indicated that VR training was as effective as conventional methods.

A study by Ikbali Afsar et al. [33] reported better outcomes in the interventional group, with significant differences observed between the experimental and control groups in terms of FMA-UE and BBT scores ($p < 0.05$). Similarly, Wang et al. [28] concluded that the motor function of the affected upper limbs, as measured with the WMFT scale, was significantly improved in the experimental group compared to the control group ($p < 0.01$). Additionally, the WMFT time was significantly shorter in the experimental group (3.29 ± 0.82 seconds) than in the control group (4.24 ± 0.57 seconds) post-intervention. El-Kafy et al. [31] found comparable results, stating that patients in the experimental group showed more favorable improvement than those in the control group in terms of the ARAT ($p < 0.01$), WMFT ($p < 0.01$), and WMFT-Time ($p < 0.01$) assessments. They concluded that the use of combined therapy (VR and conventional rehabilitation) provides greater benefits to upper limb function than conventional treatment alone. Kiper et al. [32] also reported that the experimental group demonstrated better improvement on the FMA-UE scale ($p < 0.001$), FIM ($p < 0.001$), National Institutes of Health Stroke Scale ($p \leq 0.014$), and ESAS ($p \leq 0.022$) compared to the control group. Kiper et al. [32] further assessed kinetic parameters such as time ($p < 0.001$), speed ($p < 0.001$), and peak ($p < 0.001$) during the execution of the requested task, finding significant differences compared to control participants. Lee et al. [27] reported that members of the VRBT group exhibited significant improvements in upper extremity function and muscle strength ($p < 0.05$). Lastly, Chen et al. [29] described a significant improvement in the ARAT ($Z = 2.088$, $p = 0.04$) and FMA-UE ($Z = -2.338$, $p = 0.02$) scales in the VR group compared to the control group.

Three studies reported nonsignificant results [11,26,30]. Kwon et al. [26] observed improvements in both groups post-intervention, and the difference between them was not statistically significant ($p > 0.05$). The effect size of the change in the examined variable was medium (Cohen $d = 0.48$) for the FMA. Similarly, Saposnik et al. [11] found that the WMFT performance time improved from baseline to post-intervention for both groups. Specifically, they observed a decrease in the median time from 43.7 seconds (interquartile range [IQR], 26.1–68.0 seconds) to 29.7 seconds (IQR, 21.4–45.2 seconds), representing a 32.0% reduction, for the VRWii group, compared to a change from 38.0 seconds (IQR, 28.0–64.1 seconds) to 27.1 seconds (IQR, 21.2–45.5 seconds), representing a 28.7% reduction, for the patients treated with recreational activity. However, they noted no significant difference in hand function ($p = 0.314$), grip strength ($p = 0.713$), or motor performance ($p = 0.469$) between the groups at the end of the intervention, except for gross manual hand dexterity as measured by the BBT scale ($p = 0.018$). Brunner et al. [30] reported that participants in the VR group improved on the ARAT by 12 points (standard deviation [SD], 11) from baseline to the post-intervention assessment, while the patients receiving conventional therapy improved by 13 points (SD, 10), with no significant difference ($p = 0.714$) between the control and experimental groups.

Independence in daily living

Of the included studies, 5 [11,26,30,32,33] analyzed the impact of VR therapy versus conventional therapy on ADLs. Four of these studies [11,26,30,33] reported no significant differences in ADL performance between the control and experimental groups. Supporting this, Ikbali et al. [33] found no statistical difference ($p = 0.677$) between the groups with regard to FIM score, although both the experimental (12.74 ± 2.51 to 23.74 ± 4.42) and control (13.63 ± 3.61 to 23.63 ± 4.99) groups showed improvement from baseline to post-intervention. Kwon et al. [26] also reported no significant difference in ADL between the VR and conventional therapy groups ($p > 0.05$), despite a large effect size (Cohen $d = 1.02$). Saposnik et al. [11] observed similar improvements in the Barthel index in the experimental (64.7 ± 22.4 to 83.4 ± 18.0) and control (64.2 ± 23.0 to 80.3 ± 21.7) groups. Brunner et al. [30] noted comparable improvements in FIM motor scores in the VR group (changing from 15.9 at baseline to 12.7 after intervention) and the conventional therapy group (changing from 17.5 at baseline to 13.1 after intervention) post-intervention and at follow-up. However, Kiper et al. [32] reported a significant improvement in independence in daily living associated with VR therapy, as measured with FIM (RFVE, 8.42%; conventional rehabilitation, 4.82%; $p < 0.001$).

Upper limb function: follow-up

Only 2 studies [11,30] analyzed the long-term benefits of VR

therapy among patients treated after stroke. Brunner et al. [30] observed an improvement in upper extremity function as assessed with ARAT, with a mean increase of 17 points (SD, 13) from baseline to follow-up in the VR group, which was identical to the improvement seen in the conventional therapy group. Consequently, no significant difference was found (p=0.777) between groups at follow-up when assessing all scales (ARAT, BBT, FIM, and Patient Global Impression of Change). Similarly, Saposnik et al. [11] found no significant differences at the 4-week post-intervention mark in WMFT performance time (p=0.346), Barthel index (p=0.774), FIM (p=0.848), or SIS hand function (p=0.514) between the control group (who engaged in recreational activity) and the experimental group (who received VRWii therapy).

Discussion

More than three-quarters of stroke victims experience diminished upper limb function following the event, impacting their everyday quality of life. The aim of this study was to compare motor recovery of upper limb function between interventional (VR therapy) and control (conventional therapy) groups. The interventional group received both conventional treatment and VR therapy in 7 studies [26–28,31–33], while only VR therapy was administered in 2 studies [29,30].

Of the 9 studies examined, 5 [27,28,31–33] concluded that the VR treatment group of stroke survivors experienced better outcomes than those who received conventional treatment. One study [29] reported the superiority of VR-supported exercises, while 3 studies [11,26,30] found that VR training was similarly effective to conventional methods. Most of the included studies [27–29,31–33] noted that the intervention group experienced greater improvements in upper limb function and activity than the control group. According to El-Kafy et al. [31], interventional groups using combination therapy (both virtual and traditional) saw greater enhancements in upper limb function. All trials considered reported a similar degree of improvement in ADLs between groups. Vinas-Diz and Sobrido-Prieto [35] have emphasized that VR can create environments specifically tailored for patients by modifying and controlling their engagement with their surroundings, potentially serving as a valuable adjunct or alternative to conventional therapy. In a separate comprehensive review, Zhu et al. [36] found that VR intervention is a beneficial non-pharmacological approach for enhancing cognitive and motor performance in older individuals. Wiley et al. [37] observed that the VR group performed better than the control when VR was used as an add-on to conventional therapy, concluding that VR therapy is stimulating and interesting relative to traditional rehabilitation methods. These findings aligned with those of several included studies [27,28,31–33]. Zhang et al. [38] also reported that the upper limb function in the VR group outperformed that of the conventional method, with significant differences found between the 2 groups regardless of the intervention period. This was consistent with results from other included studies [27,28,31–33], which had varied intervention durations. Separately, Zhu et al. [36] concluded that VR treatment is beneficial for improving outcomes in patients with mild cognitive impairment after stroke. A systematic review by Gao et al. [39] concluded that VR-based training did not have significant benefits over conventional methods on motor function or ADLs. However, they suggested that greater duration, frequency, and daily intensity of VR training may yield better outcomes. Chen et al. [29] reported that although the VR group showed significant improvement in upper extremity motor function and independence in day-to-day activities, the results were inconclusive regarding the benefits of VR therapy after the intervention ended. This was consistent with findings from other included studies [11,30]. The strengths of this systematic review include the exclusive inclusion of RCTs and a rigorous quality assessment of included studies using the Cochrane risk-of-bias tool. This review highlights several opportunities for future research to evaluate the long-term benefits of VR-supported exercises. However, several limitations should be addressed. Most of the included studies had small sample sizes, which limited generalizability and weakened the validity of the results. Secondly, the intervention type (immersive or non-immersive VR), duration, and frequency varied between studies, potentially influencing rehabilitation progress. The optimal frequency and duration of intervention remain to be explored.

Implications for Practice

VR-based interventions can be incorporated into clinical practice to rehabilitate the upper limb in conjunction with conventional exercises. Commercially available games, such as Nintendo Wii Sports, are readily accessible and affordable, making them a potentially appealing choice. Specialized VR programs can also be tailored to the patient's condition, enabling clinicians to effectively integrate VR treatment for rehabilitation after stroke.

Recommendations

The optimal frequency and duration of intervention using VR have yet to be determined, necessitating further research to compare the impacts of various VR interventions, including their respective frequencies and durations. Moreover, a need exists for studies with larger sample sizes to evaluate the

https://doi.org/10.24171/j.phrp.2023.0148

long-term effects of VR-supported exercises. Additionally, the suitability of these interventions following stroke warrants further investigation to ascertain which patient groups are most likely to benefit and to determine the optimal timing for initiating intervention across the stages of stroke recovery (acute, subacute, and chronic). Research should also be conducted to examine the potential adverse effects associated with VR-supported exercises.

Conclusion

Most studies included in this review suggest that incorporating VR-supported exercise alongside conventional therapy may enhance the improvement of upper limb function and activity. However, the long-term benefits of VR-supported exercises among patients after stroke remain inconclusive.

Supplementary Material

Supplementary Material 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 checklist. Supplementary data are available at https://doi.org/10.24171/j.phrp.2023.0148.

Notes

Ethics Approval
Not applicable.

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

Funding
None.

Availability of Data
The datasets used in this study are not publicly accessible; however, they can be obtained from the corresponding author upon reasonable request.

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

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https://doi.org/10.24171/j.phrp.2023.0148

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0337
Osong Public Health Res Perspect 2024;15(3):201-211

Original Article

Number of comorbidities and the risk of delay in seeking treatment for coronary heart disease: a longitudinal study in Bogor City, Indonesia

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ABSTRACT

Objectives: The aim of this study was to investigate the relationship between the number of patient comorbidities and the delays in seeking treatment for coronary heart disease (CHD).
Methods: This longitudinal study utilized secondary data from the Non-Communicable Disease Risk Factor (NCDRF) cohort study conducted in Bogor City. Individuals who participated in the NCDRF cohort study and were diagnosed with CHD within the 6-year study period met the inclusion criteria. Respondents who were not continuously monitored up to the 6th year were excluded. The final sample included data from respondents with CHD who participated in the NCDRF cohort study and were monitored for the full 6-year duration. The final logistic regression analysis was conducted on data collected from 812 participants.
Results: Among the participants with CHD, 702 out of 812 exhibited a delay in seeking treatment. The risk of a delay in seeking treatment was significantly higher among individuals without comorbidities, with an odds ratio (OR) of 3.5 (95% confidence interval [CI], 1.735–7.036; $p < 0.001$). Among those with a single comorbidity, the risk of delay in seeking treatment was still notable (OR, 2.6; 95% CI, 1.259–5.418; $p = 0.010$) when compared to those with 2 or more comorbidities. These odds were adjusted for age, sex, education level, and health insurance status.
Conclusion: The proportion of patients with CHD who delayed seeking treatment was high, particularly among individuals with no comorbidities. Low levels of comorbidity also appeared to correlate with a greater tendency to delay in seeking treatment.

Keywords: Comorbidity; Coronary disease; Delay in seeking treatment

Received: November 13, 2023
Revised: April 4, 2024
Accepted: April 7, 2024

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Introduction

Coronary heart disease (CHD) remains the leading cause of death globally and shows a concerning rise in developing countries [1,2]. Despite advancements in treating heart attacks and

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strokes, these advancements particularly benefit those with milder symptoms, which poses a significant challenge if they delay seeking treatment [3].

Treatment delays encompass 3 phases: the patient decision time, the transportation time, and the in-hospital time [4]. The longest delays occur in the patient decision time, defined as the period extending from the onset of symptoms to the decision to seek treatment. Significant improvements can be realized during this phase if patients promptly recognize their symptoms [3,4]. Recognizing symptoms promptly holds immense potential for reducing this delay and ultimately saving lives.

Delays in seeking timely intervention can adversely impact patient outcomes. A prolonged delay in the patient decision-making process is linked to a higher incidence of severe heart conditions, such as cardiac arrest and sudden death. Optimal results are achieved when treatment is initiated within 90 minutes of symptom onset. It is estimated that for each minute of treatment delay beyond this crucial 90-minute window, there is a proportional increase in mortality of 7.5% within a year [5].

Delays in seeking treatment remains a significant issue in controlling CHD, particularly during the decision-making phase, which often becomes the lengthiest stage in this process [4]. Furthermore, numerous factors including demographics, individual behaviors, and medical history are reported to influence CHD treatment delays. However, studies on specific factors that influence this decision-making phase, such as comorbidities, are inconsistent.

This study focused on the crucial decision-making phase of delay in CHD treatment, by investigating the potential influence of individual comorbidities (diabetes, stroke, and psychological distress) on this delay. The primary objective was to assess the association between the number of comorbidities and the delay in seeking treatment among individuals with CHD.

HIGHLIGHTS

- Delays in seeking treatment remain a problem in controlling coronary heart disease (CHD).
- Subjects with CHD and minimal comorbidities have a greater tendency to delay seeking treatment.
- Priority should be given to individuals with CHD who do not have other comorbidities, especially those <60 years old, who require a heightened awareness of their health status. Education should focus on recognizing the diverse symptoms of CHD and the potential consequences of neglecting these symptoms, emphasizing the importance of seeking treatment even when the symptoms are not severe. Educational initiatives can be effectively conducted at local health care facilities.

Materials and Methods

Study Design

This was a longitudinal study that utilized secondary data from the Non-Communicable Disease Risk Factor (NCDRF) cohort study, which was conducted in 5 subdistricts of Central Bogor District, Bogor City, Indonesia (i.e., Kebon Kalapa, Babakan Pasar, Babakan, Ciwaringin, and Panaragan). We initiated a baseline study that encompassed 3 distinct stages: in the first stage we recruited respondents in 2011, in the second stage we recruited respondents in 2012, and in the third stage we recruited respondents in 2015, and routine monitoring was carried out 3 times per year.

Participants

The population included individuals aged ≥25 years (at baseline) who were permanent residents of the 5 selected subdistricts and who participated in the NCDRF cohort study. The data sets of individuals who received a diagnosis of CHD during the NCDRF cohort study over 6 years (2017 for first-stage respondents and 2018 for second-stage respondents) were included. Respondents who had not yet undergone monitoring through the 6th year (2017/2018) were excluded.

Prior to starting the study, we calculated the minimum sample size using the application for sample size determination in health studies by Lwanga and Lemeshow [6]. We conducted hypothesis testing for 2 populations: the proportion of individuals with CHD and no comorbidities who delayed seeking treatment (p_1=45%) [7] and the proportion of individuals with CHD and comorbidities who delayed seeking treatment (p_2=30%) [8]. With a 95% confidence interval (CI) and the power of a study (1-β) of 90%, we obtained a minimum sample size of 177 participants. To increase the precision of the study, we used data from all eligible participants, analyzing the data of 812 participants.

To minimize the potential for selection bias related to the presence of other types of heart disease, the sample was chosen based on a confirmed diagnosis of CHD, established by examining the electrocardiogram (ECG) and Minnesota codes (at rest) from the NCDRF cohort study. The diagnosis was further confirmed through consultations with qualified cardiologists.

To minimize the risk of information bias, data on treatment behavior, considered a dependent variable, were obtained

https://doi.org/10.24171/j.phrp.2023.0337

from a questionnaire specifically for respondents who had been diagnosed with CHD in the NCDRF cohort study.

Data Collection

The data in this paper were obtained from the Health Development Policy Agency with the provisions and procedures that apply through www.badankebijakan.kemkes.go.id.

Variables

For this analysis we used data from a 6-year monitoring period. The dependent variable (i.e., delay in seeking treatment) was obtained from the 2017 data of respondents recruited in 2011 and the 2018 data of respondents recruited in 2012. Data on treatment seeking behaviors were obtained from a special questionnaire for respondents who had been diagnosed with CHD during monitoring for the NCDRF cohort study. This special questionnaire was first administered in 2017. In our analysis, the dependent variable was a delay in seeking treatment, which was obtained from the 6th-year evaluation. Individuals who had not sought CHD treatment by the 6th year of monitoring, were classified as having a delay in seeking treatment. Subjects who had sought CHD treatment by the 6th year of monitoring, were classified as no delay in seeking treatment. The dependent variable "delay in seeking treatment" was grouped into 2 categories, yes or no.

In this study, the main independent variable was a comorbidity other than CHD, including chronic diseases and psychological distress. In the NCDRF cohort study, apart from CHD, only diabetes mellitus (DM) and stroke data were collected during the examinations conducted every 2 years. Therefore, we analyzed the data for DM and stroke from the baseline, 2nd, 4th, and 6th year of monitoring.

Data on DM diagnoses were obtained from blood glucose level examinations, which included a fasting blood glucose (FBG) and 2-hour postprandial blood glucose (2HPPBG). A diagnosis of DM was established if the FBG was ≥126 mg/dL or the 2HPPBG was ≥200 mg/dL [9] at the baseline, 2-year, 4-year, or 6-year blood glucose examination. Data on stroke diagnoses were obtained from neurological examinations performed by neurologists. Stroke status was assigned if the participant had a stroke diagnosis at the baseline, 2-year, 4-year, or 6-year examinations.

Psychological distress was measured annually using the 20-question self-reporting questionnaire (SRQ-20) to assess symptoms experienced by the respondent in the past month. There was a minimum cutoff of 6 "yes" answers [10,11] to determine psychological distress. Data on psychological distress were obtained from the 6-year evaluation because it could change over time, and because what participants felt in the past 30 days could influence their decision to seek treatment. The reliability of the SRQ-20 in this study was confirmed by a Cronbach alpha of 0.780. This value (0.780) was higher than the "r" table value (0.070), indicating that all questionnaire items related to psychological distress were reliable.

The main independent variable (a comorbidity other than CHD) included chronic diseases (DM and stroke) and psychological distress. We grouped this variable into 3 categories based on the number of comorbidities: (1) no comorbidity; (2) 1 comorbidity (psychological distress alone, DM alone, or stroke alone); and (3) ≥2 comorbidities (psychological distress and DM; psychological distress and stroke; DM and stroke; or psychological distress, DM, and stroke). We included psychological distress in the comorbidity variable because it has a bidirectional relationship with chronic diseases like CHD, diabetes, and stroke. Psychological distress can be a risk factor for chronic diseases, and the long-term nature of chronic diseases can trigger psychological distress [12,13].

The covariate variables were age, sex, education level, occupation, marital status, and health insurance ownership. Because these sociodemographic variables largely remain unchanged, we used data from the 6th year of monitoring. Age was calculated as the difference between the year of the interview and the year of birth. The main independent variable, comorbidity, is usually found in older people [14]; therefore, we classified age as <60 years and ≥60 years. Sex was categorized as male or female. Education was classified into 2 categories: low (did not graduate from high school) or high (graduated from high school). Occupational status was categorized into 2 groups, yes (working) or no (not working). Marital status was classified into 2 categories, not married/divorced or married. Health insurance status was categorized as insured or uninsured [15].

Statistical Analysis

We analyzed the data using IBM SPSS ver. 25.0 (IBM Corp.). The proportion of participants with CHD who delayed seeking treatment for each characteristic category was assessed using crosstabulation with the chi-square test. Descriptive analysis and the proportion of delays in seeking treatment for coronary heart disease according to participant characteristics is shown in Table 1. Descriptive analysis of comorbidities in participants with coronary heart disease according to the characteristics of study participants is shown in Table 2.

Simple logistic regression analysis was used to assess the association between each characteristic and a delay in

Table 1. Descriptive analysis and the proportion of delays in seeking treatment for coronary heart disease according to participant characteristics ($n = 812$)

Variable	Total	Delay in seeking treatment		p
		Yes	No	
Age (y)				0.001[a]
< 60	560 (69.0)	499 (89.1)	61 (10.9)	
≥ 60	252 (31.0)	203 (80.6)	49 (19.4)	
Sex				0.006[a]
Female	657 (80.9)	579 (88.1)	78 (11.9)	
Male	155 (19.1)	123 (79.4)	32 (20.6)	
Education level				0.001[a]
Low (did not graduate from high school)	771 (95.0)	674 (87.4)	97 (12.6)	
High (high school graduation or above)	41 (5.0)	28 (68.3)	13 (31.7)	
Currently working				0.937
No	383 (47.2)	332 (86.7)	51 (13.3)	
Yes	429 (52.8)	370 (86.2)	59 (13.8)	
Marital status				0.279
Married	599 (73.8)	523 (87.3)	76 (12.7)	
Single/divorced	213 (26.2)	179 (84.0)	34 (16.0)	
Health insurance status				0.056
Uninsured	82 (10.1)	77 (93.9)	5 (6.1)	
Insured	730 (89.9)	625 (85.6)	105 (14.4)	
Diabetes mellitus				0.022[a,b]
No	587 (72.3)	518 (88.2)	69 (11.8)	
Yes	225 (27.7)	184 (81.8)	41 (18.2)	
Stroke				< 0.001[a,b]
No	763 (94.0)	670 (87.8)	93 (12.2)	
Yes	49 (6.0)	32 (65.3)	17 (34.7)	
Psychological distress				0.159[b]
No	741 (91.3)	645 (87.0)	96 (13.0)	
Yes	71 (8.7)	57 (80.3)	14 (19.7)	

Data are presented n (%).
[a]Statistically significant ($p < 0.05$). [b]Comorbidities analyzed in this study included diabetes mellitus, stroke, and psychological distress.

seeking treatment in the participants with CHD. Statistical significance was set at $p < 0.05$, with a crude OR and 95% CI. Multivariable logistic regression analysis was used to assess the association between comorbidities and the delay in seeking treatment for CHD, adjusted for age, sex, education level, and health insurance status. Statistical significance was set at $p < 0.05$. The crude OR and adjusted ORs for delays in seeking treatment for CHD are shown in Table 3.

Ethics Statement

This study was conducted according to the Research Ethics Commission's research protocol. Ethical approval was provided by the Health Research Ethics Commission, Health Research and Development Agency (KEPK-BPPK) (No. LB.02.01/2/KE. 108/2017 dated March 27, 2017 and No. LB.02.01/2/KE.076/2018 dated March 1, 2018). The questionnaire distributed to the respondents included an explanation of the research purpose and an online consent form that respondents could read.

Results

In the baseline study, 5,690 respondents participated in the NCDRF cohort study. Of these, 5,312 had complete data (interview, measurement, and examination data). Of the 5,312 participants with complete data, 1,204 had been diagnosed with CHD based on heart examination results using an ECG with Minnesota codes. Although 1,143 participants with CHD participated in monitoring until 2017/2018 (stage 1 and 2 respondents), 331 participants who did not attend the 6th year of monitoring were excluded. We analyzed data from the remaining 812 respondents (Figure 1).

Of the 812 participants with CHD (Figure 2), 702 (86.5%) delayed seeking treatment and only 110 (13.5%) did not delay seeking treatment. The percentage of participants who delayed seeking treatment decreased as the number of comorbidities increased. The highest proportion of participants who delayed seeking treatment was in the group without comorbidities, followed by the group with 1 comorbidity. Unfortunately, there were no data regarding

https://doi.org/10.24171/j.phrp.2023.0337

Table 2. Descriptive analysis of comorbidities in participants with coronary heart disease according to the characteristics of study participants ($n = 812$)

Variable	No. of comorbidities[b]			*p*
	None	One	Two or more	
Delay in seeking treatment				<0.001[a]
Yes	461 (89.2)	210 (84.3)	31 (67.4)	
No	56 (10.8)	39 (15.7)	15 (32.6)	
Age (y)				<0.001[a]
<60	384 (74.3)	153 (61.4)	23 (50.0)	
≥60	133 (25.7)	96 (38.6)	23 (50.0)	
Sex				0.242
Female	419 (81.0)	205 (82.3)	33 (71.7)	
Male	98 (19.0)	44 (17.7)	13 (28.3)	
Education level				0.070
Low	496 (95.9)	230 (92.4)	45 (97.8)	
High	21 (4.1)	19 (7.9)	1 (2.2)	
Currently working				0.027[a]
No	227 (43.9)	135 (54.2)	21 (45.7)	
Yes	290 (56.1)	114 (45.8)	25 (54.3)	
Marital status				0.030[a]
Married	397 (76.8)	169 (67.9)	33 (71.7)	
Single/divorced	120 (23.2)	80 (32.1)	13 (28.3)	
Health insurance status				0.509
Uninsured	57 (11.0)	21 (8.4)	4 (8.7)	
Insured	460 (89.0)	228 (91.6)	42 (91.3)	

Data are presented *n* (%).
[a]Statistically significant ($p < 0.05$). [b]Comorbidities analyzed in this study included diabetes mellitus, stroke, and psychological distress.

Table 3. Bivariate and multivariate analysis of the relationship between the number of comorbidities and a delay in seeking treatment for coronary heart disease according to participant characteristics ($n = 812$)

Variable	Bivariate		Multivariate	
	Crude OR (95% CI)	*p*	Adjusted OR (95% CI)	*p*
No. of comorbidities[b]				
None	3.983 (2.026–7.831)	<0.001[a]	3.494 (1.735–7.036)	<0.001[a]
1 Comorbidity	2.605 (1.288–5.272)	0.008[a]	2.612 (1.259–5.418)	0.010[a]
≥2 Comorbidities	1 (ref.)		1 (ref.)	
Age (y)				
<60	1.975 (1.311–2.975)	0.001[a]	1.752 (1.142–2.688)	0.010[a]
≥60	1 (ref.)		1 (ref.)	
Sex				
Female	1.931 (1.225–3.044)	0.005[a]	1.627 (1.011–2.620)	0.045[a]
Male	1 (ref.)		1 (ref.)	
Education level				
Low	3.226 (1.616–6.441)	0.001[a]	3.040 (1.472–6.280)	0.003[a]
High	1 (ref.)		1 (ref.)	
Currently working				
No	1.038 (0.694–1.553)	0.856		
Yes	1 (ref.)			
Marital status				
Married	1.307 (0.843–2.027)	0.231		
Single/divorced	1 (ref.)			
Health insurance status				
Uninsured	2.587 (1.023–6.543)	0.045[a]	2.247 (0.880–5.739)	0.091
Insured	1 (ref.)		1 (ref.)	

OR, odds ratio; CI, confidence interval; ref., reference.
[a]Statistically significant ($p < 0.05$). [b]Comorbidities analyzed in this study included diabetes mellitus, stroke, and psychological distress.

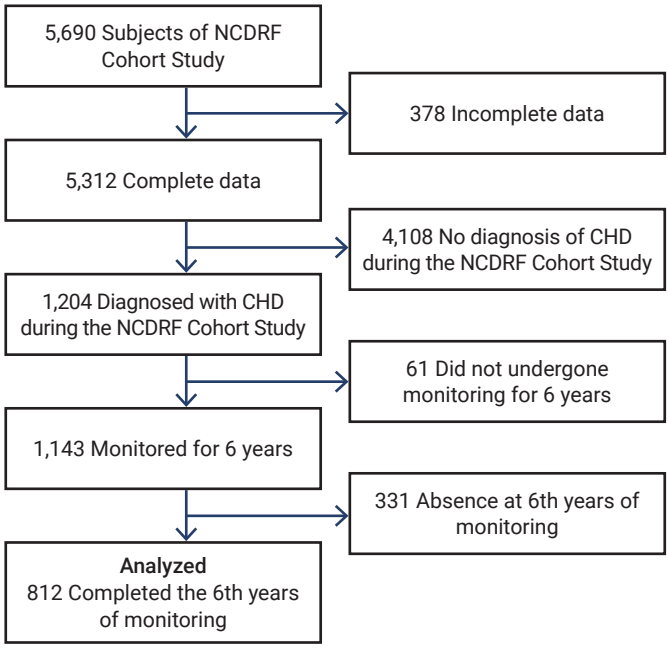

Figure 1. Flow chart of sample selection in a study of treatment delays for participants with CHD.
NCDRF, Non-Communicable Disease Risk Factor cohort study; CHD, coronary heart disease.

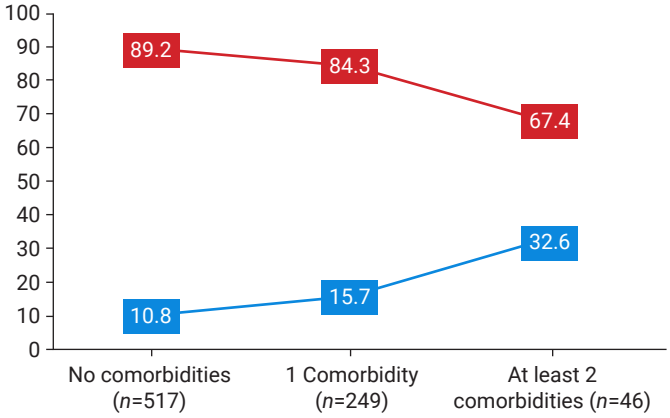

Figure 2. Proportion of participants with coronary heart disease (CHD) who delayed seeking treatment based on their number of comorbidities.
Delay-seeking treatment (red line); Not delay-seeking treatment (blue line).

the reasons why respondents did not seek treatment.

Based on the respondent characteristics (Table 1), a higher proportion of participants were aged <60 years old; female; had a low education level; were unemployed; married; had no health insurance; no DM; no stroke; and no psychological distress.

Based on crosstabulation (Table 1), the percentage of people who delayed seeking treatment was higher and statistically different for the variables of age ($p=0.001$), sex ($p=0.006$), education level ($p=0.001$), DM ($p=0.022$), and stroke ($p<0.001$). For the variables of currently working ($p=0.937$), marital status ($p=0.279$), health insurance status ($p=0.056$), and psychological distress ($p=0.159$), there were no statistically significant differences between the groups.

Initially, chronic disease and psychological distress were combined into a single variable (comorbidity) and divided into 8 categories. This was done to identify the risk of each category for delay in seeking treatment. These categories were (1) no comorbidity; (2) psychological distress alone; (3) DM alone; (4) stroke alone; (5) psychological distress and DM; (6) psychological distress and stroke; (7) DM and stroke; and (8) psychological distress, DM, and stroke. However, because of the small number of samples in several categories, the CIs were very wide and reduced the precision of the population estimate. Therefore, we reclassified the single comorbidity variable into 3 categories based on the number of comorbidities, (1) no comorbidity, (2) 1 comorbidity (psychological distress alone or DM alone or stroke alone), and (3) ≥2 comorbidities (psychological distress and DM, psychological distress and stroke, DM and stroke, or psychological distress, DM, and stroke).

The number of comorbidities according to participant characteristics is shown in Table 2. People without comorbidities were more likely to delay seeking treatment than those with comorbidities. People <60 years old who had an occupation, were married, and were uninsured were more likely to delay seeking treatment than people with comorbidities. The percentage of people without comorbidities was significantly different among these 4 groups ($p<0.05$), except for the uninsured group.

The percentage of people with 1 comorbidity was significantly higher than the participant groups with no comorbidity and ≥2 comorbidities among those who had no occupation and were single or divorced ($p<0.05$). Meanwhile, there were no significant differences among females, those with a high level of education, and those who were insured.

The percentage of people with ≥2 comorbidities was significantly higher than the participant groups with no comorbidity and 1 comorbidity among those who did not delay seeking treatment and were ≥60 years old ($p<0.05$). Meanwhile, there were no significant differences among males and those with a low level of education.

When evaluating covariate variables, we discovered that age was a confounding factor in the relationship between comorbidity and delay in seeking treatment, with a change in OR of 18.6%. Therefore, we added the age variable back to the model. Based on previous studies, we also included several factors that can influence the delay in seeking treatment in the final model: sex, education level, and health insurance status.

https://doi.org/10.24171/j.phrp.2023.0337

Bivariate analysis (Table 3) showed that the risk of delay in seeking treatment was significantly higher in the participant groups with no comorbidities (OR, 3.98; 95% CI, 2.026–7.831; $p < 0.001$) and 1 comorbidity (OR, 2.61; 95% CI, 1.288–5.272; $p = 0.008$) than those with ≥2 comorbidities. The risk of delay in seeking treatment was also significantly higher in the participant groups aged <60 years (OR, 1.98; 95% CI, 1.311–2.975; $p = 0.001$), female (OR, 1.93; 95% CI, 1.225–3.044; $p = 0.005$), with a low education level (OR, 3.23; 95% CI, 1.616–6.441; $p = 0.001$), and uninsured (OR, 2.59; 95% CI, 1.023–6.543; $p = 0.045$). Employment status and marriage status were not statistically significant (OR < 1, $p > 0.05$).

Multivariate analysis revealed that participants with CHD were at risk of delay in seeking treatment, with an OR of 3.5 (95% CI, 1.735–7.036; $p < 0.001$) for those with no comorbidities and an OR of 2.6 (95% CI, 1.259–5.418; $p = 0.010$) for those with 1 comorbidity when compared to those with ≥2 comorbidities (adjusted for age, sex, education level, and insurance status).

Discussion

In our analysis, the data on treatment seeking for the early monitoring periods were limited and our results only reflect the delays in seeking CHD treatment for the 6th year of monitoring. In addition, the reasons for the delays in seeking treatment remain unclear due to the absence of relevant data. However, we tried to elevate the internal validity of this study by minimizing the possibility of selection and information bias and by controlling for confounders.

Of the 812 participants with CHD in the study sample, 702 (86.5%) either did not seek treatment or delayed it. This was higher than in the study by Venkatesan et al. [7], which reported that 44.08% of patients with acute myocardial infarction (AMI) delayed seeking treatment. This discrepancy could be due to the fact that the study by Venkatesan et al. [7] was conducted in a hospital setting and patients who delayed treatment may not have reported it (i.e., underreporting). The data used in our study was collected from the community, resulting in a higher proportion of reported delays in seeking treatment than in the hospital study.

It is important to note that, in our study, participants without the 3 specified comorbidities were not necessarily free of other diseases. All participants in our sample were diagnosed with CHD during the cohort study examination and, in our research, individuals classified as having no comorbidities were considered to be solely affected by CHD.

Our analysis revealed that among participants with CHD, there was a 3.5-fold higher risk (95% CI, 1.735–7.036; $p < 0.001$) of delay in seeking treatment for those with no comorbidities and a 2.6-fold higher risk (95% CI, 1.259–5.418; $p = 0.010$) for those with 1 comorbidity than those with ≥2 comorbidities. This relationship remains statistically significant even after adjusting for age, sex, education level, and insurance status. Interestingly, our community-based research aligns with the hospital-based research of Stafford et al. [16], which showed that, over a 2-year study period, 3 times as many people with multiple comorbidities were admitted to the hospital than people with a single comorbidity. In other words, Stafford et al. [16] showed that people with a single comorbidity had a greater tendency to delay seeking treatment than those with multiple comorbidities.

A study exploring the relationship between the number of comorbidities, autonomic modulation, and quality of life in patients with coronary artery disease found that a higher level of pain was associated with an increased number of comorbidities [17]. Interestingly, pain serves as a motivating symptom, and in cases of acute coronary syndrome (ACS) and acute stroke, severe pain is linked to reducing the delay in seeking treatment [3].

Our study indicated that a lower comorbidity level was associated with a greater likelihood of delay in seeking treatment and was consistent with other research revealing that individuals experiencing mild chest pain were 10 times more likely to delay the decision to seek treatment (OR, 10.05; 95% CI, 6.50–15.54) [18]. The nature of symptom presentation plays a crucial role in prehospital delay. Continuous or high levels of symptom intensity predict a shorter prehospital delay, while intermittent or low-intensity symptoms tend to predict a longer delay [19]. Reduced pain levels, absence of chest pain, or experiencing chest pain alone are factors associated with delayed treatment seeking in people with AMI [7].

Patients with limited awareness of their symptoms may underestimate the severity of their condition, leading to delays in seeking treatment [20]. A study conducted in Tanzania suggested that many individuals delayed seeking treatment due to a lack of understanding about their diagnosis or treatment, a lack of awareness regarding the severity of their symptoms, or concerns about the side effects of medication [21].

Our study found an inverse relationship between the number of comorbidities and the risk of delaying treatment. In other words, patients with a greater number of comorbidities were less likely to delay seeking treatment. As mentioned previously, our findings concur with the study by Stafford et al. [16], which indicated that people with multiple conditions are less likely to delay seeking treatment. However, our results diverge from the study by Banharak et al. [22], which reported that various conditions and

symptoms, including diabetes, hypertension, heart failure, stroke, impaired gait, angina pectoris, current smoking, and a history of hospitalization, were associated with prolonged treatment delays.

Our study identified that participants with CHD who had a single comorbidity (diabetes alone, stroke alone, or psychological distress alone) were 2.6 times more likely to delay seeking treatment than those who have ≥2 comorbidities. This finding is statistically significant and aligns with Beza et al. [18] who reported a longer prehospital delay among ACS patients with diabetes. The study by Mohan et al. [23] also showed a longer prehospital delay among myocardial infarction patients who had diabetes (OR, 1.3), although this result was not statistically significant ($p = 0.317$).

Another study investigated the role of personality in AMI. It found that women with AMI and a type D personality (TDP) who have a tendency to experience negative emotions and to inhibit the expression of emotions, were generally less likely to delay seeking treatment compared to those without TDP (OR, 0.28; 95% CI, 0.08–0.98) [24]. However, depression may increase the delay in seeking treatment among participants with CHD [25]. Generalized anxiety disorders or panic disorders are typically associated with higher levels of care-seeking behaviors, while social anxiety disorder or specific phobias (e.g., blood-injection-injury phobia) may lead to decreased, delayed, or inconsistent healthcare utilization [26]. This delay is due to their fear of depending on others, rather than due to inadequate knowledge or fear of their disease [27]. Unfortunately, in our study, we did not analyze specific comorbidities, such as stroke, diabetes, or psychological distress.

Regarding age, we found that individuals <60 years old were 1.75 times more likely to delay seeking treatment than those aged ≥60 years. This finding is consistent with the study by Okunrintemi et al. [28], which showed that younger patients may be inclined to deny their medical condition. Older patients with atherosclerotic cardiovascular disease are more likely to have accepted their diagnoses and, therefore, be more willing to comply with suggested medical therapies. Patierno et al. [29] reported that participants with CHD who denied their illness had a longer delay in seeking treatment than those who acknowledged their illness. However, our findings were inconsistent with the study by Mohan et al. [23], which showed that participants with CHD and aged >60 years, tended have a prehospital delay 1.6-fold higher (95% CI, 1.048–2.487; $p = 0.030$) than those aged ≤60 years. Several studies also concurred with Mohan et al., that older people tend to delay seeking treatment [22,30,31]. This difference may be because most participants in our study were <60 years old and underwent the ECG examination as part of routine screening in the cohort study, rather than in response to a disturbance in their health. Therefore, they might tend to ignore or deny the diagnosis of the ECG examination.

Our study showed that women were more likely to delay seeking treatment than men. This result is consistent with a qualitative study in Iran of 39 women with first-time ACS that found women tended to delay seeking help when their symptoms were mild and developed gradually. Women often underestimated the symptoms and attributed them to noncardiac causes, making them more likely to delay seeking treatment for CHD symptoms [32,33]. Our findings were also in line with a study of Pakistani society, which showed that, because women are overburdened with their family and household responsibilities, many are unaware of or fail to pay attention to symptoms. Women may not prioritize their health, resulting in delays in seeking treatment [34]. However, our findings differ from those of Walsh and Joynt [35], who found that women with stroke were 0.66 times as likely to experience treatment delays as males ($p = 0.04$). The difference is likely due to the fact that stroke symptoms can be more severe and sudden, making them harder to ignore than CHD, which is often without symptoms.

Our findings on education level align with Banharak et al. [22], suggesting that individuals with lower education levels may be more likely to delay treatment. Conversely, Mosleh et al. [36] found that participants with higher education were at risk of delaying treatment. This difference may be due to several factors. Although those with low levels of education might have limited knowledge of CHD symptoms and a decreased awareness of their health circumstances, participants with higher education might exhibit a tendency to self-diagnose or delay seeking professional help for various other reasons.

Regarding possession of health insurance, we found that uninsured participants were 2.25 times more likely to delay seeking treatment, although this association was not statistically significant. Other studies found that, among uninsured participants with CHD, there is a tendency to delay treatment due to the cost [22,36] and that uninsured patients are at risk for longer delays in presenting to the hospital (adjusted OR, 1.38; 95% CI, 1.17–1.63; $p < 0.001$) [15]. Patients with cardiovascular disease face a long-term disease course, often without symptoms, as well as the presence of comorbidities, all of which require lifelong treatment with frequent medications [37]. While previous studies highlight the financial barriers associated with being uninsured, our findings may reflect the impact of Indonesia's governmental

https://doi.org/10.24171/j.phrp.2023.0337

health insurance program (Badan Penyelenggara Jaminan Sosial Kesehatan, BPJS). The health insurance program provided by the Indonesian government through BPJS specifically caters to the needs of disadvantaged individuals, as indicated by data from the Indonesian Social Service. Therefore, participants in this study could still get health services even though they were not obligated to pay themselves, thereby mitigating the financial barrier to seeking treatment.

Our study found that participants with CHD but without additional comorbidities were more likely to delay seeking treatment, with age as a confounding factor (i.e., most participants aged <60 years do not have other comorbidities). Widayanti et al. [38] found that sociocultural context plays a vital role in shaping the individual's concept of health and disease. In Indonesia, individuals often perceive themselves as healthy if they can carry out their daily activities without significant disruption, leading to a delay in seeking treatment until the disease worsens [38]. This delay is due to health beliefs and perceptions, including adaptive emotions that lead the individual to respond to illness by considering that the symptoms experienced are usual and will disappear soon [39]. Among patients with AMI, denial of their illness is common, particularly during the first few hours and even the first day after chest pain first occurs. Although this unconscious physiological response allows patients to cope with and overcome their anxiety and fear, it often leads to delays in seeking treatment, as patients attribute their symptoms to causes other than cardiac problems [40]. Our study showed that younger persons aged <60 years who did not experience disturbing symptoms and tended to be less aware of their health status, were more likely to deny their medical diagnosis and consequently delay seeking treatment.

Conclusion

A high proportion of participants with CHD delayed seeking treatment. Our findings indicate that the level of comorbidity played a significant role in this delay. Specifically, low levels of comorbidity were associated with a higher likelihood of delaying seeking treatment.

Implications

This study suggests that healthcare providers should be aware of the increased risk of delayed treatment among patients with CHD and ≤1 comorbidity. These patients may require additional support and education to encourage them to seek treatment promptly when experiencing symptoms of CHD.

Recommendation

These findings highlight the importance of health workers in Puskesmas and cadres in Posbindu, providing increased attention to people diagnosed with CHD, including those without other comorbidities and those who do not feel any symptoms. To effectively manage the prevalence and mortality rates associated with CHD, particularly among persons aged <60 years, it is crucial to raise public awareness by educating them about the following critical aspects: (1) the diverse nature and levels of intensity of CHD symptom presentation; (2) the significance of seeking medical intervention even if they do not perceive substantial disruptions to their overall health, to prevent the progression of their condition; and (3) the potentially severe consequences that may arise from neglecting CHD symptoms [19].

Notes

Ethics Approval

This study was conducted according to the Research Ethics Commission's research protocol. Ethical approval was provided by the Health Research Ethics Commission, Health Research and Development Agency (KEPK-BPPK) (No. LB.02.01/2/KE. 108/2017 dated March 27, 2017 and No. LB.02.01/2/KE.076/2018 dated March 1, 2018). The questionnaire distributed to the respondents included an explanation of the research purpose and an online consent form that respondents could read.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

This published article and its supplementary files include all data generated or analyzed during this study. The data supporting this study's findings are available from the Data Management Laboratory of the Health Development Policy Agency, Ministry of Health of Indonesia. Data can be made available after approval of a written request to the Data Management Laboratory at: datin.bkpk@kemkes.go.id

Authors' Contributions

Conceptualization: ST, LI, WR, SI, II; Data curation: all authors; Formal analysis: ST, LI, SI, TW, WR, NS; Investigation: ST, LI, WR, TW, AML, NS, ASP; Methodology: ST, LI, WR, TW, SI, II; Supervision: all authors; Validation: ST, LI, WR, TW; Visualization: ST, LI, TW; Writing–original draft: all authors; Writing–review & editing: all authors. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank all parties who helped in the process of creating this scientific paper, the technical staff from the Data Management Laboratory of the Center for Data and Information, namely the Ministry of Health of the Republic of Indonesia as the data provider, and other individuals who facilitated the realization of this research.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2024.0021
Osong Public Health Res Perspect 2024;15(3):212-220

Original Article

Periodontitis and non-communicable diseases in a Brazilian population, a cross-sectional study, Vila Velha-ES, Brazil

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ABSTRACT

Objectives: The objective of this study was to examine the hypothesis that periodontal disease is associated with chronic non-communicable diseases.
Methods: In this cross-sectional study, we evaluated the periodontal health condition of the population, based on the community periodontal index, as well as the number of missing teeth and the presence of systemic health conditions. We quantified the association between oral health and the presence of chronic diseases using simple logistic regression, adjusting for confounding factors including age, smoking, and overweight.
Results: The study population consisted of 334 volunteers, aged between 19 and 81 years. In patients over 45 years old, periodontal disease was found to be significantly associated with hypertension and diabetes. Furthermore, in female patients, periodontal disease was significantly associated with hypertension, diabetes, and cancer.
Conclusion: Our findings indicate that periodontal disease is positively and significantly associated with both arterial hypertension and diabetes, independent of potential confounding factors.

Keywords: Chronic disease; Cross-sectional studies; Diabetes mellitus; Health services research; Hypertension; Periodontitis

Received: January 17, 2024
Revised: April 11, 2024
Accepted: April 16, 2024

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Introduction

Periodontal disease is an infectious condition that includes both gingivitis and periodontitis. Gingivitis, characterized by inflammation of the gums and subsequent bleeding, is considered

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an early stage of the disease. Over time, periodontitis can develop, with changes including the accumulation of plaque and calculus, bacterial dysbiosis, the formation of periodontal pockets, gingival recession, tissue destruction, and alveolar bone loss, all of which may result in tooth loss [1]. The progression of periodontitis involves an intricate interplay between the oral microbiome and the host. This complexity is due in part to the extensive bacterial diversity found within the subgingival space, as well as the host's immune response [2]. Additionally, periodontal inflammation can have systemic consequences by promoting low-grade inflammation throughout the body, adversely affecting various organs [3].

In the 1990s, the term "periodontal medicine" was introduced by Offenbacher et al. [4]. In the early 21st century, dentists began to alert their patients to the potential link between periodontal disease and various systemic conditions. However, during this time, the relationship was often described in a unidirectional manner, suggesting that periodontal disease could contribute to the development of systemic diseases. Currently, the interaction between periodontal disease and systemic diseases is understood to be bidirectional, with each condition having the potential to influence the other [5]. Notably, periodontitis is garnering substantial attention as a possible risk factor for cardiovascular disease and type 2 diabetes. It has also been shown to be associated with adverse pregnancy outcomes, respiratory diseases, kidney diseases, certain cancers, and even Alzheimer's disease [6].

Recent evidence from intervention studies suggests that local treatment of periodontitis can improve markers of comorbidity, including blood pressure, glycemic index, and C-reactive protein (CRP) levels. Understanding the impact of disseminated periodontal pathogens on certain extraoral pathologies and examining the systemic inflammation associated with periodontitis, including the adaptation of bone marrow hematopoietic progenitors, may offer new therapeutic strategies to mitigate the risk of comorbidities linked to periodontitis. In this context, Fischer et al. [7] reported a decrease in systemic inflammation markers related to cardiovascular disease (including interleukin 1, interleukin 6, and fibrinogen), along with improvements in CRP, total cholesterol, and triglyceride levels, following 6 months of non-surgical periodontal therapy. A contributing factor to this independent association may be the capacity of periodontitis to induce low-grade systemic inflammation, which can influence the development of comorbidities. This may occur through mechanisms such as the ulcerated epithelium of periodontal pockets allowing bacteria and their products (e.g., lipopolysaccharide or proteases) to enter the bloodstream, leading to bacteremia [8]. Additionally, the innate immune system—including mature innate immune cells, their progenitor populations, and the inflammatory mediators they secrete—may represent a link between periodontitis and systemic diseases [9].

HIGHLIGHTS

- Recent evidence from intervention studies suggests that local treatment of periodontitis can improve several markers of comorbidity, including blood pressure, glycemic index, and C-reactive protein levels.
- A positive association was observed between periodontal disease, hypertension, and diabetes among individuals over 45 years old, independent of potential confounding factors. Furthermore, a greater severity of periodontal disease was associated with higher rates of hypertension and diabetes.
- Our study underscores the importance of a multidisciplinary approach in the care of public health service users, highlighting the substantial interrelationship between oral health and systemic health.

The objective of this exploratory-descriptive study was to evaluate the hypothesis that periodontal disease is positively associated with several chronic non-communicable diseases with substantial prevalence in the population. Additionally, we aimed to determine whether a greater severity of periodontal disease strengthens this association.

Materials and Methods

Design

This exploratory-descriptive study employed a cross-sectional design to investigate the periodontal health status, number of missing teeth, and systemic health conditions among residents of 3 Family Health Strategy units in the municipality of Vila Velha, ES (Ataíde, Jardim Colorado, and Divino Espírito Santo), from 2021 to 2023. Exclusion criteria encompassed an age of under 18 years and the absence of more than 14 teeth, as the latter condition would render the assessment of periodontal health conditions unfeasible.

Variables

Two professional evaluators were calibrated to minimize bias in diagnosing oral health conditions, achieving a kappa index of 0.87 [10,11]. Periodontal health status was assessed using the community periodontal index (CPI), as proposed by the World Health Organization (WHO). The

CPI is used to evaluate the presence of bleeding, calculus, and shallow and deep periodontal pockets, referencing a sextant examination. Probing was performed with a WHO-recommended model 621 periodontal probe featuring a 0.5-mm spherical tip, which is considered atraumatic and comparatively reliable for detecting gingival bleeding. Additionally, a colored band indicated measurements from 3.5 to 5.5 mm. The scores from each sextant were summed to yield a total score, which was regarded as a measure of the individual's periodontal health. General health data were sourced from the citizen's electronic medical record, eSus (managed by the Ministry of Health), supplemented by a thorough anamnesis that included measurements of blood pressure and capillary blood glucose. We evaluated variables such as arterial hypertension, diabetes, ischemic events (acute myocardial infarction and stroke), arthritis, neurological issues (dementia, Parkinson disease, Alzheimer disease, and cognitive impairment), and cancer. Potential confounding factors, including smoking, overweight, age, and sex, were also considered.

The criteria for arterial hypertension were a measurement exceeding 140/90 mmHg or the use of antihypertensive drugs. For diabetes, the criterion was a fasting blood glucose level exceeding 125 mg/dL.

In terms of confounding factors, individuals who consumed 5 or more cigarettes per day were classified as smokers, whereas overweight was defined as having a body mass index exceeding 25 kg/m^2.

Data Analysis

The associations between periodontal disease and the presence of chronic non-communicable diseases were quantified using simple logistic regression, adjusted for confounding factors such as age, smoking, and overweight. This analysis enabled the association of comorbidity outcomes, including hypertension, diabetes, ischemic events, arthritis, neurological issues, and cancer, with the total periodontal index and the number of missing teeth.

Statistical Analysis

To classify the severity of periodontal disease and the extent of tooth loss, the study population was divided into quartiles based on the CPI. Using odds ratios (ORs), we could then assess whether a higher risk of these comorbidities was associated with a greater severity of periodontal disease and/or a higher number of missing teeth. Statistical analyses were conducted using IBM SPSS ver. 24.0 (IBM Corp.), with an alpha significance level set at 5% for all tests. Data were described in terms of observed frequency and percentage. The sample size was calculated based on the population of the municipality of Vila Velha, which is approximately 467,000 inhabitants according to the CENSO 2022 (administered by the Brazilian Institute of Geography and Statistics) [12]. With a margin of error of 5% and a confidence level of 95%, the minimum initial sample size was determined to be 246; however, we chose to expand our sample to obtain a more robust analysis.

Ethics Approval

This project received approval from the Ethics Committee of the Health Sciences Center (CCS-UFES), as documented in Opinion No. 5,048,518, dated October 20, 2021. Subsequently, the Vila Velha City Hall authorized its execution, under process No. 42,621/2021. All participating volunteers underwent a clinical and periodontal examination conducted by a trained and qualified professional, following the completion of an informed consent form.

Results

The study population consisted of 334 volunteer participants from 3 Family Health Units in the city of Vila Velha, ES (UESF Ataíde, UESF Jardim Colorado, and UESF Divino Espírito Santo), comprising 106 men and 228 women. The participants ranged in age from 19 to 81 years, with a median age of 45 years (Table 1). Table 2 summarizes the oral health condition of the study population, while Table 3 presents the data regarding systemic health conditions. In patients aged 45 years or older, significant associations were found between the total periodontal index and both hypertension and diabetes. Specifically, patients with a periodontal index of 9 to 13 exhibited about 3.5 times the odds of having hypertension as those with an index of 1 to 2, while those with an index of 14 or higher had an OR for hypertension of approximately 2.9. Furthermore, patients with a periodontal index of 9 to 13 were around 5.8 times as likely to have diabetes as those with an index of 1 to 2 (Table 4). In female patients,

Table 1. Distribution of participants by age group and sex (n = 334)

Study population	Value
Age group (y)	
≤ 44	169 (50.6)
≥ 45	165 (49.4)
Sex	
Female	228 (68.3)
Male	106 (31.7)

Data are presented as n (%).

https://doi.org/10.24171/j.phrp.2024.0021

Table 2. Distribution of participants by total periodontal index and no. of lost teeth

Oral health condition	Value
Total periodontal index (*n* = 320)	
1–2	82 (25.6)
3–8	86 (26.9)
9–13	71 (22.2)
≥14	81 (25.3)
Lost teeth (*n* = 332)	
None	108 (32.5)
1–2	78 (23.5)
3–5	76 (22.9)
≥6	70 (21.1)

Data are presented as *n* (%).

Table 3. Distribution of participants by comorbidity (*n* = 334)

Systemic health condition	Value
Hypertension	
No	209 (62.6)
Yes	125 (37.4)
Diabetes	
No	303 (90.7)
Yes	31 (9.3)
Ischemic events	
No	319 (95.5)
Yes	15 (4.5)
Smoking	
No	274 (82.0)
Yes	60 (18.0)
Overweight	
No	294 (88.0)
Yes	40 (12.0)
Arthritis	
No	320 (95.8)
Yes	14 (4.2)
Neurological problems	
No	293 (87.7)
Yes	41 (12.3)
Cancer	
No	308 (92.2)
Yes	26 (7.8)

Data are presented as *n* (%).

Table 4. Associations of comorbidities with total periodontal index in patients over 45 years of age

Dependent variable	Total periodontal index	*p*[a]	OR (95% CI for OR)
Hypertension	1–2		1 (ref.)
	3–8	0.548	1.346 (0.510–3.549)
	9–13	0.011	3.532 (1.333–9.362)
	≥14	0.022	2.917 (1.170–7.270)
Diabetes	1–2		1 (ref.)
	3–8	0.532	1.758 (0.300–10.310)
	9–13	0.029	5.800 (1.193–28.203)
	≥14	0.262	2.522 (0.500–12.713)
Ischemic events	1–2		1 (ref.)
	3–8	Empty	
	9–13	0.755	0.725 (0.096–5.451)
	≥14	0.869	1.160 (0.200–6.729)
Arthritis	1–2		1 (ref.)
	3–8	0.260	3.636 (0.385–34.377)
	9–13	0.479	2.308 (0.228–23.311)
	≥14	0.443	2.400 (0.256–22.489)
Neurological problems	1–2		1 (ref.)
	3–8	0.795	1.279 (0.200–8.190)
	9–13	0.441	1.959 (0.354–10.838)
	≥14	0.104	3.709 (0.765–17.982)
Cancer	1–2		1 (ref.)
	3–8	0.941	1.055 (0.257–4.324)
	9–13	0.415	0.519 (0.107–2.509)
	≥14	0.222	2.140 (0.631–7.260)

OR, odds ratio; CI, confidence interval; ref., reference; empty, ORs were not calculated.
[a]Adjusted simple logistic regression for the age group of 45 years and older; $p < 0.05$ were considered to indicate statistical significance.

the total periodontal index was significantly associated with hypertension, diabetes, and cancer. Women with a periodontal index between 9 and 13 faced approximately 2.8 times the odds of having hypertension as those with an index of 1 to 2, while women with an index of 14 or higher exhibited an approximate OR of 5.5. Furthermore, relative to women with an index of 1 to 2, those with a periodontal index between 9 and 13 were 5.7 times as likely to have diabetes, and those with an index of 14 or higher were 8.7 times as likely to have cancer (Table 5). No significant association was observed between the total periodontal index and any comorbidity in male patients, suggesting that the presence of comorbidities in men is independent of this index. Similarly, no significant association was found between the total periodontal index and comorbidities among smokers and overweight patients, allowing us to eliminate the effects of these potential confounding factors. The number of missing teeth was significantly associated with hypertension and diabetes in female patients. Women with 1 to 2 missing teeth were 3.8 times as likely to have hypertension as those with all their teeth. The likelihood of hypertension was even greater for participants with more teeth absent, with an OR of around 10.5 for those with 3 to 5 missing teeth and 12.3 for those with 6 or more. Additionally, women with 6 or more missing teeth were 9.3 times as likely to have diabetes as those with a full set of teeth (Table 6). In male patients, a significant association was also found between the number of missing teeth and hypertension. Men with 6 or more missing teeth were 4.7 times as likely to have hypertension as those with all

Table 5. Association of comorbidities with total periodontal index in female patients

Dependent variable	Total periodontal index	p[a)]	OR (95% CI for OR)
Hypertension	1–2		1 (ref.)
	3–8	0.420	1.458 (0.584–3.640)
	9–13	0.029	2.828 (1.115–7.172)
	≥14	<0.001	5.505 (2.234–13.562)
Diabetes	1–2		1 (ref.)
	3–8	0.622	1.548 (0.272–8.810)
	9–13	0.032	5.684 (1.159–27.879)
	≥14	0.287	2.500 (0.462–13.521)
Ischemic events	1–2		1 (ref.)
	3–8	0.843	0.754 (0.046–12.353)
	9–13	0.304	3.341 (0.335–33.306)
	≥14	0.222	4.000 (0.431–37.080)
Arthritis	1–2		1 (ref.)
	3–8	0.211	4.016 (0.454–35.520)
	9–13	0.531	2.178 (0.191–24.844)
	≥14	0.222	4.000 (0.431–37.080)
Neurological problems	1–2		1 (ref.)
	3–8	0.553	1.421 (0.445–4.538)
	9–13	0.315	1.846 (0.558–6.110)
	≥14	0.092	2.634 (0.854–8.120)
Cancer	1–2		1 (ref.)
	3–8	0.310	3.161 (0.342–29.197)
	9–13	0.304	3.341 (0.335–33.306)
	≥14	0.045	8.711 (1.048–72.420)

OR, odds ratio; CI, confidence interval; ref., reference.
[a)]Adjusted simple logistic regression for female participants; $p<0.05$ were considered to indicate statistical significance.

Table 6. Association of comorbidities with the number of lost teeth in female patients

Dependent variable	Lost teeth	p[a)]	OR (95% CI for OR)
Hypertension	None		1 (ref.)
	1–2	0.012	3.792 (1.348–10.666)
	3–5	<0.001	10.446 (3.884–28.096)
	≥6	<0.001	12.311 (4.467–33.926)
Diabetes	None		1 (ref.)
	1–2	0.447	2.029 (0.327–12.593)
	3–5	0.148	3.450 (0.643–18.506)
	≥6	0.005	9.324 (1.940–44.811)
Ischemic events	None		1 (ref.)
	1–2	0.781	1.327 (0.181–9.734)
	3–5	0.795	1.302 (0.178–9.547)
	≥6	0.359	2.352 (0.378–14.645)
Arthritis	None		1 (ref.)
	1–2	0.781	1.327 (0.181–9.734)
	3–5	0.261	2.706 (0.477–15.348)
	≥6	0.189	3.209 (0.564–18.277)
Neurological problems	None		1 (ref.)
	1–2	0.962	1.026 (0.356–2.955)
	3–5	0.394	1.531 (0.575–4.075)
	≥6	0.341	1.632 (0.595–4.472)
Cancer	None		1 (ref.)
	1–2	0.077	7.143 (0.809–63.052)
	3–5	0.080	7.000 (0.793–61.769)
	≥6	0.057	8.333 (0.941–73.782)

OR, odds ratio; CI, confidence interval; ref., reference.
[a)]Adjusted simple logistic regression for female participants; $p<0.05$ were considered to indicate statistical significance.

their teeth intact (Table 7). The number of missing teeth exhibited no statistically significant associations with the comorbidities of smokers and overweight patients, allowing us to rule out the influence of these potential confounding factors.

Discussion

Atherosclerotic cardiovascular disease (ACD) refers to a group of conditions affecting the heart and blood vessels, including coronary heart disease (manifesting as angina or acute myocardial infarction), cerebrovascular disease (such as stroke or transient ischemic attacks), and peripheral arterial disease. Sharma [13] identified a consistent link between periodontitis and new ACD cases, noting that this association was especially pronounced in adults under 65 years old and in men. Our findings support these observations, as we detected a significant association with arterial hypertension in individuals over 45 years old and in those with a CPI between 9 and 13 ($p=0.011$) or a CPI of 14 or higher ($p=0.022$). A meta-analysis by Bahekar et al. [14], which synthesized data from 5 cohort studies encompassing 86,092 patients, indicated that people with periodontal disease are 1.14 times as likely to develop coronary heart disease compared to periodontally healthy individuals, even when accounting for common risk factors such as smoking. In case-control studies involving 1,423 patients, these researchers observed an even stronger association (OR, 2.22). Our research aligns with these findings, demonstrating a significant link between periodontal disease and high blood pressure, independent of shared risk factors like smoking and overweight. However, our study did not establish a significant connection with ischemic events. Upon calculating the ORs, we found that participants with more advanced periodontal disease (specifically, those with a CPI between 9 and 13 and those with a CPI greater than 14) had ORs of 3.53 and 2.91, respectively—figures exceeding those reported by Bahekar et al. [14]. Zhao et al. [15] reported similar findings in a cohort of university students, with a positive association between periodontal disease and hypertension (OR, 1.28). Although this value is lower than our result, it can be attributed to the younger age of the population studied.

https://doi.org/10.24171/j.phrp.2024.0021

Table 7. Association of comorbidities with the number of lost teeth in male patients

Dependent variable	Lost teeth	p[a)]	OR (95% CI for OR)
Hypertension	None		1 (ref.)
	1–2	0.979	0.986 (0.341–2.846)
	3–5	0.081	2.670 (0.886–8.046)
	≥6	0.008	4.655 (1.483–14.606)
Diabetes	None		1 (ref.)
	1–2	0.373	0.359 (0.038–3.420)
	3–5	0.877	0.868 (0.145–5.195)
	≥6	0.470	1.737 (0.389–7.756)
Ischemic events	None		1 (ref.)
	1–2	0.998	0
	3–5	0.683	1.800 (0.107–30.355)
	≥6	0.079	7.579 (0.790–72.680)
Arthritis	None		1 (ref.)
	1–2	Empty	
	3–5	Empty	
	≥6	Empty	
Neurological problems	None		1 (ref.)
	1–2	0.345	3.273 (0.280–38.244)
	3–5	0.289	3.789 (0.322–44.531)
	≥6	0.732	1.636 (0.097–27.511)
Cancer	None		1 (ref.)
	1–2	0.345	3.273 (0.280–38.244)
	3–5	0.132	6.000 (0.582–61.842)
	≥6	0.079	7.579 (0.790–72.680)

OR, odds ratio; CI, confidence interval; ref., reference; empty, ORs were not calculated.

[a)]Adjusted simple logistic regression for male participants; $p < 0.05$ were considered to indicate statistical significance.

Another cross-sectional study that supports our findings was conducted by Machado et al. [16] and included 1,064 patients. The findings indicated that individuals with moderate and severe periodontitis were more likely to be diagnosed with high blood pressure, with ORs of 2.60 and 2.20, respectively, relative to those without the condition. The data underscore the concept that patients with periodontitis, especially those with more severe gingival inflammation and deeper periodontal pockets, face a higher risk of changes in systolic and diastolic blood pressure. Similarly, our study revealed that the upper quartiles of the CPI exhibited stronger associations with arterial hypertension (OR, 3.53 for the 3rd quartile and OR, 2.91 for the 4th quartile) than the lower quartiles, which included patients with better periodontal health.

A study conducted by De Angelis et al. [17], employing a methodological design very similar to the present research, also yielded results that align with our findings. They enrolled 533 volunteers over the age of 65 years, with both male and female participants. The authors concluded that both high CPI values and significant tooth loss were positively associated with the likelihood of developing cardiovascular disease. These associations persisted after adjusting for other comorbidities and lifestyle factors such as alcohol consumption and smoking. In a study of postmenopausal North American women, Gordon et al. [18] identified a positive and statistically significant correlation between blood pressure levels and periodontal health. They determined that more severe periodontal disease was associated with higher blood pressure. Additionally, they found a positive correlation between tooth loss and hypertensive disease, with an OR of 1.14 for those missing more than 5 teeth. Our findings corroborate the link between arterial hypertension and periodontal index in the 3rd ($p = 0.029$; OR, 2.82) and 4th ($p < 0.001$; OR, 5.50) quartiles of CPI among women. Concerning tooth loss, our data indicate comparatively high odds of hypertension in the groups missing 1 to 2 teeth ($p = 0.012$; OR, 3.79), 3 to 5 teeth ($p < 0.001$; OR, 10.44), and 6 or more teeth ($p < 0.001$; OR, 12.31). Byun et al. [19] also analyzed the association between periodontal disease and cardiovascular diseases, conducting a large study with approximately 170,000 participants. They concluded that periodontal disease is a significant risk factor for acute myocardial infarction (OR, 1.34) and stroke (OR, 1.35). Alhadainy et al. [20] assessed the link between oral diseases and self-reported history of stroke in an elderly US population. Of all oral health variables examined, only the number of missing teeth was significantly associated with stroke history. Compared to patients missing 0 to 3 teeth, individuals missing 4 to 10 teeth had an OR of 1.72 (95% confidence interval [CI], 1.36–2.29), while those missing 11 to 27 teeth had an OR of 2.40 (95% CI, 2.24–2.69) and exhibited a significantly higher likelihood of reporting a stroke. The OR was highest for edentulous patients, who had 28 missing teeth (OR, 3.09; 95% CI, 2.97–3.12). Our results did not show a significant relationship between ischemic events (stroke and acute myocardial infarction) and periodontal disease. Regarding hypertension, Mendes et al. [21] previously demonstrated an association between high blood pressure and tooth loss. Their cross-sectional study of 10,576 patients revealed a positive and statistically significant link between extensive tooth loss (defined as the absence of more than 10 teeth) and systemic arterial hypertension, even after adjusting for common risk factors such as smoking. Similarly, our findings indicated a positive association between tooth loss and high blood pressure in both women and men. Additionally, we noted an association between tooth loss and diabetes exclusively in female participants; women with 6 or more missing teeth had a significantly increased risk of diabetes (OR, 9.32; $p = 0.005$). The significance of this indicator underscores the concept that advanced

periodontal disease can lead to the loss of many teeth, and the absence of teeth may thus be indicative of the chronicity of the condition.

The association between tooth loss and blood pressure measurements was investigated in a study by Hosadurga et al. [22]. In this cross-sectional study involving 270 volunteers, the authors identified a significant positive association between the absence of more than 10 teeth and elevated systolic blood pressure. In contrast, a study by Almoznino et al. [23], which included approximately 132,000 individuals aged 18 to 50 years, did not find a significant link between periodontal disease and high blood pressure. However, when analyzing CRP levels resulting from periodontal inflammation, the authors noted a marginally positive association with hypertension (OR, 1.01). These findings diverge from those of our study and most of the literature, as previously mentioned. A potential reason for this discrepancy could be the age range of the population in their study, which consisted mainly of young adults. In contrast, our results align with most evidence published on this topic. The diversity and broad age range of our study population underscore the importance of our findings. The robustness of the data—gathered through clinical evaluations, self-reports, and medical record reviews—lends considerable credibility and allows for comparison, as these methods are commonly employed in various studies addressing this issue.

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to a hereditary and/or acquired deficiency in insulin production, action, or both. The association between this disease and periodontitis has been documented since the 1960s. However, the specific mechanism linking diabetes mellitus and periodontal disease remains to be fully elucidated [24]. To clarify the relationship between these conditions, Stohr et al. [25] conducted a systematic review with meta-analysis, which revealed a positive prospective bidirectional association between periodontal disease and diabetes mellitus. Our study identified a significant correlation between periodontal disease and diabetes only in those with a periodontal index between 9 and 13 (the 3rd quartile). This group was 5.8 times as likely to develop diabetes ($p = 0.029$) as those in the first quartile. Another recent systematic review examined the epidemiological link between periodontitis and type 2 diabetes mellitus across 4 electronic databases, identifying 53 observational studies. The results showed that the adjusted prevalence of type 2 diabetes mellitus was significantly higher in patients with periodontitis (OR, 4.04; $p < 0.001$) and vice versa (OR, 1.58; $p < 0.001$). Severe periodontitis was associated with a 53% higher incidence of type 2 diabetes mellitus ($p < 0.001$), a finding that was generally consistent. In contrast, the impact of mild periodontitis was less pronounced (relative risk, 1.28; $p = 0.007$). These findings led the authors to conclude a bidirectional relationship between diabetes and periodontitis [26]. In a randomized clinical trial, Rapone and colleagues [27] assessed the correlation between periodontal treatment and glycemic control in patients with type 2 diabetes. Following 6 months of follow-up among 187 patients, they concluded that non-surgical periodontal therapy reduces levels of glycated hemoglobin and CRP. Similar outcomes were reported by Qureshi et al. [28]. Furthermore, a cross-sectional study involving 11,429 participants by Ghanem and Nagy [29] found that self-perceived poor oral health was a risk factor for diabetes (OR, 1.35; 95% CI, 1.04–1.75). This study underscores the importance of incorporating oral health measures into comprehensive diabetes management approaches.

Corbella et al. [30] hypothesized that periodontitis may be an independent risk factor for cancer development, both locally and at distant sites, due to the persistent chronic inflammation of periodontal tissues. These researchers observed a significant positive correlation between periodontal disease and various cancers, including those of the digestive tract, pancreas, prostate, breast, uterus, lung, and hematological system, as well as grouped esophagus/oropharynx cancer and non-Hodgkin lymphoma. Ma et al. [31] reported similar findings in a meta-analysis of epidemiological studies, lending support to the theory that periodontitis is linked to the development of esophageal cancer, prostate cancer, hematological cancer, and melanoma of the skin. These results underscore the importance of early prevention and treatment of periodontitis. Liu et al. [32] used a large database to demonstrate that individuals with periodontitis were at higher risk of developing gastric cancer compared to those with good oral health (OR, 1.13; 95% CI, 1.04–1.23; $I^2 = 0.00\%$; $p < 0.01$). Additionally, Li et al. [33] sought to explore the bidirectional causal relationship between periodontitis and 4 types of urological tumors—kidney, prostate, bladder, and testicular cancers—using a Mendelian randomization approach. They found significant positive genetic correlations between periodontitis and kidney cancer (OR, 1.287; 95% CI, 1.04–1.594; $p = 0.020$). We similarly identified a positive association between periodontal disease in the highest quartile, defined as a periodontal index of 14 or higher, and the incidence of cancer in study volunteers ($p = 0.045$; OR, 8.71). However, we did not distinguish between cancer types.

The primary limitations of our study stem from its cross-sectional design, which precludes the ability to infer causality

https://doi.org/10.24171/j.phrp.2024.0021

or assess the mechanisms underlying the relationship between periodontal disease and the chronic diseases under investigation. Another notable limitation is the potential for underdiagnosis of periodontitis resulting from our decision to employ the CPI methodology.

Conclusion

Our findings indicate that periodontal disease is positively and significantly associated with arterial hypertension and diabetes mellitus in those over 45 years old, independent of confounding factors such as smoking and overweight. Furthermore, greater severity of periodontal disease is associated with higher rates of hypertension and diabetes. Additionally, among women, severe periodontitis constitutes a risk factor for cancer, while tooth loss is implicated as a risk factor for high blood pressure and diabetes.

To further explore the topic and establish a causal link between oral health conditions and chronic non-communicable diseases, additional research is necessary. We propose a methodology that includes measuring inflammatory cytokines and detecting periodontal pathogens in atheroma plaques and neoplastic tissues using polymerase chain reaction. These studies could help elucidate the mechanisms underlying the association between periodontal disease and overall systemic health. Moreover, observational studies with long-term follow-up in specific populations could be conducted to evaluate the incidence of chronic diseases and correlate these findings with the oral health status of individuals.

Overall, our study underscores the importance of a multidisciplinary approach in the care of public health service users, considering the substantial interrelationship between oral health and systemic health. This approach is crucial for improving the effectiveness and efficiency of the therapies proposed.

Notes

Ethics Approval

This project received approval from the Ethics Committee of the Health Sciences Center (CCS-UFES), as documented in Opinion No. 5,048,518, dated October 20, 2021. Subsequently, the Vila Velha City Hall authorized its execution, under process No. 42,621/2021. All participating volunteers underwent a clinical and periodontal examination conducted by a trained and qualified professional, following the completion of an informed consent form.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

All data generated or analyzed during this study are included in the published article. Requests for additional data can be directed to the corresponding author.

Authors' Contributions

Conceptualization: GVdM, ACRF, CCJ; Data curation: GVdM, BFSdM; Formal analysis: GVdM, CCJ; Investigation: GVdM, BFSdM; Methodology: GVdM, CCJ, ACRF; Project administration: GVdM; Resources: CCJ; Software: LHSP; Supervision: CCJ, ACRF; Validation: LHSP; Visualization: GVdM; Writing–original draft: GVdM; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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https://doi.org/10.24171/j.phrp.2024.0021

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0273
Osong Public Health Res Perspect 2024;15(3):221-228

Original Article

The association between living arrangements and health-related quality of life in Korean older people: a nationwide repeated cross-sectional study

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ABSTRACT

Objectives: This study investigated the association between living arrangements and health-related quality of life (HRQoL) in older people.
Methods: A secondary analysis was conducted of 6,153 participants (aged ≥60 years) from the seventh Korean National Health and Nutrition Examination Survey (2016 to 2018). HRQoL was measured using the 3-level version of the EuroQol 5-dimensional questionnaire. The chi-square test, t-test, and multiple regression were used, applying sampling weights for the analysis.
Results: The proportion of respondents living alone was 18.0%, with a higher prevalence among women and older age groups ($p < 0.001$). The overall HRQoL was lower in groups living alone than in groups living with others ($p < 0.001$). Older people living alone showed higher impairments in all dimensions of the 3-level version of the European Quality of Life 5-Dimensional Questionnaire (EQ-5D-3L) than those living with others, including mobility ($p < 0.001$), self-care ($p < 0.001$), usual activities ($p < 0.001$), pain/discomfort ($p < 0.001$), and depression/anxiety ($p < 0.001$). Problems with mobility were most prevalent (42.8%), followed by pain/ discomfort (41.9%) in respondents living alone. Living alone was significantly associated with a lower HRQoL index score ($b = -0.048$, $p < 0.001$) after adjusting for age, gender, education, exercise, perceived stress, and perceived health status.
Conclusion: Living alone was negatively associated with HRQoL. Based on this study, future care planning for older people should consider their living arrangements. The need to strengthen and expand care programs targeting those living alone should also be addressed.

Keywords: Living arrangements; Older adults; Quality of life; Residence characteristics

Received: October 3, 2023
Revised: February 13, 2024
Accepted: February 16, 2024

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Introduction

The United Nation's Decade of Healthy Ageing (2030) highlights a concerted action in response

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to global population aging. It is also closely linked with the Sustainable Development Goal 3, which seeks to "ensure healthy lives and promote well-being for all at all ages" [1]. By 2050, more than 40% of the Korean population will be aged ≥65 years, a sharp rise from 10.8% in 2010 [2]. With a rapidly ageing population, healthy ageing and care for older people have become major concerns in the Republic of Korea.

According to the National Statistical Office, the proportion of older persons living alone in the Republic of Korea was 16.0% in 2000, increasing to 19.6% in 2020 [3]. It has been reported that older people living alone have significantly poorer physical and mental health, health behaviors, and worse quality of life than those living with a family member [4,5]. Furthermore, older people living alone are more likely to present with self-reported illness and use health services when they are sick [6]. As age advances, older people tend towards multi-morbid diseases and are likely to have more complex care needs [7]. Understanding the health status of the older population in each country is important so that effective national health and social service plans can be formulated. However, little is known about the epidemiological characteristics of older people living alone and whether living alone itself is associated with health-related quality of life (HRQoL) after adjusting for age and other socioeconomic variables.

The 3-level version of the European Quality of Life 5-Dimensional Questionnaire (EQ-5D-3L) is a suitable instrument to measure health status among older populations and has been used in a number of studies [8]. The EQ-5D-3L questionnaire is also known as a useful predictor of mortality and of first hospitalization among older populations [9].

The main purpose of this study was to determine the HRQoL of older people according to their living arrangement status using the EQ-5D-3L. The specific aims were (1) to investigate the prevalence of different living arrangements according to the subjects' sociodemographic characteristics, (2) to compare the EQ-5D-3L index scores by sociodemographic characteristics according to living arrangements, (3) to compare the EQ-5D-3L by gender and living arrangement, (4) to determine whether living arrangements are associated with quality of life after adjusting for sociodemographic variables.

HIGHLIGHTS

Various factors contribute to well-being late in life, including "ageing in place" and with whom one ages. This study found that living alone was associated with poorer health-related quality of life, including higher rates of pain, depression, and anxiety as well as greater limitations on mobility, self-care, and routine activities. The findings of this study emphasize that, when planning care for older people, living arrangements should be considered and social and health services strengthened for those living alone.

Materials and Methods

Data Sources and Participants

We conducted a secondary analysis of data obtained from the seventh Korean National Health and Nutrition Examination Survey (KNHANES VII, 2016 to 2018) [10]. The KNHANES is a nationwide periodic cross-sectional survey of the health and nutrition of a nationally representative sample of community residents. Further details on the KNHANES VII are described in the raw data use guidance of the KNHANES VII [10]. From 31,689 randomly sampled individuals, 24,269 people participated in this national survey across all ages (a response rate of 76.6%). For the present study, 6,153 KNHANES participants aged ≥60 years were included for analysis based on demographic characteristics, health behaviors, health status, and the EQ-5D-3L.

Measurements

HRQoL: EQ-5D-3L

The KNHANES uses the EQ-5D-3L to assess health status. The EQ-5D-3L was developed by the EuroQol Group and has been widely used as a concise, generic instrument for measuring, comparing, and valuing health status across disease areas [11]. The EQ-5D-3L consists of the 5 dimensions of mobility, self-care, usual activities, pain/discomfort, and anxiety/depression and has 3-level answers ("no problem", "some problems", and "severe problems") for each dimension. The EQ-5D-3L is easy to apply as a self-administered survey. A Korean version of the EQ-5D-3L was developed, and its reliability and validity were tested according to a procedure recommended by the EuroQol group [12]. Lee et al. [13] established a population-based preference weighting for the Korean EQ-5D-3L using a representative population sample [13], and the Korean EQ-5D-3L index scores were calculated using these population weightings. A higher score typically represents a better health status [14].

In this study, the sociodemographic characteristics included gender, age, living arrangement (living with others or living alone), region (urban or rural), education level (elementary, middle school, or high school), marital status (never married, married, or divorced/separated), and income (low, lower-

https://doi.org/10.24171/j.phrp.2023.0273

medium, upper-medium, or high). Current smoking status (yes or no), high-risk drinking (yes or no), and walking over 30 minutes daily (yes or no) were included as health behavior variables. Perceived stress (little or much) and perceived health status (1, very poor; 2, poor; 3, moderate; 4, good; 5, very good) were included as health status variables.

Statistical Methods

Statistical analyses were conducted using SAS software ver. 9.4 (SAS Institute Inc.). Survey procedures for the complex sampling design were applied to estimate nationally representative statistics. The chi-square test was used to compare the general characteristics and EQ-5D-3L index scores according to living arrangement, and multiple linear regression analysis was conducted to investigate if living arrangements influenced the EQ-5D-3L index scores after adjusting for covariates. A p-value less than 0.05 indicated statistical significance.

Ethics Statement

This study was approved by the Institutional Review Board (IRB) of Jeju National University (IRB No: JJNU-IRB-2022-078). Informed consent was waived by the IRB since this secondary analysis used anonymized data.

Results

Living Arrangement by Sociodemographic Characteristics

Of the 18.0% of respondents who lived alone, the prevalence was higher in women and in older age groups ($p < 0.001$): 11.70% in men (11.1% 60–64 years, 32.0% >80 years) and 23.0% in women (11.9% 60–64 years, 41.2% >80 years) (Table 1). More participants in lower income brackets lived alone (30.3%) than those with higher incomes (5.5%, $p < 0.001$), and a higher percentage of rural residents lived alone (22.4%) than urban residents (16.7%, $p < 0.001$).

HRQoL by Living Arrangement

The comparison of HRQoL (measured by EQ-5D-3L index score) according to living arrangement is presented in Table 2. Overall, the HRQoL was lower in groups living alone than

Table 1. Distribution of the general characteristics of older Korean adults according to living arrangement

Characteristic	Total (n = 6,153)		Living with others (n = 4,876, 82.0%)		Living alone (n = 1,277, 18.0%)		X^2	p
	W %	SE	W %	SE	W %	SE		
Age (y)							171.52	<0.001
60–64	31.2	0.8	88.5	1.0	11.5	1.0		
65–69	22.5	0.6	86.6	1.0	13.4	1.0		
70–74	17.4	0.5	82.0	1.3	18.0	1.3		
75–79	16.5	0.6	74.1	1.6	25.9	1.6		
≥80	12.3	0.5	68.1	2.3	31.9	2.3		
Gender							104.77	<0.001
Man	44.5	0.6	88.3	0.8	11.7	0.8		
Woman	55.5	0.6	77.0	1.0	23.0	1.0		
Marital status							2,304.14	<0.001
Not married	0.9	0.1	18.8	5.6	81.2	5.6		
Married	70.1	0.8	97.0	0.3	3.0	0.3		
Divorced/separated	29.0	0.8	47.8	1.6	52.2	1.6		
Education							87.24	<0.0001
Elementary school	44.7	1.0	76.9	1.0	23.1	1.0		
Middle school	40.6	0.9	85.2	0.9	14.8	0.9		
High school	14.6	0.8	89.3	1.2	10.7	1.2		
Income							317.13	<0.001
Low	25.2	0.8	69.7	1.5	30.3	1.5		
Low middle	24.1	0.7	77.2	1.3	22.8	1.3		
Upper middle	24.8	0.7	86.0	1.0	14.0	1.0		
High	25.9	1.0	94.5	0.7	5.5	0.7		
Region							13.82	<0.001
Urban	78.4	2.0	83.3	0.8	16.7	0.8		
Rural	21.6	2.0	77.6	1.4	22.4	1.4		

W %, weighted %, applied sampling weight; SE, standard error.

Table 2. Health-related quality of life in older Korean adults (as measured by EQ-5D-3L index scores) according to living arrangement

Variable	Category	Living with others		Living alone		t	*p*
		n	Mean±SE	*n*	Mean±SE		
Age (y)	60–64	1,409	0.944±0.003	216	0.899±0.011	3.67	<0.001
	65–69	1,228	0.924±0.004	229	0.888±0.010	3.08	0.002
	70–74	977	0.902±0.005	259	0.860±0.012	3.48	0.001
	75–79	760	0.867±0.007	302	0.836±0.013	1.73	0.084
	≥80	502	0.835±0.010	271	0.808±0.014	1.43	0.154
Gender	Man	2,317	0.932±0.003	349	0.896±0.009	4.01	<0.001
	Woman	2,559	0.888±0.003	928	0.839±0.007	6.04	<0.001
Marital status	Not married	11	0.875±0.050	47	0.777±0.042	1.24	0.215
	Married	4,182	0.920±0.002	150	0.928±0.010	-0.42	0.677
	Divorced/separated	682	0.854±0.007	1,080	0.849±0.006	0.62	0.534
Education	Elementary school	2,079	0.872±0.004	758	0.820±0.008	5.49	<0.001
	Middle school	2,038	0.931±0.003	402	0.901±0.007	4.35	<0.001
	High school	725	0.950±0.004	106	0.931±0.010	1.61	0.109
Income	Low	997	0.886±0.006	516	0.825±0.009	5.13	<0.001
	Low middle	1,126	0.909±0.005	403	0.850±0.010	5.20	<0.001
	Upper middle	1,277	0.915±0.004	248	0.911±0.009	0.92	0.360
	High	1,452	0.920±0.004	104	0.906±0.016	1.01	0.314
Region	Urban	3,676	0.914±0.003	892	0.867±0.006	7.22	<0.001
	Rural	1,200	0.889±0.005	385	0.823±0.010	4.80	<0.001
Smoking	No	4,345	0.908±0.003	1,112	0.854±0.006	8.11	<0.001
	Yes	507	0.923±0.006	155	0.884±0.015	2.76	0.006
High-risk drinking	No	4,555	0.907±0.003	1205	0.856±0.006	8.30	<0.001
	Yes	296	0.932±0.008	63	0.894±0.018	1.90	0.058
Walking	No	3,054	0.893±0.003	852	0.831±0.007	7.09	<0.001
	Yes	1,786	0.938±0.003	407	0.907±0.007	4.73	<0.001
Perceived stress	Moderate	3,906	0.925±0.002	1,007	0.882±0.006	7.02	<0.0001
	High	941	0.844±0.007	258	0.761±0.015	4.78	<0.001
Perceived health status	Very poor	378	0.716±0.014	181	0.660±0.017	2.17	0.030
	Poor	962	0.849±0.005	303	0.778±0.012	4.74	<0.001
	Moderate	2,416	0.933±0.003	591	0.916±0.005	3.11	0.002
	Good	891	0.968±0.003	155	0.961±0.006	1.39	0.165
	Very good	228	0.970±0.005	47	0.944±0.017	1.00	0.318

EQ-5D-3L, 3-level version of the European Quality of Life 5-Dimensional Questionnaire; SE, standard error.

in groups living with others. The HRQoL decreased in both groups as age advanced. The HRQoL was significantly lower in those aged ≤74 years and living alone than in those aged ≤74 years and living with others. However, for those ≥75 years, there were no significant differences in HRQoL between the 2 groups. The HRQoL in men was higher than in women, though the scores were significantly lower for those living alone in both genders ($p<0.001$). According to marital status, those who had never married and lived alone showed the lowest HRQoL. However, the scores were not significantly different based on living arrangement in the subgroups of marital status. Lower education levels were associated with lower HRQoL scores, and mean scores were significantly lower in the group living alone than in the group living with others for the same education-level subgroups. Income levels followed a pattern much like the levels for education. Those who lived alone in rural areas had lower HRQoL scores than those living alone in urban areas. However, in both urban and rural dwellers, the mean HRQoL scores of those living alone were significantly lower than those living with others.

The Prevalence of Health Problems in the EQ-5D-3L By Living Arrangement

The EQ-5D-3L index scores according to living arrangement are shown in Figure 1. Older people living alone showed higher impairments in all dimensions of the EQ-5D-3L than those living with others including mobility ($p<0.001$), self-care ($p<0.001$), usual activities ($p<0.001$), pain/discomfort ($p<0.001$), and depression/anxiety ($p<0.001$). For respondents living alone, problems with mobility were most prevalent

https://doi.org/10.24171/j.phrp.2023.0273

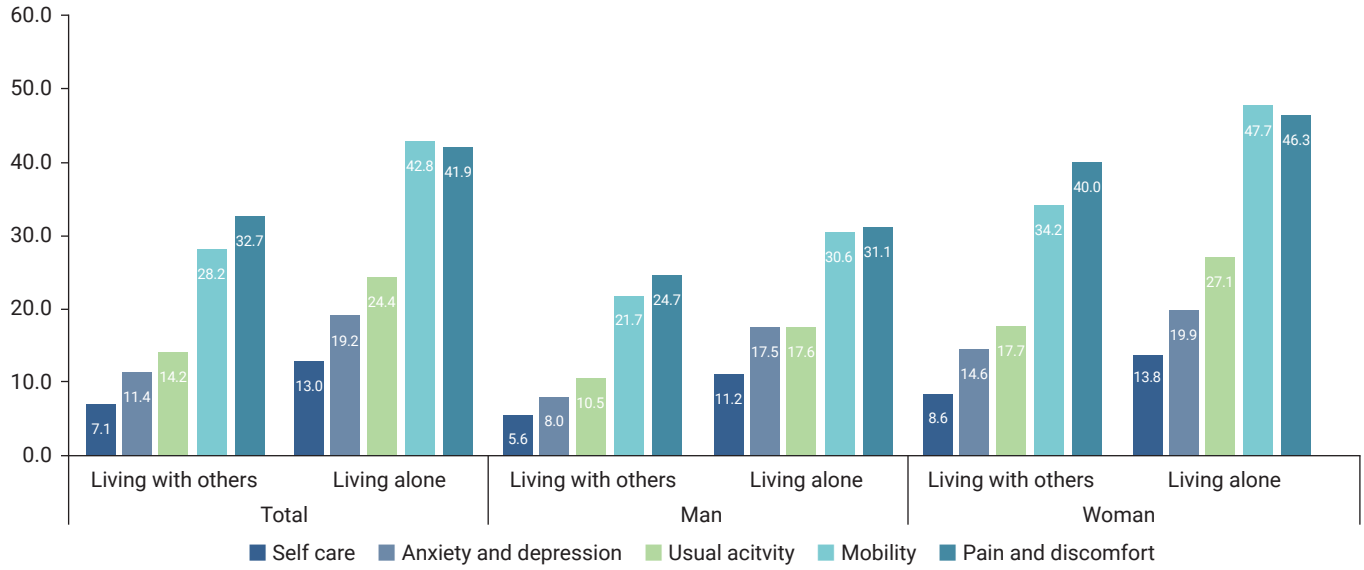

Figure 1. The prevalence of health problems (EQ-5D-3L index scores) according to gender and living arrangement in older Korean adults. EQ-5D-3L, 3-level version of the European Quality of Life 5-Dimensional Questionnaire.

(42.8%), followed by pain/discomfort (41.9%). Conversely, for those living with others, pain/discomfort was the most common problem (32.7%), followed by mobility (28.2%). In both groups, respondents reporting problems in self-care were in the lowest percentage (13.0% for living alone; 7.1% for living with others). Overall, pain/discomfort was the most frequently reported complaint (total 34.3%, men 25.4%, women 41.4%), followed by mobility (total 30.8%, men 22.7%, women 37.3%). Relatively few respondents had problems with self-care (total 8.2%, men 6.2%, women 9.8%).

Factors Influencing HRQoL

Multiple regression was used to evaluate the impact of living arrangement on HRQoL using EQ-5D-3L index scores (Table 3). After adjusting for the significant variables of HRQoL (age, gender, education, exercise, perceived stress, and perceived health status), living arrangement was a significant influencing factor for HRQoL. Living alone was significantly associated with a lower HRQoL ($b = -0.048$, $p < 0.001$). Age was also significantly associated with a lower HRQoL ($b = -0,171$, $p < 0.001$), and women showed a significantly lower HRQoL than men ($b = -0.044$, $p < 0.001$). Those with a lower education level had a lower HRQoL than those with a higher education ($b = -0.054$, $p = 0.004$). People who walked >30 minutes daily showed a higher HRQoL than those who did not. Perceived stress and subjective health status were also significantly associated with EQ-5D-3L index scores ($p < 0.001$) (Table 3).

Discussion

In this study, 18.0% of people over 60 years of age reported living alone (women, 23.0% vs. men, 11.7%). As urbanization and nuclearization of the family have increased in the Republic of Korea, the overall number of households has increased steadily, with single household occupancy being the most prevalent household type (30.2%) in 2019 [3].

Globally, the percentage of people living alone varies by both age and gender [15]. Across Europe and North America, there are much larger differences in the incidence of living alone in later life (75–79 years old) with Switzerland, the Netherlands, and the United Kingdom recorded as the highest. The incidence of living alone is reported to be 2 to 3 times higher in women than in men [15]. Understanding the distribution and trends in living alone among older people by age and gender can contribute to better estimations of long-term care needs and inform policies that meet the evolving needs of older people.

Living alone had a significant association with HRQoL as measured by EQ-5D-3L index scores. Nearly half of people aged ≥60 years reported having health problems, and those living alone demonstrated significantly lower EQ-5D-3L index scores than those living with others. Using multiple regression analysis, living arrangements were found to be significantly associated with EQ-5D-3L index scores. The impact of living arrangements on long-term care needs has been previously reported [16]. Previous studies have also presented evidence that those who belong to single-person households experience lower health status [4]. Living with

Table 3. Multiple regression analysis of the impact of living arrangement (alone vs. with others) on HRQoL using EQ-5D-3L index scores

Variable	*b*	beta	t	*p*
Age (y)	−0.004	−0.171	−11.28	<0.001
Living arrangement (ref. with others)	−0.018	−0.048	3.77	<0.001
Gender (ref. man)	−0.013	−0.044	3.49	<0.001
Education				
High school	Ref.			
Elementary	−0.016	−0.054	−2.93	0.004
Middle school	0.005	0.017	1.17	0.243
Health	0.058	0.374	23.46	<0.001
Walking				
Yes	Ref.			
No	0.025	0.083	−7.62	<0.001
Stress				
Much	Ref.			
Little	−0.049	−0.133	9.09	<0.001

HRQoL, health-related quality of life; EQ-5D-3L, 3-level version of the European Quality of Life 5-Dimensional Questionnaire; ref., reference.

a spouse in the household provided the greatest health protection [16], and living alone had the most negative effect on health for older people [17]. The lower quality of life for older people living alone may be due to a lack of social support. Previous studies have found that older people with poor social support showed a lower quality of life or psychological health [18], while those living with family fared better than older people living alone in terms of subjective health status, depression, and life satisfaction [19]. Living arrangements can be related to both social support and social networks as well as care support [20]. Older people living alone may be a vulnerable population, and that should be taken into consideration when making policies for long-term care and interventions. Older people obtain emotional and social support primarily through family and community; therefore, raising the level of social support for those living alone by strengthening the community support system can be a solution.

This study found that EQ-5D-3L index scores decreased rapidly with advancing age and that the prevalence of health problems across all dimensions of the EQ-5D-3L was higher in men than in women, regardless of the living arrangement. Previous studies also reported a significant decline in HRQoL with age [16,21], and that the HRQoL was higher in men with poorer health status and greater mobility limitations than in women [22]. The proportion of women living alone increases with age, presumably because women live longer than men. Mouodi et al. [17] suggested that the influence of living arrangements on health differed by gender, demonstrating that health problems were higher in older people who lived alone, and that health status according to living arrangement was different for men and women. This study found that women living alone had a lower quality of life; thus efforts to target interventions more specifically to them are needed.

As previously reported by Mangen et al. [8], the most common health-related complaints identified in the present study were pain/discomfort and mobility problems. Therefore, it is necessary to prioritize pain management and mobility support services for the growing older population. Previous studies found that the prevalence of health problems reported in the EQ-5D-3L increased with age, and that mobility problems occurred most frequently in the eldest population [8]. Anxiety and depression were reported more frequently in this study than in other European studies, which found anxiety/depression to be the least prevalent [21]. One reason for the higher prevalence of anxiety/depression in the Republic of Korea might be poverty. The old-age poverty rate in the Republic of Korea has been reported highest among the Organization for Economic Co-operation and Development (OECD) countries in 2016 at >45% [23]. Depression and suicide mortality were also serious challenges, with the Republic of Korea demonstrating the highest suicide mortality rate among the OECD countries at 24.6 per 100,000 persons in 2017 [24]. The high old-age poverty rate in the Republic of Korea might also be related to the fact that the pension system has not fully matured, and it is hoped that various social systems will mature to reduce poverty over time.

Multiple regression analysis of the EQ-5D-3L index scores suggested a need to target future health and well-being interventions towards women, older people living alone, those with lower education and poor perceived health status, those with poor health behavior (notably those not walking >30 minutes a day), and those with perceived high

https://doi.org/10.24171/j.phrp.2023.0273

levels of stress. Previous studies also found that gender, age, stress, perceived health, and physical activity were significant factors affecting quality of life [25,26]. Perceived health status had the greatest effect on HRQoL in this study. Among the sociodemographic variables, the effect of age on HRQoL was much greater than gender or the level of education, as demonstrated in previous studies [27]. Understanding older people as a potentially vulnerable population may lead to developing more responsive and better-integrated policies and interventions. Preventive and supportive community care services that enhance quality of life can have a positive impact and better support older people.

A strength of this study was the relatively large sample; thus, the results can be seen as representative of the general older population of the Republic of Korea. In addition, the data was collected by well-trained interviewers for the KNHANES. However, some limitations should be considered. First, we cannot attribute a causal relationship to the findings because the study design was based on a cross-sectional survey. Second, there might be recall bias because the data were collected retrospectively using questionnaires. Third, although the EQ-5D-3L focused on 5 dimensions of HRQoL, it did not measure all dimensions of quality of life. Further studies are recommended to expand on this aspect. In addition, because the EQ-5D-3L is a generic questionnaire for assessing quality of life, any correlations between existing disease and quality of life were not investigated in this study. Fourth, the EQ-5D-3L index scores in this study may have been overestimated in relation to the actual life quality of older Koreans since older people living in residential facilities were not included in the KNHANES. Fifth, because this study used data from the Republic of Korea only, it cannot be generalized to the populations of other countries.

Conclusion

Approximately 1 in 5 older people in this sample were living alone, and the HRQoL was lower in groups living alone than in groups living with others. Living alone was negatively associated with the HRQoL after adjusting for age, gender, education, exercise, perceived stress, and perceived health status. Therefore, it is important to take living arrangements into consideration when formulating policies on healthy aging. Devising innovative living arrangements, especially those that enhance interactions between older and younger populations, can play an essential role in healthy aging and improve the HRQoL for older people.

Notes

Ethics Approval

This study was approved by the IRB of Jeju National University (No: JJNU-IRB-2022-078) and was performed in accordance with the principles of the Declaration of Helsinki.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

This research was supported by the 2022 scientific promotion program funded by Jeju National University.

Availability of Data

The datasets generated and/or analyzed during the current study are available in the Korean National Health and Nutrition Examination Survey (KNHANES VII) repository (https://knhanes.kdca.go.kr/knhanes/eng/index.do).

Authors' Contributions

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

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https://doi.org/10.24171/j.phrp.2023.0273

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0251
Osong Public Health Res Perspect 2024;15(3):229-237

Original Article

Prevalence and patterns of post-COVID-19 symptoms in recovered patients of Delhi, India: a population-based study

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ABSTRACT

Objectives: Post-coronavirus disease 2019 (COVID-19) symptoms were widely reported. However, data on post-COVID-19 conditions following infection with the Omicron variant remained scarce. This prospective study was conducted to understand the prevalence, patterns, and duration of symptoms in patients who had recovered from COVID-19.

Methods: A prospective study was conducted across 11 districts of Delhi, India, among individuals who had recovered from COVID-19. Study participants were enrolled, and then returned for post-recovery follow-up at 3 months and 6 months interval.

Results: The mean age of study participants was 42.07 years, with a standard deviation of 14.89 years. The majority of the participants (79.7%) reported experiencing post-COVID-19 symptoms. The most common symptoms included joint pain (36.0%), persistent dry cough (35.7%), anxiety (28.4%), and shortness of breath (27.1%). Other symptoms were persistent fatigue (21.6%), persistent headache (20.0%), forgetfulness (19.7%), and limb weakness (18.6%). The longest duration of symptom was observed to be anxiety (138.75 ± 54.14 days), followed by fatigue (137.57 ± 48.33 days), shortness of breath (131.89 ± 60.21 days), and joint pain/swelling (131.59 ± 58.76 days). At the first follow-up visit, 2.2% of participants presented with abnormal electrocardiogram readings, but no abnormalities were noticed during the second follow-up. Additionally, 4.06% of participants exhibited abnormal chest X-ray findings at the first follow-up, which decreased to 2.16% by the second visit.

Received: September 14, 2023
Revised: February 26, 2024
Accepted: March 6, 2024

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Conclusion: The most frequently reported post-COVID-19 symptoms were joint pain, dry cough, anxiety and shortness of breath. These clinical symptoms persisted for up to 6 months, with evidence of multi-system involvement. Consequently, findings highlighted the need for long-term follow-up during the post-COVID-19 period.

Keywords: Coronavirus infection; COVID-19; Post-acute COVID-19 syndrome; SARS-CoV-2; Severe acute respiratory syndrome

Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first spotted in China in December 2019, led to a global pandemic. This pandemic led to a rapid increase in both cases and deaths, as well as overwhelming healthcare infrastructure [1]. On May 5, 2023, the World Health Organization (WHO) announced the end of COVID-19 as a public health emergency of international concern, with over 768 million cases and more than 6.9 million deaths reported to date [2]. The pandemic spurred numerous epidemiological studies aimed at understanding the transmission of the disease, as well as prevention and control strategies. The variability of the disease—influenced by the circulating viral strains—and a limited understanding of its pathophysiology led to the exploration of multiple treatment options during the peak of the outbreak. The broad clinical spectrum of SARS-CoV-2 further complicated the understanding of the disease due to its potential for multiorgan involvement [3,4].

Acute COVID-19 infection reportedly lasts up to 4 weeks before recovery [5]. However, growing evidence suggested that signs and symptoms of COVID-19 infection persist even after recovery in a substantial proportion of cases. The condition characterized by the persistence of symptoms after the initial infection and recovery from COVID-19 has been termed "long COVID," "post-COVID-19 syndrome," and "post-acute COVID-19 syndrome" [5]. No consensus has yet been reached on the characteristics defining this condition. The British Medical Association defines a syndrome as a "set of medical signs and symptoms which are correlated with each other and associated with a particular disease" [6]. More specifically, the UK National Institute of Health and Care Excellence defines post-COVID-19 syndrome as "a cluster of signs and symptoms that develop during or after an infection consistent with COVID-19, continue for more than 12 weeks and are not explained by an alternative diagnosis" [5]. Long-term symptoms of COVID-19 can include cardiopulmonary symptoms (a decline in respiratory function, fibrosis, pleural involvement, myocarditis, pericardial effusion, etc.), post-COVID-19 thrombosis, immune-mediated manifestations (arthritis, myositis, pancreatitis, etc.), and other skin, neurological, renal, hematological, endocrine, and systemic manifestations [5,7]. The presence of persistent COVID-19 symptoms has reportedly significantly affected the mental health and emotional well-being of patients [8].

To date, research into post-COVID-19 syndrome has been limited, with particularly scant data on long-term outcomes among recovered patients in emerging economies [8,9]. Under this backdrop, we designed a study to evaluate the post-COVID phenomenon or symptomatology in patients who have recovered from COVID-19 in Delhi, India. The aim of this research was to ascertain the prevalence, patterns, and duration of symptoms in these recovered individuals.

HIGHLIGHTS

In this study, out of 413 participants studied, 79.7% experienced post-coronavirus disease 2019 syndrome. In which, most frequently reported symptoms were fatigue (34.1%), bodyache (24.4%), dry cough (23.4%), and anxiety (20.9%). Loss of appetite persisted for the longest duration, with a median of 156.50 days, whereas reduced taste was the shortest-lived symptom (median 93 days). Forgetfulness lasted significantly longer amongst participants with comorbidities than those without comorbid conditions. Overall, biochemical parameters, peripheral smear results and electrocardiogram and chest X-ray findings indicated improvement at subsequent follow-up visits.

Materials and Methods

Study Design

A prospective observational study was conducted across 11 districts of Delhi among individuals who had recovered from COVID-19. Study participants were enrolled after recovery, then returned for follow-up at interval of 3 months and 6 months.

Study Area and Period

The study was conducted during October 2021 to July 2023, across 11 districts of Delhi, India.

https://doi.org/10.24171/j.phrp.2023.0251

Inclusion Criteria

The inclusion criteria encompassed adult individuals (aged 18 years and older) with a history of mild, moderate, or severe COVID-19 in the prior 6 months, diagnosed at least 14 days before study enrollment.

Exclusion Criteria

The study excluded pregnant women, individuals with a history of reinfection with COVID-19 and patients at the terminal stage of any disease.

Sample Size and Sampling Technique

The reported prevalence of long COVID is 22%. Given this prevalence rate, a 95% confidence level, a relative precision of 4.4%, and accounting for a 20% allowable error and a 20% loss to follow-up, the sample size was calculated to be 410 [10]. Based on the per-protocol principle, the analysis of the collected data was based on the second follow-up sample size of 369 participants.

Data Collection Procedure

The study participants were diagnosed with COVID-19 between April 2022 and September 2022. We obtained a daily list of patients with COVID-19 who had recovered—including those who had been hospitalized and those who had managed their illness through home isolation—from the Directorate General of Health Services, Government of NCT, Delhi. The data were cleaned by removing records of individuals with missing addresses or contact numbers, as well as those under 18 years old. Subsequently, participants meeting the inclusion criteria were contacted via phone calls, followed by household visits. They were enrolled in the study after providing written informed consent for prospective follow-up at baseline, 3 months, and 6 months after recovery. During participant interviews, a detailed medical history was recordrd, along with information about their past COVID-19 episode. A comprehensive history was recorded for all participants at each household visit. For the baseline assessment, we collected sociodemographic characteristics of the participants using an interview schedule. These data included age, sex, education, socioeconomic status, healthcare-seeking behavior during the COVID-19 episode, baseline comorbidity details, and treatment profile at their household. Additionally, a standardized and validated WHO case report form for post-COVID-19 conditions was used to gather participants' information [11]. A multidisciplinary team of doctors reviewed the medical histories and performed investigations and clinical assessments of the study participants. This was done to provide recommendations for further biochemical investigations, referrals, and treatment as needed. Blood samples were collected with participant consent and tested in a government-approved laboratory. The analyses were conducted using the Vitros 5600 integrated clinical chemistry and immunoassay analyzer and the Stago STA MAX coagulation analyzer (Tables S1 and S2).

Operational Definitions

For hospitalized patients, recovery was defined as discharge from the hospital following the completion of treatment for COVID-19. For non-hospitalized patients, recovery was indicated by a period of 14 days from symptom onset and since diagnostic confirmation using reverse transcription-polymerase chain reaction (RT-PCR) or antigen tests. Acute COVID-19 encompassed symptoms experienced up to 4 weeks after the diagnosis of SARS-CoV-2 infection [5]. Ongoing symptomatic COVID-19 referred to the presence of symptoms 4 to 12 weeks following confirmed SARS-CoV-2 infection [5]. Post-COVID-19 was defined as symptoms developed during or after SARS-CoV-2 infection that persisted for more than 12 weeks [5]. Long COVID referred to signs and symptoms that continued for more than 4 weeks, encompassing both the ongoing symptomatic COVID-19 and post-COVID-19 syndrome subgroups [5]. Mild disease was defined as upper respiratory tract symptoms without shortness of breath or hypoxia [5]. Moderate disease was characterized by upper respiratory tract symptoms accompanied by shortness of breath or hypoxia, necessitating the administration of supplemental oxygen at home. These findings included a respiratory rate (RR) greater than 24 breaths per minute, breathlessness, and an oxygen saturation (SpO_2) of 90% to less than 93% on room air [5]. Severe cases were those involving hospitalization with COVID-19 infection, characterized by an RR above 30 breaths per minute, breathlessness, and an SpO_2 below 90% on room air [5].

Statistical Analysis

The data were entered into Excel (Microsoft) and analyzed using IBM SPSS ver. 25.0 (IBM Corp.). Results were presented as frequencies and proportions for categorical variables, median (interquartile range [IQR]) for non-normally distributed continuous data, and mean ± standard deviation for normally distributed continuous data. The prevalence and incidence of post-COVID-19 symptoms and consequences were assessed at baseline and during each follow-up visit. A per-protocol analysis was employed due to the lower attrition rate in the follow-up period. Standard cut-off scores were used to evaluate the presence of depression, anxiety, poor sleep quality, and suboptimal quality of life, which constituted

dependent variables. The independent variables included age, sex, severity of COVID-19, duration of hospitalization, and history of tobacco smoking and substance abuse. To compare differences in means between 2 groups, we used the Student unpaired t-test or the Mann-Whitney U test. For assessments involving more than 2 groups at the 3 time points, we conducted a repeated measures analysis of variance with the Tukey *post hoc* test or the Friedman test, depending on the data distribution. A *p*-value of less than 0.05 was considered to indicate statistical significance.

Results

The study included 413 participants, all of whom presented with mild disease. The response rate was 91.8% (379 patients) at the first follow-up visit and 89.3% (369 patients) at the second follow-up. Following the per-protocol principle, the collected data were analyzed based on the 369 patients who completed the second follow-up. The mean age of the participants was 42.07 years, with a standard deviation of 14.89 years. We examined the sociodemographic characteristics of the participants, including sex, educational status, smoking history, and alcohol consumption. Approximately half (50.1%) of the participants were male, 97.84% were literate, 92.96% had never smoked, and 9.21% reported a history of alcohol consumption. Most participants (93.5%) were vaccinated, and 26.56% had at least 1 comorbidity (Table 1). The analysis revealed significant associations between post-COVID-19 syndrome and both sex and age ($p < 0.05$).

Nearly half of the participants (47.5%) experienced long COVID symptoms, while 79.7% had post-COVID-19 symptoms (Figure 1). The most common symptoms included joint pain

Table 1. Sociodemographic profile of recovered COVID-19 patients ($n = 369$)

Characteristic	Post-COVID-19		Total	*p*
	Yes	No		
Sex				0.03
Male	46 (24.9)	139 (75.1)	185 (50.1)	
Female	29 (15.8)	155 (84.2)	184 (49.9)	
Age (y)				0.028
18–30	74 (71.2)	30 (28.8)	104 (28.2)	
31–45	94 (81.7)	21 (18.3)	115 (31.2)	
46–60	89 (80.9)	21 (19.1)	110 (29.8)	
≥61	37 (92.5)	3 (7.5)	40 (10.8)	
Smokers				0.786
Current smoker	15 (83.3)	3 (16.7)	18 (4.9)	
Past smoker	7 (87.5)	1 (12.5)	8 (2.2)	
Never smoked	272 (79.3)	71 (20.7)	343 (93.0)	
Alcohol consumption				0.684
No	266 (79.4)	69 (20.6)	335 (90.8)	
Yes	28 (82.4)	6 (17.6)	34 (9.2)	
Educational status				0.4
Illiterate	8 (100.0)	0 (0)	8 (2.2)	
Primary and middle school	26 (89.7)	3 (10.3)	29 (7.9)	
High school	25 (80.6)	6 (19.4)	31 (8.4)	
Intermediate	46 (78.0)	13 (22.0)	59 (16.0)	
Graduate and above	189 (78.1)	53 (21.9)	242 (65.6)	
Vaccination status				0.848
Vaccinated	274 (79.4)	71 (20.6)	345 (93.5)	
Unvaccinated	12 (85.7)	2 (14.3)	14 (3.8)	
Status unknown	8 (0.8)	2 (0.2)	10 (2.7)	
Morbidity profile				0.393
Any comorbidity	81 (82.7)	17 (17.3)	98 (26.6)	
Obesity	34 (89.5)	4 (10.5)	38 (10.3)	0.113
Hypertension	38 (79.2)	10 (20.8)	48 (13.0)	0.877
Diabetes	34 (87.2)	5 (12.8)	39 (10.6)	0.354
Coronary heart disease	6 (66.7)	3 (3.3)	9 (2.4)	0.326
Chronic liver disease	9 (100.0)	0 (0)	9 (2.4)	0.27

Data are presented as *n* (%).

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(36.0%), persistent dry cough (35.7%), anxiety (28.4%), and shortness of breath (27.1%). Other symptoms reported were persistent fatigue (21.6%), persistent headache (20.0%), forgetfulness (19.7%), and limb weakness (18.6%) (Table 2). The longest symptom duration was reported for anxiety, at 132 days (IQR, 97–190 days), followed by fatigue at 127 days (IQR, 97–172 days), body ache at 124 days (IQR, 89–157 days), and joint pain/swelling at 115 days (IQR, 89–180 days). Prolonged symptom duration was also noted for shortness of breath, at 112.5 days (IQR, 88.25–181.50 days); headache, at 109.5 days (IQR, 87–139.50 days); and limb weakness, at 109 days (IQR, 83–148.75 days). Anxiety and persistent dry cough had significantly longer durations in men compared to women ($p < 0.05$). Relative to the unvaccinated subgroup, limb weakness and fever lasted significantly longer in vaccinated participants ($p < 0.05$). Additional symptoms included dry cough, lasting 105.5 days (IQR, 86–146 days), and forgetfulness, lasting 100 days (IQR, 87.5–145 days). Notably, forgetfulness persisted for significantly longer in participants with any comorbidity than in those without comorbid conditions (Table 3).

Biochemical investigations were conducted for study participants at baseline, the first follow-up, and the second follow-up. The Shapiro-Wilk normality test indicated that the data were skewed in the distribution of the biochemical parameters. The analysis revealed no significant differences in the median levels of thyroid-stimulating hormone (TSH), hemoglobin A1c (HbA1c), aspartate aminotransferase/serum glutamic oxaloacetic transaminase (AST/SGOT), alanine aminotransferase/ serum glutamic pyruvic transaminase (ALT/SGPT), alkaline phosphatase (ALP), creatinine, urea, or uric acid between individuals with post-COVID-19 syndrome and those without the syndrome ($p > 0.05$). The median values for all parameters fell within normal ranges (Table 4). New instances of elevated HbA1c were detected in 3.5% of patients at the first follow-up and in 6.9% at the second follow-up. New cases of elevated TSH were observed in 9.2% of patients at the first follow-up and in 6.6% at the second visit. In liver function tests, AST/SGOT levels were raised in 4.2% of participants at the first follow-up and in 3.6% at the second follow-up. ALT/SGPT levels were elevated in 8.4% of participants at the first follow-up and in 4.8% at the second visit. Additionally, ALP levels were found to be elevated in 12.6% of participants at the first and in 7.0% at the second follow-up. For the kidney function test panel, patients were assessed regarding creatinine, urea, and uric acid levels. Creatinine levels were raised in 0.8% of patients at the first follow-up and in 1.1% at the second visit. Elevated urea levels were found in only 2.5% of patients at the first and 1.6% at the second follow-up. Similarly, high uric acid levels impacted just 1.9% of patients at the first follow-up and 1.4% at the second follow-up appointment.

A peripheral blood smear was conducted for patients at baseline, the first follow-up, and the second follow-up. Transformed lymphocytes and activated monocytes were observed in 1.1% of the participants at the first follow-up and 0.8% at the second appointment. An abnormal absolute neutrophil count was detected in the peripheral smears of 16.5% of patients at the first follow-up and 11.7% at the second. In turn, an abnormal absolute eosinophil count was noted in 7.9% of participants at the first follow-up and in 4.6% at the second. Electrocardiogram (ECG) results were

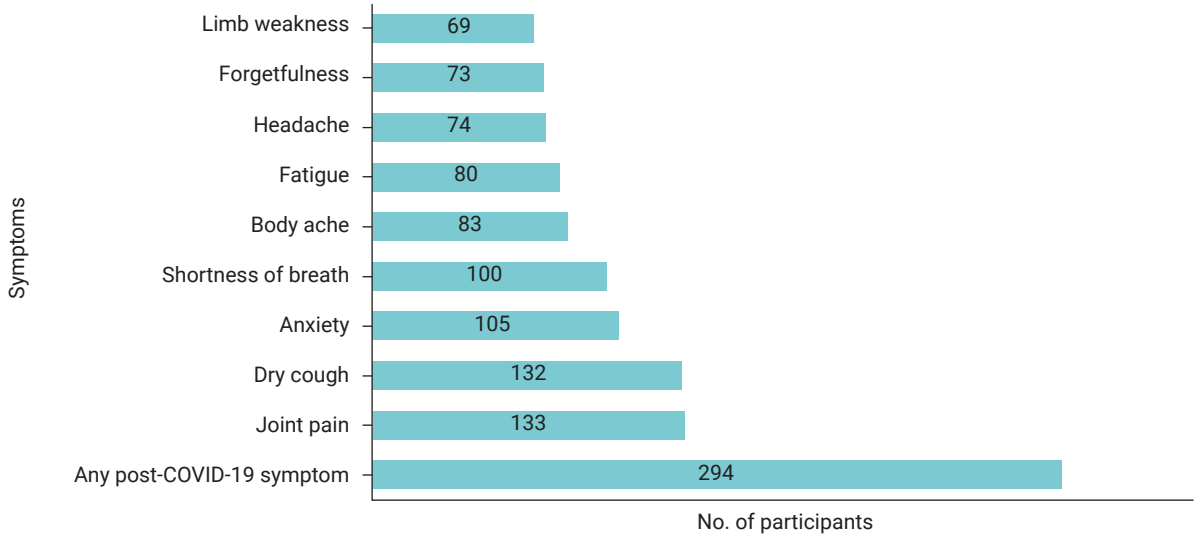

Figure 1. Pattern of post-COVID-19 symptoms ($n = 369$).

Table 2. Distribution of study participants on the basis of prevalence of post-COVID-19 symptoms (*n* = 369)

Symptoms	Baseline (*n* = 413)	Follow-up 1		Follow-up 2		PCoVS
		Prevalence	Incidence	Prevalence	Incidence	Prevalence
Any symptom	196 (47.5)	228 (61.8)	107 (29.0)	229 (62.1)	39 (10.6)	294 (79.7)
Anxiousness	41(9.9)	84 (22.8)	70 (19.0)	43 (11.7)	14 (3.8)	105 (28.4)
Join pain/swelling	40 (9.7)	75 (20.3)	67 (18.2)	89 (24.1)	47 (12.7)	133 (36.0)
Persistent fatigue	67 (16.2)	66 (17.9)	44 (11.9)	26 (7.0)	11 (3.0)	80 (21.6)
Shortness of breath	28 (6.8)	52 (14.1)	38 (10.3)	74 (20.1)	45 (12.2)	100 (27.1)
Persistent body pain	46 (11.1)	46 (12.5)	40 (10.8)	48 (13.0)	31 (8.4)	83 (22.4)
Weakness in limbs	40 (9.7)	48 (13.0)	40 (10.8)	34 (9.2)	17 (4.6)	69 (18.6)
Forgetfulness	17 (4.1)	38 (10.3)	34 (9.2)	44 (11.9)	30 (8.1)	73 (19.7)
Persistent dry cough	46 (11.1)	38 (10.3)	30 (8.1)	112 (30.4)	84 (22.8)	132 (35.7)
Persistent headache	27 (6.5)	37 (10.0)	32 (8.7)	45 (12.2)	33 (8.9)	74 (20.0)

Data are presented as *n* (%).
PCoVS, post-COVID-19 symptoms.

Table 3. Duration of post-COVID-19 symptoms in study participants

Symptoms	Mean ± SD (no. of days)	Median (IQR, no. of days)
Anxiousness (*n* = 105)	138.75 ± 54.14	132 (97–190)
Persistent Fatigue (*n* = 80)	137.57 ± 48.33	127 (97–172)
Shortness of breath (*n* = 100)	131.89 ± 60.21	112.5 (88.25–181.50)
Joint pain/swelling (*n* = 133)	131.59 ± 58.76	115 (89–180)
Persistent body pain (*n* = 83)	127.48 ± 47.44	124 (89–157)
Persistent dry cough (*n* = 132)	121.01 ± 56.48	105.5 (86–146)
Weakness in limbs (*n* = 69)	120.91 ± 49.34	109 (83–148.75)
Forgetfulness (*n* = 73)	116.47 ± 52.86	100 (87.5–145)
Persistent headache (*n* = 74)	114.74 ± 42.68	109.5 (87–139.50)

SD, standard deviation; IQR, interquartile range.

abnormal in 2.2% of the participants at the first follow-up, with diagnoses including blocks (37.5%), bradycardia (25.0%), low voltage complexes (12.5%), Q waves (12.5%), and arrhythmias (12.5%). No ECG abnormalities were reported at the second follow-up visit. At the first follow-up, 4.06% of participants presented with abnormal chest X-ray findings, which were identified in the parenchymal (66.7%), pleural (26.7%), and mediastinal (6.7%) regions. At the second follow-up, 2.16% had abnormal findings, with involvement of the parenchymal (62.5%), pleural (12.5%), and mediastinal (25.0%) regions (Figure 2).

Discussion

Our study clarifies the potential long-term symptoms experienced by patients diagnosed with COVID-19. To our knowledge, this is one of the first community-based prospective cohort studies to evaluate post-COVID-19 syndrome in India. Within our participant cohort, the prevalence of post-COVID-19 syndrome was alarmingly high, with nearly two-thirds reporting 1 or more post-COVID symptoms. This finding aligns with research by Huang et al. [12] in China in 2020, where approximately two-thirds (68%) of participants reported at least 1 post-COVID-19 symptom. In contrast, a prospective cohort study by Selvakumar et al. [13] in 2023 in Norway found that nearly half (48.5%) of participants experienced post-COVID-19 syndrome, a lower rate than our findings. This discrepancy may be attributed to differences in the age profiles of the participants, which ranged from 12 to 25 years in the Norwegian study, as well as the prevalence of the B.1.1.7 (Alpha) variant of SARS-CoV-2 in that region in 2020. In contrast, our study coincided with the predominance of the Omicron variant of SARS-CoV-2. Another investigation by Naik et al. [10] in 2021 examined the clinical features and risk factors associated with post-COVID-19 signs and symptoms in a Northern Indian population. There, only 9.9% of participants reported symptoms 12 weeks following COVID-19 infection. The difference in findings might stem from the larger sample size of the latter study and the dominance of the SARS-CoV-2 Delta variant during their research period. Additional studies conducted around the world with similar objectives have reported a higher prevalence of post-COVID-19 syndrome (Table 1) [14,15].

In the present study, 25.2% of participants were diagnosed with 1 or more comorbidities at baseline, a figure that was 26.56% at the second follow-up visit. This finding is consistent with a study by Menges et al. [16] in 2021, which found that 34% of participants reported at least 1 chronic comorbidity at baseline. Our study indicated that 12.1% of participants had hypertension and 9.7% had diabetes mellitus. By comparison, a study by Cioboata et al. [17] in 2022 reported 36% of participants as exhibiting hypertension and 14.52% as having diabetes. The discrepancy may be attributable to the Alpha

https://doi.org/10.24171/j.phrp.2023.0251

Table 4. Association between biochemical parameters and post-COVID-19 syndrome

Biochemical parameter	First follow-up			Second follow-up		
	Post-COVID-19 symptoms		*p**	Post-COVID-19 symptoms		*p**
	Present	Absent		Present	Absent	
HbAlC (>6.5%)	5.4 (0.9)	5.3 (0.6)	0.334	5.35 (1.2)	5.2 (0.4)	0.241
TSH (>4.68 mIU/L)	2.98 (2.16)	2.78 (2.01)	0.385	3.07 (1.83)	2.99 (2.11)	0.284
AST (>59 IU/L)	33 (12)	33 (14)	0.943	33 (14)	34 (15)	0.993
ALP (>126 IU/L)	95 (34)	100 (40)	0.235	98 (36)	101.5 (34)	0.298
ALT (>50 IU/L)	30 (22)	29 (20)	0.420	32 (21)	30 (21)	0.364
Creatinine (>1.3 mg/dL)	0.7 (0.3)	0.8 (0.2)	0.103	0.80 (0.2)	0.80 (0.2)	0.233
Urea (>43 mg/dL)	23 (10)	25 (9)	0.428	26 (7)	25 (5)	0.177
Uric acid (8.5 mg/dL)	5.3 (2.15)	5.35 (2.2)	0.710	5.45 (2.0)	5.1 (2)	0.518

HbAlC, hemoglobin A1c; TSH, thyroid-stimulating hormone; AST, aspartate aminotransferase; ALP, alkaline phosphatase; ALT, alanine aminotransferase.
*$p<0.05$ was considered statistically significant.

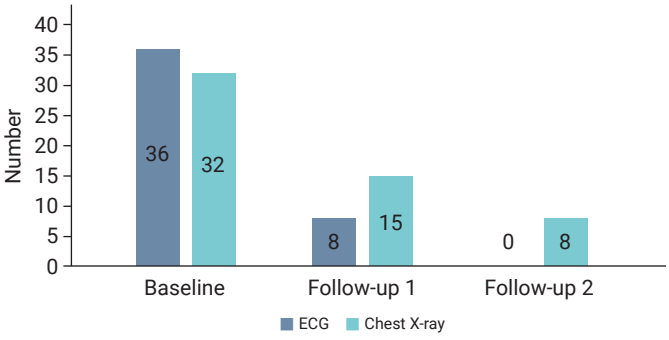

Figure 2. No. of participants with electrocardiogram (ECG) and chest X-ray abnormalities ($n=369$).

strain of COVID-19, which was present during the period of the latter study. We found no statistically significant relationship between the incidence of post-COVID-19 syndrome and the presence of any comorbidity. A 2023 study by Falsetti et al. [18] analyzed 3 years of data to assess whether the burden of comorbidities correlates with COVID-19 outcomes. Their nationwide analysis suggested that the development of post-COVID-19 symptoms was associated with a higher burden of comorbidities ($p<0.0001$). However, differences in sample size and study period, as well as the involvement of a different COVID-19 strain, could explain the variations in these findings. In our study, 93.5% of participants had received 2 doses of a COVID-19 vaccine at the time of baseline data collection. This aligns with national data from India, which indicated a vaccination coverage of 90% in 2022–2023 [19]. We observed no significant difference in post-COVID-19 symptoms between the vaccinated and unvaccinated cohorts (Table 1).

In the present study, we found no significant differences in liver function or kidney function parameters between the groups of patients with and without post-COVID-19 syndrome. The reported values were comparable between the groups. This is consistent with the findings of Alfadda et al. [20] in 2022, who investigated clinical and biochemical parameters in individuals 6 months post-recovery from COVID-19. Furthermore, we observed a decrease in lymphocyte count from baseline to the second follow-up in our study. Gameil et al. [21] in 2021 reported similar findings when assessing long-term biochemical residue after COVID-19 recovery. Additionally, we noted a decline in monocyte counts in participants at follow-up, which aligns with the findings of Ruenjaiman et al. [22] in their study on the response of innate immune cells after recovery from SARS-CoV-2 infection. At the 3-month follow-up, 4.06% of our participants presented with abnormal chest X-ray findings. This is notably lower than the 50.4% reported by Fogante et al. [23], who observed chest X-ray abnormalities in participants at the same follow-up interval. The discrepancy between these findings may be attributed to differences in methodology; specifically, Fogante et al. [23] focused on hospitalized patients, which could account for the higher rate of abnormalities. In our cohort, only 2.2% of participants exhibited ECG changes at the first follow-up, with no ECG changes observed at the second follow-up. Ovrebotten et al. [24] conducted a study assessing changes in cardiac structure and function 3 to 12 months after hospitalization for COVID-19 and found that, regardless of the severity of the initial illness and persistent dyspnea, cardiac structure and function remained unchanged.

A major strength of our study is the confirmation that all participants tested positive for SARS-CoV-2 via RT-PCR testing, thus minimizing the potential for misclassification. The data collection form employed in our study adhered to the standard format established by the WHO and underwent rigorous pretesting. The field staff responsible for conducting the interviews were well-trained and possessed a strong

understanding of COVID-19. Although the sampling technique was robust in minimizing bias, its generalizability to larger populations may be limited by the small sample size.

On that note, our study has several limitations. First, the absence of baseline clinical profiles for the participants prior to COVID-19 infection represents a major drawback. Second, since participants self-reported their symptoms experienced after COVID-19, a potential exists for recall bias. Third, we were unable to determine whether the post-COVID-19 symptoms were attributable to COVID-19 vaccination, reinfection, or post-COVID-19 syndrome. Furthermore, while it would have been possible to include a variety of factors—such as data from the acute phase—in data collection from the patients' medical histories, we limited the scope to the after-effects of COVID-19 infection to keep the study manageable. Finally, although we presume that the Omicron variant was predominant during the study period, we lack definitive evidence to confirm this.

Conclusion

In our study, the most common post-COVID-19 symptoms were joint pain, dry cough, anxiety, and shortness of breath. The most frequently reported comorbidities were obesity, hypertension, and diabetes. The longest median duration of symptoms was observed for loss of appetite, followed by anxiety and shortness of breath. Both sex and age were significantly associated with the presence of post-COVID-19 syndrome. Participants with any comorbidity in conjunction with post-COVID-19 syndrome exhibited a significantly longer duration of forgetfulness than participants without comorbid conditions. Additionally, we noted a general trend of significant improvement in ECG and chest X-ray findings at subsequent follow-ups.

The study findings suggest that clinical symptoms persist in participants for up to 6 months, with multi-system involvement observed during the post-COVID-19 period. Consequently, this necessitates long-term, regular follow-up appointments. As the post-COVID-19 sequelae are not well-defined, it is crucial to continue researching the long-term effects of COVID-19 to better understand how to treat and prevent post-COVID-19 syndrome. In conclusion, our study supports the use of a comprehensive healthcare approach to holistic patient management.

Supplementary Material

Table S1. Distribution of study participants according to biochemical evaluations; **Table S2.** Hematological and biochemical reference values. Supplementary data are available at https://doi.org/10.24171/j.phrp.2023.0251.

Notes

Ethics Approval

The Institutional Ethics Committee at MAMCand Associated Hospitals in New Delhi granted approval for the study, as documented in letter No. F.1/IEC/MAMC/87/05/2021/No526. All potential participants were informed about the study's purpose, potential risks, and benefits. They were assured of confidentiality and informed that they had the option to withdraw from the study at any time if they wished. Written informed consent was obtained from all participants. For medical/telehealth consultations and follow-up appointments, participants were referred to LNH.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

The research received support from the Indian Council of Medical Research, Department of Health Research, Ministry of Health and Family Welfare, Government of India. This support was provided in the form of funding through grant No. CTU/ Cohort study/17/1025/2021/ECD. We express our gratitude for this assistance, which was instrumental in advancing our study and enhancing our comprehension of these key issues.

Availability of Data

The data supporting the findings of this study are available upon request from the corresponding author. They are not publicly accessible due to privacy or ethical restrictions.

Authors' Contributions

Conceptualization: NB, MMS, SM, TA, SuS, SG; Data curation: HS, GS, US; Formal analysis: HS, SM, GS; Funding acquisition: NB, MMS; Investigation: HS, GS, SR, AB, TA, BG, SaS, MK, SuS, BK, NK; Methodology: NB, MMS, SM, TA, SuS, SG; Project administration: NB, MMS, HS, GS, SR, AB; Resources: NB; Software: HS, SM, GS; Supervision: NB, MMS, HS, SM, GS, SR, AB; Validation: NB, MMS; Writing–original draft: NB, MMS, HS, SM, GS; all authors. All authors read and approved the final manuscript.

Additional Information

This study was preprinted in medRxiv on August 25, 2023 (https://doi.org/10.1101/2023.08.25.23294654).

Acknowledgements

The authors would like to thank Dean, MAMC for providing support in the course of the conduction of the study. We also express thanks to the team of Doctors For You, Radiology Department LNH and NDTB, Delhi for providing necessary diagnostic support.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0394
Osong Public Health Res Perspect 2024;15(3):238-247

Original Article

Effects of aquatic versus land-based exercise on irisin and fibroblast growth factor 21 expression and triiodothyronine and free fatty acid levels in elderly women

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ABSTRACT

Objectives: This study investigated the impacts of exercise on irisin and fibroblast growth factor 21 (FGF-21) expression, as well as triiodothyronine (T_3) and free fatty acid (FFA) levels in elderly women.

Methods: Thirty women aged 65 to 70 years (10 per group) were randomly assigned to aquatic exercise, land exercise, and control groups. The aquatic and land groups engaged in 3 exercise sessions per week (60 min/session) for 16 weeks. The intensity was progressively increased every 4 weeks.

Results: Irisin and FGF-21 levels significantly increased in the aquatic exercise group. In the post-test, the aquatic exercise group had the highest irisin levels. Significant findings were observed for irisin and FGF-21 for the main effect between aquatic and band exercise groups ($p < 0.05$ for both), the main effect between measurement times ($p < 0.01$ and $p < 0.001$, respectively), and the interaction effect ($p < 0.05$ and $p < 0.001$, respectively). The irisin level was significantly higher in the aquatic than in the land group 30 minutes after the last session ($p < 0.05$). In both exercise groups, T_3 levels were significantly higher 30 minutes after the final session ($p < 0.05$) than before the program. The FFA level was significantly higher in the aquatic exercise group than the others. In the aquatic group, FFA levels were significantly higher 30 minutes after both the first ($p < 0.01$) and the last ($p < 0.001$) session compared to pre-program values.

Conclusion: Differences in exercise type and environment can promote fat metabolism by stimulating hormonal changes that induce brown fat activity and browning.

Keywords: Aquatic exercise; Fibroblast growth factor 21; Free fatty acid; Irisin; Triiodothyronine

Received: January 3, 2024
Revised: February 29, 2024
Accepted: March 6, 2024

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Introduction

Reductions in muscle mass and strength associated with aging lead to decreased energy

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expenditure and fat metabolism [1]. These changes promote a gradual accumulation of body fat [2] and represent a major contributor to the onset of obesity and metabolic diseases, including type 2 diabetes. As muscle function declines and type 2 diabetes develops, conditions such as insulin resistance, hypertension, and dyslipidemia are likely to arise [3].

Human fat can be functionally categorized into 2 types: white and brown fat [4]. White adipose tissue (WAT) can store a substantial amount of energy as triglycerides, which are then released as free fatty acids (FFAs) to provide energy during periods of high demand [5]. While this fat tissue helps insulate the body and protects organs from impact injuries [6], its excessive accumulation can lead to obesity. In contrast, brown adipose tissue (BAT) plays a crucial role in regulating body temperature and expending energy through thermogenesis in response to heat, cold, and exercise [7]. This is largely due to the abundance of mitochondria expressing uncoupling protein 1 (UCP1), which has thermogenic functions. Consequently, heat is produced via the uncoupling pathway without generating adenosine triphosphate (ATP) [8]. This process enhances the rate of energy consumption by facilitating fatty acid oxidation and increasing exothermic reactions [9]. The heat produced is then circulated throughout the body [10], which is valuable for maintaining core body temperature and for the prevention and reduction of obesity [11].

Unlike conventional BAT, WAT can be converted into brown fat through various stimuli. This transformation process is known as browning, and the resulting cells are termed "brite" or "beige" adipocytes [12]. These cells facilitate the clearance of glucose and FFAs from the body through their expression of the *UCP1* gene, which modulates thermogenesis in a similar process to that of conventional brown fat [9].

The hormones irisin and fibroblast growth factor 21 (FGF-21) promote BAT formation and induce the browning of WAT [13]. Irisin, a myokine, is secreted by muscles in response to shivering from cold exposure [14] or exercise [15]. It facilitates browning [16] and can serve as an indicator of muscle mass [17]. Irisin activates UCP1 in the inner mitochondrial membranes to generate thermal energy [16]. These processes enhance the body's energy expenditure through fat metabolism, leading to weight loss. Additionally, as people age, these effects are observed at lower levels in women compared to men, and in obese individuals compared to those of normal weight [16,18]. FGF-21 is secreted by the liver and adipocytes in response to physical activity or non-shivering thermogenesis [19]. This hormone contributes to body fat reduction by increasing glucose uptake [20], stimulating lipolysis [21], and triggering physiological processes such as browning, thermogenesis, and energy consumption [22]. These effects collectively increase insulin sensitivity, combat obesity, and improve metabolic health [23].

HIGHLIGHTS

- Compared to land-based resistance band exercises, aquatic exercise more effectively increases the expression or secretion of irisin, fibroblast growth factor 21, triiodothyronine, and free fatty acids. In turn, this activation enhances brown fat activity, which stimulates fat metabolism and reduces body fat mass.
- Variations in exercise type and environment can trigger hormonal changes that induce brown fat activation and fat browning. Ultimately, this process promotes fat metabolism, contributing to a cumulative effect that helps prevent and lessen obesity through fat reduction.

Triiodothyronine (T_3) is a potential transcription factor for the *UCP1* gene [14] that, in conjunction with FGF-21, may influence metabolic activity. For instance, increased T_3 secretion following exercise or sustained cold exposure can raise the resting metabolic rate [24]. The expression of irisin, FGF-21, and T_3 activates UCP1 in BAT and beige fat; this promotes lipolysis, leading to an increase in FFAs. These FFAs are then utilized as an energy source during thermogenesis, making them a primary substrate for thermogenesis in brown and beige fat [4].

Most studies investigating irisin and FGF-21 have been focused on aerobic and resistance exercises, respectively. However, limited research has compared and analyzed these 2 types of exercise concurrently. To our knowledge, no studies have yet examined differences in irisin, FGF-21, and T_3 expression between aquatic and land-based exercises while considering temperature effects. Consequently, our study was designed to investigate the differences between aquatic and land-based exercise environments. Specifically, we utilized aquatic exercises with aerobic characteristics and land exercises with resistance characteristics over a period of 16 weeks. The study objective was to compare the effects of these exercise modalities on the expression of irisin and FGF-21, as well as on the levels of T_3 and FFA, in elderly women. By identifying effective exercise strategies for obesity prevention, we hope that the findings may contribute to the extension of life expectancy.

Materials and Methods

Participants

This study was conducted in women aged 65 to 70 years from Changwon city, Republic of Korea, who did not engage in regular exercise (that is, they had performed less than 1 hour of regular exercise per week over the past year). Participants were randomly selected, with G*Power 3.1 (Christian-Albrechts-Universität) utilized to calculate the appropriate sample size. The analysis indicated that 36 participants were necessary under the following conditions: an effect size of 0.40, an alpha level of 0.05, and a power of 0.80. To account for potential dropouts, 39 individuals were initially recruited. Simple random sampling was used to assign 13 participants to each of the 3 groups: control, aquatic exercise, and land exercise. Over the course of the experiment, some participants withdrew for personal reasons or were excluded due to poor attendance. Consequently, measurements and analyses were conducted among a total of 30 participants, with 10 in each of the 3 groups.

The study procedures are detailed in Figure 1.

Physique and Body Composition

Participant height, weight, body mass index, skeletal muscle mass, and body fat percentage were measured with the participants dressed in light clothing using the X-SCAN PLUS II (Jawon Medical) bioelectrical impedance analysis device. These measurements were taken in accordance with the procedures recommended by the American College of Sports Medicine [25].

Blood Tests

For control participants, blood samples were collected and analyzed under the same methods and resting conditions at 2 time points: before and after a 16-week interval. For the aquatic and land exercise groups, samples were taken at 3 points: first, at rest prior to the 16-week program; second, 30 minutes after the first exercise session; and third, 30 minutes after the final exercise session.

Blood collection was conducted between 8 AM and 9 AM on the day of the procedure, with participants instructed to fast starting at 8 PM the previous evening. After 30 minutes of rest following the completion of the respective exercise programs, a clinical pathologist drew 10 mL of blood from the antebrachial vein of individuals in the exercise groups using a vacutainer and a disposable syringe. The blood was then placed into a serum separator tube and centrifuged at 3,000 rpm for 10 minutes using a Combi-514R centrifuge (Hanil

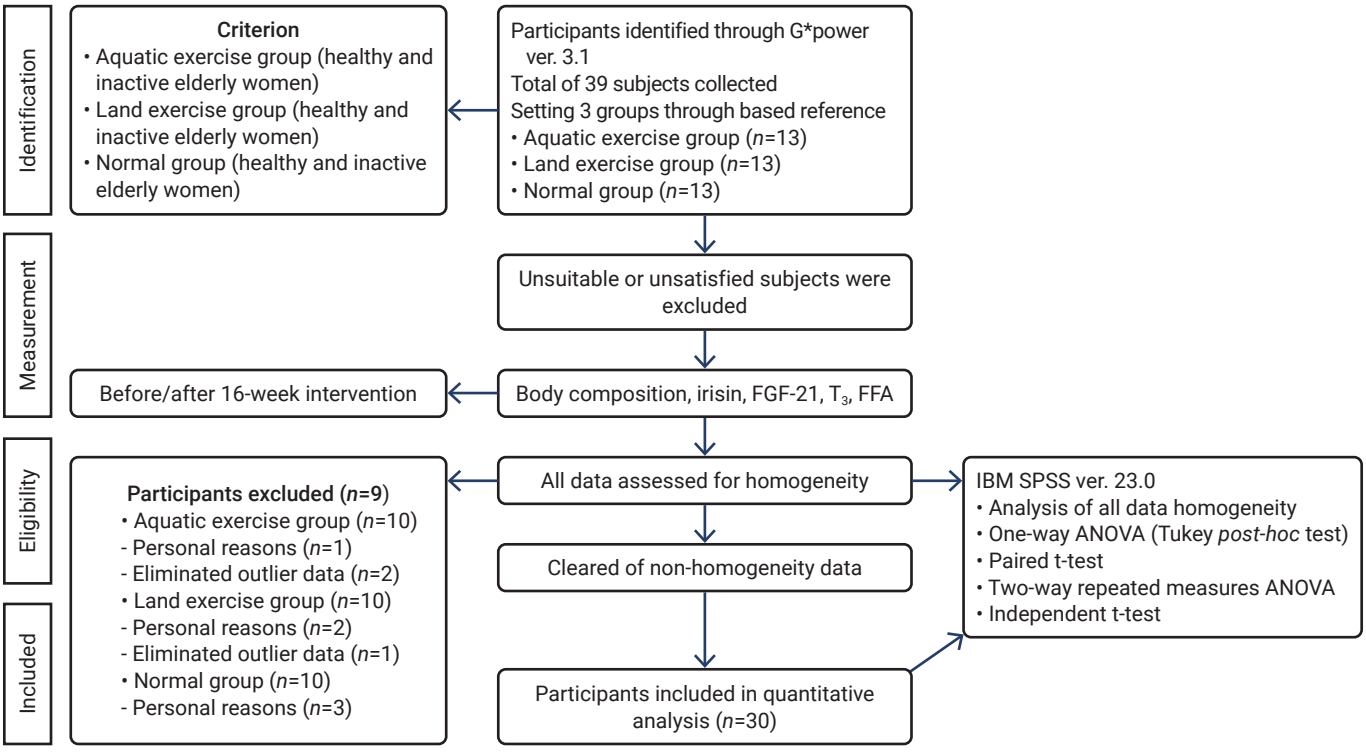

Figure 1. Study procedures.
FGF-21, fibroblast growth factor 21; T_3, triiodothyronine; FFA, free fatty acids; ANOVA, analysis of variance.

https://doi.org/10.24171/j.phrp.2023.0394

BioMed Inc.). Following centrifugation, the supernatant was decanted from the serum and transferred into a 1.5-mL microtube. These tubes were stored at −80 °C until analysis. The GC Cell Medical Foundation conducted subsequent analyses using the appropriate methods for each test.

Irisin levels were measured through an enzyme-linked immunosorbent assay (ELISA) using an irisin recombinant ELISA kit (Phoenix Pharmaceuticals). Readings were taken on a Multiskan GO spectrophotometer (Thermo Scientific) with optical density measured at 450 nm. FGF-21 levels were determined using ELISA with a human FGF-21 immunoassay kit (R&D Systems) and a microplate reader (VersaMax; Molecular Devices LLC). T_3 was quantified via an electrochemiluminescence immunoassay using Elecsys T_3 (Roche) and a Cobas 8000 analyzer (e801; Roche). FFA levels were assessed via calorimetry with a NEFA HR assay kit (FUJIFILM Wako Pure Chemicals) and a Cobas 8000 system (c702; Roche).

Aquatic Exercise Program

This study adapted the exercise program developed by the Korea Aquatic Exercise Association. The program was conducted in a swimming pool with an indoor air temperature of 30°C to 32°C, relative humidity between 65% and 70%, water temperature of 27°C ± 1°C, and water depth of 1.2 meters. Participants engaged in the exercise program 3 times per week for 16 weeks. Each session lasted 60 minutes, comprising a 10-minute warm-up, a 40-minute main exercise routine, and a 10-minute cool-down. The intensity of the exercises was progressively increased every 4 weeks, a process conducted by measuring the participant's heart rate and setting the rating of perceived exertion (RPE) using a heart rate monitor (Polar RS400sd; Polar Electro). The program's intensity levels were set at 40% to 50% heart rate reserve (HRR) with an RPE of 11 to 12 for the first 4 weeks, 50% to 60% HRR with an RPE of 13 to 14 for weeks 5 to 8, 60% to 65% HRR with an RPE of 14 to 15 for weeks 9 to 12, and 65% to 70% HRR with an RPE of 15 to 16 for the final 4 weeks (13–16) [25].

Land Exercise Program

This study employed a modified version of the resistance exercise program originally presented by the American College of Sports Medicine [25]. The program was conducted in an indoor gym maintained at a temperature of 25°C ± 1°C and a relative humidity of 50%–55%. Participants engaged in the exercise program 3 times per week for 16 weeks. Each session, performed with a TheraBand (Hygenic Corporation), lasted 60 minutes and included a 10-minute warm-up, a 40-minute main exercise routine consisting of 3 sets of 15 repetitions with rest intervals between sets, and a 10-minute cool-down period. The intensity of the exercises was progressively increased, starting with a yellow (thin) band for the first 4 weeks and transitioning to a red (medium) band for weeks 5 through 16 [26]. The length of the band was adjusted every 4 weeks to match the participants' increasing adaptability and strength. Additionally, the RPE was used to estimate exercise intensity at the beginning of the program and was adjusted every 4 weeks. The target intensity levels were set at 40% to 50% of HRR (RPE, 11–12) for the first 4 weeks, 50% to 60% HRR (RPE, 13–14) for weeks 5 to 8, 60% to 65% HRR (RPE, 14–15) for weeks 9 to 12, and 65% to 70% HRR (RPE, 15–16) for weeks 13 to 16. The control group was instructed to continue with their usual activities without starting any new exercise programs.

Data Analysis

For this study, data processing was conducted using IBM SPSS ver. 23.0 (IBM Corp.). Descriptive statistics for each group were analyzed according to measurement items, and group homogeneity for each variable was assessed using the Levene test. To determine the average differences between groups before and after the 16-week exercise regimen, a paired t-test and 1-way analysis of variance (ANOVA) were utilized. Subsequent post hoc analysis was conducted using the Tukey test. The interaction between measurement times for the aquatic and land exercise groups was examined using 2-way repeated measures ANOVA. The average differences between these groups were assessed using an independent t-test, with the significance (alpha) level set at 0.05.

Ethics Approval

Before the experiment began, the study (derived from PNU IRB/2017_68_HR) received approval from the Institutional Human Research Committee. Participants were fully informed of the purpose of the research, and only those who willingly agreed to participate submitted their consent.

Results

Participant Characteristics and Body Composition

The demographic characteristics of the study participants are detailed in full in Table 1. For all women, we measured body composition and the levels of irisin, FGF-21, T_3, and FFA before the initiation of the 16-week aquatic or land-based exercise program and again at its conclusion.

Irisin Level

Table 2 presents the variations in irisin levels within and between the groups. Within the aquatic exercise group, a

Table 1. Changes in body composition after 16 weeks of aquatic and land exercise

	Control group (n = 10)		Aquatic exercise group (n = 10)		Land exercise group (n = 10)	
	Before	After	Before	After	Before	After
Age (y)	68.59 ± 1.83		68.34 ± 1.64		67.71 ± 1.35	
Height (cm)	151.40 ± 4.50		151.87 ± 3.41		149.72 ± 7.78	
Weight (kg)	56.36 ± 5.11	56.41 ± 4.88	55.78 ± 5.07	55.53 ± 4.87	58.47 ± 5.75	58.43 ± 5.76
LBM (kg)	33.88 ± 2.49	33.86 ± 2.38	34.51 ± 2.60	34.85 ± 2.61	33.72 ± 3.06	33.77 ± 2.44
BFM (kg)	20.35 ± 3.21	20.47 ± 2.64	19.15 ± 3.36	18.51 ± 3.09*	22.64 ± 5.40	22.63 ± 5.48
%BF (%)	35.94 ± 3.20	36.19 ± 2.12	34.17 ± 3.54	33.19 ± 3.40**	38.37 ± 6.29	38.31 ± 6.29

Values are presented as mean ± standard deviation.
LBM, lean body mass; BFM, body fat mass; %BF, percentage of body fat.
*p < 0.05, **p < 0.01.

Table 2. Changes in irisin and FGF-21 levels throughout 16 weeks of aquatic and land exercise

Variable	Group (n = 10)	First	Second	Third	Change	Paired t-test	F	
Irisin (ng/mL)	CG	166.67 ± 15.44		164.37 ± 14.85	−2.30 ± 13.27	−0.548	Group (G)	4.480*
	AEG	169.64 ± 12.93	185.67 ± 17.19	198.43 ± 21.31	28.80 ± 29.29	3.108*	Time (T)	5.909**
	LEG	172.70 ± 12.63	176.86 ± 12.32	175.87 ± 15.89	3.17 ± 19.44	0.516	G×T	3.717*
	F	0.483		9.715**	5.846**			
	Post-hoc	NS		CG, LEG < AEG	CG, LEG < AEG			
FGF-21 (pg/mL)	CG	95.29 ± 18.19		93.93 ± 21.62	−1.36 ± 22.54	−0.191	Group (G)	4.651*
	AEG	91.14 ± 9.57	100.35 ± 9.57	111.83 ± 12.56	20.69 ± 14.83	4.413*	Time (T)	14.885***
	LEG	92.46 ± 9.15	94.07 ± 7.53	94.06 ± 7.34	1.60 ± 5.25	0.964	G×T	11.066***
	F	0.267		4.686*	5.690**			
	Post-hoc	NS		CG, LEG < AEG	CG, LEG < AEG			

Values are presented as mean ± standard deviation.
FGF-21, fibroblast growth factor 21; first, before the 16-week exercise program; second, 30 minutes after the first exercise session; third, 30 minutes after the last exercise session; CG, control group; AEG, aquatic exercise group; LEG, land exercise group; NS, non-significant.
*p < 0.05, **p < 0.01, ***p < 0.001.

significant increase was observed (p < 0.05). When comparing the 3 groups, significant differences were observed in the final exercise period (p < 0.01) and in the magnitude of change (p < 0.01). Post hoc analysis revealed that both the irisin level after the last exercise session and the magnitude of change were higher in the aquatic exercise group compared to the control and land exercise groups.

The main effects were significant both between groups (p < 0.05) and across measurement times (p < 0.01), as was the interaction effect (p < 0.05). Thirty minutes following the last exercise session, the irisin level was significantly higher in the aquatic exercise group than in the land exercise group.

FGF-21 Level

Table 2 presents the intra- and inter-group changes in FGF-21 levels. Within the aquatic exercise group, a significant increase in FGF-21 was observed (p < 0.05). When comparing the 3 groups, significant differences emerged in the final exercise session (p < 0.05) and the magnitude of change (p < 0.01). Post hoc analysis revealed that the aquatic exercise group displayed higher values for both the final exercise session and the magnitude of change compared to the control and land exercise groups.

The main effects were significant both between groups (p < 0.05) and across measurement time point (p < 0.001), as was the interaction effect (p < 0.001). Thirty minutes following the last exercise session, the FGF-21 level was significantly higher in the aquatic exercise group than in the land group.

T_3 Level

The changes in T_3 levels over time within and between the aquatic and land exercise groups, as well as the interaction effect, are depicted in Figure 2. Significant differences were observed between measurement points, and a significant interaction effect was found (p < 0.05). However, no significant difference was observed between the groups. In both aquatic and land exercise groups, T_3 levels were significantly elevated 30 minutes after the final exercise session (p < 0.05) compared to measurements taken before the exercise program.

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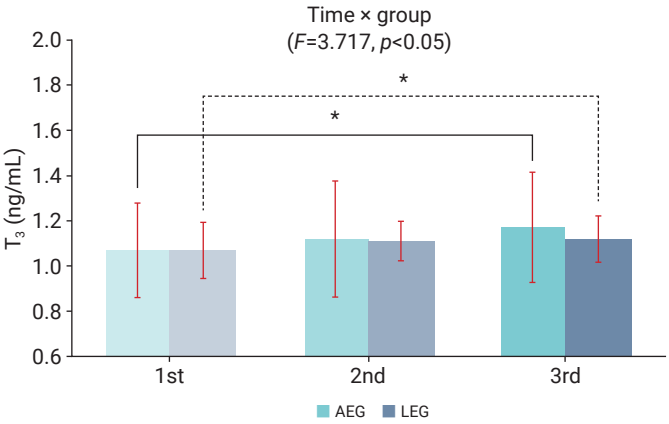

Figure 2. Two-way repeated measures analysis of variance of triiodothyronine (T_3) levels throughout the 16-week aquatic and land exercise programs. 1st, before the 16-week exercise program; 2nd, 30 minutes after the first exercise session; 3rd, 30 minutes after the last exercise session. *p < 0.05 compared with the first measurements.
AEG, aquatic exercise group; LEG, land exercise group.

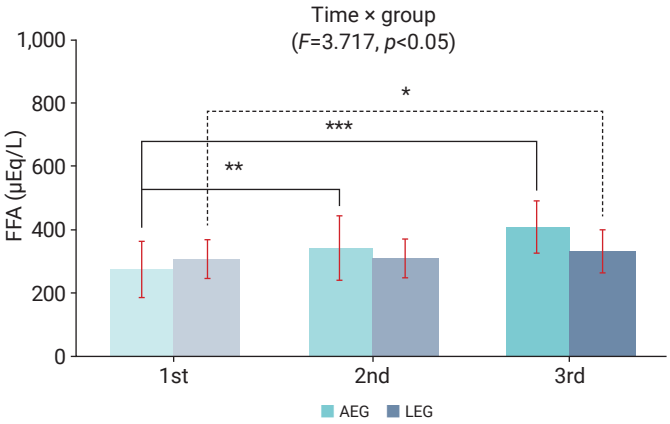

Figure 3. Two-way repeated measures analysis of variance of free fatty acid (FFA) levels throughout the 16-week aquatic and land exercise programs. 1st, before the 16-week exercise program; 2nd, 30 minutes after the first exercise session; 3rd, 30 minutes after the last exercise session. *p < 0.05, **p < 0.01, ***p < 0.001 compared with the first measurements.
AEG, aquatic exercise group; LEG, land exercise group.

FFA Level

The changes in FFA levels over time within and between the aquatic and land exercise groups, as well as the interaction effect, are depicted in Figure 3. Significant differences were observed between measurement points (p < 0.05); however, no significant differences were found between the groups, nor was a significant interaction effect observed. In the aquatic exercise group, FFA levels were significantly higher 30 minutes after both the first exercise session (p < 0.01) and the last session (p < 0.001) relative to pre-program values. In the land exercise group, FFA levels were significantly higher 30 minutes after the initial exercise session (p < 0.05) compared to the pre-program baseline.

Discussion

This study examined the effects of a 16-week aquatic exercise and land-based exercise program on elderly women, specifically investigating the levels of irisin and FGF-21, which are known to induce brown fat activity and browning.

The present findings indicate that levels of irisin and FGF-21 varied according to the timing of blood sample collection. Previous research has indicated that irisin levels temporarily increase after exercise, then stabilize [27] at 125 minutes after moderate-intensity endurance exercise and at 15 minutes following high-intensity interval exercise [28]. Irisin typically peaks 60 minutes after engaging in aerobic and resistance exercises [29]. Meanwhile, FGF-21 levels return to a stable state 60 minutes after exercise [30], with a physiological half-life of less than or equal to 2 hours [31]. In light of these earlier findings, the present study was designed to collect blood samples 30 minutes after exercise. The results confirmed that sampling blood at this time point results in elevated levels of irisin and FGF-21 compared to their stabilized levels, aligning with the findings reported by Kim and Kim [32].

Irisin is expressed in response to various forms of exercise, including aerobic exercise [33,34] and resistance training [35,36]. Kim and Kim [32] observed that elderly women participating in a 16-week aquatic exercise program displayed increased levels of irisin. Similarly, Lee et al. [37] found that a 12-week dance sports program aimed at elderly women with obesity also led to elevated irisin levels. Additionally, Lee and So [38] reported that treadmill walking exercises resulted in higher irisin levels in elderly women. Bostrom et al. [16] noted a similar increase following a 10-week aerobic exercise program in healthy adults.

Irisin secretion is higher after high-intensity exercise than after low-intensity activity [39] and can be further increased by high-intensity sprinting [33,34]. Therefore, aerobic exercise is likely to correspond to varying levels of irisin expression based on the intensity of the exercise. In this study, the significant difference observed in the aquatic exercise program can be explained as follows. Although the intensity of the final exercise session was only moderate to high, at 65% to 70% HRR with an RPE of 15 to 16, this level of intensity was as effective as high-intensity exercise for the participants. For instance, the step exercise required participants to hop repeatedly for an hour to relatively fast-tempo music, demanding considerable effort from those

with lower stamina.

Furthermore, the higher levels of irisin observed during aquatic exercise compared to land exercise in this study may stem from several factors. The water temperature for aquatic exercise was 27°C ± 1°C versus the indoor air temperature for land exercise (25°C ± 1°C). Notably, however, water is approximately 800 times denser than air, with a thermal conductivity around 25 times greater. Consequently, the increased energy expenditure required to maintain body temperature during aquatic exercise likely had a major impact. Furthermore, additional research is warranted to explore how other characteristics of water, such as buoyancy, hydrostatic pressure, and resistance, might affect irisin levels.

Previous studies investigating the effects of resistance exercise on irisin levels have reported increased levels in several contexts: following a 12-week land resistance exercise program among elderly women [40], a 12-week weight training regimen for men aged 20 to 30 years [41], and an 8-week resistance training routine in individuals with obesity [42]. However, research by Moraes et al. [43] indicated that resistance exercise in hemodialysis patients and healthy male and female adults aged 40–50 years did not lead to a significant change in irisin levels. Additionally, Norheim et al. [44] found that resistance exercise either maintained or reduced irisin levels. Collectively, these studies suggest that resistance exercise may result in an increase or no change in irisin concentration.

Resistance exercise alters irisin expression based on the method and intensity of the exercise. However, it does not influence irisin excretion, as the land exercise in this study was characterized by low intensity and high repetitions, which enhance muscle endurance rather than muscle hypertrophy associated with high-intensity, low-repetition exercise. Given that resistance exercise yields inconsistent results varying by sex, age group, and exercise intensity, future studies should establish and apply specific criteria tailored to these factors.

Previous studies have examined the impact of exercise on FGF-21 levels. Kim et al. [23] found that a single session of either moderate-intensity (50% maximal oxygen consumption [VO_2 max]) or high-intensity (80% VO_2 max) treadmill running significantly increased FGF-21 concentrations in men in their 20s. This increase was more pronounced following high-intensity exercise compared to low-intensity exercise. Additionally, Lee and Kim [45] observed that treadmill running at low intensity (50% VO_2 max) and medium intensity (75% VO_2 max) also led to a significant elevation in FGF-21 levels in men in their 20s, with the medium-intensity exercise producing a greater increase.

FGF-21 secretion increases during high-intensity exercise [46]. The intensity of the activity correlates with increased activity in the adenosine monophosphate-activated protein kinase-FGF21 signaling pathway [47], and expression levels rise with the extension of a medium-intensity exercise regimen over a relatively long term [45]. These findings indicate that exercise intensity influences FGF-21 expression. In this study, by progressively increasing exercise intensity every 4 weeks, and by engaging in a medium- or high-intensity aquatic exercise regimen for an extended duration of 16 weeks, a significant impact was observed. Notably, a significant difference in FGF-21 expression was seen in the aquatic exercise group, in contrast to the land exercise group. This discrepancy may be attributed to differences in the energy metabolism systems of the 2 exercise modalities. Aquatic exercise, which is predominantly aerobic, utilizes fat as its main energy source. In contrast, the land exercise used in this study involved resistance training, which relies primarily on glucose for energy.

Irisin and FGF-21 expression are influenced by the exercise environment and temperature [30,42,48–50]. The following summarizes previous research on the impact of exercise environment on irisin levels. Lee et al. [42] demonstrated that a single hour of cold exposure induced shivering and elevated irisin levels in 10 healthy adults. Seebacher and Glanville [49] observed no change in the expression of PGC1-α, a transcription factor for irisin, in mice subjected to 12 °C or 22 °C. However, the presence or absence of exercise at each temperature did impact expression levels. Notably, expression was higher in mice that exercised at 12 °C [49]. Kim [48] conducted a comparison between aquatic and land-based exercises in elderly women, finding that those in the aquatic exercise group exhibited higher irisin levels.

Several previous studies have investigated the impact of the exercise environment on FGF-21 levels. Lee et al. [42] demonstrated that a single 1-hour exposure to cold induced shivering and elevated irisin levels in 10 healthy adults. Hanssen et al. [50] observed that continuous cold exposure for 6 hours daily over a 10-day period led to a significant rise in irisin concentration among healthy men. Additionally, Jeon et al. [30], who studied a group of scuba divers, noted that a 1-time exposure to cold water increased irisin concentrations. These findings suggest that variations in the temperature of the exercise environment can influence the expression of irisin and FGF-21.

Heat conduction in water is approximately 25 times greater than in air, which can adversely impact temperature homeostasis during aquatic exercises [51]. Consequently, additional energy is needed to maintain body temperature

https://doi.org/10.24171/j.phrp.2023.0394

[32]. Nevertheless, mild exposure to cold can stimulate energy expenditure with minimal impacts on other parts of the body [11].

In this study, levels of irisin and FGF-21 were significantly altered following aquatic exercise, while no significant changes were noted after land-based exercise. The aquatic exercise program was conducted in a swimming pool at a controlled temperature of 27 °C ±1 °C (medium-temperature water). In this case, irisin and FGF-21 were likely secreted to counteract the reduction in core body temperature, which is a response to the high thermal conductivity of water that promotes non-shivering thermogenesis [50]. Perspiration increases during land exercise, which here consisted of a 1-hour session set to music with a tempo of ≤70 beats per minute, performed in an indoor fitness center with an ambient temperature of 25 °C ±1 °C and 50% to 55% relative humidity. This resulted in a more pronounced decrease in plasma volume. In this study, the timing of blood collection—30 minutes after exercise completion—may have influenced the measurement of changes in irisin and FGF-21 concentrations due to the body's fluid redistribution for homeostatic balance.

Furthermore, irisin—an exercise-induced myokine—is expressed at levels 40% higher in skeletal muscle fibers than in type II (fast-twitch) and type I (slow-twitch) fibers [52]. In this study, the predominant muscle fiber type utilized during land exercise was type II, while type I fibers were primarily engaged during aquatic exercise.

Exercise impacts levels of both T_3 and FFAs. Miller et al. [53] observed that exercise prompts an increase in thyroid-stimulating hormone release, which in turn stimulates the secretion of T_3. Similarly, Hackney et al. [54] found that both high-intensity training and endurance exercise elevated T_3 levels in participants, suggesting that exercise can influence thyroid hormone activity. Additionally, Goto et al. [55] reported that 60 minutes of continuous exercise at 60% VO_2 max intensity led to an increase in FFAs. Complementing these findings, Kim et al. [56] demonstrated that both intermittent and continuous fast walking exercises increased FFA levels 30 minutes after exercise in female college students, a result that aligns with the findings of the present study.

Coggan et al. [57] reported that the rise in serum T_3 concentration observed immediately after exercise is influenced by norepinephrine and the activation of adrenaline. In this study, the difference in the elevated T_3 levels during the recovery period compared to the levels stabilized 30 minutes post-exercise may result from excess post-exercise oxygen consumption. Additionally, the high FFA levels following both types of exercise indicate that fat metabolism as an energy source during the latter portion of the exercise session can mobilize more energy. This suggests a potential for body fat reduction during the post-exercise recovery period.

The stress of cold exposure and exercise activates the sympathetic nervous system, and the activity of T_3 induces lipolysis in both BAT and WAT. This process of lipid metabolism elevates the FFA concentration [22]. Additionally, Cuevas-Ramos et al. [21] found that PPAR-α, a transcription factor critical for FGF-21 expression, is stimulated by FFAs and that lipolysis leads to the expression of FGF-21. These findings imply that sympathetic nerve stimulation results in increased FFA levels and a subsequent rise in FGF-21 secretion due to lipolysis. The present study confirmed that levels of T_3 (which activates the sympathetic nervous system), as well as FFAs and FGF-21, were highest following the final exercise session. Thus, an increase in FFA levels is closely related to the increased expression of FGF-21.

Irisin, FGF-21, and T_3, which are secreted during shivering, non-shivering thermogenesis, and exercise, activate BAT and induce browning [16,22]. This process enhances triglyceride consumption through lipid metabolism. Consequently, the increase in energy expenditure through thermogenesis may help prevent weight gain [9,58] and combat obesity. However, further research is needed to investigate the expression levels of irisin, FGF-21, T_3, and FFA under various exercise conditions, including ambient and water temperatures, program content, and the specific goals of aquatic and land-based exercise regimens.

Our study has certain limitations. First, due to the relatively small sample size (n=10 per group), it may be challenging to generalize the results to broader populations. Second, participants necessarily engaged in additional, unrecorded activities of daily living, which could have confounded the results. Third, further research is required to confirm our findings; additionally, future studies should compare the effects of aquatic and land exercise programs on various subgroups (obese and non-obese individuals, men and women, etc.) categorized by age or type of exercise, and develop effective aerobic and land exercise programs for different age groups. Additionally, safety concerns for the elderly female participants prevented full exposure to cold temperatures during aquatic exercises. Future studies should consider the exercise environment from multiple perspectives.

Conclusion

This study demonstrates that levels of irisin, FGF-21, T_3, and FFAs were highest 30 minutes following the final exercise session. The data suggest that variations in the expression

of irisin and FGF-21, as well as fluctuations in T_3 and FFA levels, could be attributed not only to the heightened energy expenditure from exercise but also to differences in the exercise environment. Specifically, aquatic exercise may be more effective than land-based activity in enhancing the secretion of irisin and FGF-21.

Different exercise types and environments can enhance fat metabolism by triggering hormonal changes that activate brown fat and encourage the conversion of white to brown fat. This process can ultimately aid in preventing and lessening obesity through body fat reduction.

Further research is needed to ascertain how the expressions of irisin and FGF-21, as well as the levels of T_3 and FFAs, can be elevated by modifying exercise environment conditions—including ambiance, water temperature, and program content—to prevent and ameliorate obesity.

Notes

Ethics Approval

Before the experiment began, the study (derived from PNU IRB/2017_68_HR) received approval from the Institutional Human Research Committee. Participants were fully informed of the purpose of the research, and only those who willingly agreed to participate submitted their consent.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

This work was supported by a 2-year research grant from Pusan National University.

Availability of Data

The datasets are not publicly available but can be obtained from the corresponding author upon reasonable request.

Authors' Contributions

Conceptualization: DWK, DYK; Data curation: KSH, MKK; Formal analysis: DWK, SHK, MKK; Investigation: DWK, SHK, MKK; Methodology: DWK, SHK, DYK; Project administration: DYK; Resources: SHK, MKK; Software: SHK, MKK; Supervision: DWK; Validation: DWK, DYK; Visualization: SHK, MKK; Writing–original draft: DWK, DYK; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0228
Osong Public Health Res Perspect 2024;15(3):248-259

Original Article

A *Mycobacterium bovis* outbreak among exhibition animals at a zoo in the Republic of Korea: the first contact investigation of zoonotic tuberculosis

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ABSTRACT

Objectives: Between July 2, 2021, and September 20, 2022, a *Mycobacterium bovis* outbreak occurred among exhibition animals at a zoo in the Republic of Korea. This study was conducted to assess the likelihood of *M. bovis* transmission to human contacts through a contact investigation and to implement preventive treatment for latent tuberculosis infection (LTBI).

Methods: In this descriptive study, the Korea Disease Control and Prevention Agency conducted a contact investigation, which included interviews, interferon-gamma release assay (IGRA) tests, and chest X-rays. Contacts underwent IGRA testing on 2 occasions: initial testing of 29 contacts (15 in the first cluster of infection and 14 in the second cluster) and follow-up testing of the 15 contacts in the first cluster.

Results: The study included 29 participants, 18 of whom were male (62.1%) and 11 female (37.9%). The mean participant age was 37.3 years (standard deviation, 9.6 years). In the initial IGRA tests, 6 of the 29 participants tested positive, indicating a prevalence of 20.7%. Following prolonged exposure, 1 additional positive case was detected in follow-up testing, raising the prevalence of LTBI to 24.1%. None of the contacts had active tuberculosis. Among the 7 individuals with positive results, 2 (28.6%) underwent treatment for LTBI.

Conclusion: This study faced challenges in confirming the transmission of *M. bovis* infection from infected animals to humans in the Republic of Korea. Nevertheless, adopting a One Health approach necessitates the implementation of surveillance systems and infection control protocols, particularly for occupational groups at high risk of exposure.

Keywords: Contact investigation; Interferon-γ release assay; *Mycobacterium bovis*; Zoonotic tuberculosis

Received: August 15, 2023
Revised: January 31, 2024
Accepted: February 12, 2024

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Introduction

Zoonotic tuberculosis (TB) is a form of TB that affects humans and is caused by *Mycobacterium bovis*, a member of the *Mycobacterium tuberculosis* complex (MTBC) [1–3]. *M. bovis* is responsible for the majority of TB cases in cattle and also infects a wide range of domestic and wild mammal species, leading to a chronic and progressive bacterial disease [4–6]. Transmission of *M. bovis* can occur among animals, from animals to humans, and from humans to animals, although human-to-human transmission is rare [7]. Humans can contract *M. bovis* through the inhalation of aerosols when in close contact with infected cattle or their carcasses. In addition, infection is associated with consuming unpasteurized dairy products or raw meat from infected cattle [8,9].

In 2020, the World Health Organization estimated that out of 10 million new cases of active TB, 140,000 (1.4%) were zoonotic TB, with approximately 11,400 (8.1%) resulting in death [10]. The United States Centers for Disease Control and Prevention reported in 2014 that *M. bovis* caused about 1.6% of all cases of TB in humans [11]. However, these global estimates are imprecise due to the lack of routine surveillance data from both human and animal populations [12]. In the Republic of Korea, no cases of *M. bovis* infection have yet been reported among Korean-born individuals [13,14]. The absence of effective surveillance programs hampers the accurate assessment of the disease's burden. Moreover, the detection and identification of *M. bovis* cases in humans are particularly challenging given the current TB management systems for humans and livestock in the Republic of Korea. This difficulty arises because information and data are managed separately for different infected groups (humans, livestock, and exhibition animals), without an integrated approach.

From July 2, 2021, to September 20, 2022, a zoo in the Republic of Korea experienced an outbreak of *M. bovis* among its exhibition animals. In January 2021, out of 56 animals on display, 6 consecutive deaths occurred and were attributed to respiratory symptoms. Following these incidents, 43 of the remaining 50 animals tested positive for animal TB using polymerase chain reaction (PCR) testing. In response to the outbreak, the zoo's health manager initiated an investigation into individuals who had been exposed to *M. bovis* and carried out TB screenings for both direct and indirect contacts. However, these examinations were not conducted in accordance with the protocol set forth in the Korean national guidelines for TB [15].

According to international research on risk factors for *M. bovis* infection in humans, such infection is closely associated with occupational exposure [8,16–18]. Transmission through occupational exposure can happen by inhaling aerosols exhaled by infected animals or humans [17]. It can also occur through direct contact, often in situations where wounds or injuries are present [17]. Despite a delay in sharing outbreak information, the Korea Disease Control and Prevention Agency (KDCA) opted to initiate contact investigations and preventive measures due to concerns about the possible spread of zoonotic disease.

Therefore, the objective of this study was to assess the likelihood of *M. bovis* transmission to human contacts through contact investigation and to implement preventive treatment for latent tuberculosis infection (LTBI). The findings may enhance our understanding of the occurrence and transmission mechanisms of zoonotic TB, with the ultimate goal of aiding in the development of prevention and control strategies for high-risk occupational groups.

HIGHLIGHTS

- A *Mycobacterium bovis* outbreak occurred among exhibition animals at a zoo in the Republic of Korea from July 2, 2021, to September 20, 2022.
- The initial prevalence of latent tuberculosis infection (LTBI) was 20.7% (6 of 29 contacts), which increased to 24.1% (7 of 29) after follow-up testing.
- The 7 individuals with positive interferon-gamma release assays included 3 veterinarians, 2 maintenance staff, 1 zookeeper, and 1 laboratory technician. Of these, 2 individuals (28.6%) underwent treatment for LTBI.
- This study increases awareness of zoonotic tuberculosis, underscoring the necessity for a tuberculosis surveillance system targeting high-risk occupational groups within the One Health framework.

Materials and Methods

Outbreak Recognition

From July 2, 2021, to September 20, 2022, an outbreak of *M. bovis* occurred among exhibition animals imported from South America to a zoo located in Gyeonggi Province, Republic of Korea [19]. These animals were housed in the zoo's South American Pavilion, which is divided into 3 main sections: an outdoor exhibition space featuring llamas and guanacos, an open-air field where species such as giant anteaters and South American tapirs are kept, and an indoor exhibition hall connected to the open-air field. The

zoo is home to approximately 370 species, 144 of which are endangered. As of January 2021, the pavilion showcased a total of 56 animals. Between January and May 2021, 6 animals—2 guanacos and 4 llamas—exhibited respiratory symptoms, including coughing and labored breathing, and subsequently died. In response, the zoo restricted access to the pavilion starting on June 5, 2021, and sought assistance from the National Institute of Wildlife Disease Control and Prevention (NIWDC), under the Ministry of Environment (ME), to perform PCR tests for TB on the 23 animals that had shared habitats with those that had died. On July 2, 2021, the first cluster of *M. bovis* infection was confirmed in 5 animals, consisting of 3 guanacos and 2 South American tapirs. Following the initial cluster, sporadic cases continued to emerge, leading to the confirmation of a second large-scale cluster on September 20, 2022. This cluster included 22 animals—6 llamas, 5 guanacos, 4 capybaras, 3 maras, 2 collared peccaries, and 2 South American tapirs—testing positive for *M. bovis* (Figure 1). As result of, from July 2, 2021, to September 20, 2022, 43 of the 50 animals exhibited in the pavilion were confirmed to be infected with *M. bovis* using PCR (Figure 2). During this period, 52 animals either died or were euthanized, which included 7 that were housed in

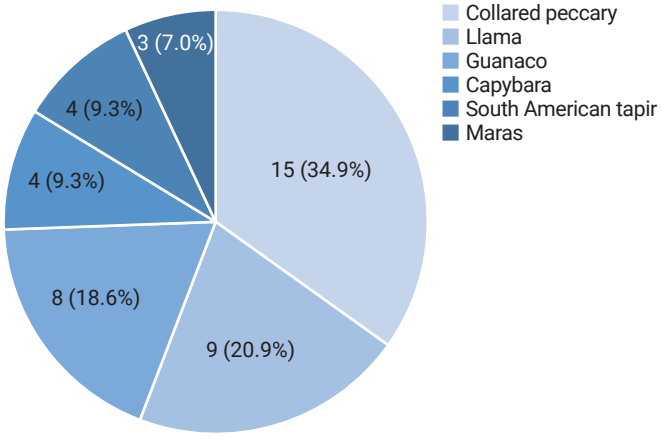

Figure 2. Distribution of animals with confirmed *Mycobacterium bovis* infection at a zoo in the Republic of Korea ($n = 43$).

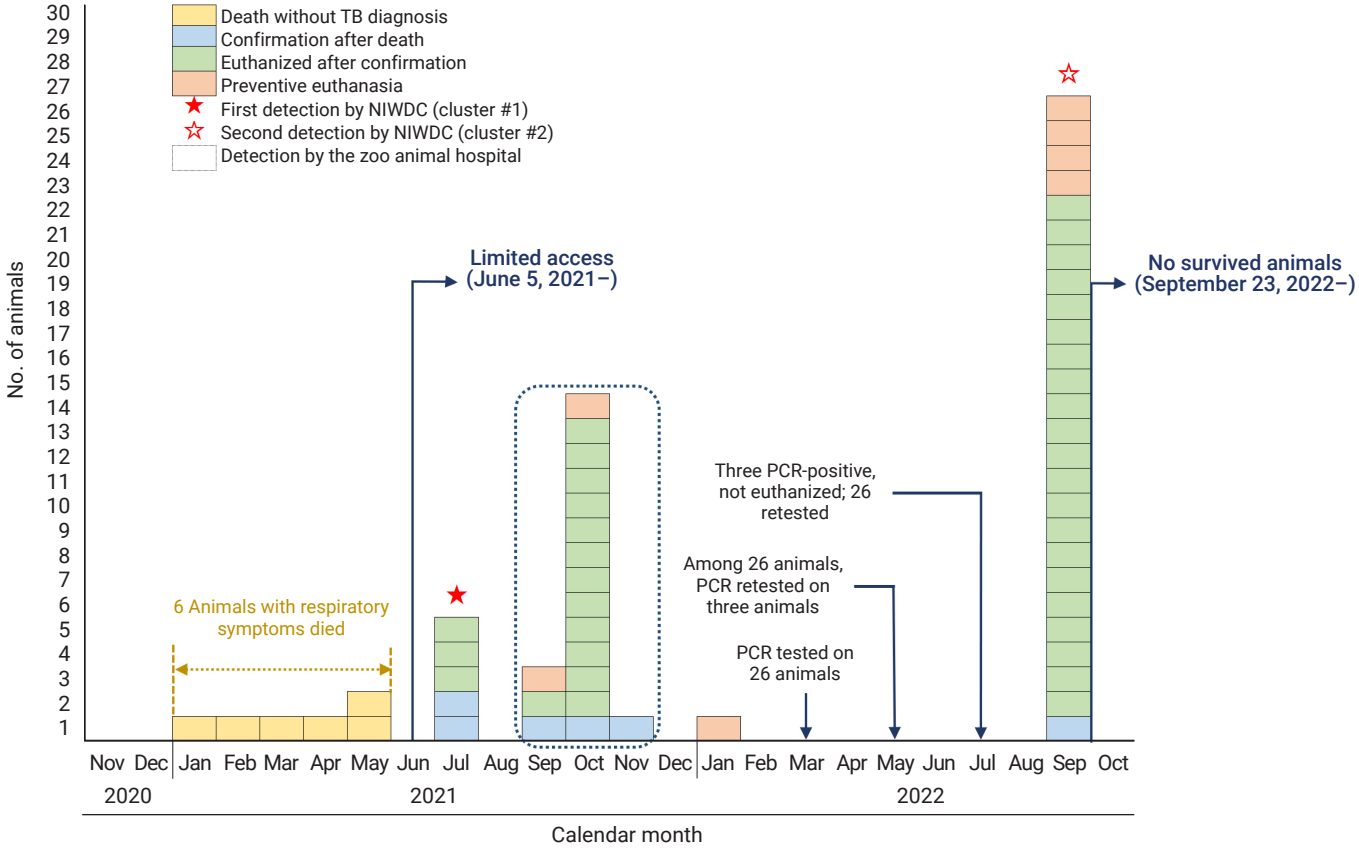

Figure 1. Epidemic curve of a *Mycobacterium bovis* outbreak among exhibition animals at a zoo in the Republic of Korea ($n = 56$).
NIWDC, National Wildlife Disease Control Center; TB, tuberculosis; PCR, polymerase chain reaction.

https://doi.org/10.24171/j.phrp.2023.0228

the same enclosures as infected animals and 2 capybara cubs. None of the animals in the South American Pavilion survived.

The zoo outbreak of *M. bovis* infection went undetected for a prolonged period. The KDCA identified the outbreak following notification from the Zoonosis Countermeasure Committee on December 23, 2022 [20]. In response, the KDCA organized a contact investigation following the protocols specified in Article 49 of the Infectious Disease Prevention and Control Act and Article 11 of the Tuberculosis Prevention Act [21,22].

Study Design and Study Population

This study is descriptive in nature, as we prospectively collected and analyzed data on contacts identified by the zoo's health manager in both July 2021 and September 2022. Additionally, the study included only an exposure group, without a non-exposure group to serve as a control.

The total number of human contacts during the outbreak period was 36, with 15 contacts identified in July 2021 and 21 contacts in September 2022. Of these, 7 individuals were identified as contacts in both clusters. Consequently, our study population comprised 29 individuals, consisting of zookeepers, veterinarians, veterinary assistants, a laboratory technician, and maintenance staff.

Case Definition

The term "zoonotic tuberculosis" is commonly used in the medical literature to describe human TB disease resulting from infection with *M. bovis* [23]. However, according to the 2023 Case Definitions for National Notifiable Infectious Diseases published by the KDCA [24], TB represents an infectious disease caused by the MTBC, the diagnosis of zoonotic TB caused by *M. bovis* is not specifically differentiated. Consequently, for the purposes of this study, we have defined zoonotic TB as a case in which a patient with TB has had *M. bovis* identified by genotype analysis conducted by the KDCA.

The term "outbreak," in the context of TB, is not commonly defined in the literature due to the disease's extended latency period [25]. Nevertheless, for the purposes of this study, we adopted this term to describe a large-scale incidence of *M. bovis* infection. Specifically, we defined an outbreak as the occurrence of 3 or more epidemiologically linked cases of confirmed *M. bovis* infection among exhibition animals between July 2021 and September 2022. This definition aligns with the guidelines for contact investigations issued by the US Centers for Disease Control and Prevention [25,26].

A "cluster" of TB cases is defined as 2 or more cases that share both epidemiological links and laboratory profiles, including genomic and drug susceptibility characteristics [25]. In the present study, culturing *M. bovis* was unsuccessful; instead, *M. bovis* infection was detected using PCR testing. To reduce variability in testing proficiency, methodology, and equipment, we categorized the cases into 2 clusters based on PCR results obtained from the NIWDC. Consequently, 2 distinct infection clusters were identified during the outbreak period: cluster #1 was recognized on July 2, 2021, and cluster #2 was detected on September 20, 2022.

This study entailed a secondary analysis of data derived from individuals identified as contacts. Evaluating the criteria used to select these contacts was not possible. Furthermore, no globally standardized definition or classification exists regarding "contact" with infected animals, which should consider variables such as the infectivity of the animals, the degree of exposure, and the proximity of contact. For the purposes of this study, "contact" was defined as an individual who had either direct or indirect contact with an infected animal, in accordance with the Korean national TB guidelines [15].

Data Collection

The zoo provided a list of 29 individuals along with the results of interferon-gamma release assays (IGRAs) and chest X-rays conducted in July 2021 and September 2022. In January 2023, we conducted a full contact investigation for these 29 individuals. Of these contacts, 8 had either resigned or been transferred. Therefore, contacts underwent IGRA tests and chest X-rays at local public health clinics either in the vicinity of the zoo or near their current residential addresses. We acquired the results of the chest X-rays and IGRA tests from these clinics and monitored the TB occurrence status among the contacts using the Integrated Tuberculosis Information System of the KDCA.

Contact Investigation

We carried out contact investigations in accordance with the Korean national TB guidelines, which included conducting interviews, IGRA testing, and chest X-rays [15]. Initially, we disseminated a survey form to local public health clinics to gather demographic information as well as epidemiological data, including occupation, department of work, exposure type, and exposure duration. Due to the extended duration of the outbreak and the risk of memory decay bias, we decided against collecting information on symptoms.

In July 2021, the zoo conducted IGRA tests and chest X-rays for the 15 contacts in cluster #1. For the 21 contacts in cluster #2, testing in September 2022 included only chest X-rays, with the tuberculin skin test (TST) and IGRA omitted. Consequently, in January 2023, a thorough contact investigation was

initiated. This process included IGRA testing for all contacts from both clusters, for a total of 29 individuals.

LTBI testing of contacts

LTBI testing was conducted among contacts with normal chest radiograph findings to confirm their immune response to TB bacteria [27]. The current standard for diagnosing LTBI involves either TST or IGRA. In our study, we utilized IGRA due to its high specificity for LTBI diagnosis. This assay detects the cellular immune response to specific MTBC antigens, such as early secreted antigenic target-6 and culture filtrate protein-10, which are produced from genes in the RD1 region of the MTBC DNA [28,29].

In July 2021, the zoo initiated IGRA testing of 15 individuals from cluster #1, utilizing the QuantiFERON TB Gold Plus test (QFT-Plus; Qiagen). These contacts visited a private specialized diagnostic facility for blood sample collection. The test results were interpreted in accordance with the manufacturer's instructions and subsequently communicated to each individual.

In January 2023, we carried out IGRA among 27 individuals. This included initial tests for 14 individuals in cluster #2 and follow-up tests for 13 individuals from cluster #1, excluding 2 who had tested positive initially. A total of 21 participants were tested using the STANDARD TB-Feron enzyme-linked immunosorbent assay (SD Biosensor), while the remaining 6 underwent testing with the QuantiFERON TB Gold Plus (QFT-Plus). The use of different test products stemmed from the varying practices of local public health clinics. The testing procedures were almost identical, with the primary difference being the use of 2 TB antigen tubes (TB1 and TB2) in the QuantiFERON TB Gold Plus kit [30]. Whole blood samples were collected in heparinized vacutainer tubes, following the sequence of nil tube, TB antigen tubes (including TB1 and TB2 for QFT-Plus), and mitogen tube. After thorough mixing, the tubes were transferred to the laboratory at the Gyeonggi Province Institute of Health and Environment for analysis. The results were interpreted using uniform criteria for determining positive, negative, and indeterminate outcomes, adhering to the guidelines provided by the manufacturers.

Clinical evaluation and testing for TB infection in contacts

The contacts were evaluated for symptoms and signs consistent with TB, and chest radiographs were performed to detect active pulmonary TB. When TB infection was suspected based on chest radiograph findings and respiratory symptoms, sputum microbiological tests, including sputum smear and culture, were scheduled.

Preventive treatment for LTBI and follow-up

The local public health clinics carried out monthly medical examinations and blood tests to identify potential side effects in contacts who had received preventive treatment. We tracked the incidence of TB among contacts with the aid of a digital TB surveillance system. In their duties as field workers, zoo staff members are required to participate in an annual national health examination that incorporates chest radiography to screen for active TB.

Data Analysis

The IGRA was administered to contacts on 2 separate occasions, with the results for each test presented individually. The prevalence of LTBI was determined by calculating the proportion of individuals who tested positive for IGRA within the study population. Conversion to positive IGRA was characterized by an initial negative IGRA result followed by a positive result upon follow-up.

The baseline characteristics of all enrolled contacts are presented using the mean and standard deviation for continuous variables, and frequencies and percentages for categorical variables. Descriptive statistics were used to compare the prevalence of LTBI among contacts according to sex, age, and occupation. Microsoft Excel ver. 2016 (Microsoft Corp.) was employed for the analysis.

Ethics Statement

This study was reviewed and approved by the institutional review board of the KDCA (No: KDCA-2023-04-10-PE-01) and conducted in accordance with the principles of the Declaration of Helsinki. It entailed a full contact investigation in accordance with the Tuberculosis Prevention Act and the Korean national guidelines for TB control, using data provided by the zoo. The requirement for informed consent was waived, as contacts signed separate consent forms for IGRA testing and LTBI treatment.

Results

Outbreak Investigation

The indoor area of the South American Pavilion, designed to mimic a tropical climate, is exceptionally hot and humid. The absence of adequate ventilation and humidity control in this space heightens the risk of TB infection. We hypothesized that the risk of transmission could affect not only the animals but also staff members who interacted with the infected animals and the contaminated environment. However, during the outbreak, staff members followed personal hygiene practices, such as wearing masks and practicing hand hygiene, due to the coronavirus disease 2019 pandemic.

https://doi.org/10.24171/j.phrp.2023.0228

They also donned disposable personal protective equipment, including KF94 masks, protective clothing rated at Ingress Protection levels 5–6, nitrile gloves, and rubber safety shoes while performing autopsies. Consequently, the likelihood of transmission from the animals or the environment to the staff was deemed relatively low.

Contact Investigation

Characteristics of the study population

The study included 29 participants, 18 of whom were male (62.1%) and 11 female (37.9%). The mean age of the participants was 37.3 years, with a standard deviation of 9.6 years. We stratified their baseline characteristics by occupation. Zookeepers were the most numerous, with 10 individuals (34.5%), followed by 9 veterinarians (31.0%), 7 maintenance staff (24.1%), 2 veterinary assistants (6.9%), and 1 laboratory technician (3.4%). Zookeepers engaged in daily contact with animals for 1 hour while performing breeding tasks and assisted with specimen collection during the outbreak. Veterinarians were in contact with animals for about 1 hour weekly during medical procedures that involved collecting specimens, such as blood and nasal swabs. One veterinarian also had direct contact with animal tissues during autopsies. The maintenance staff were responsible for irregular duties, including the disposal of feces and sewage and the replacement of pipelines (Table 1).

IGRA

In the initial IGRA results, 6 of the 29 participants tested

Table 1. Baseline characteristics of the study population by occupation ($n = 29$)

Characteristic	Total	Zookeepers	Veterinarians	Veterinary assistants	Laboratory technician	Maintenance staff
Total	29 (100.0)	10 (34.5)	9 (31.0)	2 (6.9)	1 (3.4)	7 (24.1)
Sex						
Male	18 (62.1)	5 (50.0)	4 (44.4)	1 (50.0)	1 (100.0)	7 (100.0)
Female	11 (37.9)	5 (50.0)	5 (55.6)	1 (50.0)	0 (0)	0 (0)
Age (y)	37.3 ± 9.6	32.6 ± 10.5	37.0 ± 9.6	30.0 ± 5.0	34.0 ± 0.0	46.9 ± 4.1
Age group (y)						
20–29	8 (27.6)	6 (60.0)	1 (11.1)	1 (50.0)	0 (0)	0 (0)
30–39	8 (27.6)	2 (20.0)	4 (44.4)	1 (50.0)	1 (100.0)	0 (0)
40–49	9 (31.0)	1 (10.0)	3 (33.3)	0 (0)	0 (0)	5 (71.4)
50–59	4 (13.8)	1 (10.0)	1 (11.1)	0 (0)	0 (0)	2 (28.6)
Family TB history						
None	28 (96.6)	10 (100.0)	8 (27.6)	2 (100.0)	1 (100.0)	7 (100.0)
Yes	1 (3.4)	0 (0)	1 (3.4)	0 (0)	0 (0)	0 (0)
History of TB treatment						
None	29 (100.0)	10 (100.0)	9 (100.0)	2 (100.0)	1 (100.0)	7 (100.0)
Yes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Workplace						
Zoo field	17 (58.6)	10 (100.0)	0 (0)	0 (0)	0 (0)	7 (100.0)
Zoo animal hospital	12 (41.4)	0 (0)	9 (100.0)	2 (100.0)	1 (100.0)	0 (0)
Type of activity						
Breeding, specimen collection	9 (31.0)	9 (90.0)	0 (0)	0 (0)	0 (0)	0 (0)
Only breeding	1 (3.4)	1 (10.0)	0 (0)	0 (0)	0 (0)	0 (0)
Medical evaluation, specimen collection	7 (24.1)	0 (0)	7 (77.8)	0 (0)	0 (0)	0 (0)
Only specimen collection	1 (3.4)	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)
Autopsy, testing for disease	2 (6.9)	0 (0)	1 (11.1)	0 (0)	1 (100.0)	0 (0)
Medical assistance	2 (6.9)	0 (0)	0 (0)	2 (100.0)	0 (0)	0 (0)
Feces and sewage disposal	7 (24.1)	0 (0)	0 (0)	0 (0)	0 (0)	7 (100.0)
Amount of time of exposure						
1 h per day	10 (34.5)	10 (100.0)	0 (0)	0 (0)	0 (0)	0 (0)
1 h per week	8 (27.6)	0 (0)	8 (88.9)	0 (0)	0 (0)	0 (0)
1 h per month	2 (6.9)	0 (0)	1 (11.1)	0 (0)	1 (3.7)	0 (0)
30 min per month	2 (6.9)	0 (0)	0 (0)	2 (100.0)	0 (0)	0 (0)
Unmeasurable	7 (24.1)	0 (0)	0 (0)	0 (0)	0 (0)	7 (100.0)

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

positive, yielding a prevalence of 20.7%. Subsequently, of the 13 individuals from cluster #1 with initial indeterminate or negative results, 1 person tested positive on follow-up due to prolonged exposure. Consequently, the prevalence of LTBI rose to 24.1% (7 of 29), as shown in Figure 3.

Initial IGRA

The initial IGRA was administered to a total of 29 individuals. In cluster #1, the 15 contacts were tested at the time of exposure. The 14 contacts in cluster #2 underwent the test 24 weeks following their last exposure.

Among the 15 contacts in cluster #1, 2 individuals tested positive, representing an LTBI prevalence of 13.3%. The sex distribution of the positive cases was evenly split, with 1 male and 1 female contact. The positive cases had a higher mean age, at 46.0 ± 4.0 years, compared to 35.6 ± 9.2 years for the negative cases. Both of the individuals with positive results were veterinarians, which corresponds to a 33.3% prevalence of LTBI within this occupational group.

Among the 14 contacts in cluster #2, 4 tested positive, resulting in a 28.6% prevalence of LTBI. Regarding sex distribution, LTBI was diagnosed in 3 male and 1 female. The mean age of the contacts was 37.7 ± 10.1 years, with no meaningful difference between the IGRA-positive and IGRA-negative groups. When examining LTBI prevalence by occupation, veterinarians had the highest proportion at 33.3%, followed by maintenance staff at 28.6% and zookeepers at 25.0%. One veterinarian reported a family history of TB, but this individual fortunately received a negative IGRA result.

In summary, of the 29 individuals who underwent initial IGRA testing, 6 tested positive, indicating a 20.7% prevalence of LTBI. cluster #2 exhibited a higher prevalence of IGRA positivity at 28.6%, compared to 13.3% in cluster #1. Notably, the mean age of IGRA-positive individuals in cluster #1 was higher (46.0 ± 4.0 years) than in cluster #2 (37.3 ± 9.7 years). Additionally, veterinarians constituted the largest proportion (33.3%) of IGRA-positive individuals in both clusters, as

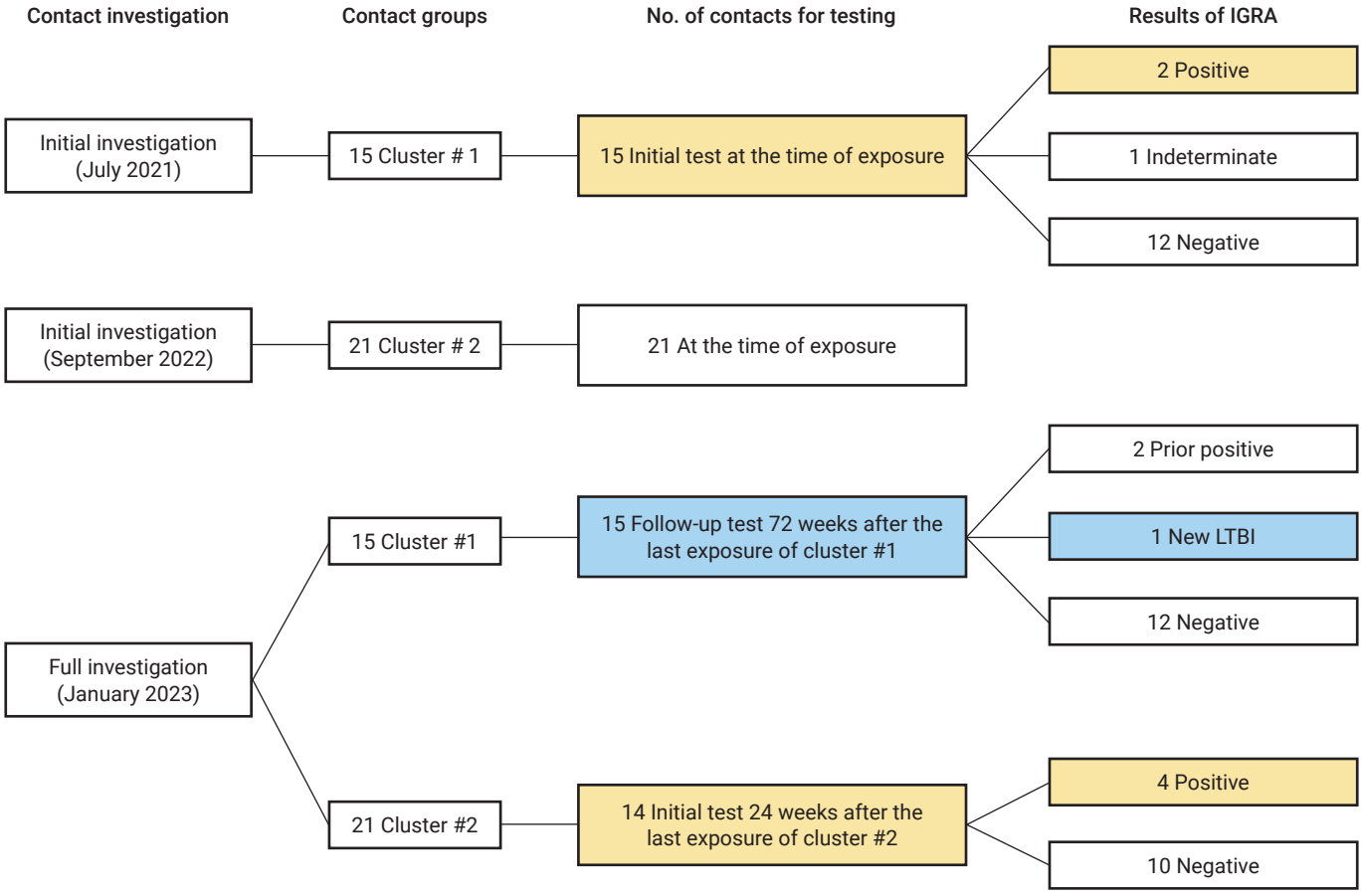

Figure 3. Investigation of contacts with animals infected with *Mycobacterium bovis* at a zoo in the Republic of Korea. Seven staff members in cluster #2 experienced dual exposure during the *M. bovis* outbreak period.
IGRA, interferon-gamma release assay; LTBI, latent tuberculosis infection.

https://doi.org/10.24171/j.phrp.2023.0228

Table 2. Distribution of exposed contacts in Clusters #1 and #2 ($n = 29$) based on initial IGRA results

Characteristic	Cluster #1				Cluster #2		
	Total	Positive	Negative	Indeterminate	Total	Positive	Negative
Total	15 (100.0)	2 (13.3)	12 (80.0)	1 (6.7)	14 (100.0)	4 (28.6)	10 (71.4)
Sex							
Male	9 (60.0)	1 (11.1)	7 (77.8)	1 (11.1)	9 (64.3)	3 (33.3)	6 (66.7)
Female	6 (40.0)	1 (16.7)	5 (83.3)	0 (0)	5 (35.7)	1 (20.0)	4 (80.0)
Age (y)	36.9 ± 9.1	46.0 ± 4.0	35.6 ± 9.2	34.0 ± 0.0	37.7 ± 10.1	37.3 ± 9.7	37.9 ± 10.3
Age group (y)							
20–29	4 (26.7)	0 (0)	4 (100.0)	0 (0)	4 (28.6)	1 (25.0)	3 (75.0)
30–39	5 (33.3)	0 (0)	4 (80.0)	1 (20.0)	3 (21.4)	1 (33.3)	2 (66.7)
40–49	4 (26.7)	1 (25.0)	3 (75.0)	0 (0)	5 (35.7)	2 (40.0)	3 (60.0)
50–59	2 (13.3)	1 (50.0)	1 (50.0)	0 (0)	2 (14.3)	0 (0)	2 (100.0)
Occupation							
Zookeeper	6 (40.0)	0 (0)	6 (100.0)	0 (0)	4 (28.6)	1 (25.0)	3 (75.0)
Veterinarian	6 (40.0)	2 (33.3)	4 (66.7)	0 (0)	3 (21.4)	1 (33.3)	2 (66.7)
Veterinary assistant	2 (13.3)	0 (0)	2 (100.0)	0 (0)	0 (0)	0 (0)	0 (0)
Laboratory technician	1 (6.7)	0 (0)	0 (0)	1 (100.0)	0 (0)	0 (0)	0 (0)
Maintenance staff	0 (0)	0 (0)	0 (0)	0 (0)	7 (50.0)	2 (28.6)	5 (71.4)

Data are presented as *n* (%) or mean ± standard deviation.
IGRA, interferon-gamma release assay.

shown in Table 2.

Follow-up IGRA

The zoo did not perform follow-up testing 8–10 weeks after the initial IGRA for contacts in cluster #1 who initially tested negative. However, the KDCA opted to conduct follow-up IGRA for these contacts, despite 72 weeks having elapsed since the initial test. Of the 15 contacts, 1 individual was newly diagnosed with LTBI, in addition to the 2 who had tested positive initially. The new positive case is a 34-year-old laboratory technician who had received an indeterminate result on the initial test. Therefore, among the 7 individuals with prolonged exposure during the outbreak, 3 tested positive either during the initial testing or follow-up testing (Table 3).

Clinical evaluation and chest radiograph

Contacts were physically examined to detect signs and symptoms of active TB; however, none displayed evidence of the disease. All chest radiographs showed normal findings.

Preventive treatment and follow-up

Among the 7 contacts diagnosed with LTBI, 2 (28.6%) consented to undergo treatment, while 5 (71.4%) declined. The prescribed LTBI treatment regimen consisted of a 3-month course of combined isoniazid and rifampin. Both individuals who commenced treatment completed it successfully, without experiencing adverse effects. As of September 30, 2023, no active TB cases among the 29 contacts had been reported in the KDCA's digital TB surveillance system. In preparation for potential TB infections among contacts, the NIWDC is preserving tissue samples from animals with confirmed *M. bovis* infection to facilitate epidemiological linkage by genetic analysis.

Discussion

The KDCA conducted a comprehensive investigation into the *M. bovis* outbreak among exhibition animals that extended from July 2, 2021, to September 20, 2022. They also performed IGRA testing and chest X-rays for zoo staff at risk of *M. bovis* transmission.

In this study, the prevalence of LTBI among contacts was 20.7% (6 of 29) in initial testing and 24.1% (7 of 29) in follow-up evaluation. A thorough review of the international literature revealed several factors that complicated a comparison of our research results with data from other countries. Zoonotic TB is strongly associated with economic and sociocultural factors, such as the consumption of unpasteurized dairy products and the sharing of living spaces with animals [11]. However, in the Republic of Korea, the sale of raw milk is prohibited by the Livestock Products Sanitary Control Act (Articles 4 and 32) [31], and the living spaces of livestock and humans are strictly separated. In the present study, LTBI testing was conducted to prevent secondary cases of TB. The mean age of our study population was 37.3 ± 9.6 years. Therefore, we compared our findings with the 35–49-year-old subgroup within group A (representing workers at

Table 3. Distribution of exposed contacts in Cluster #1 ($n = 15$) based on follow-up IGRA results

Characteristic	Total	Cluster #1		
		Prior positive	Positive	Negative
Total	15 (100.0)	2 (13.3)	1 (6.7)	12 (80.0)
Sex				
Male	9 (60.0)	1 (11.1)	1 (11.1)	7 (77.8)
Female	6 (40.0)	1 (16.7)	0 (0)	5 (83.3)
Age (y)	36.9±9.1	46.0±4.0	34.0±0.0	35.6±9.2
Age group (y)				
20–29	4 (26.7)	0 (0)	0 (0)	4 (100.0)
30–39	5 (33.3)	0 (0)	1 (20.0)	4 (80.0)
40–49	4 (26.7)	1 (25.0)	0 (0)	3 (75.0)
50–59	2 (13.3)	1 (50.0)	0 (0)	1 (50.0)
Occupation				
Zookeeper	6 (40.0)	0 (0)	0 (0)	6 (100.0)
Veterinarian	6 (40.0)	2 (33.3)	0 (0)	4 (66.7)
Veterinary assistant	2 (13.3)	0 (0)	0 (0)	2 (100.0)
Laboratory technician	1 (6.7)	0 (0)	1 (100.0)	0 (0)

Data are presented as *n* (%) or mean±standard deviation.
IGRA, interferon-gamma release assay.

postpartum care centers, social welfare institutions and educational facilities) from a large-scale LTBI study using IGRA, conducted by the government of the Republic of Korea in 2017–2018 [32]. Despite the limited number of contacts, the prevalence of LTBI in our study resembles that of the general population (19.9%) reported in the reference literature. Prior research indicates that as individuals age, their pulmonary immune responses deteriorate, contributing to an increased susceptibility to TB [33]. Age has been identified as the variable most strongly influencing the prevalence of LTBI, which increases with advancing years [32]. However, in our study, the largest number of LTBI cases (3 individuals) was found in the 40–49-year age group, followed by 2 individuals in the 30–39-year age group and 1 individual each in the 20–29-year and 50–59-year groups. Age appeared to exhibit no direct correlation with the incidence rate of LTBI. Regarding the distribution of IGRA positivity by occupation, 3 of the 7 contacts with positive results were veterinarians, followed by 2 maintenance staff members, 1 zookeeper, and 1 laboratory technician. Unfortunately, only 2 individuals (28.6%) underwent treatment for LTBI, as the veterinarians and a laboratory technician in the high-risk exposure group refused treatment due to their asymptomatic status and considerations related to pregnancy.

Zoonotic TB is clinically and pathologically indistinguishable from TB caused by *M. tuberculosis* [22]. This disease requires careful management because *M. bovis* is inherently resistant to pyrazinamide, a crucial first-line medication [1], and may be more deadly than *M. tuberculosis*, as it more frequently results in miliary and central nervous system TB [3]. Differentiating among the members of the MTBC is essential for epidemiological studies in human cases and for the appropriate treatment of patients with TB. However, the genetic sequence homogeneity within the MTBC exceeds 99.9%, which poses challenges in distinguishing between individual strains [34]. International studies suggest that the likelihood of *M. bovis* transmission from animals to humans is low [21]. However, the risk of infection is higher among certain occupational groups [16–18]. Notably, the occupational risk for *M. bovis* exposure is greater for individuals working in enclosed spaces with close contact during animal examination, treatment, or handling, which increases the potential for transmission [17,35]. As a preventive measure, workers should consistently wear personal protective equipment, and workspaces should enforce proper ventilation and infection control measures. Additionally, high-risk occupational groups, including veterinarians performing necropsies, abattoir workers, livestock farmers, and dairy farmers, should regularly monitor their health status [36]. Other occupational groups at risk of contracting *M. bovis* from animals include laboratory workers, as well as those employed in wildlife parks, zoos, and animal houses [36]. However, following the implementation of the Tuberculosis Prevention Act in the Republic of Korea [21], zoo staff members have not been subjected to mandatory LTBI examinations. Prior to the *M. bovis* outbreak at the zoo, these workers had not undergone TST or IGRA testing. Furthermore, contact with contaminated water or soil represents a meaningful route of transmission, as *M. bovis* can survive in the environment for

https://doi.org/10.24171/j.phrp.2023.0228

extended periods after being expelled in aerosols [20,37,38]. To eliminate the source of infection, early detection of preclinical *M. bovis* infection in animals and the prompt removal of all infected animals are necessary [39,40].

In the Republic of Korea, the Animal Plant Quarantine Agency (APQA) is working to eliminate TB from cattle through a "test-and-slaughter" strategy [41]. These preventive measures have contributed to a gradual decrease in animal TB cases. However, demand is rising for more sophisticated preventive policies, such as vaccination, to ensure animal health. ME has introduced the First Comprehensive Plan for Zoo Management (2021–2025) to improve zoo management practices [42]. Despite these efforts, no legal framework currently governs screening for zoonotic infectious diseases, including TB, when animals are imported for exhibition or introduced to new facilities. In terms of human health, local public health clinics evaluate individuals who have been in contact with cattle diagnosed with LTBI using the TST or IGRA. These clinics perform chest radiographs to check for TB but do not conduct LTBI examinations, and they report their findings to the KDCA [14]. However, sometimes inspections and reporting fail to occur due to ineffective information sharing between regional human and animal health departments. The situation described in this report underscores the importance of recognizing TB as a zoonotic disease and provides a direct impetus for promoting intersectoral cooperation among the human, animal, and environmental health sectors in the Republic of Korea. To this end, the tripartite organizations (the KDCA, APQA, and ME) launched the Joint Epidemiological Investigation Manual for Zoonotic TB on May 31, 2023, to enhance collaborative responses across relevant sectors [43]. A key meeting took place on June 27, 2023, to improve coordination and communication between national and regional authorities responsible for human and animal health. The KDCA has also established a new act to facilitate the collection of digital data, which will be linked to the Integrated Tuberculosis Information System and the Korea Animal Health Integrated System [44]. The KDCA's Third National Strategic Plan for Tuberculosis Control (2023–2027) includes the implementation of a TB pathogen genotyping surveillance system, targeting high-risk occupational groups such as veterinarians and zookeepers [45]. Additionally, on June 20, 2023, the organization updated its case report forms to screen occupational risk groups among patients with TB [46].

This study had several limitations. First, we could not determine whether individuals who tested positive for IGRA were infected with *M. bovis*, since IGRA returns a positive result for TB bacterial antigens within the MTBC. Second, due to the small sample size, we cannot rule out the possibility of underestimation or overestimation of the IGRA results. Additionally, the small sample size precluded the performance of statistical significance testing of the IGRA results by variable. Third, given the prolonged incubation period of TB, it is difficult to definitively exclude the chance that individuals who tested positive for IGRA may have acquired the infection before the outbreak. Moreover, as the testing interval increases, so does the likelihood of exposure to various sources, which complicates the generalization of IGRA test results. Therefore, we recommend collecting additional data from high-risk occupational groups to identify cases of TB and LTBI and to conduct a risk factor analysis for *M. bovis* infection.

Conclusion

To our knowledge, this is the first report to document a contact investigation and the use of IGRA to test human contacts of exhibition animals with confirmed *M. bovis* infection. In this Korean study, confirming transmission of *M. bovis* from infected animals to humans was challenging due to the limited number of contacts and a prevalence of LTBI resembling that of the general population. Nonetheless, within the framework of a One Health approach, a clear need exists for surveillance systems and infection control protocols tailored to groups at high risk of occupational exposure. The insights gained from this study are anticipated to inform the development of improved policies and prevention strategies for zoonotic TB.

Notes

Ethics Approval

This study was reviewed and approved by the institutional review board of the KDCA (No: KDCA-2023-04-10-PE-01) and conducted in accordance with the principles of the Declaration of Helsinki. It entailed a full contact investigation in compliance with the Tuberculosis Prevention Act and the Korean national guidelines for tuberculosis control, using data provided by the zoo. The requirement for informed consent was waived, as contacts signed separate consent forms for IGRA testing and LTBI treatment.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

This study was supported by the KDCA of the Republic of Korea (Grant No: 6136-303-210-01).

Availability of Data

All data generated or analyzed during this study are included in the published article. Additional data can be requested from the corresponding author.

Authors' Contributions
Conceptualization: HYL, YK; Data curation: HYL, YK; Formal analysis: HYL; Investigation: HYL, YK, JK; Methodology: HYL, YK, SEL, JK; Project administration: YK, SEL, HC; Software: HYL; Visualization: HYL; Writing–original draft: HYL; Writing–review & editing: all authors. All authors read and approved the final manuscript.

Acknowledgements
We extend our deepest gratitude to the zoo staff and the officials at the local public health clinics for their invaluable cooperation with the contact investigation.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2023.0216
Osong Public Health Res Perspect 2024;15(3):260-264

Brief Report

Replication kinetics and infectivity of SARS-CoV-2 Omicron variant sublineages recovered in the Republic of Korea

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ABSTRACT

Objectives: We analyzed the correlation between the infectivity and transmissibility of the severe acute respiratory syndrome coronavirus 2 Omicron sublineages BA.1, BA. 2, BA.4, and BA.5.
Methods: We assessed viral replication kinetics and infectivity at the cellular level. Nasopharyngeal and oropharyngeal specimens were obtained from patients with coronavirus disease 2019, confirmed using whole-genome sequencing to be caused by the Omicron sublineages BA.1, BA.2, BA.4, or BA.5. These specimens were used to infect Vero E6 cells, derived from monkey kidneys, for the purpose of viral isolation. Viral stocks were then passaged in Vero E6 cells at a multiplicity of infection of 0.01, and culture supernatants were harvested at 12-hour intervals for 72 hours. To evaluate viral replication kinetics, we determined the cycle threshold values of the supernatants using real-time reverse transcription polymerase chain reaction and converted these values to genome copy numbers.
Results: The viral load was comparable between BA.2, BA.4, and BA.5, whereas BA.1 exhibited a lower value. The peak infectious load of BA.4 was approximately 3 times lower than that of BA.2 and BA.5, while the peak load of BA.2 and BA.5 was about 7 times higher than that of BA.1. Notably, BA.1 demonstrated the lowest infectivity over the entire study period.
Conclusion: Our results suggest that the global BA.5 wave may have been amplified by the higher viral replication and infectivity of BA.5 compared to other Omicron sublineages. These analyses could support the rapid assessment of the impact of novel variants on case incidence.

Keywords: Infectivity; Omicron; Replication kinetics; SARS-CoV-2; Transmissibility

Received: August 4, 2023
Revised: February 14, 2024
Accepted: April 2, 2024

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Introduction

Since the first case of the Omicron variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was detected in late November 2021, it has triggered another major global wave

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of coronavirus disease 2019 (COVID-19). The World Health Organization designated Omicron as a variant of concern following an emergency meeting, due to evidence of its rapid transmission [1]. In the Republic of Korea, Omicron was first identified in December 2021 and has since become the dominant variant (https://www.kdca.go.kr/board/board.es?mid=a20501010000&bid=0015&list_no=720694&cg_code=&act=view&nPage=1). Researchers studying the Omicron variant have identified at least 30 mutations in the genes that encode spike proteins, with additional mutations also reported [2–4]. Based on these mutations, several sublineages have been established, including BA.1, BA.2, BA.3, BA.4, and BA.5. The mutations in the Omicron spike protein have been associated with increased transmissibility, immune evasion, and enhanced binding to angiotensin-converting enzyme [5–9], suggesting that Omicron sublineages may be evolving to become more transmissible. In this study, we aimed to investigate the correlation between the infectivity and transmissibility of the Omicron sublineages BA.1, BA.2, BA.4, and BA.5, which have been responsible for distinct COVID-19 waves in the Republic of Korea. We assessed this relationship by examining viral replication kinetics and cellular infectivity.

HIGHLIGHTS

- This study explored the correlation between infectivity and transmissibility among various Omicron sublineages.
- The predominant variant, BA.5, demonstrated a growth advantage as well as immune evasion.
- The high levels of viral replication and infectivity of BA.5 played a key role in the corresponding global wave of coronavirus disease 2019.

Materials and Methods

Specimen Collection and Real-Time Reverse Transcription Polymerase Chain Reaction for SARS-CoV-2

Swab specimens from patients infected with the Omicron sublineages BA.1, BA.2, BA.4, and BA.5 were collected in the Republic of Korea (Table S1). These specimens were subjected to RNA extraction and subsequent real-time reverse transcription polymerase chain reaction (RT-PCR), as previously described [10]. In brief, RNA was extracted from 140 μL of each sample using a Viral RNA Mini Kit (Qiagen) in accordance with the manufacturer's instructions. Real-time RT-PCR was then performed on the extracted RNA to determine the cycle threshold values for the SARS-CoV-2 target gene. All specimens were processed in a biosafety cabinet in accordance with the biosafety guidelines of the Korea Disease Control and Prevention Agency for COVID-19.

Viral Genome Sequencing

For full-genome sequencing, complementary DNA was amplified from total RNA using the QIAseq SARS-CoV-2 Primer Panel and the QIAseq FX DNA Library UDI Kit (Qiagen). Libraries were prepared with the Nextera DNA Flex Library Prep Kit (Illumina), and sequencing was performed on a MiSeq instrument with the MiSeq reagent kit V2 (Illumina), achieving an average genome coverage exceeding 1,000× for all samples. The reads were trimmed and mapped to the reference genome Wuhan-Hu-1 (MN908947.3; GenBank) using CLC Genomics Workbench version 20.0.3 (CLC Bio) [11]. Lineages and clades were assigned using NextClade (v1.7.1) [12] and PANGOLIN [4].

Virus Isolation

Samples were combined with a 1:1 mixture of nystatin (10,000 U/mL) and penicillin-streptomycin (10,000 U/mL) at a 1:4 ratio and incubated at 4 °C for 1 hour. The samples were then centrifuged at 400×*g* for 10 minutes, and the resulting supernatant was used as the inoculum. Vero E6 cells were cultured in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin at 37 °C in 5% CO_2. Twelve hours before infection, the cells were seeded at a density of 1×10^5 cells/well in 12-well plates. On the day of infection, the medium was replaced with DMEM containing 2% fetal bovine serum. A 100-μL aliquot of each sample was used to infect the cells. After 5 days of culture, the cells were harvested. The supernatant was centrifuged at 600×*g* for 10 minutes before collection. This procedure was repeated for secondary infections. Five days after infection, the supernatant was harvested, centrifuged at 600×*g* for 10 minutes, and collected. To quantify viral replication, RNA was extracted from the supernatants of the secondary infections and assessed for the presence of SARS-CoV-2 using real-time RT-PCR.

Viral Replication Kinetics and Infectivity

Vero E6 cells were seeded in 6-well plates at a density of 3×10^5 cells/well. After 12 to 18 hours, the cells were infected at a multiplicity of infection of 0.01 and incubated for 1 hour at 37 °C with frequent agitation. Following incubation, the inoculum was removed, and the cell monolayers were washed once before being incubated in fresh complete medium. Supernatants were harvested at 12, 24, 36, 48, 60, and 72 hours post-infection and stored in aliquots at −80 °C. Viral

replication kinetics and infectivity were analyzed using 4 replicates.

Plaque Assay

Vero E6 cells were seeded as described in the preceding section and subsequently exposed to 10-fold serial dilutions of the supernatants for 1 hour at 37 °C with frequent agitation. After incubation, the inoculum was removed, and the monolayers were washed once with Minimum Essential Medium containing 5% fetal bovine serum, 1% penicillin, and 0.5% agarose. The plates were then incubated for 3 days at 37 °C in an atmosphere of 5% CO_2. After incubation, the plaques were fixed with 4% formaldehyde for 4 hours and subsequently stained with 10% crystal violet for 30 minutes.

Copy Number Measurement

Plasmids carrying the SARS-CoV-2 E gene were utilized as positive controls. A standard curve was established using plasmid concentrations and the following regression equation: y=−3.5705x+39.055, where "y" represents the cycle threshold and "x" denotes the copy number. Viral copy numbers were determined using this equation and expressed as $\log_{10}$ copies/mL [13].

Statistical Analysis

Descriptive analysis was performed using SAS ver. 9.4 (SAS Institute) to compare viral RNA replication copy numbers, as determined by real-time RT-PCR, with viral titers measured by plaque assays among SARS-CoV-2 Omicron variants. Statistical significance was established at 0.05 for all copy numbers and titers.

Ethics Statement

The study received approval from the institutional review board (IRB) of the Korea Disease Control and Prevention Agency (approval number: 2020-03-01-P-A) and was designated as a public health service during the pandemic. Consequently, the IRB waived the requirement for written informed consent from the participants, as outlined in the document "Laboratory Response to COVID-19."

Results

The viral load gradually increased over time, peaking at 72 hours. The peak values were as follows: BA.1, 2.1×10^{11} $\log_{10}$ copies·mL^{-1}; BA.2, 6.8×10^{11} $\log_{10}$ copies·mL^{-1}; BA.4, 6.2×10^{11} $\log_{10}$ copies·mL^{-1}; BA.5, 5.6×10^{11} $\log_{10}$ copies·mL^{-1}. The viral loads for BA.2, BA.4, and BA.5 were similar, whereas BA.1 exhibited a lower value (Figure 1A).

The released infectious particles were titrated to examine viral infectivity over time. The infectious load also increased gradually, reaching its peak at 60 hours (BA.1, 1.6×10^{6} $\log_{10}$ plaque-forming units [PFU]·mL^{-1}; BA.2, 1.1×10^{7} $\log_{10}$ PFU·mL^{-1}; BA.4, 4.0×10^{6} $\log_{10}$ PFU·mL^{-1}; BA.5, 1.1×10^{7} $\log_{10}$ PFU·mL^{-1}). The peak infectious load of BA.4 was approximately 3 times lower than that of BA.2 and BA.5, while the peak load of BA.2 and BA.5 was 7 times higher than that of BA.1. Notably, BA.5 exhibited a high load at both 48 and 72 hours, while BA.1 demonstrated the lowest infectivity over the entire study period (Figure 1B).

Discussion

In this study, we sought to understand the characteristics

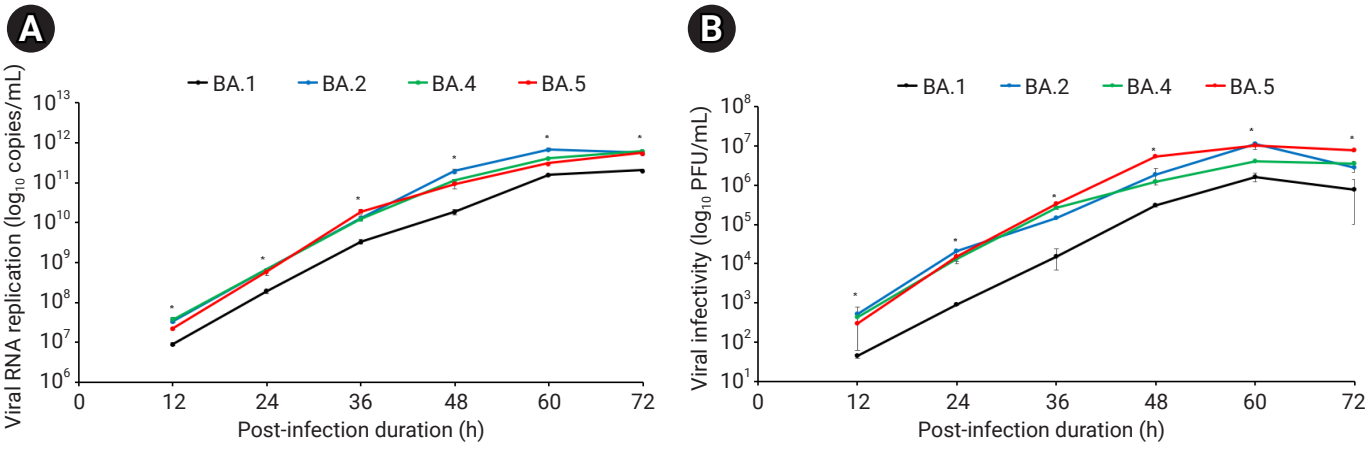

Figure 1. Viral replication kinetics and infectivity in Vero E6 cells infected at a multiplicity of infection of 0.01 for 72 hours. Culture supernatants were harvested at the specified time points. (A) Viral RNA replication copy numbers were determined using real-time reverse transcription polymerase chain reaction. (B) Viral titers were measured through plaque assays. Statistical significance was established at $p<0.05$ for all data (1-way analysis of variance). PFU, plaque-forming unit.

https://doi.org/10.24171/j.phrp.2023.0216

of variants driving waves of COVID-19 by comparing the replication kinetics and infectivity of Omicron sublineages implicated in current and past waves in the Republic of Korea. Relative to BA.1—the first Omicron sublineage—all subsequent sublineages exhibited over 10-fold higher infectivity, indicating that SARS-CoV-2 is adapting to its host and evolving to become more transmissible. Furthermore, BA.2 has been found to have higher viral infectivity than BA.1 in the Calu-3 cell line [14]. However, in this study, BA.4 demonstrated lower infectivity than BA.2 and BA.5, suggesting it is less likely to trigger a global wave of COVID-19. In contrast, the viral infectivity of BA.5 was 3 times greater than that of BA.2 at both 48 and 72 hours after infection, a characteristic that appears key in driving the corresponding global wave. The detection of BA.5 infection was 35.1% faster than that of BA.2 infection, echoing findings from a previous study that reported higher transmissibility for BA.5 [15]. BA.5 also displayed a growth advantage (56%) over BA.4 (17%), BA.2 (−17%), and BA.1 (−36%), which may be attributed to its intrinsic viral advantage, increased transmission, immune escape, and prolonged infectious period. Therefore, BA.5 is expected to remain the predominant variant for some time [16]. Furthermore, BA.5 has been shown to induce neutralizing antibody titers that are 7.5-fold lower than those elicited by BA.1, contributing to immune response evasion and lower efficacy of certain antibody treatments [17,18].

Limitations

SARS-CoV-2 cultures can be performed using various cell lines, including Calu-3, A549, and others. However, Vero E6 cells are commonly utilized for the culture analysis of SARS-CoV-2. Consequently, we used Vero E6 cells in our study. Nevertheless, this choice may represent a limitation; further analysis utilizing various cell lines is warranted.

Conclusion

The recent global wave of BA.5 may be partly due to its higher viral replication and infectivity compared to other Omicron sublineages. However, analyzing replication and infectivity at the cellular level does not provide definitive evidence of how these properties influence the magnitude of a real-world outbreak. Therefore, it is necessary to further examine population-level immunity and epidemiological relationships to better understand the immune evasion properties of variants.

Supplementary Material

Table S1. Deposit number of patients infected with 4 severe acute respiratory syndrome coronavirus 2 Omicron variant sublineages in the Republic of Korea. Supplementary data are available at https://doi.org/10.24171/j.phrp.2023.0216.

Notes

Ethics Approval

All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Declaration of Helsinki, including its later amendments or comparable ethical standards. The study received approval from the IRB of the Korea Disease Control and Prevention Agency (approval number: 2020-03-01-P-A) and was designated as a public health service during the pandemic. Consequently, the IRB waived the requirement for written informed consent from the participants, as outlined in the document "Laboratory Response to COVID-19."

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

This work was supported by the Korea Disease Control and Prevention Agency (grant number: 6300-6331-301).

Availability of Data

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request. The whole-genome sequences of SARS-CoV-2 are accessible in the Global Initiative on Sharing All Influenza Data database (accession IDs: EPI_ISL_6959993, 13086512, 13086515, and 13086516).

Authors' Contributions

Conceptualization: JMK, JER, EJK; Funding acquisition: CKY, EJK; Investigation: JMK, DK; Methodology: JMK, DK; Software: JMK; Validation: all authors; Writing–original draft: all authors; Writing–review & editing: all authors. All authors read and approved the final manuscript.

Additional Contributions

The authors extend their gratitude to everyone who assisted with the collection and transportation of patient specimens, as well as to the staff at the Korea Disease Control and Prevention Agency, local government officials, and private testing agencies for their contributions to controlling COVID-19.

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https://doi.org/10.24171/j.phrp.2023.0216

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Enacted January 1, 2010
Last revised June 1, 2024

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- **Introduction** should provide concise yet sufficient background information about the study to provide the readers with a better understanding of the study, avoiding a detailed literature survey or a summary of the results.
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text, kept to a minimum, and should not be repeated. Supplementary materials and other details can be separately presented in an appendix. The authors should state the statistical method used to analyze the results (statistical significance of differences) with the probability values given in parentheses.

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Please refer to the following examples.

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

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Appendix and Supplemental Data

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

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After the paper has been accepted for publication, the author(s) should submit the final version of the manuscript. The names and affiliations of the authors should be double-checked, and if the originally submitted image files were of poor resolution, higher-resolution image files should be submitted at this time. Symbols (e.g., circles, triangles, squares), letters (e.g., words, abbreviations), and numbers should be large enough to be legible on reduction to the journal's column widths. All symbols must be defined in the figure caption. If references, tables, or figures are moved, added, or deleted during the revision process, renumber them to reflect such changes so that all tables, references, and figures are cited in numeric order.

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

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Tables and Figures

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

References

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