

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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How to transform the medical care system after the COVID-19 pandemic

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After the coronavirus disease 2019 (COVID-19) pandemic crisis subsided, social restrictions were lifted and daily life began to return to normal. However, several issues in the healthcare sector that had been dormant are now resurfacing. The post-pandemic situation has not served as a “strategic inflection point” to improve our healthcare system—instead, we are witnessing a reversion to the previous state, as if the crisis never occurred. As Professor Lawrence Gostin, who visited last month, has been pointed out in his book, *Global Health Law*, a comprehensive COVID-19 response involves a medical component, a public health strategy, and an approach that addresses social determinants. To find fundamental solutions, we need good governance, social commitment, and a focus on human rights, considering equity as the primary lever for closing gaps and envisioning a transformation toward a sustainable healthcare system. However, the shortage of medical personnel looms large, overshadowing all other discussions.

First, we must acknowledge the significance of investigating excess mortality [1]. According to the 2022 Cause of Death Statistics from Statistics Korea, 31,280 deaths were attributed to COVID-19 (8.4% of all deaths that year). The mortality rate soared by 522.8% to 61.0 per 100,000 people compared to the previous year, with the highest rates observed in individuals over 80 years of age. The rise in deaths from Alzheimer disease (45.6%), diabetes (24.9%), hypertensive diseases (24.2%), pneumonia (17.3%), and cerebrovascular diseases (12.6%) may be viewed as indirect consequences, although the particularly sharp increase in Alzheimer’s-related deaths is difficult to categorize in this way. The potential for undiagnosed COVID-19 cases in nursing homes, long-term care hospitals, and mental health hospitals should also be considered. Excess mortality is estimated at 8.7%, potentially reflecting challenges in healthcare access, emergency care systems, and intensive care management [2]. Therefore, in addition to addressing diagnostic challenges, it is necessary to identify discrepancies between projected and actual figures to develop effective solutions.

Second, after the COVID-19 pandemic, there have been concerns regarding the shortage of physicians for essential medical care, regional disparities in physician distribution, and related excess mortality. Discussions aimed at resolving these issues have been ongoing since 2013, but progress has been hindered by differing definitions of the problem and perceptions of its urgency. Statistics and various indicators must be developed, and it is necessary to integrate and manage medical college education, resident training, and continuing medical education to determine the appropriate number of medical doctors needed by our society. The prolonged COVID-19 pandemic has also highlighted the importance of ensuring the

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stability and functionality of primary care even during outbreaks of highly pathogenic infections. The primary care system should involve holistic management encompassing the initial diagnosis, treatment, recovery, and prevention of secondary infections in the household, as well as the responsible management of chronic diseases such as diabetes and hypertension through outpatient and home visits. However, the current training is predominantly hospital-centered. Additionally, the use of hospital residents as inexpensive labor rather than for educational purposes has distorted the medical care system. Approximately 3,000 residents have left hospitals over the past 10 years due to shortened education terms, exacerbating the workload in university hospitals [3]. Instead of relying on residents, more specialists should be employed. The policy of subsidizing residents' wages has led hospitals to refrain from hiring specialists, resulting in a decline in academic development and a decrease in the quality of education due to heavy workloads. This issue, which is separate from the medical school quota debate, could be addressed by increasing the number of supervising specialists in hospitals. This issue is also tied to the quality of healthcare and the need to employ essential medical personnel such as primary care providers, as some opt to work in local healthcare without formal training. Restructuring undergraduate and resident education to prioritize community-centered primary healthcare education and training, rather than focusing on university hospitals, and supporting this system—including night consultations, home visits, medical consultations, emergency transportation, referral systems, and livelihood support—will transform primary care into a patient- and community-centered system.

Third, improvement is needed in the traditional vertical health program, which has focused on constructing and operating single disease control, such as tuberculosis hospital before 2000, but in COVID-19 isolation and quarantine purpose hospital to address single health issues. During the COVID-19 pandemic, the scarcity of patient isolation facilities resulted in the repurposing of public hospital beds as isolation wards. After the COVID-19 crisis subsided, these hospitals struggled to resume their roles as central regional or district hospitals. The reorganization of hospital functions to accommodate infectious patients led to the discharge or transfer of patients with other chronic conditions and reduced activity in some departments. Therefore, medical personnel were reassigned to different departments or left their positions. However, post-crisis, the inability to recruit new medical staff prevented these hospitals from restoring their previous functionality and fully meeting regional medical needs. Theoretically, hospitals dedicated to

infectious diseases are beneficial for containing outbreaks and treating critically ill infectious patients. However, when outbreaks expand, the patient load can exceed their capacity, leading to collateral deaths and making it challenging for these hospitals to continue functioning post-crisis. Therefore, future improvements in this area should be considered, along with improvements in the isolation and quarantine method and diagnostic test system, which is centered around public health centers and operates separately from the traditional medical care system. In 2010, the tuberculosis management system transitioned from a public health center-based program to a public-private mix that involves private hospitals. This transition did not increase the number of tuberculosis management personnel in public health centers, but rather integrated them into general hospitals. The approach of managing symptomatic patients and their families with tracing, testing, isolation, quarantine, and chemoprophylaxis hospital-based approach has provided valuable lessons for the management of other infectious diseases, including those that are drug-resistant and severe respiratory disease, as well as the prevention of healthcare-associated infections. Despite its public health importance and effectiveness, the isolation-centered management system in COVID-19 pandemic has struggled to respond during periods of high demand, limiting its utility for diverse patient management. Therefore, developing strategies to overcome these limitations is necessary.

Notes

Ethics Approval

Not applicable.

Conflicts of Interest

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Global prevalence of enterobiasis in young children over the past 20 years: a systematic review and meta-analysis

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ABSTRACT

Parasitic infections are the most common diseases worldwide, and enterobiasis is a common parasitic infection in children. Various studies have reported on the prevalence of *Enterobius vermicularis* in different regions of the world. However, no study has gathered and analyzed this data systematically. Our systematic review and meta-analysis investigated the overall prevalence of *E. vermicularis* among children globally. Data were extracted from 4 available databases for studies published from January 2002 to April 2022. The quality of the included studies was scored based on the standard Strengthening the Reporting of Observational Studies in Epidemiology. A random-effect model was chosen to calculate the pooled prevalence and corresponding 95% confidence interval (CI) according to the degree of heterogeneity in the included studies. Thus, 40 publications (42 data sets) that included 3,279 children with enterobiasis met all criteria and were included in the analysis. The meta-analysis showed that heterogeneity among the included studies was high ($Q=4,399.35$, $I^2=99.96\%$; $df=41$; $p<0.001$). The pooled global prevalence of enterobiasis among the studied children was 12.9% (95% CI, 8.2%–17.7%). Our systematic review and meta-analysis estimated that, for the past 20 years, 12.9% of children around the world have been infected with *E. vermicularis*.

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Keywords: Child; Enterobiasis; Enterobius; Prevalence

Introduction

Enterobiasis or oxyuriasis, a nematode infection caused by *Enterobius vermicularis* (*E. vermicularis*, pinworm, oxyure) is common among children and their family members [1]. *E. vermicularis* is a cosmopolitan parasite and one of the most common parasitic infections in many countries [2]. The World Health Organization reported that the prevalence of enterobiasis in children is between 4% and 28% [3]. It has been estimated that approximately 200 million people are infected worldwide, and over 30% of cases are children aged 5 to 10 years [4]. The prevalence of oxyuriasis among children has been reported as 2.5% to 45% in Latin America [5,6], 18% in Norway [7], 18.5% in the Republic of Korea [8], 17.2% in Iran [9], and 2.9% in north-central Ethiopia [10].

Some patients with enterobiasis are asymptomatic, while others, especially children, may show symptoms such as perianal pruritus, restlessness, loss of appetite, malnutrition, anemia, insomnia, and irritability. Ectopic enterobiasis can penetrate the kidneys and fallopian tubes, leading to severe health disorders and even death [11,12].

Although there are multiple ways to transmit enterobiasis, including the fecal-oral route, inhalation, auto-infection, and retrograde infection [13], the main route of transmission for *E. vermicularis* is direct contact between infected and uninfected individuals. Therefore, children in crowded environments such as kindergartens, schools, orphanages, and mental institutions are most susceptible to this infection [14]. The prevalence of this infection is mainly related to public health and personal hygiene [9]. Therefore, surveying enterobiasis infections in children can help us assess personal, familial, and social health status. The identification and prevention of pinworm transmission among children can promote infection control and benefit the health of children and the community [9].

There are many publications regarding the prevalence of *E. vermicularis* infection among children. However, there are no comprehensive studies describing the status of enterobiasis infection in children globally. In the present systematic review and meta-analysis, we investigated the global pooled prevalence of *E. vermicularis* among young children.

Materials and Methods

Design

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines [15]. It also followed recommendations from the Cochrane Collaboration

HIGHLIGHTS

- We analyzed data on 60,167,000 children from 42 data sets
- The global prevalence of *Enterobius vermicularis* among the children studied was 12.9%.
- The highest prevalence rate of *E. vermicularis* infection (34.7%) was observed in the primary school group.

Handbook of Systematic Reviews [15] for the systematic, transparent, and reproducible investigation of scientific evidence.

Search Strategy

Two independent investigators (S.D., E.K.L.) searched 4 international databases (PubMed, Scopus, Science Direct, and ProQuest) for published studies that investigated the prevalence of *E. vermicularis* in children. English language publications between 2002 and 2022, with mesh terms ("*Enterobius vermicularis*" OR *E. vermicularis* OR Oxyuris OR pinworm OR roundworm OR seatworm) AND (Prevalence OR Rate) AND (Children OR Preschool OR Kindergarten), were collected. In addition, the reference lists of the identified articles were manually searched. We included studies conducted in the last 2 decades (01 January 2002 to 24 April 2022), published in English, and relevant to the aim of the study.

Eligibility Criteria

The eligibility criteria for the study were determined using the population, intervention, comparison, outcome, and study classification design (Table 1). The inclusion criteria were (1) cross-sectional studies that estimated the positive rate of *E. vermicularis* in children, (2) studies published online between January 2002 and April 2022, (3) original research papers, (4) studies published in English, (5) articles with full text, (6) studies that provided the total sample size and positive samples, and (7) studies that had a clear test method. Articles that did not meet these criteria were excluded. In addition, we contacted the corresponding authors to obtain more information if the data was incomplete. Reviews, case reports, letters to editors, and commentaries were not included in the study, but were used to enhance the search sensitivity to include any missed studies.

Table 1. Eligibility criteria for a systematic review and meta-analysis of the global prevalence of enterobiasis in children

Category	Inclusion	Exclusion
Population	Studies that reported the prevalence of <i>Enterobius vermicularis</i> exposure in children population	Studies that reported the prevalence of <i>E. vermicularis</i> exposure in adults or appendicitis cases
Exposure	Children with enterobiasis	
Comparator	None	
Outcomes	True seroprevalence of children exposed to enterobiasis worldwide	
Study type	Cross-sectional	

Study Selection

The 2-step study selection process included a title/abstract reading phase and a full-text reading phase. All search records were imported to EndNote software ver. X7.0.1 (Clarivate). All duplicates and irrelevant papers were excluded after the screening of titles/abstracts by 2 independent reviewers (S.D., E.K.L.). Any discrepancies were resolved by a third reviewer (S.A.H.). In the second phase, the full-text articles were downloaded and meticulously evaluated and discussed with 2 other reviewers and, if necessary, a third reviewer was consulted. Study selection was performed in accordance with the PRISMA flowchart.

Data Extraction

The data in each study were extracted independently by 2 reviewers (S.D., E.K.L.), using Microsoft Excel ver. 16.39 (Microsoft Corp.). Discrepancies were resolved by a third reviewer (A.M.). We included information on title, first author, year of publication, continent and country, sample size, number of positive samples, subject age and gender, and diagnostic method.

Quality Assessment

The quality of the included studies was scored using the standard Strengthening the Reporting of Observational Studies in Epidemiology (STROBE). A STROBE score of 25.6 to 34 indicated a high-quality study, and studies with scores of 16.6 to 25.5 and ≤ 16.5 were considered moderate and low quality, respectively. The articles included in our meta-analysis were deemed to have acceptable quality.

Data Analysis

We used Stata ver. 15.0 (Stata Corp.) for all statistical analyses. The heterogeneity among studies was calculated using both the Cochran Q test and the I^2 statistic, with a cutoff at 50% to define a statistically significant degree of heterogeneity. The random-effect model was chosen according to the degree of heterogeneity in the included studies to calculate the pooled prevalence and corresponding 95% confidence interval (CI). To investigate the effect of different variables

on heterogeneity, we did a subgroup analysis stratifying participants based on continent, age group, and publication decade. In addition, a funnel plot and an Egger test were used to assess the publication bias of selected studies.

Ethics Approval

This study received approval from the Qazvin University of Medical Sciences Ethical Committee Iran under the contract no. IR.QUMS.REC.1401.291.

Results

A flow chart of the study search and selection process for inclusion is shown in Figure 1. In our study, a total of 3,508 publications were searched. After removing duplicate articles, the titles and abstracts of the remaining 3,097 publications were screened. Finally, a total of 117 articles were selected for full paper review, of which 40 publications (42 data sets) fulfilled all criteria and were suitable for inclusion in the analysis. Among 60,176 children surveyed in these 40 publications, 3,279 children had enterobiasis.

Most of the included publications originated in Asia (32 out of 42, 76.2%). Five were from South America (11.9%), 2 from Africa (4.8%), 2 from Europe (4.8%), and 1 from North America (2.4%). Among the countries, Iran and The Republic of Korea had the most studies with 8 (19.0%) and 7 (16.7%) studies, respectively. The main characteristics of the included studies are summarized in Table 2 [8,11,16–53].

Among the included studies, 14 investigated oxyuriasis in kindergarten children, 14 investigated preschool populations, and the remaining 7 studies investigated oxyuriasis in general child populations. Four were studies of children referred to medical clinics. Two studies were based on primary school children, and 1 studied child in a random population.

The diagnostic methods used to detect *E. vermicularis* infection across studies were: cellophane tape swab (33 articles), cellophane anal swab (1 article), formalin-ethyl acetate concentration (3 articles), cellophane tape and formalin-ether concentration (1 article), microscopic formalin-ether

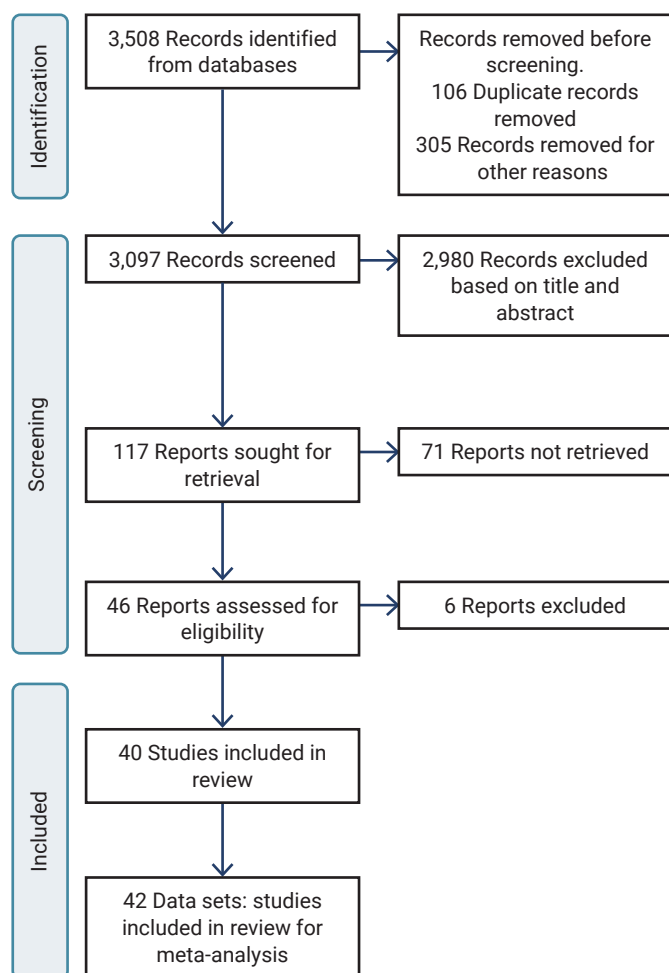


Figure 1. Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) flowchart of literature selection to analyze the worldwide prevalence of enterobiasis in children.

concentration (1 article), the Lutz method (1 article), the Kato-Katz-direct smears (1 article), and the Kato-Katz method (1 article).

The meta-analysis showed that heterogeneity among the included studies was very high ($Q=4,399.35$; $I^2=99.96\%$; $df=41$; $p<0.001$). Since the heterogeneity was significant, a random-effects model was used to estimate the pooled prevalence of enterobiasis among kindergarten and preschool children globally. In addition, the funnel plot and bias coefficient diagram did not show the presence of publication bias ($b=-1.25$; 95% CI, -0.0002 to 4.69 ; $p=0.21$) (Figure 2).

Using the random effect method, the pooled global prevalence of enterobiasis among the children studied was 12.9% (95% CI, 8.2%–17.7%). The global pooled and weighted prevalence of enterobiasis among children based on geographic location was as follows (Figure 3): Europe 24.9% (95% CI, 0%–64%), South America 14.3% (95% CI, 0%–36.4%), Asia 13%

Table 2. Characteristics of studies investigating the prevalence of enterobiasis in children

No.	Study	Publication year	Country	Population group	Age group (y)	Sample size (n)	Positive (n)	Diagnostic methods	STROBE score
1	Changsap et al. [17]	2002	Thailand	Primary school children	5, 6	1,139	255	Cellophane tape swab	17.5
2	Song et al. [18]	2003	Republic of Korea	Preschool children	≤5	552	35	Cellophane tape swab	26
3	Nithikathkul et al. [19]	2003	Thailand	Children	1–6	42	5	Cellophane tape swab	20
4	Tashima and Simoes [20]	2004	Brazil	Children	1–4	420	1	Cellophane tape swab	18
5	Park et al. [8]	2005	Republic of Korea	Kindergartens and primary schools	3–6	365	70	Cellophane tape swab	23
6	Tang and Luo [21]	2005	China	Children	2–6	1,749	136	Kato-Katz method	17
7	Cazorla et al. [22]	2006	Venezuela	Preschool and school children	2–6	241	143	Cellophane tape swab	26
8	de Carvalho et al. [23]	2006	Brazil	School children	0–6	279	28	Cellophane tape swab	25
9	Kang et al. [16]	2006	Republic of Korea	Preschool children	0–6	1,507	119	Cellophane tape swab	21
10	Remm [24]	2006	Estonia	Nursery school children	1–6	372	167	Cellophane tape swab	29
11	Muge et al. [25]	2008	Turkey	Preschool children	5, 6	797	37	Cellophane tape anal swab	19
12	Kim et al. [26]	2010	Republic of Korea	Kindergartens children	1–5	695	96	Cellophane tape swab	26
13	Goncalves et al. [27]	2011	Brazil	Preschool children	0.5–6	133	2	Lutz method	26
14	Hong et al. [28]	2011	Republic of Korea	Preschool children	0–6	5,704	209	Cellophane tape swab	25
15	Lee et al. [29]	2011	Republic of Korea	Preschool children	2–6	896	52	Cellophane tape swab	28
16	Rashid et al. [30]	2011	Bangladesh	Children	1–6	52	8	Cellophane tape swab	25

(Continued to the next page)

Table 2. Continued

No.	Study	Publication year	Country	Population group	Age group (y)	Sample size (n)	Positive (n)	Diagnostic methods	STROBE score
17	Masucci et al. [31]	2011	Italy	Patient population	<5	387	1	Formalin-ethyl acetate concentration	32
18	Sayasone et al. [32]	2011	Laos	Random population	<5	116	3	Formalin-ethyl acetate concentration	34
19	Canete et al. [33]	2012	Cuba	Children who attend a day care center	0.5–5	104	2	Cellophane tape swab	31
20	Chu et al. [34]	2012	Taiwan	Preschool children	1–6	6,661	30	Cellophane tape swab	21
21	Ebrahimzadeh et al. [35]	2014	Iran	Preschool children among kindergartens	1–6	907	219	Cellophane tape swab	21
22	Jiang and Li [36]	2013	China	Kindergarten children	1–6	1,088	12	Cellophane tape swab	20
23	Kim et al. [37]	2013	Republic of Korea	Kindergarten children	1–6	3,422	205	Cellophane tape swab	22
24	Hegazy et al. [38]	2014	Egypt	Preschool children	2–6	500	17	Cellophane tape swab	25
25	Chai et al. [39]	2015	Myanmar	Primary school children	5–6	761	359	Cellophane tape swab	28
26	Ventur Doni et al. [40]	2015	Turkey	Children	0–6	333	126	Cellophane tape swab	32
27	Li et al. [11]	2015	China	Children	2–6	508	295	Cellophane tape swab	22
28	Afrakhteh et al. [41]	2016	Iran	Kindergarten and preschool children	2–6	462	33	Cellophane tape swab	20
29	Amiri et al. [42]	2016	Iran	Kindergarten children	2–6	126	28	Cellophane tape swab	25
30	Anuar et al. [43]	2016	Malaysia	Kindergarten and preschool children	1–6	136	17	Cellophane tape swab	25
31	Haratipour et al. [44]	2016	Iran	Kindergarten children	4–6	1,850	230	Cellophane tape and formalin-ether concentration	23
32	Mansourian et al. [45]	2016	Iran	Kindergarten children	2–6	175	61	Cellophane tape swab	24
33	Shahdoust et al. [46]	2016	Iran	Individuals referred to medical centers	0–6	820	4	Microscopic formalin-ether concentration	25
34	Wang et al. [47]	2016	China	Preschool children	2–6	510	65	Cellophane tape swab	27
35	Wang et al. [47]	2016	China	Preschool children	2–6	1,734	89	Cellophane tape swab	27
36	Sakari et al. [48]	2017	Kenya	Preschool children	2–5	361	3	Kato Katz-direct smears	33
37	Chen et al. [49]	2018	Taiwan	Kindergarten and preschool children	1–6	22,776	57	Cellophane tape swab	24
38	Dudlova et al. [50]	2018	Slovakia	Preschool children	3–6	159	8	Cellophane tape swab	24
39	Goes et al. [51]	2019	Brazil	Preschool children	1–6	121	1	Formalin-ethyl acetate concentration	25
40	Khazaal et al. [52]	2020	Iraq	Schools, kindergartens, pediatric hospitals	2–6	216	24	Cellophane tape swab	22
41	Tavan et al. [53]	2020	Iran	Kindergarten children	4–6	500	15	Cellophane tape swab	21
42	Tavan et al. [53]	2020	Iran	Kindergarten children	4–6	500	12	Cellophane tape swab	21

STROBE, Strengthening the Reporting of Observational Studies in Epidemiology.

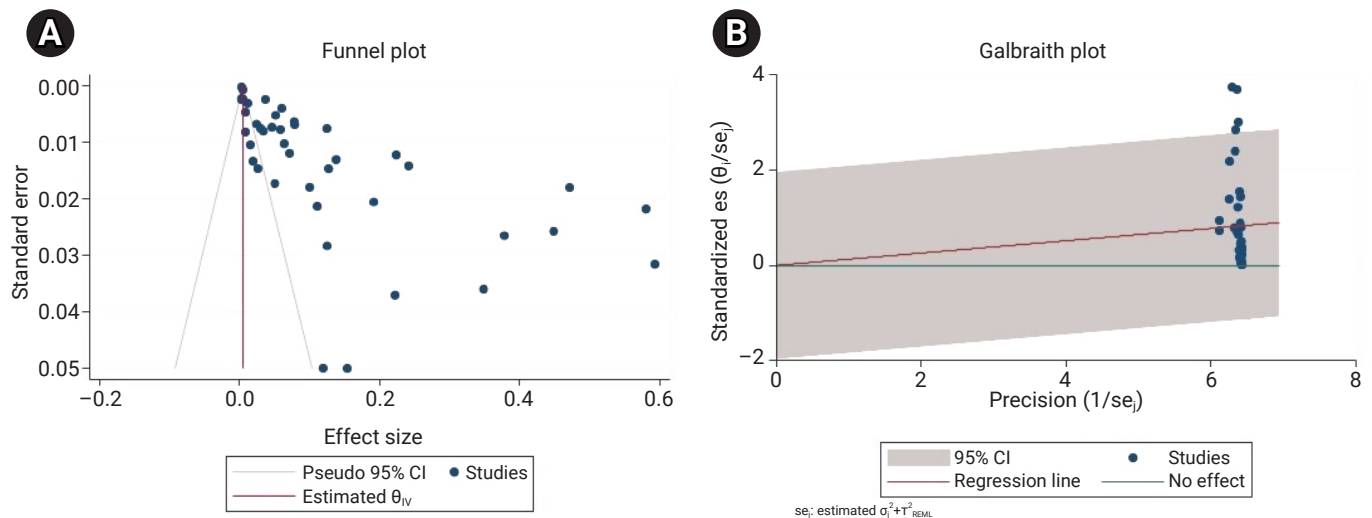


Figure 2. Bias assessment plots for the global prevalence of enterobiasis among the subjects studied. (A) Funnel plot for detecting publication bias. (B) Galbraith plot for detecting publication bias. CI, confidence interval.

(95% CI, 8.1%–17.9%), Africa 2% (95% CI, 0%–4.5%), and North America 1.9% (95% CI, 0%–4.6%).

According to our meta-analysis, the primary school group exhibited the highest pooled prevalence of *E. vermicularis* infection, with a prevalence rate of 34.7% (95% CI, 10.5%–59%), followed by 20.2% (95% CI, 4.9%–35.5%) in the general child population group, 14.1% (95% CI, 0%–34.7%) in children referred to medical clinics, 11.2% (95% CI, 5.9%–16.5%) in kindergarten children, 8.2% (95% CI, 0.6%–15.8%) in preschool children, and 2.6% (95% CI, 0%–5.5%) in the random population group.

Discussion

To the best of our knowledge, this is the first systematic review and meta-analysis to pool the global prevalence of enterobiasis in children. The current review included studies published between 2002 and 2022 that reported on the epidemiology of *E. vermicularis* infection in children worldwide. According to our review results, the global pooled estimated of children infected with enterobiasis was 12.9 %.

Several regional studies on the prevalence of *Enterobius* infection have been conducted [4], showing enterobiasis prevalence among school children as follows: approximately 55% in China, 8.8% in Thailand, 47.2% in Myanmar, 4.4% in the Republic of Korea, and 19.3% in Kyrgyzstan (Asia); 26.3% in Tanzania, 1.7% in Angola, and 11.7% in Nigeria (Africa); 35% in Chile and 19% in Argentina (South America); and 17.4% in Germany.

Pinworm infection is transmitted through direct contact

with infected persons or objects. The worm is transmitted through ingestion of eggs, from the anus to the finger, fingernails, or hands when a patient scratches the perianal area where the gravid female worms emerge and deposit eggs [54]. Indirect aerosol transmission has also occurred in humans; the microscopic eggs can be released in the air and inhaled with dust [55]. Socioeconomic status, personal hygiene habits, and the environment are also important factors in the spread or prevention of *E. vermicularis* infection [56], contributing to its significance as a public health problem.

Our data revealed a higher prevalence of *E. vermicularis* in younger children who lacked knowledge and understanding of this infection, its risk factors, and prevention. The major risk factors for enterobiasis include the personal hygiene habits of children, such as thumb-sucking and putting toys into their mouths; the overcrowded conditions in schools, kindergartens, and childcare centers; and inadequate sanitation. The risk factors for pinworm infection may vary in different countries [9,54]. According to a study conducted in Yemen, war led to increased pollution in the water and food supplies and worsened sanitary disposal systems and housing. These circumstances, along with a lack of health awareness by the majority of parents and affected children were the main risk factors for disease [4].

The diagnosis of enterobiasis is based on finding adult worms or eggs using the scotch tape technique or by stool examination, which is less sensitive. The scotch tape technique is the gold standard method for detection of enterobiasis in children because it is practical, easy, and inexpensive [13,57]. The fact that the worm's eggs are sticky and adhere to the

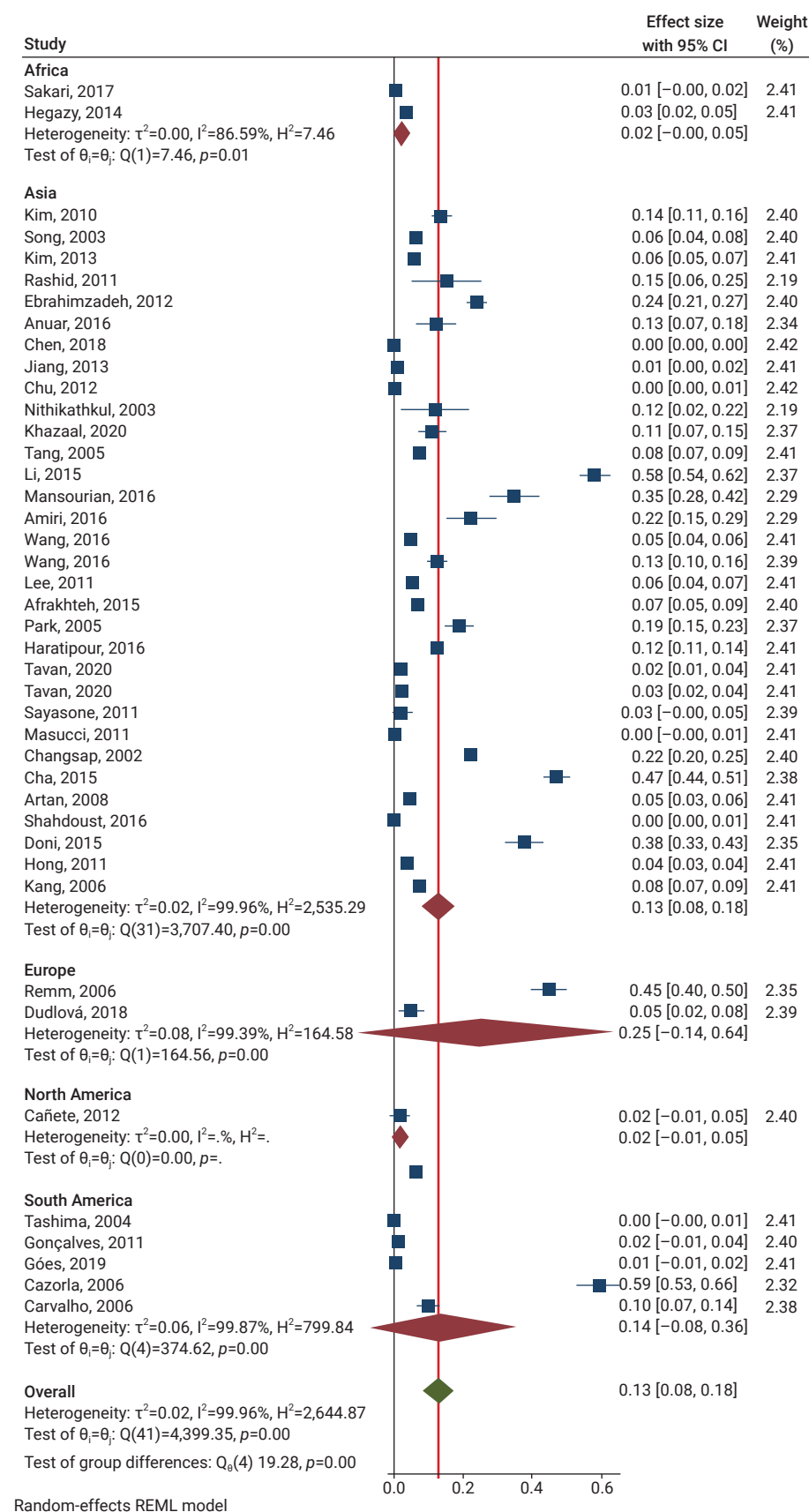


Figure 3. Forest plot of the global prevalence of enterobiasis among the subjects studied using the random effects method.
CI, confidence interval; REML, residual or restricted maximum likelihood.

perianal skin [4] is consistent with the lower prevalence rates we found in studies that detected eggs in stool samples rather than using the scotch tape technique.

For effective infection control and health promotion in children, it is important to identify the factors that help identify parasite transmission as well as aid in its prevention [9]. Although *Enterobius* is susceptible to some anthelmintic drugs including mebendazole and albendazole, these drugs only kill the adult worm and are not effective against eggs and larvae. The main point is that medical treatment is not sufficient to cure and control enterobiasis and does not prevent re-infection [13].

To reduce prevalence rates, effective health promotion includes increasing the awareness of the child and family. Because of the high prevalence of re-infection with enterobiasis, health education and screening programs must be provided for the children, teachers, and parents who gather in crowded places like schools, kindergartens, and daycare centers [9]. One of the most effective control strategies for *Enterobius* infection is to promote knowledge among families, especially mothers and children, and change their hygiene behaviors [56].

Various studies have confirmed that increasing knowledge and the hygiene practice levels of families is a suitable control strategy. In Egypt, the Republic of Korea, and Cameroon, decreasing prevalence rates have been reported as a result of health education provided to mothers, the distribution of educational brochures to the children and their families, and visual educational cards, respectively [16,56,58]. As a result of control and screening programs, a decreasing trend in the prevalence rate of *E. vermicularis* infection has been reported in some countries [9]. In the Republic of Korea and Greece, the prevalence rates dropped from 17.1% to 7.9% and 22.1% to 5.2%, respectively. In Turkey, another study reported the infection rate from 1985 to 2000 was 45.9%, while it was 16% from 2000 to 2008, similar to our findings [16,59–61].

Our systematic review and meta-analysis had certain limitations: (1) lack of uniform sample size, (2) differing diagnostic methods with varied sensitivity and specificity, and (3) heterogeneity in our review results due to the heterogeneity of different articles. These factors may have biased the prevalence of *E. vermicularis* infection in the study population.

Conclusion

Although there were many country-specific studies on the prevalence of enterobiasis in children, there were no comprehensive studies describing the status of enterobiasis infection in children globally. Our systematic review and

meta-analysis estimated that 12.9% of children around the world are infected with *E. vermicularis*. Health education for children and their families is a cost-effective and safe control strategy that can decrease the burden of enterobiasis infection. Infection, especially enterobiasis, increases the financial burden of preventative medicine programs, part of which must be paid by the government. Therefore, it is necessary that health policymakers establish effective screening and training programs to eliminate enterobiasis.

Notes

Ethics Approval

This study received approval from the Qazvin University of Medical Sciences Ethical Committee Iran under the contract no. IR.QUMS.REC.1401.291.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

All data generated or analyzed during this study are included in this published article.

Authors' Contributions

Conceptualization: SD, EKL; Data curation: BH, KT; Software: SAH, AJ; Interpretation of data: AM, AT; Writing—original draft: SAH, BH, KT, AJ; Writing—review & editing: AM, SD, EKL, AT. All authors read and approved the final manuscript.

Additional Contributions

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The value of CDC42 effector protein 2 as a novel prognostic biomarker in liver hepatocellular carcinoma: a comprehensive data analysis

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ABSTRACT

Objectives: The prognostic significance of CDC42 effector protein 2 (CDC42EP2) and its association with tumor-infiltrating immune cells (TIICs) have not been explored in liver hepatocellular carcinoma (LIHC). This study aims to assess the potential prognostic value of CDC42EP2 by conducting a comprehensive analysis of online databases pertaining to LIHC.

Methods: We evaluated the potential of CDC42EP2 as a prognostic biomarker by utilizing online databases such as TIMER, GEPIA2, KM, OSlihc, HPA, and LinkedOmics.

Results: In LIHC, we observed that the mRNA and protein expression of CDC42EP2 were upregulated compared to normal tissues. Upregulated CDC42EP2 expression was associated with a worse prognosis based on the clinicopathological characteristics of patients with LIHC. Furthermore, CDC42EP2 was positively associated with TIICs. In the co-expression and functional enrichment analyses of CDC42EP2, 11,416 genes showed positive associations with CDC42EP2 while 8,008 genes showed negative associations. CDC42EP2-related co-expression genes were involved in protein localization to the endoplasmic reticulum, translational initiation, and RNA catabolic processes in gene set enrichment analysis-Gene Ontology (GSEA-GO), and regulated the ribosome, spliceosome, and primary immune deficiency in the GSEA-Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. In a survival map, 23 and 17 genes that exhibited positive associations with CDC42EP2 showed a significant hazard ratio (HR) for overall survival and disease-free survival, respectively.

Conclusion: Our findings demonstrated that CDC42EP2 is a novel prognostic biomarker and a potential tumor immune therapeutic target in patients with LIHC.

Keywords: Immune cells; Liver hepatocellular carcinoma; Mutation; Prognosis

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Introduction

In 2020, liver cancer was identified as the third leading cause of cancer-related deaths worldwide, presenting a significant global health challenge. By 2025, it is estimated that there

will be 1 million cases. Liver hepatocellular carcinoma (LIHC) is the most prevalent histological subtype of liver cancer [1]. LIHC ranks among the most common malignant tumors globally and is the primary cause of death in patients with cirrhosis [2,3]. It is associated with well-defined risk factors, such as hepatitis B and C viruses, excessive alcohol consumption, metabolic syndrome, and diabetes [4]. Over the past decade, significant progress in biochemical, pathological, and technical methods has enhanced the early diagnosis and treatment of LIHC, leading to better survival rates. These advancements have shed light on the molecular pathogenesis of LIHC; however, the therapeutic options currently available are still limited. The cure rate for LIHC is disappointingly low due to its aggressive nature, high recurrence rate, increased risk of metastasis, and poor response to chemotherapy. Additionally, achieving an early, definitive diagnosis of LIHC is often difficult. The majority of patients present with advanced-stage LIHC and have a dismal prognosis [5]. Despite recent progress in understanding the molecular etiology of LIHC, which has led to the development of new approved drugs, treatment options for advanced stages are scarce. Consequently, there is a pressing need for novel therapies for LIHC. The development of a new prognostic biomarker is crucial to enhance early diagnosis and improve the survival rates of patients with LIHC. In recent years, tumor immunotherapy has emerged as a promising new therapeutic strategy for LIHC.

The immune system plays a crucial role in controlling cancer progression [6]. A previous study has demonstrated that tumor-infiltrating immune cells (TIICs) can help the host resist the development of cancer cells and solid tumors [7]. TIICs are a significant focus in cancer research [8]. The density and type of TIICs have a strong correlation with clinical outcomes of tumors and the efficacy of immunotherapy [9–12]. Some studies have highlighted the characteristics of the immune response and its link to prognosis [13,14]. The prognostic importance of TIICs and immune molecules, such as tumor-associated dendritic cells, macrophages, and natural killer cells, has been particularly noted in LIHC [15–17]. The TP53 mutation is common across various cancers and is associated with clinical prognostic outcomes [18,19]. However, the mechanisms by which TP53 mutations influence the relationship between CDC42 effector protein 2 (CDC42EP2) and TIICs remain unclear. CTNNB1 mutations occur in about 19% to 26% of LIHC patients, and these mutations are linked to immunological exclusion. Yet, systematic studies of TIICs in LIHC with CTNNB1 mutations and the mechanism by which these mutations cause immunological exclusion have not been conducted. In this context, we examined the correlation between CDC42EP2 and TIICs based on

HIGHLIGHTS

- Upregulated CDC42 effector protein 2 (CDC42EP2) is correlated with poor prognosis, depending on various clinicopathological factors in liver hepatocellular carcinoma (LIHC).
- CDC42EP2 is positively associated with tumor-infiltrating immune cells (TIICs) such as B cells, dendritic cells, macrophages, neutrophils, CD4+T cells, and CD8+T cells in LIHC.
- CDC42EP2 is associated with TP53 and CTNNB1 mutations and is related to TIICs of TP53 and CTNNB1 mutations in LIHC.
- CDC42EP2 is a novel prognostic biomarker that provides insight into potential tumor immune therapeutic targets for patients with LIHC.

the mutation statuses of TP53 and CTNNB1 in LIHC. Copy number alterations (CNAs) represent one of the most frequent genetic variations in the human genome and are key molecular mechanisms in the pathogenesis of human diseases [20]. They can lead to the activation of oncogenes and the inactivation of tumor suppressor genes across various cancers. Consequently, CNAs are implicated in the pathogenesis of a wide range of cancers and play a crucial role in the molecular mechanisms of autoimmune and infectious diseases [21,22]. CNAs are also considered prognostic biomarkers for numerous diseases [23–26]. Understanding the mechanisms underlying TIIC-related prognoses and CNAs in LIHC is essential. However, the specific mechanisms connecting TIIC-related prognoses and CNAs in LIHC have yet to be elucidated. Therefore, the discovery of novel biomarkers associated with TIICs in LIHC could be instrumental in enabling early detection through the identification of specific immune mechanisms.

In the present study, we aimed to evaluate the potential prognostic value of CDC42EP2 by analyzing its associations with clinicopathological features and TIICs in LIHC. We examined CDC42EP2 expression and its prognostic implications in LIHC using publicly available databases, including the Tumor Immune Estimation Resource (TIMER, <https://cistrome.shinyapps.io/timer/>), UALCAN (<http://ualcan.path.uab.edu>), Gene Expression Profiling Interactive Analysis 2 (GEPIA2, <http://gepia2.cancer-pku.cn/#index>), Kaplan-Meier (KM, <http://kmplot.com/plotter>), and OSlihc (<http://bioinfo.henu.edu.cn/DatabaseList.jsp>). Additionally, we evaluated CDC42EP2 protein levels in liver cancer through the Human Protein Atlas (HPA, <https://www>).

proteinatlas.org/) database. Employing the TIMER database, we explored the relationship between CDC42EP2 and TIICs, as well as the prognostic significance of their combined expression in LIHC. We also examined the correlations between TIICs and CDC42EP2-related gene mutations, as well as between TIICs and CNAs. Promoter methylation changes across various clinicopathological characteristics were investigated using the UALCAN database. The Linked Omics (<http://www.linkedomics.org/>) database facilitated our analysis of CDC42EP2's biological functions and co-expression patterns in LIHC. Furthermore, we utilized the GEPIA2 database to evaluate the prognostic relevance of genes associated with CDC42EP2 in a range of cancer types, including LIHC. Our research indicates that CDC42EP2 may serve as a viable target for therapeutic intervention and immunotherapy in LIHC patients. We aim to establish its utility as a prognostic biomarker for this disease.

Materials and Methods

TIMER Database Analysis

TIMER is an online tool for analyzing immune cell infiltration across various cancer types. It leverages data from 10,897 samples within The Cancer Genome Atlas (TCGA) to estimate the abundance of immune infiltrates [27]. Our analysis focused on the expression and survival rates associated with CDC42EP2, as well as its clinical data. We examined the relationship between CDC42EP2 expression and TIICs, along with the prognostic significance of this association. Additionally, we explored the correlations between gene mutations (specifically TP53 and CTNNB1) and TIICs, as well as the relationships among CNAs, TIICs, and LIHC.

UALCAN Database Analysis

UALCAN is a database that utilizes TCGA level 3 RNA sequencing and clinical data from 31 cancer types. It enables users to analyze the relative expression of specific genes across both tumor and normal samples, as well as within various tumor subgroups. These subgroups can be categorized based on a range of clinicopathological features, including tumor stage, grade, race, sex, histological subtype, age, nodal metastasis, and TP53 mutation status [28]. Additionally, UALCAN offers tools to examine gene methylation levels. We analyzed the expression levels of CDC42EP2 in both tumor and normal tissues and explored the clinicopathological significance of CDC42EP2, along with its promoter methylation profiles. To assess the relationship between CDC42EP2 expression and promoter methylation, we utilized the plot module for cancer genomics, employing both Spearman and Pearson tests for our evaluation.

Immunohistochemistry Staining Analysis

HPA is a database that provides information on the distribution of proteins across various human tissues and cells [29]. For our analysis of CDC42EP2 proteomic expression levels, we obtained immunohistochemistry (IHC) images from the HPA specific to LIHC. We categorized the proteomic expression level of CDC42EP2 as “not detected,” “low,” “medium,” or “high.” This classification was determined by assessing the staining intensity and the proportion of cells that were stained.

KM Plotter Database Analysis

The KM plotter was used to estimate the effect of 54,000 genes on the prognosis of 21 common types of cancer [30,31]. This database comprises gene chip and RNA sequencing data sourced from repositories such as the Gene Expression Omnibus. Consequently, we assessed the prognostic significance of CDC42EP2 expression in patients with LIHC. Hazard ratios (HRs) with 95% confidence intervals (CIs) and log-rank *p*-values were calculated. A *p*-value of less than 0.05 was considered to indicate a statistically significant difference.

OSlihc Database Analysis

The OSlihc database serves as a platform for researchers to discover new prognostic biomarkers and may provide the opportunity to create novel targeted therapies for various cancers. Survival outcomes, such as overall survival (OS), disease-free interval (DFI), the progression-free interval (PFI), and disease-specific survival (DSS) were obtained to evaluate the prognostic value of CDC42EP2 in OSlihc [32].

LinkedOmics Database Analysis

LinkedOmics is a publicly accessible portal that contains data for 32 cancer types from TCGA [33]. It offers a distinctive resource for biologists and clinicians to explore multi-omics data related to cancer. The Kyoto Encyclopedia of Genes and Genomes (KEGG) is an online resource designed for the systematic analysis of gene functions and genomic information. Gene Ontology (GO) analysis categorizes gene functions into terms related to cellular components (CC), biological processes (BP), and molecular functions (MF). This categorization aids researchers in examining gene expression information from a network perspective. Pathway analyses facilitated by these tools provide detailed insights into signal transduction, transport, metabolism, and various other cellular activities. We conducted analyses of GO-BP and KEGG pathways using the “LinkInterpreter” module [33]. The ranking criterion was set at a false discovery rate of less than 0.05, and we carried out 500 simulations.

GEPIA2 Analysis

GEPIA2 was used to integrate clinical data from 9,736 tumors and 8,587 normal tissues derived from the TCGA and GTEx projects. The “Expression Analysis” module facilitated the conduction of clinical staging and survival analysis. We produced KM curves for OS and disease-free survival (DFS) associated with CDC42EP2 across 33 types of cancer, including LIHC, using the median value as the cut-off. Additionally, GEPIA2 offers a heat map that displays the survival analysis outcomes for various cancer types [34]. The survival results are presented as KM curves, accompanied by HRs and *p*-values obtained from the log-rank test. The threshold for significance in the Student *t*-test was set at a *p*-value of 0.05.

Statistical Analysis

For our analysis of gene expression data, we utilized the TIMER and UALCAN databases. We employed online tools such as the KM plotter, TIMER, OSlihc, and GEPIA2 to generate KM curves. The survival outcomes are presented as HRs with

p-values from the log-rank test. A *p*-value of less than 0.05 from the log-rank test was considered to indicate a significant difference in survival times. The TIMER database was also used to assess the correlation between gene expression levels and immune signature scores, applying Spearman's correlation coefficients. All data were sourced from publicly accessible databases, and the analyses were conducted using online tools. All reported results include *p*-values derived from the log-rank test, with values below 0.05 deemed to indicate statistical significance.

Results

mRNA Expression Analysis of CDC42EP2 in LIHC

Figure 1 presents a flowchart of this study. To assess the differential expression of CDC42EP2 between tumor and normal tissues, we analyzed CDC42EP2 levels in various cancer types, including LIHC, using the TIMER database. We found that CDC42EP2 expression was elevated in LIHC, bladder

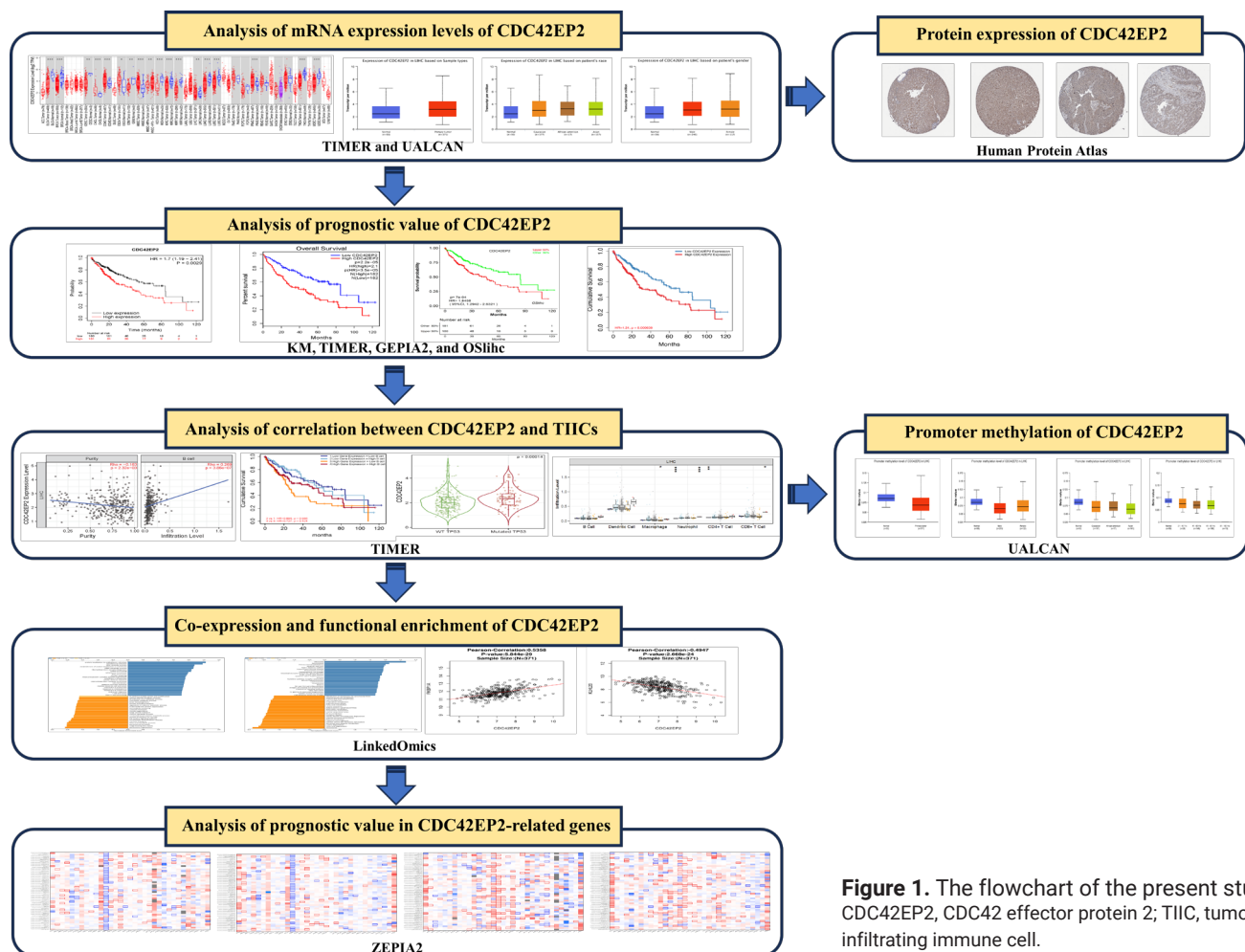


Figure 1. The flowchart of the present study. CDC42EP2, CDC42 effector protein 2; TIIC, tumor-infiltrating immune cell.

choleangiocarcinoma, colon adenocarcinoma, esophageal carcinoma, head and neck squamous cell carcinoma (HNSC), prostate adenocarcinoma, thyroid carcinoma, and uterine corpus endometrial carcinoma when compared to normal tissues. Conversely, CDC42EP2 expression was reduced in bladder urothelial carcinoma (BLCA), breast invasive carcinoma, cervical squamous cell carcinoma and endocervical adenocarcinoma, glioblastoma multiforme, kidney chromophobe, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, THCA, and UCEC relative to normal tissues, as shown in Figure 2A. We also examined the relationship between CDC42EP2 expression and various clinicopathological features in LIHC, including race, sex,

histological subtype, age, stage, grade, lymph node metastasis status, and TP53 mutations. Our findings revealed a significant association between CDC42EP2 expression and primary tumor, race (Caucasian and Asian), sex, histological subtype (LIHC, hepatocarcinoma [HCC-CC]), age, grade (II, III, IV), stage (I, II, III), and TP53 mutations in LIHC. However, there was no correlation between CDC42EP2 expression and lymph node metastasis in LIHC, as depicted in Figure 2B. In summary, our data indicate that CDC42EP2 expression is upregulated in LIHC.

Protein Expression of CDC42EP2 in LIHC

To evaluate CDC42EP2 expression at the protein level, we analyzed CDC42EP2 protein expression levels using the HPA

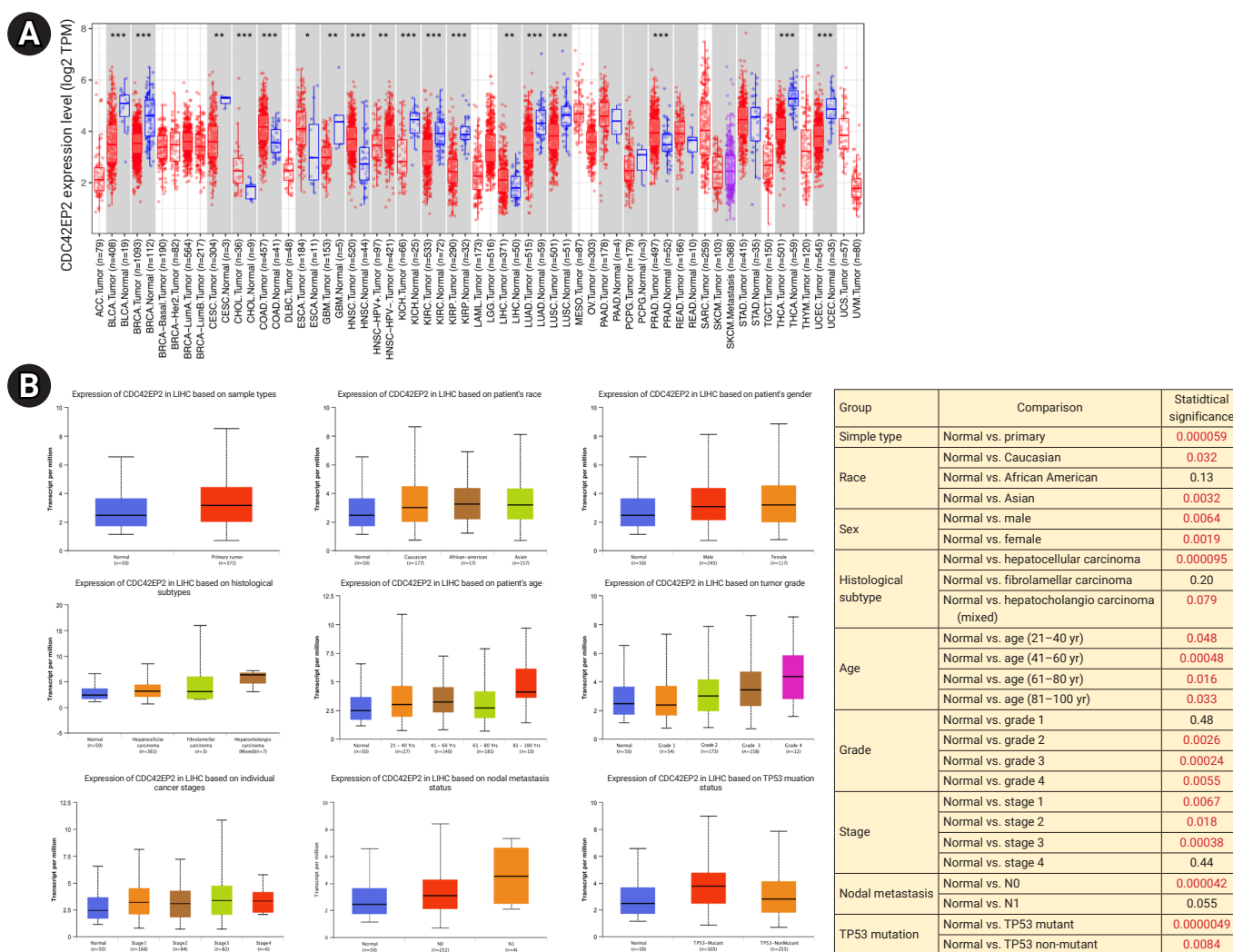


Figure 2. mRNA expression levels of CDC42 effector protein 2 (CDC42EP2) in liver hepatocellular carcinoma (LIHC). (A) High or low expression of CDC42EP2 in tumor tissues compared with normal tissues using the TIMER database. (B) The expression of CDC42EP2 according to the presence of various clinicopathological characteristics compared with normal tissues using the UALCAN database.

database. IHC images from the HPA indicated that CDC42EP2 protein expression was not detectable in normal liver tissue. In contrast, CDC42EP2 protein expression was significantly elevated in LIHC tissues compared to normal tissues. Additionally, low levels of CDC42EP2 were observed in HCC-CC (Figure 3). Our findings demonstrate that CDC42EP2 is overexpressed at both the transcriptional and translational levels in patients with LIHC.

Prognostic Value of CDC42EP2 Expression in LIHC

To investigate the potential prognostic value of CDC42EP2 expression, we utilized the KM plotter, TIMER, GEPIA2, and OSlihc databases. Survival rates, including OS, DFI, PFI, and DSS, were analyzed according to the expression of CDC42EP2 in LIHC cells. Our findings indicated that increased CDC42EP2 expression was associated with significantly reduced OS (KM plotter: HR, 1.7; $p=0.0029$) (Figure 4A) (GEPIA2: HR, 2.1; $p=0.000022$) (Figure 4B). Furthermore, elevated CDC42EP2 expression was linked to poor prognosis in LIHC, as evidenced by OS (HR, 1.85; $p=0.0007$), DFI (HR, 1.36; $p=0.0474$), PFI (HR, 1.41; $p=0.0222$), and DSS (HR, 1.78; $p=0.0124$) (Figure 4C). Additionally, increased CDC42EP2 expression was associated with poor prognosis in LIHC across various demographics, including age (HR, 1.31; $p=0.000638$), sex (HR, 1.31; $p=0.000595$), and race (HR, 1.33; $p=0.000454$), as well as DSS (HR, 1.35; $p=0.000488$) (Figure 4D). To further confirm the prognostic significance of CDC42EP2 in LIHC, we examined the relationship between CDC42EP2 expression and the clinicopathological characteristics of LIHC patients using the OSlihc database. The clinicopathological features are depicted in Figure S1. The results demonstrated that increased CDC42EP2 expression was associated with poorer OS in both males (HR, 1.76; $p=0.0141$) and females (HR, 1.92; $p=0.027$), as well as in Asians (HR, 3.62; $p=0.0002$) and Caucasians (HR,

1.65; $p=0.0351$). Additionally, elevated CDC42EP2 expression was linked to worse prognosis in stage II (HR, 3.74; $p=0.0047$), stage III (HR, 2.52; $p=0.0035$), grade II (HR, 1.83; $p=0.0228$), and grade III (HR, 2.24; $p=0.0132$) LIHC. Increased CDC42EP2 expression also correlated with poorer DFI in Asians (HR, 1.57; $p=0.0035$) and PFI in females (HR, 1.73; $p=0.039$) and Asians (HR, 2.09; $p=0.0026$). Moreover, it was associated with worse PFI in Asians (HR, 4.65; $p=0.0011$), stage II (HR, 6.33; $p=0.0168$), stage III (HR, 2.10; $p=0.0492$), and grade III (HR, 3.17; $p=0.0066$). Furthermore, increased CDC42EP2 expression correlated with poor prognosis in BLCA, HNSC, and uveal melanoma (Figure S2). Taken together, our results show that upregulated CDC42EP2 expression predicts a poor prognosis of LIHC.

Analysis of the Correlations between CDC42EP2 Expression and TIICs in LIHC

Subsequently, we focused on correlations between CDC42EP2 expression and TIICs in LIHC using the TIMER database. CDC42EP2 was positively correlated with the infiltration levels of B cells ($r=0.269$, $p=0.0000000386$), dendritic cells ($r=0.443$, $p<0.0000000000000001$), macrophages ($r=0.261$, $p=0.0000000845$), neutrophils ($r=0.075$, $p=0.148$), CD4+ T cells ($r=0.229$, $p=0.0000174$), and CD8+ T cells ($r=0.15$, $p=0.00529$) in LIHC (Figure 5A). Next, we explored the association between CDC42EP2 expression, prognosis, and TIICs in LIHC. Our findings indicated that high CDC42EP2 expression combined with low B cell infiltration was linked to a poorer prognosis compared to low CDC42EP2 expression with low B cell infiltration. Similarly, high CDC42EP2 expression with low dendritic cell infiltration was associated with a poorer prognosis than low CDC42EP2 expression with low dendritic cell infiltration. A worse prognosis was also observed with high CDC42EP2 expression and high macrophage infiltration compared to low CDC42EP2 expression and low macrophage

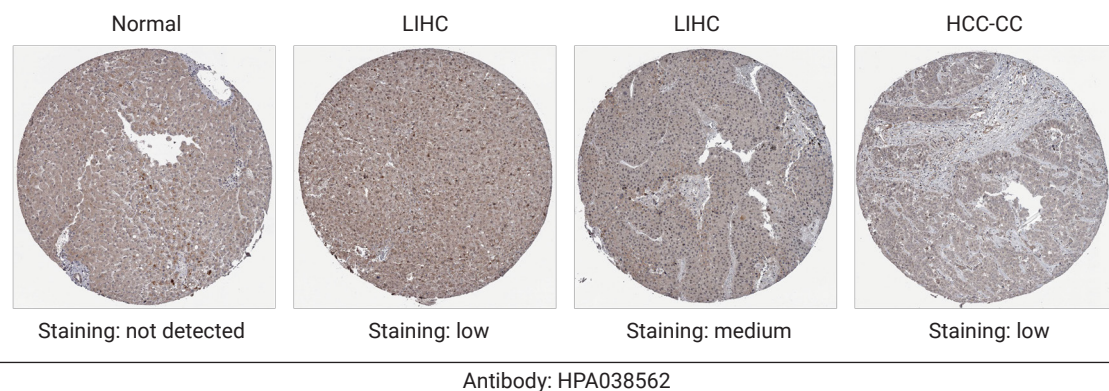


Figure 3. Protein expression levels of CDC42 effector protein 2 (CDC42EP2) in liver hepatocellular carcinoma (LIHC). Protein expression of CDC42EP2 was analyzed using the Human Protein Atlas (HPA) database. HCC-CC, hepatocholangiocarcinoma.

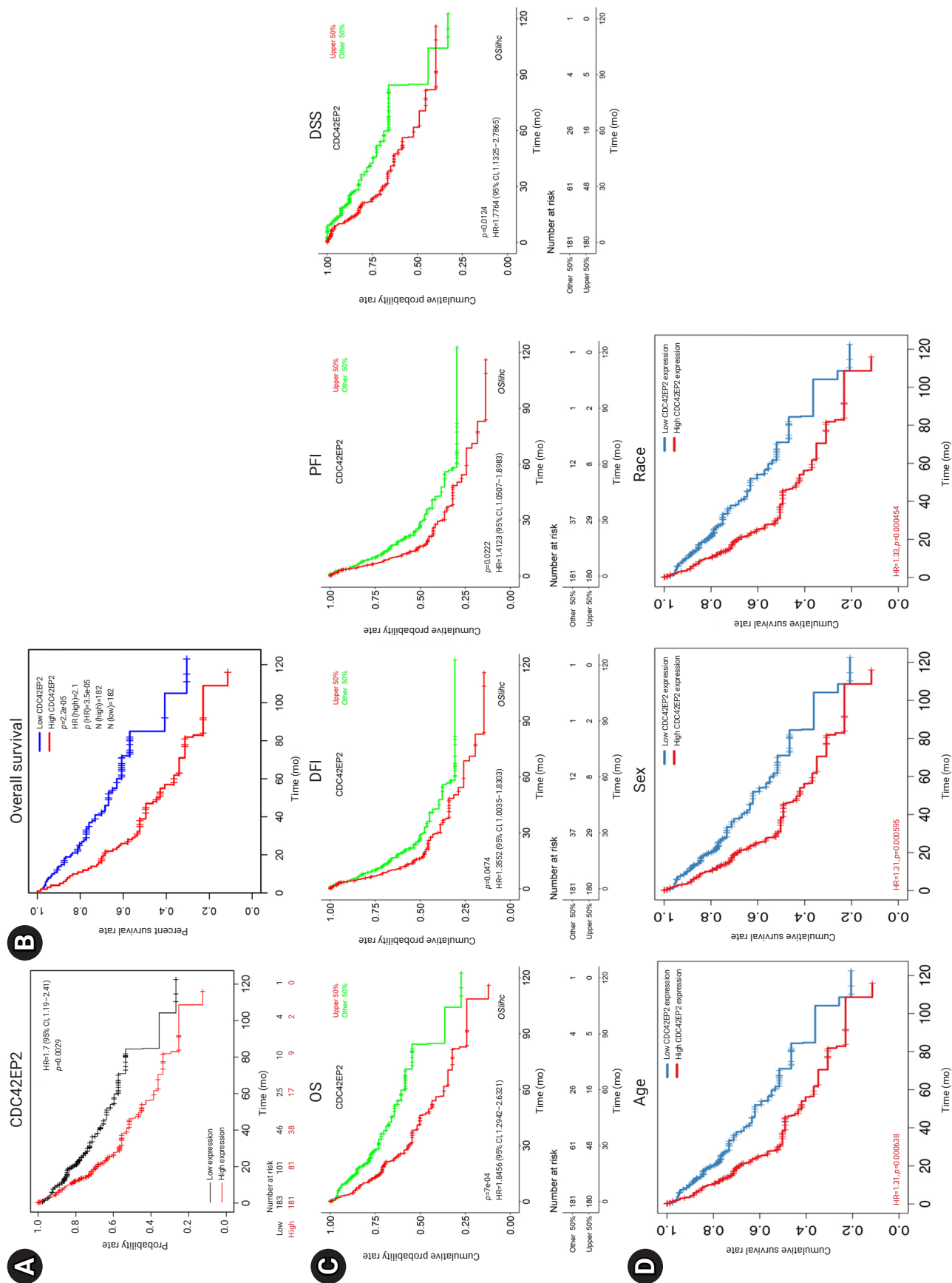


Figure 4. Prognostic significance of CDC42 effector protein 2 (CDC42EP2) expression in liver hepatocellular carcinoma (LIHC). The prognostic value of CDC42EP2 expression was analyzed using the Kaplan-Meier (KM) plotter (A), GEPIA2 (B), OS/IC (C), and TIMER (D). OS, overall survival; DFI, disease-free interval; PFI, progression-free interval; DSS, disease-specific survival.

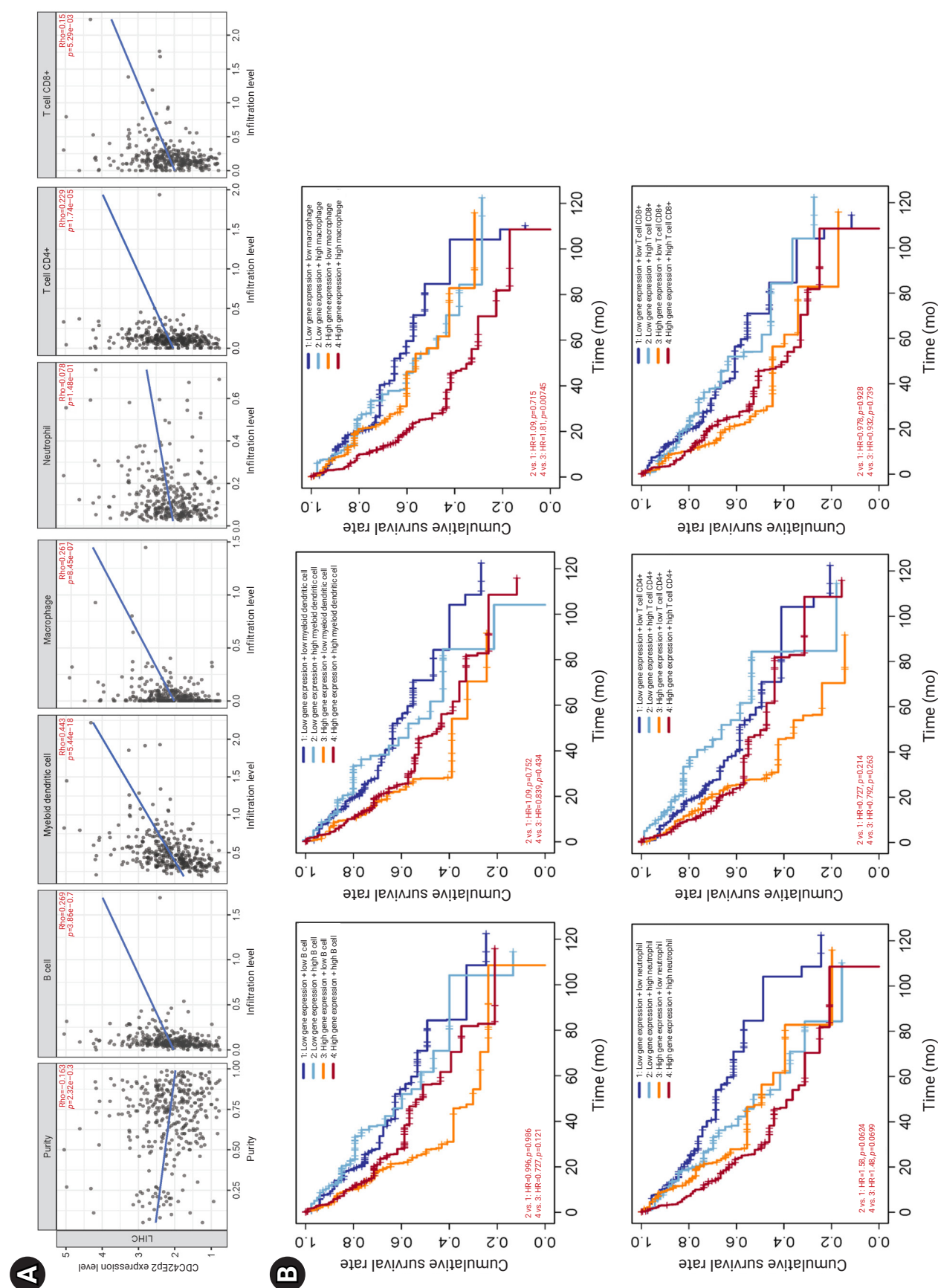


Figure 5. Correlation between CDC42 effector protein 2 (CDC42EP2) expression and tumor-infiltrating immune cells (TICs) in liver hepatocellular carcinoma (LIHC). (A) Scatterplots showing the correlation between CDC42EP2 and TICs, including B cells, dendritic cells, macrophages, neutrophils, CD4+ T cells, and CD8+ T cells were analyzed using the TIMER database. (B) The prognostic value between CDC42EP2 expression and TICs was analyzed using the TIMER database.

infiltration. Additionally, high CDC42EP2 expression with high neutrophil infiltration levels was linked to a poorer prognosis than low CDC42EP2 expression with low neutrophil infiltration. For CD4+ T cells, high CDC42EP2 expression with low infiltration was associated with a worse prognosis than low CDC42EP2 expression with high infiltration. Finally, high CDC42EP2 expression with low CD8+ T cell infiltration was linked to a poorer prognosis than low CDC42EP2 expression with low CD8+ T cell infiltration (Figure 5B). In summary, our results suggest that increased CDC42EP2 expression is associated with TIICs and may influence tumor prognosis in LIHC.

Analysis of the Correlations between CDC42EP2 Expression and Gene Mutations in LIHC

Correlations between gene mutations and CDC42EP2 expression in LIHC cells were investigated using the TIMER database. Increased expression of CDC42EP2 was observed in cells with TP53 ($p=0.00014$) and CTNNB1 ($p=0.012$) mutations (Figure 6A). We also examined the relationship between TP53 mutations and TIICs in LIHC patients. TP53 mutations were associated with higher levels of dendritic cells, macrophages, and neutrophils compared to the wild-type (WT) counterparts. Conversely, CTNNB1 mutations resulted in a reduced presence of B cells, dendritic cells, macrophages, neutrophils, and CD4+ T cells when compared to WT (Figure 6B). Additionally, we analyzed the TIICs in relation to CNAs of CDC42EP2 in LIHC. The findings indicated that CDC42EP2 CNAs were linked to changes in macrophages, neutrophils, and CD8+ T cells (Figure 6C). In summary, our data suggest a significant association between CDC42EP2 and mutations in TP53 and CTNNB1, as well as a notable correlation with the TIIC profiles of these mutations in LIHC.

Promoter Methylation Analysis for CDC42EP2 in LIHC

We further investigated whether the expression of CDC42EP2 is affected by promoter methylation using the UALCAN platform. In LIHC, we found that CDC42EP2 expression is significantly associated with promoter hypomethylation in the primary tumor, race (African-American and Asian), sex (male), age groups (41–60 years, 61–80 years), tumor grade (II, III), tumor stage (I, II), and the presence of TP53 mutations. However, there was no significant relationship between promoter hypomethylation of CDC42EP2 and lymph node metastasis, as shown in Figure 7. These findings suggest that elevated expression of CDC42EP2 in LIHC may be a consequence of promoter hypomethylation.

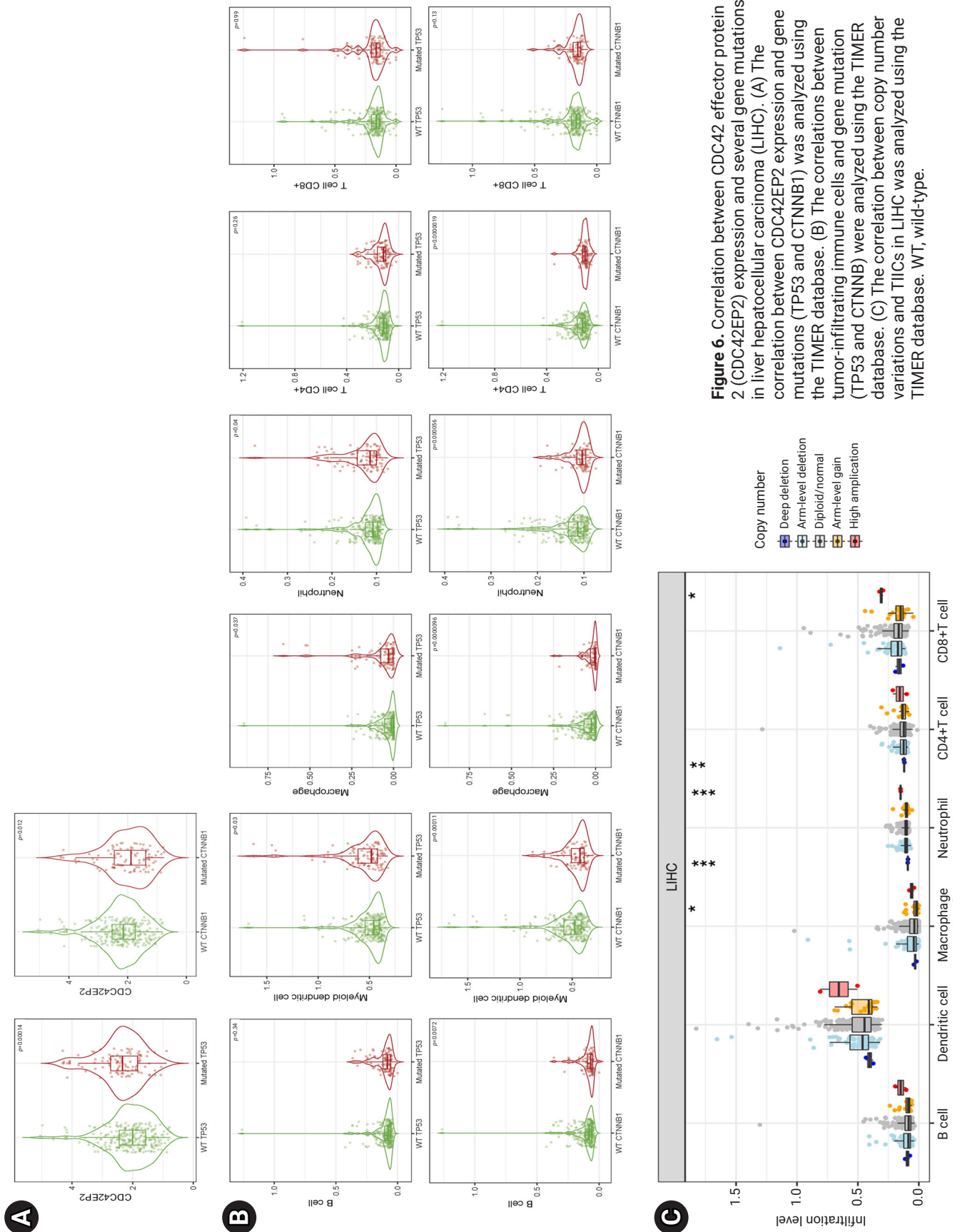
Co-Expression and Functional Enrichment Analysis of CDC42EP2 Expression in LIHC

To explore the potential biological role and pathway of CDC42EP2 expression in LIHC, we assessed the co-expression of CDC42EP2 using the LinkedOmics database. A total of 11,416 genes demonstrated a positive association with CDC42EP2, indicated by dark red dots, while 8,008 genes exhibited a negative association, represented by dark green dots (Figure 8A). Heat maps were utilized to highlight the top 50 genes positively and negatively associated with CDC42EP2 (Figures 8B and C). The top five positively correlated genes included FKBP1A ($r=0.5358$, $p=5.844e-29$), EMP3 ($r=0.5255$, $p=1.008e-27$), LGALS2 ($r=0.5212$, $p=3.201e-27$), PLEKHO1 ($r=0.5166$, $p=1.055e-26$), and CMTM7 ($r=0.5053$, $p=1.965e-25$) (Figure 8D). Conversely, CDC42EP2 was negatively correlated with KLHL20 ($r=-0.4947$, $p=2.668e-24$), TOM1L1 ($r=-0.488$, $p=1.365e-23$), NNT ($r=-0.4782$, $p=1.34e-22$), C1orf25 ($r=-0.4757$, $p=2.37e-22$), and NFIC ($r=-0.4648$, $p=2.773e-21$) (Figure 8E). A comprehensive list of genes co-expressed with CDC42EP2 is provided in Table S1.

We identified biological process categories using gene set enrichment analysis (GSEA) based on GO. The analysis revealed that genes co-expressed with CDC42EP2 are implicated in processes such as protein localization to the endoplasmic reticulum, translational initiation, and RNA catabolic processes (Figure 8F; Table S2). Additionally, a GSEA analysis of the KEGG pathways indicated that the co-expressed genes are predominantly enriched in ribosomes, spliceosomes, and primary immunodeficiency (Figure 8G; Table S3). In summary, our results suggest that CDC42EP2 may influence the prognosis of LIHC by modulating the global transcriptome.

Prognostic Value of CDC42EP2-Related Genes in LIHC

We investigated the prognostic value of CDC42EP2-associated genes in LIHC by analyzing data from the ZEPHA2 database. These CDC42EP2-associated genes appear to be high-risk factors for LIHC. Within the group of genes positively associated with CDC42EP2, 23 exhibited a high HR for OS (Figure 9A), while 17 demonstrated a high HR for DFS (Figure 9B). Conversely, among the genes negatively associated with CDC42EP2, MIA3 was associated with a low HR for OS (Figure 9C), and MYO18A was linked to a high HR for DFS (Figure 9D). CDC42EP2 was found to have a high HR in various cancer types, and genes negatively associated with CDC42EP2 generally showed a low HR across different cancers (Figure S3). Therefore, CDC42EP2 and its related genes are of prognostic importance in a range of cancers, including LIHC.



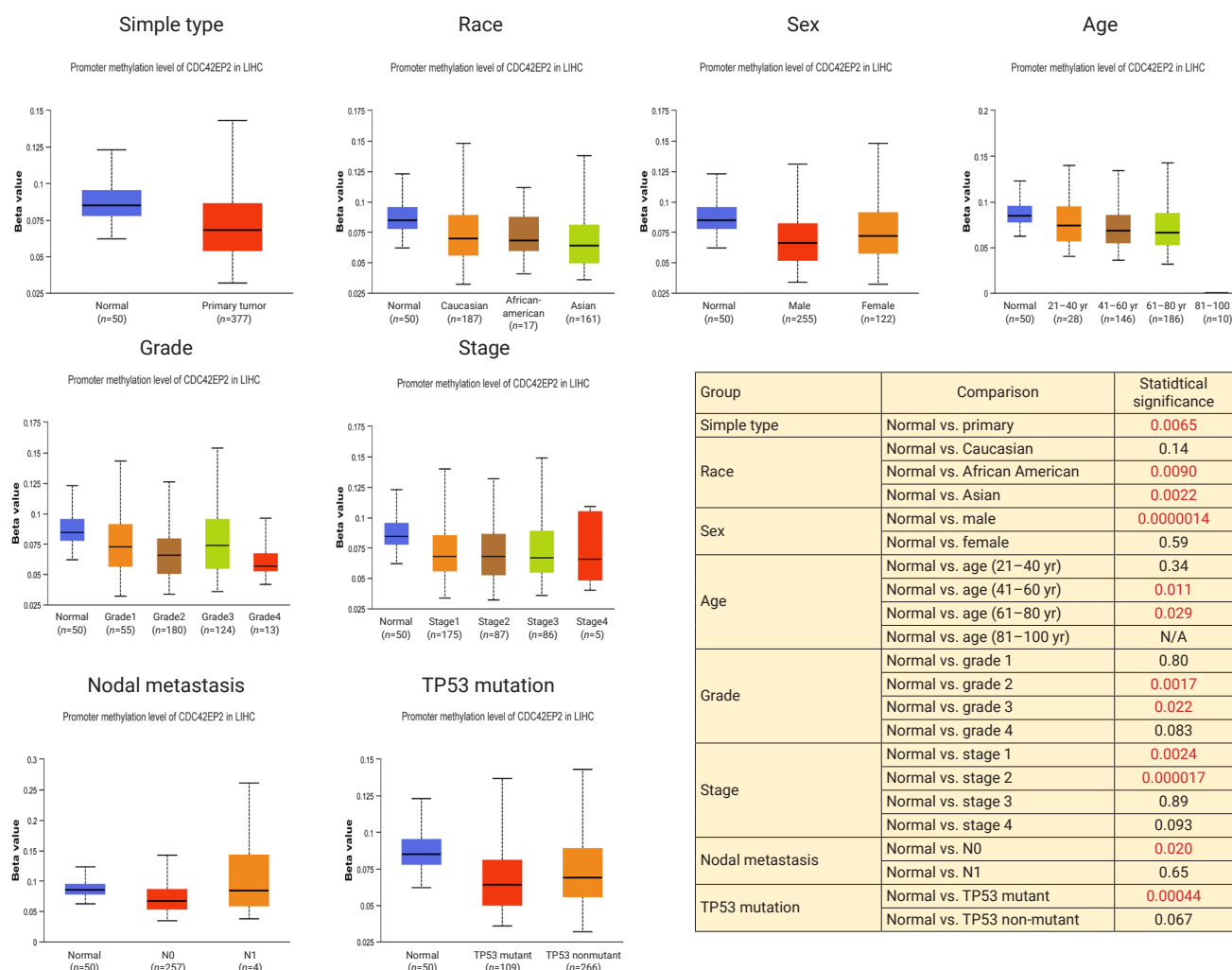


Figure 7. Correlation between CDC42 effector protein 2 (CDC42EP2) expression and promoter methylation in liver hepatocellular carcinoma (LIHC). The correlation between CDC42EP2 expression and promoter methylation was analyzed using the UALCAN database.

Discussion

Liver cancer is a leading cause of mortality worldwide [35,36]. LIHC, the most common pathological form of liver cancer, often develops from chronic liver inflammation and fibrosis [37,38]. LIHC represents approximately 85% to 90% of liver cancer cases [35]. The prognosis for LIHC is extremely poor, largely because it is typically diagnosed at a late stage and there are limited therapeutic options available [37,39]. Therefore, there is an urgent need to identify useful biomarkers for the diagnosis and prognosis of LIHC, as well as treatment targets.

The immune system plays a critical role in controlling cancer growth, and the activity of immune cells may actually promote cancer progression [6,40,41]. The tumor immune

microenvironment (TIME), governed by TIICs, is a key player in cancer development and progression [42]. TIICs contribute to the evolution of cancer cells and tumors [7], and their quantity and type are directly linked to clinical outcomes [10,11]. Numerous studies have highlighted the characteristics of different immune cell types and their connections with prognosis [13,14], as well as the prognostic significance of TIICs and immune molecules in LIHC [15–17]. These findings underscore the importance of TIICs as critical prognostic markers and potential therapeutic targets. Therefore, TIICs may significantly influence the prognosis of LIHC patients. The TP53 gene is a renowned tumor suppressor involved in a range of anti-cancer processes, including apoptosis, senescence, cell cycle arrest, DNA repair, and the autophagy response [19]. Mutations in TP53 are the most common genetic

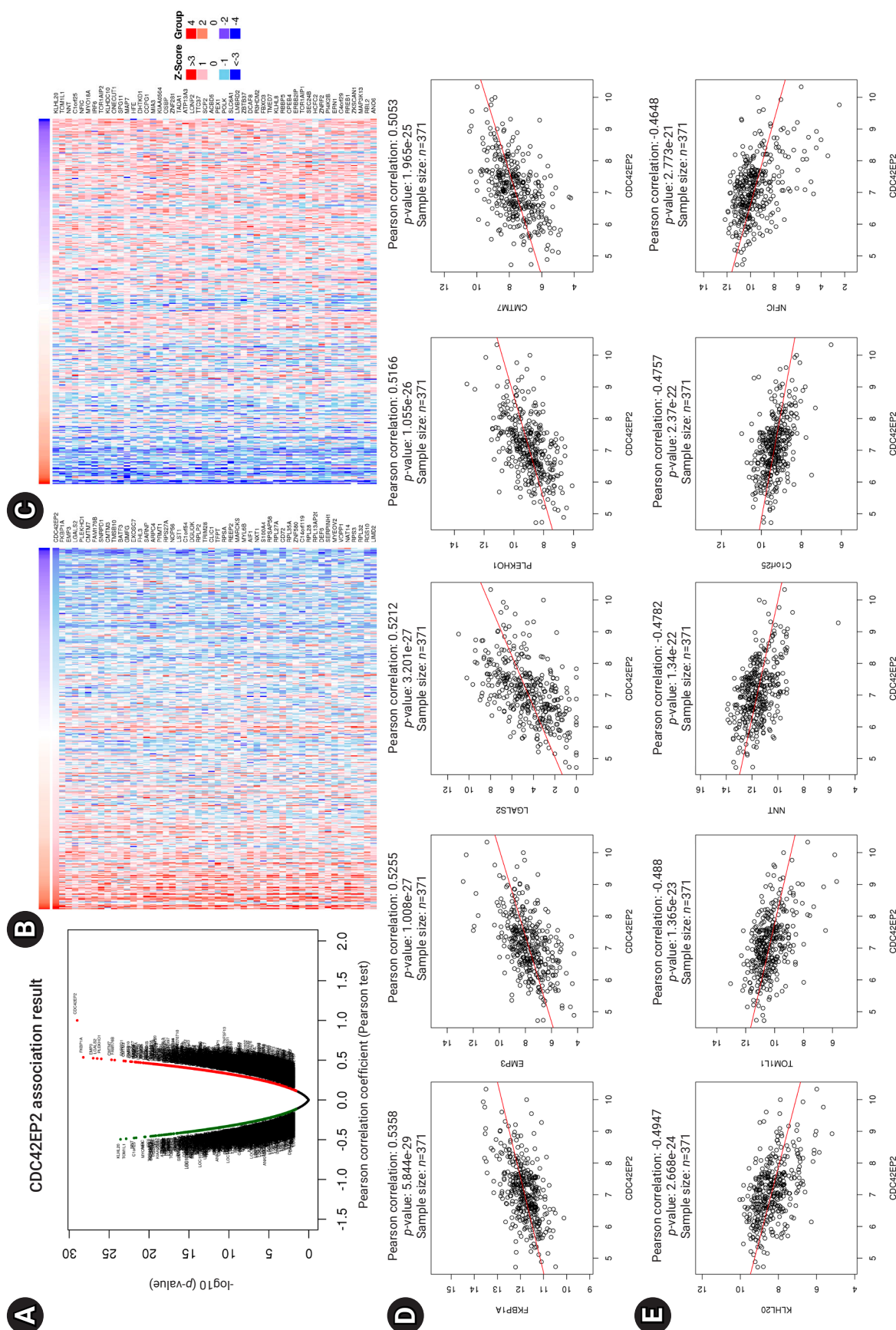


Figure 8. Co-expression and functional enrichment for CDC42 effector protein 2 (CDC42EP2) in liver hepatocellular carcinoma (LIHC). CDC42EP2 co-expression genes and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) terms were analyzed using LinkedOmics database. (A) Highly correlated genes for CDC42EP2 expression were assessed by the Pearson test in the LIHC cohort. Heat maps showing the top 50 genes positively and negatively correlated with CDC42EP2 expression in LIHC. Red indicates positively correlated genes (B) and blue indicates negatively correlated genes (C). Correlation with the top 5 positively related genes (D) and negatively related genes (E) with CDC42EP2 in LIHC. (F) Enriched biological processes related to CDC42EP2 co-expressed genes by gene set enrichment analysis (GSEA). (G) KEGG pathway analysis for CDC42EP2 co-expressed genes indicate a false discovery rate (FDR) ≤ 0.05 and light blue and orange indicate FDR > 0.05 . (Continued to the next page).

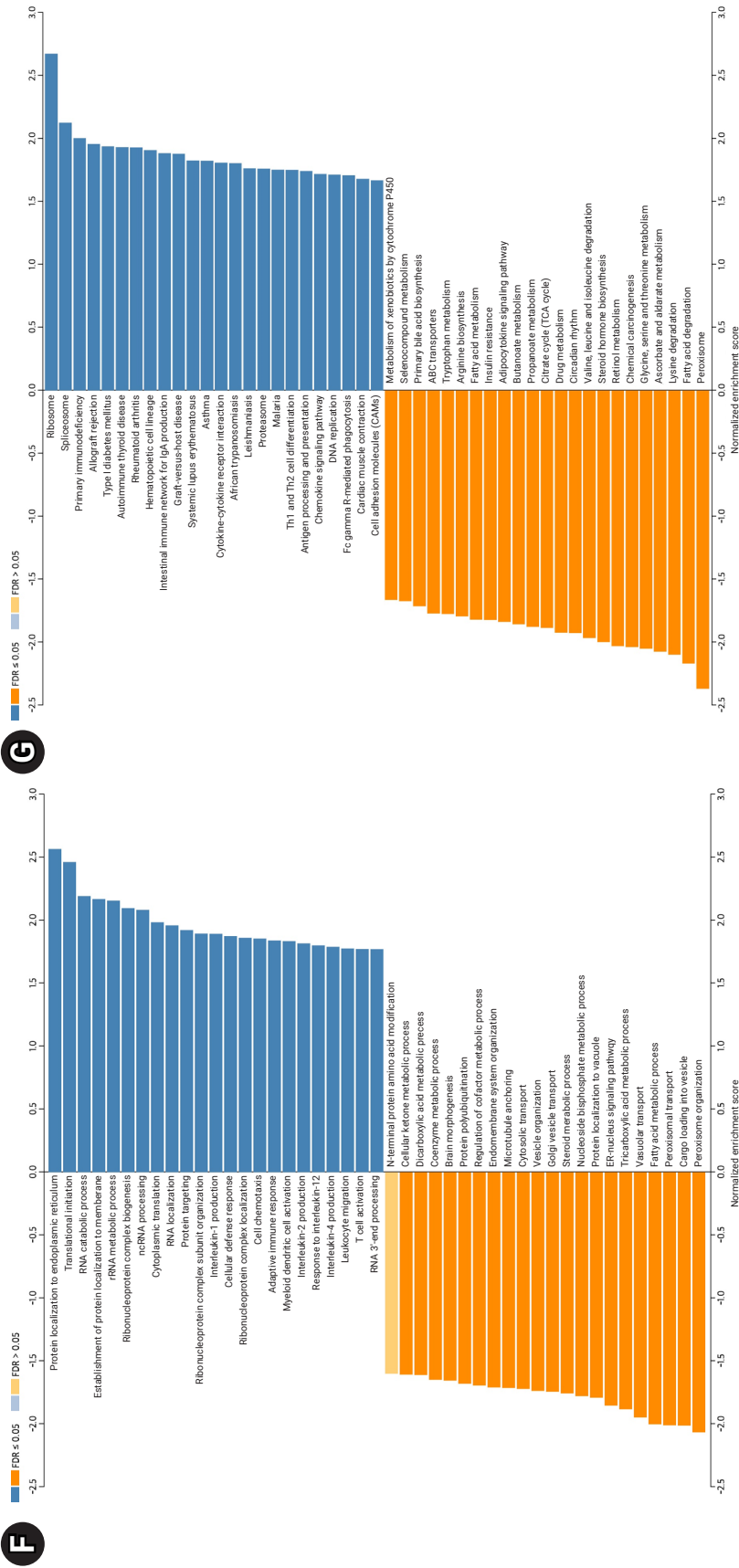


Figure 8. (Continued; caption shown on previous page).

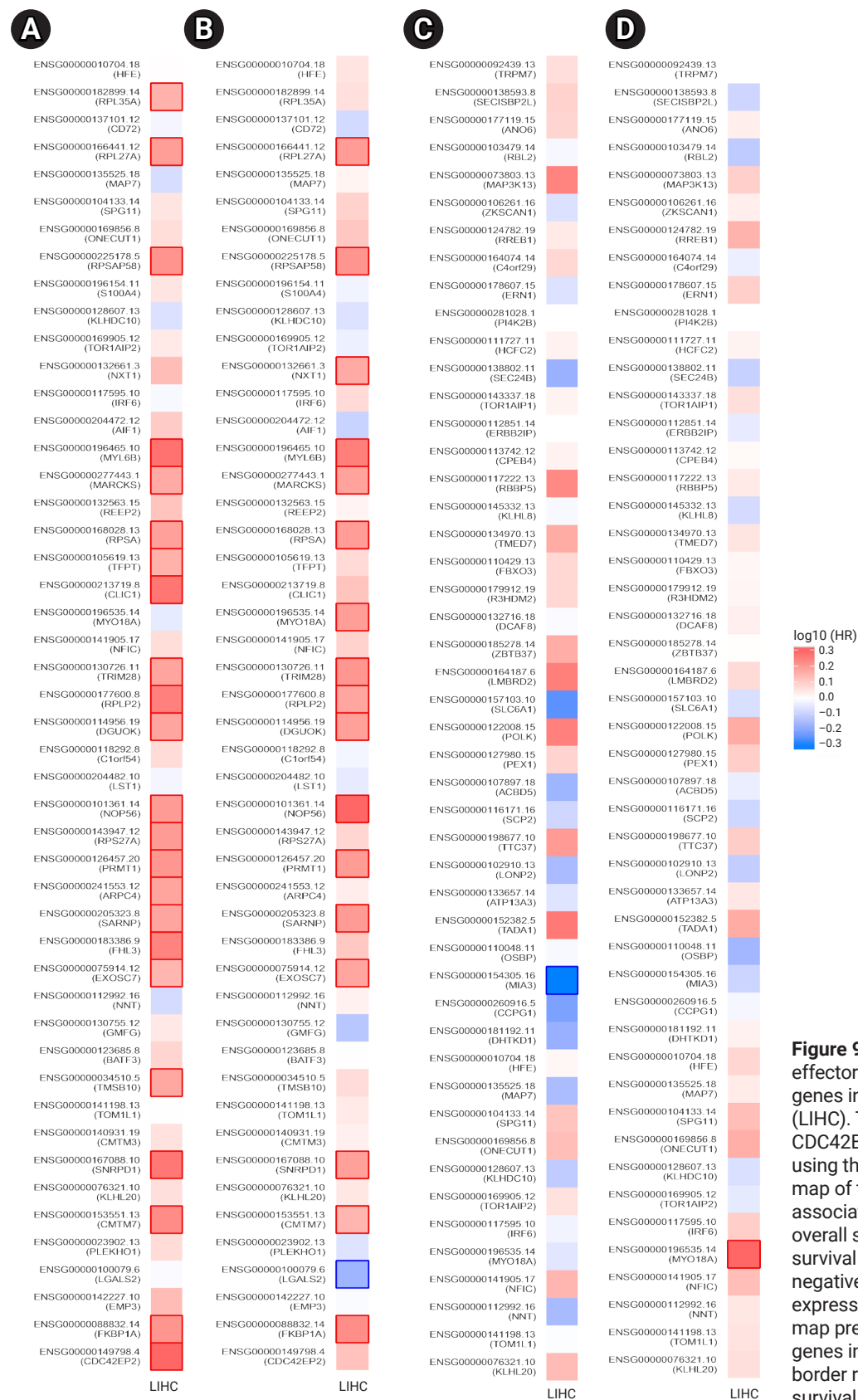


Figure 9. Prognostic value of CDC42EP2 effector protein 2 (CDC42EP2)-related genes in liver hepatocellular carcinoma (LIHC). The prognostic significance of CDC42EP2-related genes was analyzed using the GEPIA2 database. Survival map of the genes that exhibited positive associations with CDC42EP2 with overall survival (OS) (A) and disease-free survival (DFS) (B). Survival map of genes negatively associated with CDC42EP2 expression for OS (C) and DFS (D). Heat map presenting the log₁₀ (HR) of the genes in LIHC. A square with a bold border represents a *p*-value < 0.05 in the survival analysis. HR, hazard ratio.

alterations in human cancers, leading to uncontrolled cell proliferation and oncogenic activity [43,44], and are linked to poor outcomes in various cancer types [18,19,45]. The interplay between p53 mutations and immune system regulation has been extensively explored, particularly p53's role in tumor immune regulation [46]. However, the mechanisms by which TP53 mutations affect the interaction between CDC42EP2 and TIICs remain unexplored. CTNNB1 is essential for the development of liver cancer and represents one of the most frequent mutations [47]. These mutations often occur at phosphorylation sites, leading to the accumulation of nuclear beta-catenin and the activation of the Wnt signaling pathway [48]. Mutations in CTNNB1 in LIHC are linked to immune exclusion and a poor prognosis. Yet, studies focusing on TIICs in the context of mutant CTNNB1 in LIHC are lacking. CNAs are among the most common genetic changes in the human genome and play a vital role in the molecular pathology of human diseases [20]. CNAs are associated with the activation of oncogenes and the suppression of tumor suppressor genes across various cancers, and they are instrumental in cancer development. Furthermore, CNAs have been identified as potential prognostic biomarkers in a range of diseases [23–26]. However, the specific mechanisms underlying TIIC-related prognoses and CNAs in LIHC remain unclear.

In this study, we investigated the mRNA expression levels of CDC42EP2 in LIHC. We found that CDC42EP2 expression was upregulated in LIHC. Various clinicopathological factors were associated with this upregulation. While the CDC42EP2 protein was undetectable in normal tissues, its expression was significantly higher in LIHC tissues. Increased CDC42EP2 levels were linked to a poorer prognosis in LIHC, with this correlation being influenced by different clinicopathological factors. Our data strongly indicate that elevated CDC42EP2 could serve as a novel prognostic biomarker for LIHC. We also examined the relationship between CDC42EP2 and TIICs in LIHC. Our findings showed a positive correlation between CDC42EP2 expression and the presence of various TIICs, including B cells, dendritic cells, macrophages, neutrophils, CD4+ T cells, and CD8+ T cells. Moreover, increased CDC42EP2 levels were associated with a poorer prognosis. When analyzing TIICs in the context of CDC42EP2-related gene mutations and CNAs, we discovered that CDC42EP2 is linked to mutations in TP53 and CTNNB1, as well as to the TIICs associated with these mutations in LIHC. Additionally, CDC42EP2 significantly affects promoter hypomethylation in relation to clinicopathological factors. In investigating the co-expression and potential biological role of CDC42EP2 in LIHC, we identified 11,416 genes that showed a positive correlation with CDC42EP2, while 8,008 genes

had a negative correlation. This suggests that CDC42EP2 has a widespread impact on the transcriptomic landscape of LIHC. This study also explored the prognostic significance of CDC42EP2-related genes in LIHC. Both CDC42EP2 and its related genes hold prognostic value in various cancer types, including LIHC. Numerous studies, including our own previous work, have confirmed the association between high gene expression and poor prognosis, echoing the findings of this study [49–53]. These collective results highlight the limitations of big data analyses and underscore the necessity for further research to understand the functional implications of these genes.

In conclusion, our findings demonstrate that upregulated expression of CDC42EP2 correlates with poor prognosis and TIICs in LIHC. Consequently, CDC42EP2 represents a novel prognostic biomarker that offers insights into potential tumor immune therapeutic targets for LIHC patients. Further studies are required to investigate the detailed mechanisms of the CDC42EP2 gene as a potential prognostic biomarker through *in vitro* and *in vivo* research.

Supplementary Material

Figure S1. Correlations between CDC42 effector protein 2 (CDC42EP2) and clinicopathological characteristics in liver hepatocellular carcinoma (LIHC); **Figure S2.** Prognostic value of CDC42 effector protein 2 (CDC42EP2) expression in several cancers; **Figure S3.** Prognostic value of CDC42 effector protein 2 (CDC42EP2)-related genes in various types of cancer; **Table S1.** CDC42EP2 co-expressed genes; **Table S2.** GO terms for CDC42EP2 co-expressed genes; **Table S3.** Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation for CDC42EP2 co-expressed genes. Supplementary data are available at <https://doi.org/10.24171/j.phrp.2023.0229>.

Notes

Ethics Approval

Not applicable. Written informed consent was obtained for publication of this study and accompanying images.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Availability of Data

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

Authors' Contributions







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

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The effect of the COVID-19 pandemic on the trends and characteristics of natural and unnatural deaths in an urban Sri Lankan cohort viewed through retrospective analysis of forensic death investigations from 2019 to 2022

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ABSTRACT

Objectives: The coronavirus disease 2019 (COVID-19) pandemic has had a severe impact on global health. Apart from the disease itself, the strict restrictions and lockdowns enforced to minimize its spread have also substantially disrupted personal and public health.

Methods: An analysis of forensic autopsy investigations was conducted between 2019 and 2022 on a selected urban population in Colombo, Sri Lanka, assessing the effects of the COVID-19 pandemic on mortality within these communities.

Results: During the COVID-19 restrictions, there was a 2.5-fold increase in the total number of deaths, with a significantly higher percentage of female deaths than before. The majority of these deaths were due to cardiovascular causes, while COVID-19-related deaths ranked third overall. The highest proportion of COVID-19 deaths occurred among unvaccinated females. The monthly frequency of deaths from traffic accidents, poisoning, and asphyxiation decreased, while deaths from blunt trauma, sharp trauma, burns, and immersion increased. There was also a rise in blunt homicides and a greater number of femicides during the COVID-19 restrictions than in the pre-pandemic period. A significantly higher percentage of males who received the COVID-19 vaccine died from cardiovascular causes compared to those in the unvaccinated group.

Conclusion: The significant changes in mortality demographics and causes of death within this community during the COVID-19 restrictions underscore the disruption in healthcare, health-seeking behavior, and social interactions during this period. The vulnerability of individuals residing in highly urbanized areas with lower socioeconomic status, particularly women, is brought into sharp focus.

Keywords: Death rates; Premature mortality; Public health

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Introduction

Coronavirus disease 2019 (COVID-19) was first reported in Wuhan, Hubei Province, China, on December 12, 2019. Shortly thereafter, the Wuhan Municipal Health Commission officially reported a cluster of severe pneumonia cases [1–3]. This marked the beginning of a global crisis caused by a novel and highly contagious coronavirus, known as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The symptoms of COVID-19 range from mild fever, cough, and shortness of breath to more severe and potentially fatal pneumonia and respiratory distress [4,5]. By June 2023, the global death toll had exceeded 6.9 million, with over 760 million people infected [6]. COVID-19 transmission by asymptomatic carriers is a particular concern [7,8]. In response, the world has undergone dramatic shifts in social behavior and lifestyle. Preventive measures such as the use of respiratory masks and face shields, hand sanitization, social distancing, travel restrictions, and community lockdowns have been enforced worldwide.

By the time the World Health Organization declared the outbreak a pandemic on March 11, 2020 [9], the Sri Lankan government had already established a national action committee. This committee, composed of government officials, public health administrators, and military officials, was tasked with planning and implementing strategies to control the disease [10]. Law enforcement and military personnel enforced mandatory quarantine, an island-wide curfew, and travel restrictions after several cases were detected and the first death due to COVID-19 occurred in March 2020 [11–13]. By May 2020, the number of cases had reached 1,000, with a case fatality rate of 1% (Figure 1). This

HIGHLIGHTS

- The mortality rates of natural categories of death increased during the coronavirus disease 2019 (COVID-19) restrictions, indicating disruptions to health care.
- A significantly higher percentage of females died during the period of lockdown, and the majority of COVID-19 deaths were in females.
- Higher frequencies of blunt homicides and femicides reflect interpersonal conflicts and domestic violence during the COVID-19 pandemic.
- Deaths due to COVID-19 were seen mostly among residents in overcrowded, urban communities and in those who were not vaccinated.

figure included the first reported case of post-mortem detection of SARS-CoV-2 through reverse-transcription polymerase chain reaction (RT-PCR) in a forensic autopsy [14]. Despite the emergence of 3 distinct clusters of COVID-19 by the end of October 2020 [15–17], the case fatality rate remained below 0.5%. On November 13, 2020, a new government circular mandated RT-PCR testing of all deaths within the Western province [18]. This resulted in all home deaths being sent to medicolegal institutions within the country for COVID-19 screening. Autopsies were only conducted after nasopharyngeal swabs were confirmed negative. The number of COVID-19 cases continued to rise, and by January 1, 2021, Sri Lanka had recorded a total of

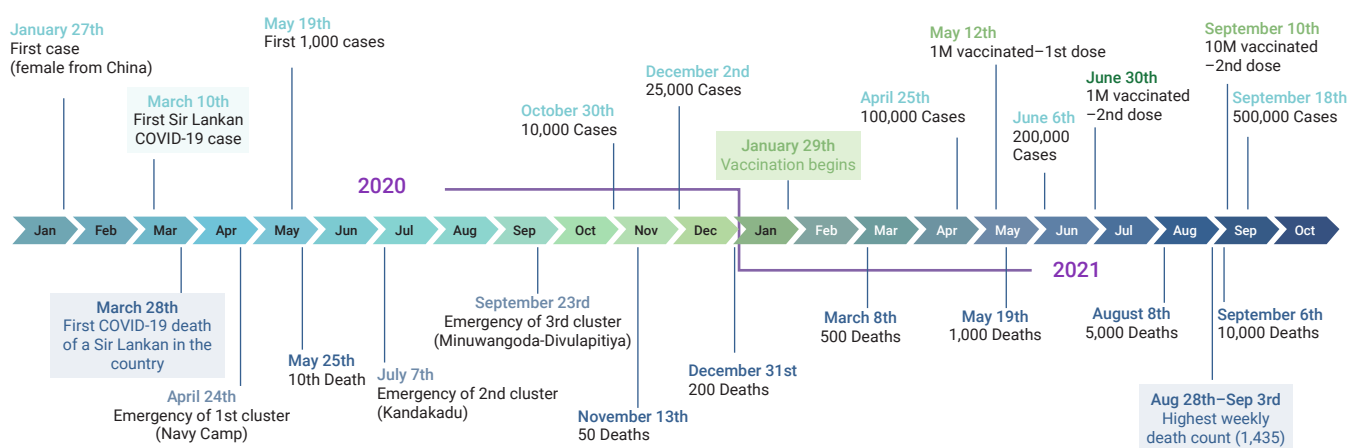


Figure 1. Timeline of important events related to COVID-19 transmission in Sri Lanka from January 2020 to October 2021.

43,299 cases with 204 deaths [19].

Sri Lanka initiated its vaccination program in late January 2021, initially targeting frontline healthcare workers and security personnel. The public vaccination campaign began in early March, achieving coverage of over 1 million individuals by May 2021. However, following the New Year celebrations in April, a third wave of infections struck, pushing the death toll past 3000 by mid-2021 [20]. Travel restrictions and public place closures were intermittently enforced, and specific areas with high case rates were isolated. The highest weekly death toll (1,435) was recorded between August 28 and September 3, which brought the overall death toll to 10,000 [21]. This third wave was attributed to the Delta variant, which increased the case fatality rate from 0.6% to 3% [22]. However, a significant decline in the daily death toll and case density was observed from mid-September. By the end of October, the government officially lifted travel restrictions and curfews [23]. Most public and private services gradually resumed normal operations in the following months, and the requirement for PCR screening of deaths was also removed. COVID-19 deaths continued at a significantly reduced rate, and aside from a minor surge in mid-February, very few deaths were reported in 2022.

Numerous secondary social, economic, and political factors have played a role in disrupting lifestyles and overall health and well-being during the COVID-19 pandemic [24]. Beyond government-imposed social restrictions, apprehension and mistrust among individuals also led to increased distancing, psychosocial discord, and isolation. Social stigma emerged as a powerful determinant influencing the reporting and presentation of those affected by COVID-19 [25]. In line with the global trend of disbelief, conspiracy theories, and myths related to the pandemic [26,27], Sri Lanka also grappled with many misconceptions about COVID-19. One contentious issue was the mandate to cremate all COVID-19 victims within 24 hours, which sparked considerable social, political, and religious controversy [28–31]. These factors collectively resulted in a health burden surpassing the actual pathogenicity of SARS-CoV-2.

Many studies have demonstrated an increase in mortality during the pandemic, primarily due to cardiovascular and cerebrovascular causes [32–35]. The presence of co-morbidities, particularly cardiovascular disease, is a significant risk factor for mortality [36]. Nearly all studies and reviews have highlighted the disruption of healthcare, unequal access to healthcare services, and the collapse of social and communal infrastructure during the pandemic period [37–39]. Reports have also described escalated levels of domestic violence and abuse during lockdown periods, further exacerbating the

psychosocial health of the affected communities [40–42].

Within this context, we conducted a retrospective analysis of the forensic autopsies performed at the Department of Forensic Medicine and Toxicology (DFMT) of the University of Colombo. This department has been exclusively providing medicolegal services to 6 police areas within the Colombo district for over 50 years (Figure 2).

The aims of our study included (1) determining whether there were any shifts in the patterns of natural and unnatural deaths within this community during the COVID-19 restrictions, compared to the periods before and after, (2) establishing the frequency of deaths attributed to COVID-19, and (3) contrasting the mortality trends between individuals who received the COVID-19 vaccine and those who did not.

Materials and Methods

Study Setting and Case Selection

Cases were selected from the forensic autopsies conducted at the DFMT from January 1, 2019 to December 31, 2022. We only selected cases that fell within the jurisdiction of the department's 6 police areas, which serve fixed populations with distinct regional and communal characteristics, constituting approximately one-eighth of the Colombo district population. Depending on their geographic location relative to the city limits of Colombo, this cohort could be divided into 3 groups of residents living within 5 km, 10 km, and 25 km, respectively. The first 2 subgroups are highly urbanized [43]. There is little variation in socioeconomic standards among the 6 police divisions, except for a higher proportion of slum dwellings in the group living within 5 km. Sudden natural deaths, as well as all types of unnatural and suspicious deaths that occur within this community, are referred to the DFMT for forensic autopsies. Information about the deceased, including the circumstances surrounding death, past medical conditions, and vaccination history, is obtained from next of kin, medical records, eyewitnesses, and attending police officers. Forensic pathologists conduct autopsies, and upon completion, a medical certificate of cause of death is issued, including an opinion on whether the death is natural, accidental, suicidal, or homicidal. In some situations, cases remain under investigation until further tests can be conducted or are deemed as deaths due to unascertainable causes. This practice remained unchanged during the COVID-19 restrictions. However, when home deaths occurred within quarantined families or in lockdown areas, law enforcement personnel transported the bodies for forensic autopsies following the inquest. In these situations, the family was interviewed via telephone or video conferencing. We excluded deaths referred for a second



Figure 2. Police areas covered in this study. The 6 police areas for which medicolegal services are provided by the Department of Forensic Medicine and Toxicology, Faculty of Medicine, University of Colombo. Two police divisions each are located within 5-km, 10-km, and 25-km radii from the Colombo city limits, as depicted in the image.

autopsy or review, deaths of unidentified individuals, and skeletonized remains referred for forensic anthropological examination. For specific statistical analyses and comparisons, such as age, vaccination details, or causes of death, cases where the relevant data was not available were excluded accordingly.

Data Collection

All records, encompassing personal details, autopsy reports, medical certificates of cause of death, and digital copies of documented information from police and medical records provided to the DFMT at the time of autopsy, are preserved in the departmental database, from which relevant data were retrieved. The average number of autopsies conducted each month over the previous 8 years (2010–2018) was also determined from the departmental registers. Information regarding COVID-19 positivity was based on RT-PCR test reports issued by authorized government laboratories, which were incorporated into the autopsy reports. Only cases where COVID-19 was listed in part 1 of the cause of death form were classified as COVID-19 deaths.

Details about the epidemiology and transmission of COVID-19, the duration of lockdowns and travel restrictions, and the extent of vaccination coverage were gathered from government press releases and news media. The period from January 1, 2019 to March 20, 2020 was designated as the pre-COVID-19 era. The period of COVID-19 restrictions

was identified as spanning from March 21, 2020 to October 31, 2021. The post-COVID-19 period was defined as the period from November 1, 2021 to December 31, 2022.

The official vaccination program began on January 29, 2021, but the initial recipients were restricted to healthcare workers, security personnel, and individuals over 65 years of age. The general public did not start receiving routine vaccinations until March 2021. As such, we designated the period after March 15, 2021 as the post-vaccination period. The vaccination status of the deceased was determined based on information provided by the next of kin. If the deceased had received at least 1 dose, they were classified as part of the vaccinated group.

Statistical Analysis

We utilized Microsoft Excel ver. 15.20 (Microsoft Corp.) for managing descriptive data. IBM SPSS ver. 24.0 (IBM Corp.) was employed for comparative statistical analysis and significance tests. The Kolmogorov-Smirnov test revealed that the age distributions did not follow a normal distribution ($p < 0.001$). Consequently, the Mann-Whitney U test was used for age comparisons. We did not remove any outliers. The chi-square test was used for comparisons between groups of ordinal variables. We considered a p -value of ≤ 0.05 as statistically significant for all tests. When analyzing the broad categories of deaths, we excluded those that were still under investigation or due to

unascertainable causes. To better represent the changes in death rates within the community, we calculated the monthly frequency for each category of death.

$$\text{Monthly frequency} = \frac{\text{No. of cases in a particular category during a period}}{\text{No. of months within that period}}$$

Ethical Approval

Ethical approval for this study was granted by the Ethics Review Committee of Faculty of Medicine, University of Colombo, Sri Lanka (EC-21-055).

Results

General Demographic Characteristics

Between 2019 and 2022, the DFMT received 2,197 death referrals, of which 2,163 cases met the inclusion criteria. [Table 1](#) illustrates the breakdown of these cases before, during, and after COVID-19 restrictions, with age ranges and the sex distribution. The monthly autopsy load from 2010 to 2018 typically ranged between 25 and 35 cases. However, after the implementation of COVID-19 restrictions in March, the monthly case load dropped noticeably compared to the pre-COVID-19 period. This was followed by a significant increase starting in December 2020 ([Figure 3A](#)). During the COVID-19 restrictions, there were 1,162 deaths, accounting

for 53.7% of the total for all 4 years. After the vaccination program started in March, there were 739 deaths (33.6%) in the final 8 months of the COVID-19 restrictions ([Figure 3B](#)). There was then a swift decline in the number of cases until the end of 2022.

In all 3 periods, males constituted the majority of deaths (76.1%, 62.4%, and 76.6%, respectively). During the COVID-19 restrictions, a higher proportion of deaths occurred among females (37.6%) than in both the pre-COVID-19 (23.9%) and post-COVID-19 (23.4%) periods. This difference was statistically significant ($\chi^2(2)=48.69$, $p<0.001$). For 55 deaths (40 males and 15 females), reliable age information was not available, and these cases were therefore excluded from age comparisons. The mean (standard deviation) and median (interquartile range) ages of the total populations were 60.13 (± 18.69) and 62.00 (49.00–74.00) respectively. The Mann-Whitney U test revealed significant differences in the median age between the pre-COVID-19 and COVID-19 periods ($p<0.001$), the COVID-19 and post-COVID-19 periods ($p<0.001$), and the pre-COVID-19 and post-COVID-19 periods ($p=0.001$). In all 3 periods, the median ages of females were significantly higher than those of males ($p<0.001$) ([Table 1](#)). However, during the COVID-19 restrictions, there was a notable number of females who died at very young ages ([Figure 3C](#)).

Significant differences were also observed in the proportion

Table 1. General demographic characteristics of cases according to the 3 periods

Characteristic	Before COVID-19 restrictions (15 mo)	COVID-19 restrictions (19 mo)	After COVID-19 restrictions (14 mo)	Total
Total	569 (26.3)	1,162 (53.7)	432 (20.0)	2,163 (100.0)
Male	433 (76.1)	725 (62.4)	331 (76.6)	1,489 (68.8)
Female	136 (23.9)	437 (37.6)	101 (23.4)	674 (31.2)
Age (y)				
(Valid cases, <i>n</i>)	(557)	(1,133)	(418)	(2,108)
Total population	58.00 (44.50–68.00)	66.00 (52.00–76.50)	61.50 (47.00–72.00)	62.00 (49.00–74.00)
Male	56.00 (43.00–66.00)	61.00 (49.50–73.00)	59.00 (45.00–70.25)	59.00 (46.00–70.50)
Female	62.00 (49.00–73.00)	71.50 (58.25–82.00)	68.00 (53.75–76.00)	70.00 (56.00–79.00)
Police divisions				
Within 5 km				
Welikada	76	182	96	354
Wellampitiya	99	264	92	455
Sub-total	175 (30.8)	446 (38.4)	188 (43.5)	809 (37.4)
Within 10 km				
Mulleriyawa	97	144	43	284
Talangama	113	249	102	464
Sub-total	210 (36.9)	393 (33.8)	145 (33.6)	748 (34.6)
Within 25 km				
Athurugiriya	88	168	48	304
Nawagamuwa	96	155	51	302
Sub-total	184 (32.3)	323 (27.8)	99 (22.9)	606 (28.0)

Data are presented as *n* (%) or median (interquartile range) unless otherwise stated.

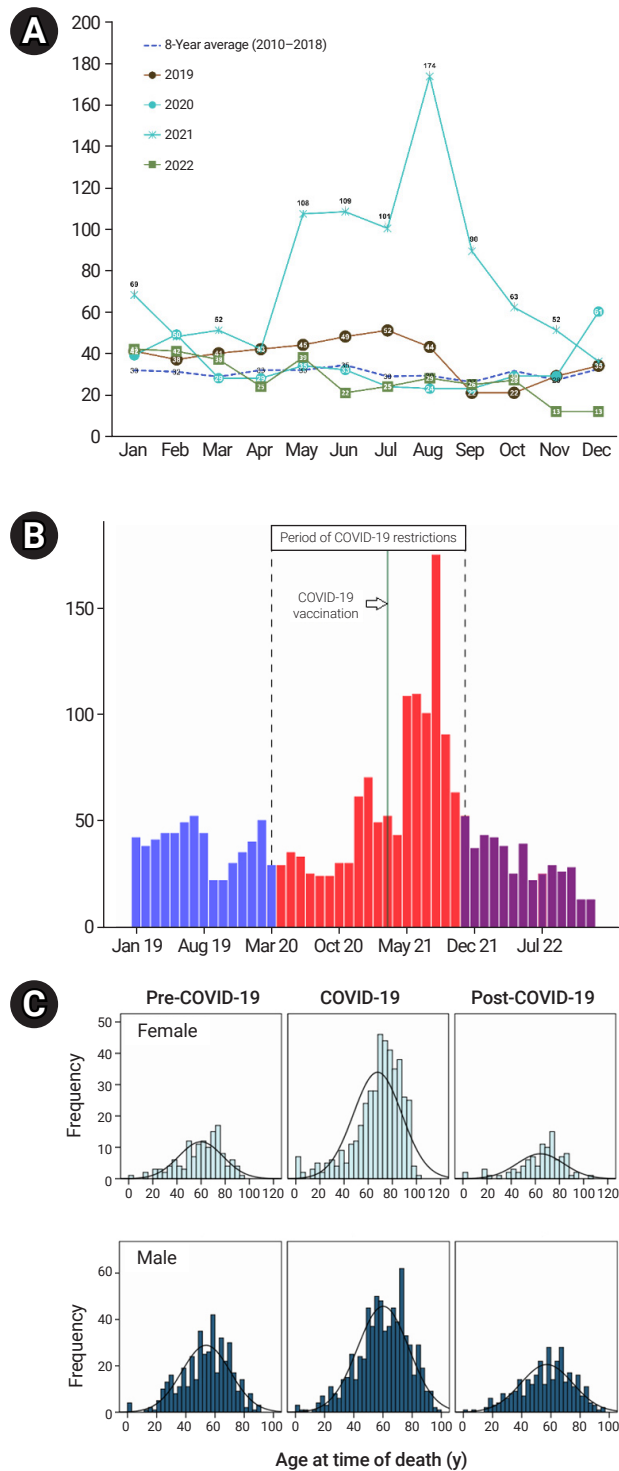


Figure 3. Monthly distribution of cases during 2019–2022. (A) Monthly autopsy load for each year compared with the monthly average during the previous 8 years (2010–2018). (B) Comparison of caseloads during the 3 periods of the study. Red represents the period of COVID-19 restrictions; Blue represents the period before and magenta represents the period after the restrictions. (C) Age distribution of deaths in males and females within each period.

of deaths before and during COVID-19 restrictions among police divisions ($\chi^2(2)=9.93$, $p=0.007$). The group located less than 5 km from the city experienced the highest number of deaths during the COVID-19 restrictions, marking a 7.6% increase from the numbers recorded prior to the pandemic.

Trends and Characteristics of Natural and Unnatural Deaths

Excluding 141 cases where the circumstances were either unascertainable or still under investigation (Figure 4A), the most common broad category of death was cardiovascular diseases (36.6%). This was followed by non-COVID-19-related infections (12.6%), and then COVID-19 deaths (9.3%). Among deaths due to unnatural circumstances, traffic fatalities were the most prevalent (7.7%), followed by asphyxiation (6.4%), which primarily included suicidal hangings, and blunt trauma (4.9%). The distribution of these broad categories of death across the 3 periods is presented in Table 2. Significant differences were observed in the proportions of natural, accidental, suicidal, and homicidal circumstances of death across the 3 periods ($\chi^2(6, n=2,022)=45.78$, $p<0.001$). Most deaths in all 3 periods were due to natural circumstances. Compared to accidents and homicides, there was a more significant reduction in the proportion of suicides during the COVID-19 restrictions.

The proportions and monthly frequencies of both natural and unnatural causes of death varied significantly across the 3 periods (Table 2; Figure 4B). During the COVID-19 restrictions, the monthly frequencies of all categories of natural deaths, with the exception of venous thrombosis, increased (Figure 4B). Chronic lung diseases and malignancies experienced more than a 2-fold increase, while chronic liver disease, chronic kidney disease, and diabetic complications showed approximately 3-fold increases. The monthly frequency of cardiovascular deaths rose from 13.2 to 21.8 during the COVID-19 restrictions, before falling to 9.1 in the post-COVID-19 period. Traffic fatalities decreased from 4.5 per month to 2.8 per month, and firearm deaths fell from 0.3 to 0.1 per month. The reduction in the proportion of traffic fatalities during the COVID-19 restrictions, from 12.0% to 4.6%, was statistically significant ($\chi^2(1)=31.10$, $p<0.001$) (Table 2). Conversely, the proportions of deaths due to blunt trauma, immersion (drowning), and burns were higher during the COVID-19 restrictions, and cases of blunt homicidal trauma were only observed during this period (Table 2). No homicidal deaths among females were reported in the pre-COVID-19 period, but 5 females were killed during the COVID-19 restrictions. In the post-COVID-19 restriction period, 90% of male homicides were due to sharp weapon trauma.

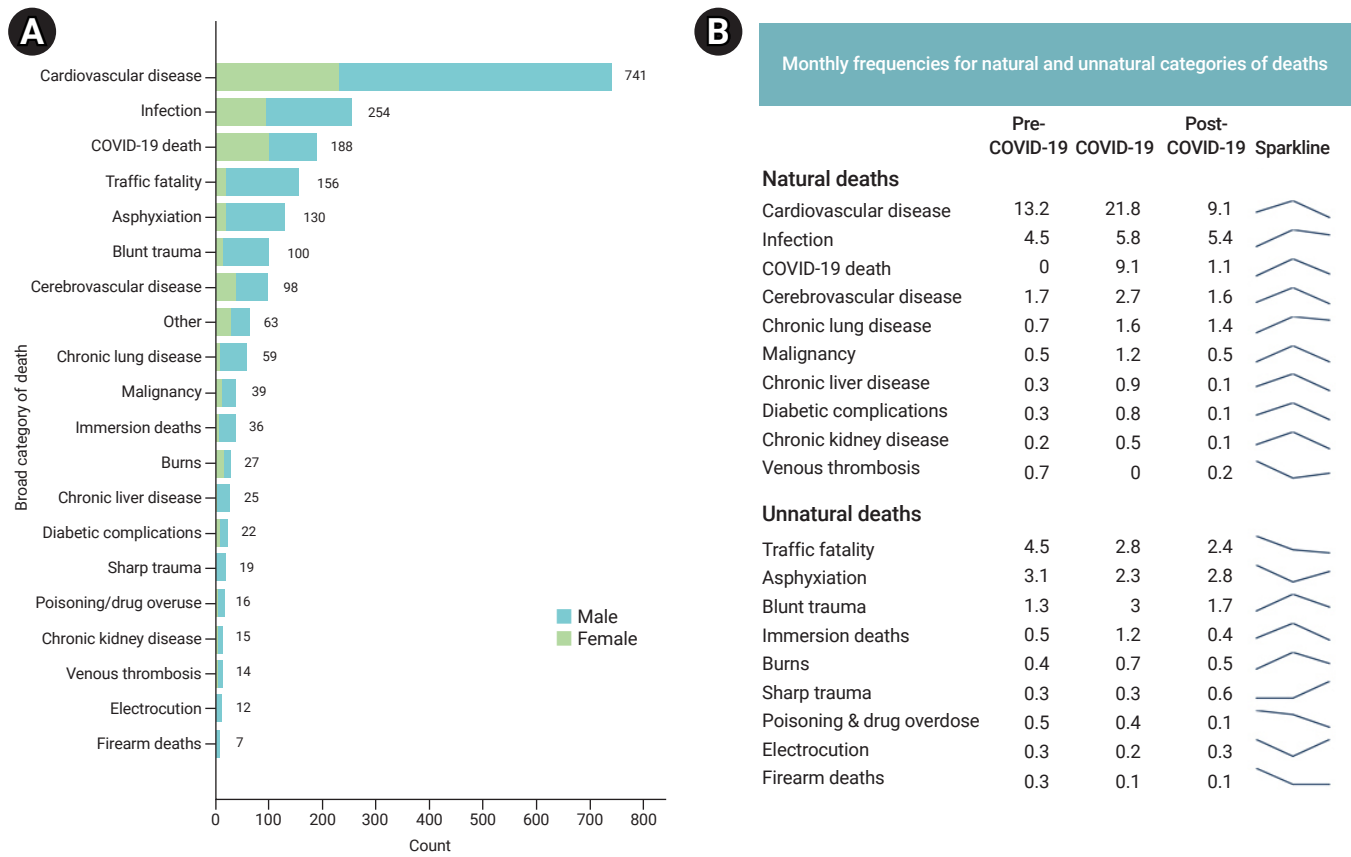


Figure 4. (A) Distribution of cases under each broad category of death in descending order. Cases that were unascertained or under investigation have been excluded. (B) Comparison of monthly frequencies for natural and unnatural categories of death across the 3 periods.

Post-mortem PCR screening for COVID-19 was conducted on 1,133 bodies from November 1, 2020 to March 4, 2022. Of these, 345 bodies tested positive. Among the positive cases, COVID-19 was directly related to the cause of death in 185 cases (53.6%), while it was considered a contributory cause in 103 cases (29.9%). The remaining cases were classified as incidental findings. There were 2 instances where the clinical history and pathological features strongly suggested COVID-19 as the cause of death, yet the post-mortem PCR results were negative. One case was classified as a COVID-19 death despite the absence of a PCR confirmation. Over 75% of the COVID-19 deaths originated from police divisions located within the < 5 km and < 10 km groups. The majority of the deceased were over 65 years old, and the proportion of females who died from COVID-19 was higher than that of males, with a female-to-male ratio of 102:86.

Natural Deaths during the Post-Vaccination Period

In the post-vaccination period, starting on March 15, 2021, there were 1,145 recorded deaths. Of these, 495 individuals (362 males and 133 females) had received some form of COVID-19

vaccination, while 381 (222 males and 159 females) had not. For 269 deaths (175 males and 94 females), vaccination data was unavailable. The most widely distributed vaccines were Sinopharm (80%) and Covishield (13.1%). Among those vaccinated, 77.3% of males and 60.9% of females had received both doses. Of the 876 deaths for which vaccination data was available, 657 were attributed to confirmed natural causes, with cardiovascular diseases being the most common, followed by COVID-19. All but 5 COVID-19-related deaths occurred during the post-vaccination period. Table 3 provides a comparison of the broad causes of death between vaccinated and non-vaccinated individuals, broken down by sex.

There was a significantly higher percentage of vaccinated males among those who died due to cardiovascular causes ($\chi^2(1) = 22.08, p < 0.001$). Their median age was not significantly different from that of the non-vaccinated males ($z = -0.67, p = 0.504$). However, the median age of vaccinated females who died of cardiovascular causes was significantly lower than that of non-vaccinated females ($z = -2.99, p = 0.003$). Of the 145 deaths attributed to COVID-19, 29 males and 26

Table 2. Causes and distribution of natural and unnatural deaths during the 3 periods among males and females

Causes of natural and unnatural deaths	Male			Female		
	Pre-COVID-19	COVID-19	Post-COVID-19	Pre-COVID-19	COVID-19	Post-COVID-19
Natural (<i>n</i> = 1,522)	257	503	211 ^{a)}	93	374	84 ^{a)}
Median age (y)	66 (55–76)					
Cardiovascular diseases (<i>n</i> = 741)	156 (60.7)	253 (50.3)	99 (46.9)	42 (45.2)	162 (43.3)	29 (34.5)
Infection (<i>n</i> = 254)	47 (18.3)	67 (13.3)	45 (21.3)	21 (22.6)	43 (11.5)	31 (36.9)
COVID-19 (<i>n</i> = 188)	0 (0)	76 (15.1)	10 (4.7)	0 (0)	96 (25.7)	6 (7.1)
Cerebrovascular disease (<i>n</i> = 98)	16 (6.2)	25 (5.0)	17 (8.1)	9 (9.7)	26 (7.0)	5 (6.0)
Chronic lung disease (<i>n</i> = 59)	9 (3.5)	23 (4.6)	17 (8.1)	1 (1.1)	7 (1.9)	2 (2.4)
Other (<i>n</i> = 62)	10 (3.9)	15 (3.0)	8 (3.8)	8 (8.6)	17 (4.5)	4 (4.8)
Malignancy (<i>n</i> = 39)	5 (1.9)	15 (3.0)	5 (2.4)	3 (3.2)	8 (2.1)	3 (3.6)
Chronic liver disease (<i>n</i> = 25)	5 (1.9)	14 (2.8)	2 (0.9)	0 (0)	4 (1.1)	0 (0)
Diabetic complications (<i>n</i> = 22)	3 (1.2)	8 (1.6)	1 (0.5)	1 (1.1)	8 (2.1)	1 (1.2)
Chronic kidney disease (<i>n</i> = 15)	2 (0.8)	7 (1.4)	1 (0.5)	1 (1.1)	3 (0.8)	1 (1.2)
Venous thrombosis (<i>n</i> = 14)	4 (1.6)	0 (0)	3 (1.4)	7 (7.5)	0 (0)	0 (0)
Accidents (<i>n</i> = 303)	82	113	64	16	21	7
Median age (y)	55 (39–68)					
Traffic fatality (<i>n</i> = 155)	57 (69.5)	48 (42.5)	30 (46.9)	10 (62.5)	6 (28.6)	4 (57.1)
Blunt trauma (<i>n</i> = 92)	16 (19.5)	41 (36.3)	22 (34.4)	2 (12.5)	9 (42.9)	2 (28.6)
Immersion deaths (Drowning) (<i>n</i> = 28)	4 (4.9)	16 (14.2)	5 (7.8)	2 (12.5)	1 (4.8)	0 (0)
Electrocution (<i>n</i> = 12)	4 (4.9)	4 (3.5)	4 (6.3)	0 (0)	0 (0)	0 (0)
Burns (<i>n</i> = 8)	0 (0)	2 (1.8)	1 (1.6)	1 (6.3)	3 (14.3)	1 (14.3)
Asphyxiation (<i>n</i> = 6)	1 (1.2)	2 (1.8)	1 (1.6)	1 (6.3)	1 (4.8)	0 (0)
Poisoning & drug overuse (<i>n</i> = 1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.8)	0 (0)
Suicides (<i>n</i> = 163)	47	41	38	13	17	7
Median age (y)	39 (28–54)					
Asphyxiation (Hanging) (<i>n</i> = 123)	38 (80.9)	34 (82.9)	33 (86.8)	7 (53.8)	7 (41.2)	4 (57.1)
Burns (<i>n</i> = 19)	1 (2.1)	3 (7.3)	2 (5.3)	4 (30.8)	6 (35.3)	3 (42.9)
Poisoning & drug overuse (<i>n</i> = 15)	6 (12.8)	3 (7.3)	2 (5.3)	1 (7.7)	3 (17.6)	0 (0)
Immersion deaths (drowning) (<i>n</i> = 4)	1 (2.1)	0 (0)	1 (2.6)	1 (7.7)	1 (5.9)	0 (0)
Sharp trauma (<i>n</i> = 1)	0 (0)	1 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)
Traffic fatality (<i>n</i> = 1)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Homicides (<i>n</i> = 34)	8	10	10	0 (0)	5	1
Median age (y)	39 (29–55)					
Sharp trauma (<i>n</i> = 18)	4 (50.0)	3 (30.0)	9 (90.0)	0 (0)	2 (40.0)	0 (0)
Firearm deaths (<i>n</i> = 7)	4 (50.0)	2 (20.0)	1 (10.0)	0 (0)	0 (0)	0 (0)
Blunt trauma (<i>n</i> = 7)	0 (0)	5 (50.0)	0 (0)	0 (0)	2 (40.0)	0 (0)
Asphyxiation (ligature strangulation) (<i>n</i> = 1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100.0)
Immersion deaths (<i>n</i> = 1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20.0)	0 (0)
Total	394 (19.5)	667 (33.0)	323 (16.0)	122 (6.0)	417 (20.6)	99 (4.9)

Data are presented as median (interquartile range) or *n* (%).

^{a)}In 3 males and 2 females who died of natural causes, the exact causes of death are pending further investigation.

females had received at least 1 dose of the COVID-19 vaccines. The highest percentage of COVID-19 deaths occurred among non-vaccinated females, although this was not statistically significant ($\chi^2(1)=1.786$, $p=0.181$). The majority of deaths occurred in individuals 65 years and older, with no significant difference found between the median ages of the vaccinated and non-vaccinated groups ($z=-0.72$, $p=0.470$). There were 10 COVID-19 deaths in those under 40 years, all of whom were non-vaccinated. No significant differences

were found in either the percentages or median ages of vaccinated and non-vaccinated males and females in other categories of natural deaths.

Discussion

Changes in the Frequency and Demography of Deaths during the COVID-19 Restrictions

The observed increase in mean and median ages of

Table 3. Comparison of the frequency and median ages among vaccinated and non-vaccinated males and females within the broad categories of natural deaths

Broad categories of natural deaths among deceased persons with vaccination details (n = 657) ^{a)}	Vaccinated males	Non-vaccinated males	Vaccinated females	Non-vaccinated females
Cardiovascular disease				
Total (281, 42.8%)	131	50	44	56
Median age (y)	64.5 (55.0–73.0)	64.0 (56.5–75.5)	69.5 (55.0–79.0)	81.5 (69.0–87.0)
COVID-19 deaths				
Total (145, 22.1%)	29	36	27	53
Median age (y)	73.0 (66.5–82.5)	69.5 (53.0–79.5)	77.0 (69.0–85.0)	78.0 (57.5–88.0)
Infections				
Total (93, 14.2%)	28	28	22	15
Median age (y)	62.5 (52.0–75.0)	62.5 (57.5–82.0)	70.0 (57.0–81.0)	72.0 (65.0–83.0)
Cerebrovascular disease				
Total (43, 6.5%)	14	11	10	8
Median age (y)	61.5 (49.0–75.0)	69.0 (46.0–77.0)	72.0 (60.0–79.0)	74.5 (65.5–85.0)
Chronic lung disease				
Total (23, 3.5%)	12	8	2	1
Median age (y)	67.0 (56.0–72.0)	61.5 (58.0–75.0)	73	-
Other				
Total (20, 3.0%)	6	7	3	4
Median age (y)	72.0 (61.0–82.0)	70.0 (49.0–84.0)	66	72.0 (51.0–90.0)
Diabetic complications				
Total (16, 2.4%)	3	6	0	7
Median age (y)	59	75.5 (67.0–84.0)	-	70.0 (44.0–78.0)
Malignancy				
Total (16, 2.4%)	5	4	1	6
Median age (y)	63.0 (58–66.0)	72.0 (63.0–80.5)	43	70.0 (37.0–74.0)
Chronic liver disease				
Total (13, 2.0%)	7	6	0	0
Median age (y)	54.0 (53.0–60.0)	43.0 (39.0–71.0)	-	-

Data are presented as median (interquartile range).

-, Median age not calculated.

^{a)}Two categories where the total number was less than 5 were excluded.

deaths during the COVID-19 restrictions is attributable to a decrease in unnatural deaths, such as traffic fatalities, which typically involve younger individuals, and an increase in sudden natural deaths among the elderly population. During the COVID-19 restrictions, there was nearly a 15% rise in the proportion of female deaths compared to the other 2 periods. This increase could be partially attributed to the high number of females who succumbed to COVID-19, as discussed in more detail below. It is widely recognized that females often face discrimination during times of disaster and crisis [44], which has been observed globally during the COVID-19 pandemic as well [45,46]. Numerous reports have also highlighted increased instances of domestic violence, sexual violence, and gender-based harassment during the COVID-19 lockdowns, all of which predominantly affect females [47,48]. In Sri Lanka, a predominantly patriarchal society, the responsibility of household duties primarily falls on females, and their health needs are often neglected. A high prevalence of domestic violence, particularly among

lower socioeconomic communities, has always been a significant issue. The occurrence of 5 femicides exclusively during the COVID-19 restrictions in our cohort further underscores the vulnerability of females during such crisis periods.

The higher percentage of deaths within a 5-km radius of Colombo, specifically from the Welikada and Wellampitiya police divisions, during the COVID-19 restrictions is noteworthy. These 2 divisions were frequently subjected to strict curfews and lockdowns during this period. Despite their proximity to major tertiary hospitals and clinics in Colombo, the stringent restrictions likely impeded access to healthcare. These areas are characterized by high levels of housing congestion and limited living space. During the lockdowns, these highly urbanized communities faced numerous challenges, including overcrowded houses, reduced ventilation, decreased sanitation, and the closure of food outlets [49]. These conditions contributed to malnutrition, lower immunity, and a decline in the overall health status

of urban residents [50]. In some instances, we found that despite exhibiting severe illness symptoms such as chest pains and headaches, the deceased had resorted to home remedies. Some even refrained from seeking medical treatment due to fear of contracting COVID-19. In contrast, residents in the more distant suburbs of Athurugiriya and Nawagamuwa, located almost 25 km away from the city limits, live in a more dispersed housing layout with ample garden space. Although these residents may not have had access to tertiary healthcare facilities during this period, they had greater freedom of movement to obtain primary and basic community healthcare services. Many households in these areas are also more self-sufficient, growing their own food, which likely contributed to a healthier lifestyle. This disparity in death rates between the 2 communities underscores the negative impacts of urbanization and overcrowding during a pandemic with airborne transmission.

The Impact of COVID-19 Restrictions on Unnatural Deaths

In our cohort, traffic fatalities decreased by nearly 50% during the lockdowns, a figure that aligns with studies reported from other regions [51,52]. Although the reduced traffic volume due to lockdowns and travel restrictions has led to fewer traffic collisions, it also encouraged higher speeds and reckless driving, resulting in more fatalities [53]. A review of road traffic fatalities across multiple countries revealed that strict lockdown measures decreased the crash fatality ratio by 0.36% [54]. The most significant reduction was observed in pedestrian deaths, with some studies indicating a decline of over 60% during the lockdown periods [55].

Sri Lanka is known for its high suicide rate [56,57]. Our study did not reveal a significant shift in the frequency of suicides during the COVID-19 restrictions. A global surge in suicides was anticipated due to social isolation, economic difficulties, and fear of infection during the COVID-19 pandemic [58–60]. However, a paradoxical decrease was observed in most countries, at least during the initial stages [61], and the predicted alarming increase in suicides did not materialize [62]. Another study conducted in Sri Lanka reported a decrease in hospital admissions due to self-harm from poisoning during the COVID-19 restrictions, but no change in the overall suicide trend [63].

Sri Lankan households typically comprise of extended families. During lockdown periods, the constant presence of family members at home may have served as a mitigating factor for suicidal tendencies. Conversely, increased family contact could potentially lead to more conflicts. This could account for the observed rise in suicidal burns, which are

generally considered impulsive suicides.

It is noteworthy that blunt homicides were only observed during the period of COVID-19 restrictions, potentially reflecting an increased frequency of interpersonal conflicts and domestic violence during lockdowns. An earlier analysis of homicides in Sri Lanka revealed that blunt and sharp weapon trauma were predominantly associated with sudden provocations and robbery homicides, while firearms were typically used in terrorist activities, contractual, and underworld killings [64]. Therefore, the heightened frequency of blunt and sharp weapon homicides during and after the period of COVID-19 restrictions is concerning, as it indicates a growing propensity for violent and aggressive behavior within the community. The COVID-19 restrictions imposed a significant economic burden on many of Sri Lanka's lower socioeconomic classes, particularly small-scale business enterprises and daily wage workers [65]. While no comprehensive study is available, an uptick in drug-related and property crimes during the pandemic has been reported [66]. The sole case of ligature strangulation homicide and many of the sharp weapon trauma homicides in our study occurred in the context of burglary or mugging, further substantiating this concern.

The majority of immersion fatalities during the COVID-19 restrictions were accidental. Interestingly, studies have reported an increase in unintentional drowning deaths during the COVID-19 pandemic [67]. This increase has been attributed to a higher participation in water-based recreational activities, which are seen as leisure activities where social distancing can be maintained. In the communities we studied, many households are situated near water sources, which families use for daily tasks such as washing and bathing. The high number of accidental immersion fatalities among males possibly reflects the frequent use of these water sources while under the influence of alcohol or drugs during the COVID-19 restrictions.

The Impact of COVID-19 on Deaths due to Natural Causes

The rise in monthly mortality rates from various natural causes reflects a hesitancy to seek hospital care for acute cardiovascular and cerebrovascular conditions, respiratory and neurological conditions, and malignancies, as documented in numerous studies [68–71]. Certain studies have identified a significant decrease in “discretionary” admissions related to cardiovascular disease, such as those for unstable angina, heart failure, chronic obstructive pulmonary disease, and dizziness/syncope. However, these studies also found that admissions for strokes and heart attacks remained constant [72]. This pattern was evident in our cohort, with family members recounting multiple instances where the

deceased had ignored non-specific symptoms like chest pain or headaches in the days leading up to their death. During the COVID-19 restrictions, the DFMT observed a high frequency of myocardial rupture cases following acute myocardial infarction among sudden at-home deaths, further substantiating this trend of avoiding emergency care during the COVID-19 pandemic.

Sri Lanka primarily operates a state-sponsored allopathic healthcare service, which is freely available to all citizens. This is supplemented by private healthcare, consisting of major private hospitals primarily located in metropolitan areas, as well as numerous private general practitioners who provide outpatient treatment in both suburban and rural regions of the country [73]. During the initial phase of the COVID-19 pandemic, state health authorities repurposed many regional hospitals to accommodate the isolation and treatment of COVID-19 patients [74]. Many routine clinics and follow-up centers were unable to operate as usual during this period. Consequently, many patients with noncommunicable diseases and those requiring surgical follow-up received their routine medication without the usual clinical assessments [75]. Some units implemented telephone consultations to reduce hospital attendance [76]. Many patients with long-term, noncommunicable diseases chose to avoid their usual clinic follow-ups. Clinic records provided by the family members of those who passed away during this period revealed that, although these patients had received their usual medications, they had not been seen or evaluated by any medical personnel for many months before their death.

Sudden, unexpected deaths were also observed among those who had sought hospital care. During the pandemic, hospital authorities faced significant challenges in maintaining continuous service provision [77,78]. To minimize transmission of COVID-19, healthcare staff were strategically scheduled, resulting in many units operating below optimal capacity. All incoming patients were initially screened for COVID-19, and those testing positive were transferred to quarantine units. These procedures inevitably led to delays in initiating acute management, postponements in investigations, a shortage of specialist staff, and extended emergency response times, as evidenced in the medical records of some hospital deaths.

Analysis of Deaths due to COVID-19

In this study, we observed a higher proportion of COVID-19-related deaths among females compared to males. In contrast, many studies have reported that males had a higher risk of mortality [79], despite the higher number of infected females [80]. The reason for our finding is unclear, although

it could reflect gender disparities in healthcare accessibility. Interestingly, a higher percentage of females had not received the COVID-19 vaccine. Although the difference was not statistically significant, the highest proportion of COVID-19 deaths occurred among unvaccinated females. This gender discrepancy in COVID-19 vaccination is a global trend, with females showing lower intent and acceptance of the vaccines [81]. Regrettably, we lacked reliable data to determine whether this trend was also present in the Sri Lankan context.

The Impact of COVID-19 Vaccination on Excess Mortality

The frequency of COVID-19-related deaths in our cohort aligned with national death rates, with the highest proportion occurring in August. Nearly one-third of those who died from COVID-19 had received at least 1 dose of the vaccine. However, we did not have data regarding the interval between vaccination and death, preventing us from establishing a clear link with vaccine efficacy. Despite reports of breakthrough COVID-19 infections post-vaccination, substantial evidence supports a decrease in hospitalization and mortality rates among vaccinated individuals [82–84]. Consistent with this, our study also found a higher proportion of COVID-19-related deaths among the unvaccinated group, further reinforcing the life-saving potential of COVID-19 vaccination.

The question of whether COVID-19 vaccination leads to increased mortality rates from other causes has been explored. The consensus is that there has been no rise in overall mortality among those who have been vaccinated [85,86]. However, given that 80% of the vaccinations in our study involved the Sinopharm vaccine, the applicability of our findings to these studies may be limited. In our cohort, a significantly higher proportion of vaccinated males died from cardiovascular causes compared to the unvaccinated group. Likewise, while the proportion of vaccinated females who died from cardiovascular causes was lower than that of unvaccinated females, the median age in the vaccinated group was notably lower. These findings necessitate further investigation in a larger population with more rigorous case selection to evaluate any potential association between COVID-19 vaccination and cardiovascular mortality. We observed no significant differences in any other non-COVID-19 categories of natural deaths in relation to COVID-19 vaccination, nor did we record any deaths directly attributable to the vaccination.

Limitations of the Study

This study examined mortality trends over the past 4 years, providing a comparative analysis of deaths before, during, and after the COVID-19 pandemic. The data collected were

derived from post-mortem registers and autopsy reports. The primary limitation of this study was data availability, particularly concerning vaccination history. Additionally, the number of cases within certain broad death categories was too small to provide reliable analysis. In the early stages of the pandemic, many families were hesitant to disclose any history of contact with COVID-19 or recent respiratory illnesses. This reluctance likely impacted the accuracy of COVID-19 death diagnoses at autopsy. It is also plausible that the composition of these communities may have changed during the COVID-19 restrictions due to various social and occupational factors. The pandemic's disruption of administrative, health, and judicial services may have influenced decisions to refer deaths for autopsy. These changes would not be reflected in the study's results. Another potential limitation is the subjectivity involved in determining causes of death. However, since most cases were discussed and reviewed among departmental specialists, this bias is likely minimal.

Conclusion

This retrospective analysis examined mortality trends within a specific urban community in Sri Lanka across 3 periods related to the COVID-19 pandemic. During the community's COVID-19 lockdown, there was an increased fatality rate from various chronic natural conditions. This increase predominantly affected highly urbanized, densely populated communities where COVID-19 restrictions were consistently and stringently enforced. In contrast, unnatural deaths presented a mixed picture. There was a decrease in fatalities from traffic accidents, firearm incidents, suicides by hanging, and poisonings. However, there was also an uptick in deaths resulting from blunt trauma, sharp weapon homicides, and drownings. COVID-19-related deaths peaked approximately 6 months after the vaccination program's initiation. The majority of these deaths were among individuals over 65, those who were not vaccinated, and females. Aside from a significantly higher proportion of cardiovascular deaths among vaccinated males, and a non-significantly higher proportion of COVID-19 deaths among non-vaccinated females, there were no other discernible differences in mortality trends between the vaccinated and non-vaccinated groups.

Notes

Ethics Approval

This study was granted ethics approval from the Ethics Review Committee of the Faculty of Medicine, University of Colombo under EC-21-055.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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None.

Availability of Data

The datasets generated during and/or analyzed during the current study are not publicly available due to them being related to forensic death investigations but are available from the corresponding author on reasonable request.

Authors' Contributions

Conceptualization: SAG; Data curation: ND, SK, BIK, HR; Formal analysis: SAG, JWG; Methodology: SAG; Project administration: ND, SK; Resources: ND, HR, SK, JWG; Supervision: SAG; Writing—original draft: SAG, SK, BIK, JWG; Writing—review & editing: all authors. All authors read and approved the final manuscript.

Additional Contributions

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Characteristics and related factors of waterborne and foodborne infectious disease outbreaks before and after the onset of the COVID-19 pandemic (2017–2021) in the Republic of Korea: a descriptive study

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ABSTRACT

Objectives: The incidence of waterborne and foodborne infectious diseases (WFIDs) continues to increase annually, attracting significant global attention. This study examined trends in WFID outbreaks in the Republic of Korea over the 5-year period before and during the coronavirus disease 2019 (COVID-19) pandemic and provided foundational data to establish measures for the prevention and control of WFID outbreaks.

Methods: We analyzed 2,541 WFID outbreaks from 2017 to 2021 (42,805 cases) that were reported through the Integrated Disease Surveillance System of the Korea Disease Control and Prevention Agency. Outbreaks were defined as the occurrence of gastrointestinal symptoms in ≥ 2 individuals within a group with temporal and regional epidemiological associations. The related factors associated with WFID outbreaks during the observation period were statistically analyzed.

Results: The total number of WFID outbreaks significantly decreased in 2020 during the COVID-19 pandemic and increased to the pre-pandemic level in 2021. Different patterns were observed for each pathogen. The incidence of *Salmonella* outbreaks more than doubled, while norovirus outbreaks decreased significantly.

Conclusion: WFID outbreaks in the Republic of Korea showed different patterns before and during the COVID-19 pandemic, influenced by infection control measures and changes in dietary consumption patterns. Outbreaks of some diseases increased, but the infection control measures applied during the pandemic resulted in a significant decrease in the overall number of WFID outbreaks. This highlights the importance of strengthening the management strategies for outbreak prevention through hygiene inspections, long-term monitoring, education, and promotion by conducting multidimensional analyses to understand the complex related factors.

Keywords: COVID-19; Diseases outbreaks; Food-borne diseases; Gastrointestinal diseases

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Introduction

Waterborne and foodborne infectious diseases (WFIDs) are caused by pathogenic bacteria and viruses. Symptoms such as diarrhea, vomiting, fever, and abdominal pain are the typical manifestations of WFIDs. The increasing incidence of WFIDs is a significant global phenomenon. In the Republic of Korea, 400 to 600 WFID cases were reported annually, with an upward trend due to factors such as dining out and institutional food service [1–3].

In 2017, WFID outbreaks involved numerous cases of shigellosis, typhoid fever, and rotavirus. Recent increases in norovirus and *Salmonella*, as well as outbreaks of enterohemorrhagic *Escherichia coli* (EHEC) and hepatitis E, have also been reported. In particular, the changes in WFIDs during pandemics, such as coronavirus disease 2019 (COVID-19), are noteworthy.

On March 11, 2020, COVID-19 was declared a pandemic by the World Health Organization. Subsequently, the nationwide implementation of nonpharmaceutical interventions in the Republic of Korea significantly reduced the weekly positivity rates for respiratory viral infections in 2020. Infection control measures, including personal hygiene and social distancing, may have also reduced the positivity rates for non-respiratory infectious diseases [4–7].

A comparative study investigating trends in the occurrence of gastrointestinal (GI) infections in the United States (US) and the Republic of Korea before and during the COVID-19 pandemic revealed a notable decline in both bacterial and viral GI infections in the US. In the Republic of Korea, only the decrease in viral GI infections was statistically significant [8].

The occurrence of WFID outbreaks has demonstrated diverse trends for individual pathogens, influenced by seasonal variations and the prolonged impact of the COVID-19 pandemic. This study analyzed and assessed temporal (2017–2021) and regional (17 municipalities and provinces in the Republic of Korea) trends in WFID occurrence, and compared the epidemiological characteristics of WFID outbreaks before and during the pandemic.

Materials and Methods

Study Population

A total of 2,541 reported outbreaks and 42,805 cases of WFIDs, reported from January 2017 to December 2021 through the Integrated Disease Surveillance System of the Korea Disease Control and Prevention Agency (KDCA), were analyzed. Data were obtained from 17 administrative regions nationwide and were classified according to the addresses of the reporting institutions.

The types of WFIDs included Class II (cholera, typhoid and

HIGHLIGHTS

- Waterborne and foodborne infectious disease (WFID) outbreaks significantly decreased during the coronavirus disease 2019 (COVID-19) pandemic, whereas bacterial and viral outbreaks increased and decreased, respectively, depending on the major pathogens.
- The infection control measures for COVID-19 resulted in different outbreak patterns across various settings. In 2020, school-related outbreaks decreased, whereas restaurant-related outbreaks remained constant. Thus, strengthening outbreak management strategies through hygiene inspections, monitoring, education, and promotion is crucial.
- During the COVID-19 pandemic, a consistent increase in WFID outbreaks coincided with an increase in food delivery services and convenience food consumption, as well as the sustained occurrence of seasonal outbreaks. Therefore, enhanced monitoring for major infectious diseases should also consider changes in dietary consumption patterns and seasonal factors. For effective prevention and control strategies, multidimensional analyses should be used to determine the predictive factors.

paratyphoid fever, bacterial dysentery, EHEC, and hepatitis A and E), Class III (*Vibrio* sepsis), and Class IV (20 other GI infections) infectious diseases. The related pathogens included 17 bacterial species (including enteroadherent *E. coli*), 6 viral species, 4 protozoal species, and *Kudoa septempunctata*. Class II and Class III infectious diseases were managed under the mandatory surveillance system, and Class IV infectious diseases (GI infections) were managed under the sentinel surveillance system implemented at 208 facilities nationwide [2].

The reported cases of WFIDs included in this study were cases in which the causative pathogens were confirmed through laboratory analysis of human samples. An outbreak was defined as a concurrent infection in ≥ 2 individuals who experienced symptoms of an enteric infection such as diarrhea and vomiting after consuming the same food. According to the operational criteria of the epidemiological investigation team, outbreaks with ≥ 7 and 2 to 6 related cases were classified as large- and small-scale outbreaks, respectively.

Data Analysis

To describe the general characteristics of WFID outbreaks and to provide temporal and regional occurrence trends, data analysis was performed using Excel ver. 2016 (Microsoft

Corp.). The WFID incidence rate per 100,000 population in each region was calculated to examine the population-standardized rates and regional variations, using the mid-year (July 1) population data from the resident population registry released by the Ministry of the Interior and Safety. To calculate WFID incidence rates, the annual population data from 2017 to 2021, as categorized by year, age group, region, and district, were used as the denominator, and the number of WFID cases in the corresponding category was used as the numerator. Meteorological data for each year were obtained from the weather data portal service provided by the Korea Meteorological Administration [9]. We analyzed trends in WFID outbreaks according to meteorological factors such as the number of days with heat waves and days with precipitation from 2017 to 2021.

IRB/IACUC Approval

This study was granted an exemption from review by the Institutional Review Board of the KDCA (IRB No: 2023-03-01-PE-A).

Results

Outbreak Status

Annual trends

The number of WFID outbreaks peaked at 600 to 700 in 2018–2019. Following the implementation of social distancing measures in February 2020 due to the COVID-19 pandemic, the number of outbreaks sharply decreased to approximately 200. Since 2021, the number of outbreaks has rebounded to the previous average level.

While WFID outbreaks decreased overall because of the COVID-19 infection control measures, different patterns were observed for specific pathogens. Among bacterial infections, the incidence rate of salmonellosis more than doubled. Among viral infections, the incidence rate of norovirus outbreaks decreased significantly.

Salmonellosis outbreaks occurred at or above the average level. In 2020, salmonellosis outbreaks accounted for 8.5% of all WFID outbreaks, which was the highest proportion during the observation period.

On average, salmonellosis outbreaks accounted for 4.2% of all WFID outbreaks during the observation period, with a consistent proportion of roughly 3% to 4% each year and a trend toward a high number of cases per outbreak. Notable outbreaks of *Salmonella* included the “chocolate cake,” “gimbap restaurant,” and “cold noodle restaurant” outbreaks in 2018 and 2021. A continued increase was expected with the increase in outdoor activities related to seasonal factors (summer), the relaxation of infection control measures, and the increased use of recreational facilities during the vacation season.

The number of norovirus outbreaks decreased significantly to 51 in 2020, half the pre-pandemic levels. However, norovirus still accounted for 21.8% of all WFID outbreaks, and this proportion remained at the pre-pandemic level. During the observation period, the average number of norovirus outbreaks accounted for 31.9% of all WFID outbreaks, ranging between 20% and 30% each year. Notable norovirus outbreaks include the PyeongChang Olympics outbreak in 2018.

Monthly trends

During the observation period, WFID outbreaks mainly

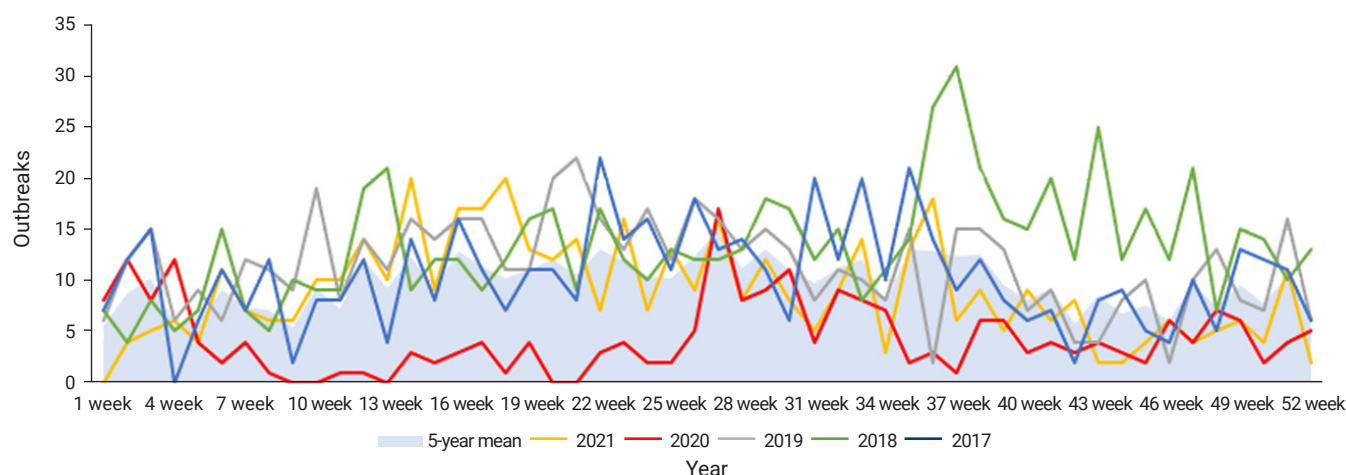


Figure 1. Weekly outbreak status of waterborne and foodborne infectious diseases before and during the COVID-19 pandemic in the Republic of Korea.

Table 1. Monthly outbreak status of waterborne and foodborne infectious diseases in the Republic of Korea before and during the COVID-19 pandemic

Month	Total (n)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2021													
Outbreaks	471	20	31	56	70	50	51	50	49	34	21	22	27
Cases	7,628	266	462	822	927	654	607	1,195	922	592	386	270	525
2020													
Outbreaks	234	40	10	3	11	7	20	42	27	17	15	20	22
Cases	3,459	285	111	135	143	24	506	780	303	159	258	443	312
2019													
Outbreaks	600	48	35	56	67	72	66	58	48	48	29	35	38
Cases	7,659	503	275	988	1,019	873	952	772	517	308	344	577	531
2018													
Outbreaks	691	28	32	61	47	62	51	69	50	95	79	62	55
Cases	16,247	178	318	1,334	848	1,213	1,068	979	1,586	2,787	970	880	872
2017													
Outbreaks	539	38	33	33	50	51	65	56	65	50	25	30	43
Cases	7,770	296	215	351	858	691	904	753	1,346	1,179	379	369	471

Values are presented as number only.

occurred between May and September of each year. In 2018, an earlier increase in the number of outbreaks than seen in the average year was observed, starting in early spring and continuing into winter. In 2020, a significant decrease in monthly outbreaks, compared with the average level, was observed (Figure 1).

Among the bacterial infections investigated, the average monthly number of salmonellosis outbreaks was 1.8, with a seasonal concentration of outbreaks during the summer (June to October). The highest number of salmonellosis outbreaks occurred in October 2017, September 2018, August 2019, August 2021, and May 2022; these also demonstrated a pattern for earlier onset over the same 5 years. In 2022, the cumulative number of salmonellosis outbreaks reached 40, approximately 2.5 times higher than the average level. This may be attributed to the relaxation of COVID-19 infection control measures and the increase in outdoor activities.

In the analysis of viral infections by month during the observation period, norovirus outbreaks predominantly occurred in early spring (January to April) and in winter (November and December). In 2020, a notable decrease in monthly outbreaks compared to a typical year was observed (Table 1).

Outbreak status by region

During the observation period of this study, the highest incidence of WFID outbreaks nationwide was recorded in 2018, with 25.1 cases per 100,000 population. The regions with the highest incidence rates, in descending order, were Sejong (58.6 cases), Gyeongsangbuk-do (51.0 cases), Seoul (36.8 cases), Jeju (34.2 cases), and Gangwon and Chungcheongnam-do

(33 cases each). Conversely, Daejeon (3.2 cases), Gwangju (4.4 cases), and Ulsan (9.8 cases) had the lowest incidence rates. In 2020, the incidence rates per 100,000 population decreased to <10 cases in most regions, with a national average of 6.4 cases, which was the lowest during the observation period. Seoul and Jeju had the lowest and highest incidence rates of 2.7 and 48.8 cases, respectively (Table 2).

Outbreak status by pathogen

During the observation period, norovirus was the most prevalent causative pathogen of WFID outbreaks, followed by *Salmonella* and *Campylobacter*. In 2020, norovirus outbreaks substantially decreased (51 cases), while the number of *Salmonella* and *Campylobacter* outbreaks remained constant or increased when compared with a typical year (Table 3).

Outbreak status by place of occurrence

During the observation period, WFID outbreaks occurred most frequently in restaurants (253 cases, 49.8%), followed by schools (including kindergartens and daycare centers; 161 cases, 31.7%) (Figure 2). While outbreaks in restaurants remained constant at 200 to 300 cases (50%–60%) per year, the number of cases significantly decreased to 123 in 2020.

When confirmed COVID-19 cases were detected within a facility, the operation of the institution's cafeteria was temporarily suspended, and packed meals were delivered by external vendors or food delivery services. This measure led to an increase in WFID outbreaks. Unlicensed packaged meal providers were identified through hygiene inspections.

The average 5-year incidence of school-related WFID outbreaks was 163 cases, which decreased significantly to 72

Table 2. Regional outbreak status of waterborne and foodborne infectious diseases in the Republic of Korea before and during the COVID-19 pandemic

Region	2017			2018			2019			2020			2021 (estimate)		
	Inc.	Pop.	IR ^{a)}	Inc.	Pop.	IR ^{a)}	Inc.	Pop.	IR ^{a)}	Inc.	Pop.	IR ^{a)}	Inc.	Pop.	IR ^{a)}
Total	539	7,770	15.0	691	13,033	25.1	592	7,630	14.7	234	3,323	6.4	470	7,874	15.2
Seoul	71	1,208	12.3	103	3,591	36.8	82	1,397	14.4	19	261	2.7	47	799	8.4
Busan	15	415	12.0	22	747	21.7	28	652	19.1	20	268	7.9	47	1,184	35.3
Daegu	21	127	5.1	19	395	16.0	17	297	12.2	11	87	3.6	10	221	9.3
Incheon	31	354	12.0	42	521	17.6	37	709	24.0	10	216	7.3	23	280	9.5
Gwangju	15	82	5.6	12	64	4.4	13	66	4.5	6	74	5.1	10	65	4.5
Daejeon	8	206	13.7	4	47	3.2	16	169	11.5	4	72	4.9	8	28	1.9
Ulsan	9	81	7.0	8	113	9.8	3	28	2.4	4	32	2.8	4	35	3.1
Sejong	4	22	7.9	3	184	58.6	5	43	12.6	4	40	11.2	7	331	89.0
Gyeonggi	131	2,485	19.3	156	2,894	22.1	118	1,583	12.0	57	1,006	7.5	111	2,017	14.9
Gangwon	39	343	22.1	29	515	33.4	26	216	14.0	8	98	6.4	24	371	24.1
Chungcheongbuk-do	22	288	18.1	28	385	24.1	49	399	24.9	11	168	10.5	24	438	27.4
Chungcheongnam-do	57	431	20.4	78	708	33.3	65	439	20.7	8	142	6.7	46	660	31.1
Jeollabuk-do	20	433	23.3	26	236	12.8	33	378	20.8	13	126	7.0	14	219	12.3
Jeollanam-do	23	180	9.5	73	431	22.9	2	7	0.4	12	100	5.4	18	168	9.2
Gyeongsangbuk-do	21	581	21.6	66	1,364	51.0	37	466	17.5	22	276	10.5	25	261	9.9
Gyeongsangnam-do	29	259	7.7	43	610	18.1	28	322	9.6	13	150	4.5	37	600	18.1
Jeju	23	275	41.9	9	228	34.2	7	120	17.9	12	329	48.8	15	197	29.1

Values are presented as number only. The reported data for years 2017–2021 are provisional. Incidence rate = (no. of cases/no. of population) × 100,000 (based on the status of the resident registered population provided by the Ministry of the Interior and Safety in 2021).

Inc., incidence; pop., population; IR, incidence rate.

^{a)}Incidence per 100,000 population.

Table 3. Outbreak status of waterborne and foodborne infectious diseases by pathogen before and during the COVID-19 pandemic in the Republic of Korea

Year	Total	Norovirus	Salmonella	Campylobacter	<i>Escherichia coli</i>	<i>Clostridium perfringens</i>	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Vibrio parahaemolyticus</i>	EHEC	HAV
2021	471	127	22	24	15	6	6	3	1	0	13
2020	234	51	19	16	20	6	4	2	1	5	1
2019	600	152	21	12	18	10	5	8	5	1	77
2018	697	178	22	19	38	16	13	4	15	0	0
2017	539	101	23	7	57	9	9	2	10	0	2

Values are presented as number only.

EHEC, enterohemorrhagic *Escherichia coli*; HAV, hepatitis A virus.

cases in 2020. In 2021, school-related outbreaks outnumbered restaurant-related outbreaks. Among the school-related outbreaks in 2021, the number of cases in daycare centers nearly tripled compared with the 5-year average, while the number of cases in primary, middle, and high schools declined.

Outbreaks in workplaces and facilities decreased in 2020 and subsequently increased. School-related norovirus outbreaks displayed a seasonal pattern, with a higher concentration in the spring (March/April) and late autumn/early winter (November/December) semesters.

Outbreak status by outbreak scale

Before COVID-19, approximately 60% of outbreaks were small-scale. In 2020, the proportion of small-scale group outbreaks increased to 64.1%, while in 2021, the proportion of large-scale outbreaks reached 53.8%. In 2020, the scale of outbreaks significantly decreased (approximately 100 people) compared to the pre-pandemic period. In 2021, the number of *Salmonella* cluster cases (> 300 people) related to *gimbap* restaurants and *milmyeon* restaurants increased.

Reported outbreak status

Results from the sentinel surveillance of WFID outbreaks

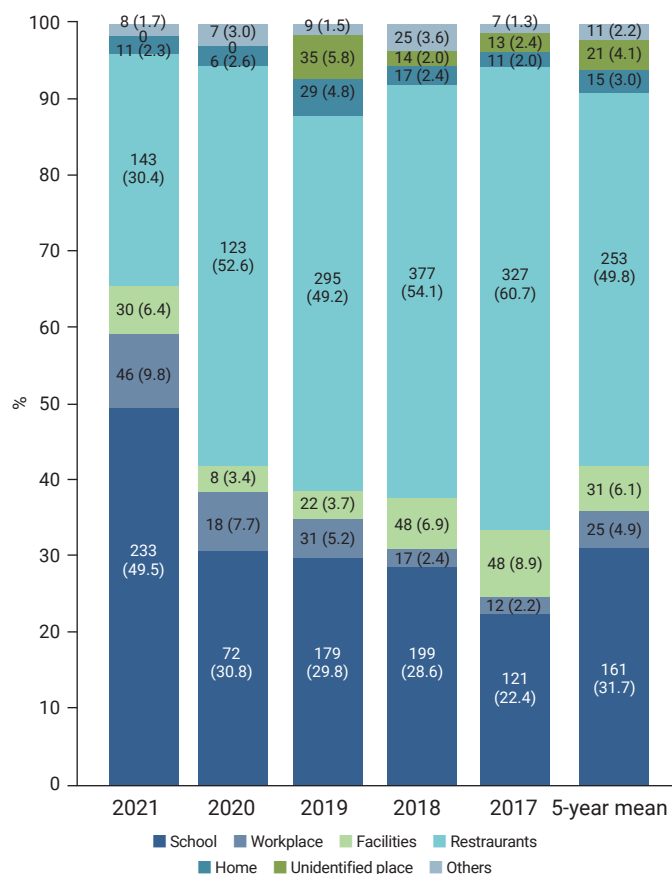


Figure 2. Outbreak status of waterborne and foodborne infectious diseases in the Republic of Korea by place of occurrence before and during the COVID-19 pandemic (2017–2021). Values are presented as number (%).

revealed a significant decrease in viral infections during the pandemic period (2020–2021), with levels half those observed before the pandemic. However, the incidence of bacterial infections was constant or even increased when compared with the pre-pandemic period.

Despite infection control measures, bacterial infections such as salmonellosis and campylobacteriosis occurred at or above average levels in 2020–2021 when compared with pre-pandemic levels. In 2022, more EHEC cases occurred than during the pre-pandemic period. Although reported cases of salmonellosis decreased nationwide during the COVID-19 pandemic, they subsequently increased to levels surpassing the usual rates.

Regarding viral infections, Group A rotavirus maintained a decreasing trend since 2019, while norovirus had a consistently high incidence each year. In 2022, the number of astrovirus, enteric adenovirus, and sapovirus outbreaks was higher than during the pre-pandemic period.

Related Factors

Suspected sources of infection

During the observation period, the most frequently suspected source of infection in WFID outbreaks was seafood (217 cases), followed by mixed foods (59 cases) and eggs and poultry (50 cases). Seafood-related outbreaks peaked around 2018, possibly because the average seawater temperature that year was the highest recorded. Eggs and poultry maintained a relatively stable infection rate of approximately 2%. Notably, an increasing trend of person-to-person (P2P) transmission was observed in 2021.

The identification rate for suspected sources of infection in WFID outbreaks was approximately 20% during 2017–2021, while unidentified sources remained at approximately 75% to 80%, regardless of COVID-19 infection (Table 4). Even when pathogens were detected in human samples, the rate of obtaining consistent test results from environmental samples (e.g., preserved food) was low. This discrepancy can be attributed to the sampling practices in restaurants, where samples are often taken from leftover food ingredients because preserved food samples are difficult to obtain, unlike in schools and companies that provide institutional food services. The sources of infection in salmonellosis outbreaks have consistently been identified as eggs and poultry, pork, bakery products, and other mixed foods such as *gimbap* (seaweed rice roll), cold noodles, and pancakes with multiple ingredients.

Meteorological factors

During the observation period, 2018 had the highest number of heatwave days (31), WFID outbreaks (697), and cases (16,247). In 2020, the number of heatwave days decreased considerably to 7.7, and the incidence of WFIDs reached a record low with 234 outbreaks and 3,459 cases. Furthermore, the number of wet days in 2018 was 10.8, which was unusually low. However, in 2020, the number of wet days nearly tripled to 28.5 (Figure 3).

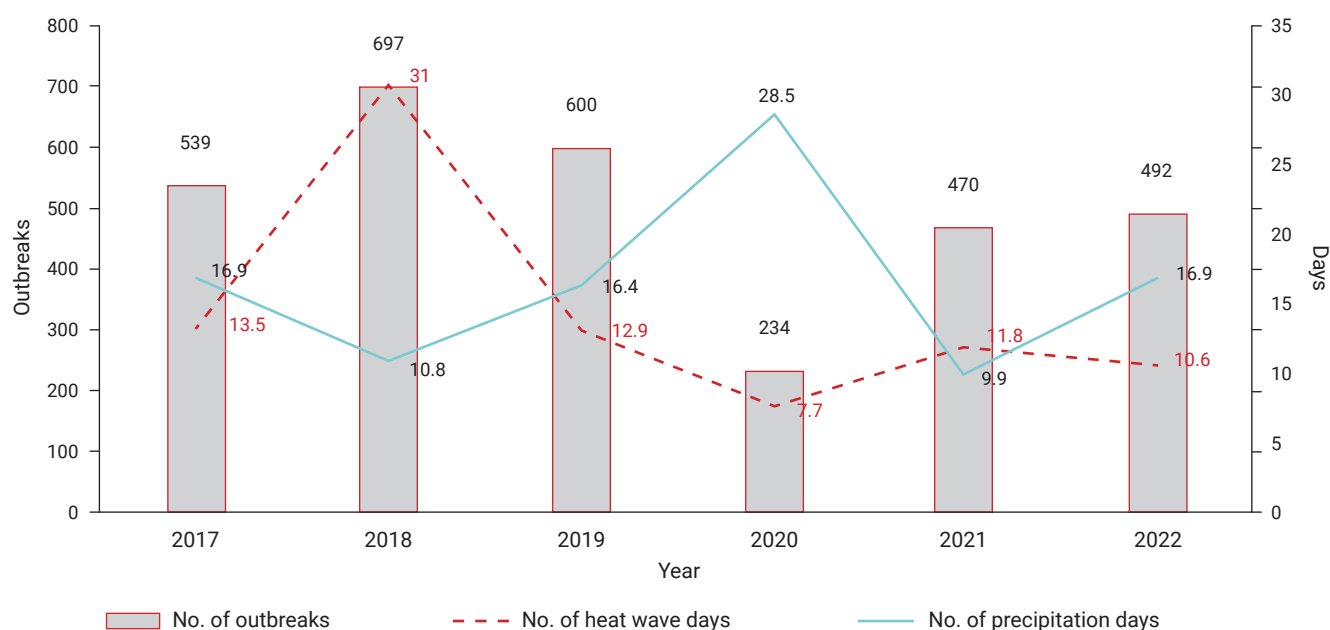
Discussion

This study examined the 5-year trends and related factors of WFID outbreaks before and during the COVID-19 pandemic. In the Republic of Korea, more than 400 to 600 group outbreaks of WFID occur every year. Over the past 10 years, the highest number of group outbreaks was confirmed in 2018, with 697 cases. Norovirus has maintained a high incidence every year, and Group A rotavirus has shown a decreasing trend every year since 2019. In 2022, astrovirus, enteric adenovirus, and sapovirus showed higher trends than before the COVID-19 outbreak.

Table 4. Outbreak status of waterborne and foodborne infectious diseases by the suspected source of infection before and during the COVID-19 pandemic in the Republic of Korea

	Year				
	2017	2018	2019	2020	2021
Total	539 (100)	697 (100)	600 (100)	234 (100)	471 (100)
Food/water					
Chocolate cake, confectionery	0 (0)	2 (0.3)	0 (0)	1 (0.4)	0 (0)
Eggs, poultry	7 (1.3)	11 (1.6)	14 (2.3)	5 (2.1)	13 (2.8)
Pork	2 (0.4)	6 (0.9)	0 (0)	0 (0)	0 (0)
Beef	2 (0.4)	1 (0.1)	1 (0.2)	0 (0)	0 (0)
Shellfish	62 (11.5)	88 (12.6)	46 (7.7)	12 (5.1)	9 (1.9)
Raw meat, raw liver	0 (0)	1 (0.1)	2 (0.3)	1 (0.4)	3 (0.6)
Kimchi, vegetables	12 (2.2)	14 (2.0)	8 (1.3)	1 (0.4)	2 (0.4)
Mixed food	8 (1.5)	15 (2.1)	6 (1.0)	13 (5.6)	17 (3.6)
Drinking water/ground water	10 (1.9)	9 (1.3)	8 (1.3)	0 (0)	2 (0.4)
Others					
Vomiting, person-to-person transmission	5 (0.9)	18 (2.6)	14 (2.3)	9 (3.8)	43 (9.1)
Infection transmitted by cooks	7 (1.3)	3 (0.4)	0 (0)	4 (1.7)	2 (0.4)
Agriculture, other water	0 (0)	1 (0.1)	0 (0)	1 (0.4)	0 (0)
Total	115 (21.3)	169 (24.2)	99 (16.5)	48 (20.5)	91 (19.3)
Unidentified	424 (78.7)	528 (75.8)	501 (83.5)	186 (79.5)	380 (80.7)

Values are presented as number (%).

**Figure 3.** Number of waterborne and foodborne infectious disease outbreaks according to heat wave days and precipitation days (2017–2022) in the Republic of Korea.

In a US study, the incidence of food poisoning during the pandemic decreased by as much as 60% [5]. The incidence rate of foodborne infection in 2020 decreased by 26%, reflecting a record-high single-year fluctuation in incidence rates in over 25 years of the FoodNet surveillance [10]. In a

UK study, the number of food poisoning cases decreased by 56.4%, from 191 to 83 cases/week [11].

In the Republic of Korea, the pre-pandemic period (2017–2019) had the highest number of WFID cases. These significantly decreased in 2020 during the pandemic. Moreover, in the

last decade (2011–2021), the lowest number of both outbreaks and cases occurred in 2020. However, an increase has been observed since the gradual relaxation of infection control measures in 2021.

In 2020, while small-scale group outbreaks increased significantly, large-scale outbreaks decreased significantly when compared to the pre-COVID-19 period. Since 2021, large-scale outbreaks related to general restaurants have increased. It is presumed that the relaxation of quarantine measures increased opportunities for gathering and face-to-face contact.

The high population density of the Seoul capital area (> 50% of the national population) makes this area more susceptible to highly communicable infectious diseases because of the high level of interaction among residents [12]. In a US study, states with earlier re-openings and fewer restrictions had the lowest rates of foodborne infectious diseases during the COVID-19 pandemic [5]. In 2020, the weekly occurrence of WFID outbreaks in the Republic of Korea remained below a typical year's weekly occurrence level, and this downward trend continued nationwide.

The incidence rate of WFID outbreaks was high in areas such as Jeju, Sejong, and Busan (Table 2), potentially as a result of mass food poisoning in those areas. The level of social distancing measures in metropolitan and non-metropolitan areas during the early stage of the COVID-19 pandemic (2020) can also be considered.

In 2020, WFID outbreaks in schools decreased, while outbreaks in daycare centers and kindergartens increased. Norovirus was identified as the primary cause of most cases of acute gastroenteritis in daycare centers and schools and was spread via P2P contact. School outbreaks were larger, lasted for less time, had more seasonal patterns, and involved fewer contacts compared with outbreaks in childcare centers [13].

P2P contact was the predominant mode of transmission for both bacterial and viral pathogens. While the transition to online classes partially restricted P2P transmission in schools, facilities like daycare centers continued to experience ongoing exposure. To accurately assess these variations, based on differences in community and school outbreak patterns and the characteristics of pathogens that primarily spread through the P2P route, more in-depth analysis and enhanced monitoring of WFID outbreak trends are necessary.

The main source of infection for norovirus is human-to-human transmission, which increased following the relaxation of COVID-19 quarantine measures and the normalization of outdoor activities and face-to-face classes at schools. In particular, this indicated that nonpharmaceutical interventions were effective in reducing the spread of

disease transmitted through close person-to-person contact. However, the impact of delayed and low reporting during the relevant period must be taken into consideration.

Salmonella is a common causative agent of WFID outbreaks in the Republic of Korea, ranking second to norovirus. Salmonellosis outbreaks have been steadily increasing due to the increased consumption of convenience foods and seasonal influences. Foodborne illnesses have a markedly higher incidence among individuals with higher education and income levels, despite their access to high-quality healthcare services, possibly due to more frequent international travel, consumption of high-risk foods, and dining out [5,10].

The incidence of bacterial diseases characteristically increases when the temperature reaches the ideal range for bacterial growth [14]. A time-series regression study using data from 10 European countries revealed a positive linear relationship between temperature and salmonellosis incidence. The strongest correlation was observed 1 week prior to disease onset. Similarly, positive correlations were found between specific temperature thresholds and the incidence of salmonellosis and campylobacteriosis [15,16]. In the Republic of Korea, the monthly and yearly isolation rates of *Salmonella* begin to increase in March, coinciding with the rise in average temperature to above 10 °C. High isolation rates are observed from June to October, when the average temperature exceeds 20 °C [14].

Notable salmonellosis outbreaks have occurred in the Republic of Korea. In 2018, contaminated egg whites, mixed into chocolate cake batter, were identified as the suspected source of infection ("chocolate cake case"). This outbreak affected schools across 12 regions nationwide, where students consumed the contaminated cakes. Other salmonellosis outbreaks linked to contaminated eggs include incidents at a *gimbap* restaurant in Gyeonggi Province, a *milmyeon* (wheat noodles) restaurant in Busan in July/August 2021, and a *naengmyeon* (cold noodles) restaurant in Gyeongsangnam-do in May 2022. In 2022, the cumulative number of salmonellosis outbreaks reached 40, displaying an earlier onset of increase than seen in a typical year, reflecting an upward trend in the number of cases [17].

In 2021, WFID outbreaks related to outdoor activities such as local festivals and gatherings increased, exhibiting different patterns compared with previous years. From a public health perspective, post-pandemic monitoring of the anticipated rebound is crucial, and includes monitoring of the public's health management behaviors, testing practices, and international travel trends [18]. Continued post-pandemic surveillance may improve our understanding of the pandemic's impact on foodborne illnesses and help develop appropriate preventive measures and strategies

for major pathogens and related food groups [9]. When analyzing and interpreting long-term post-pandemic trends, it will be important to carefully consider evaluation frame to prevention and management strategies from a more comprehensive point of view [19].

These incidents underscore the importance of adopting a multidimensional analytical approach and fostering enhanced collaboration among various departments, including the Ministries of Drug and Food Safety and Agriculture, to effectively address major foodborne bacterial infections. In addition, monitoring the shifting trends in dietary consumption patterns and healthcare utilization during the pandemic and strengthening the monitoring of seasonal variations in major infectious diseases is crucial.

Norovirus accounted for approximately 20% to 30% of group outbreaks over the past 5 years. Before 2020, pathogens such as hepatitis A and *Vibrio parahaemolyticus* tended to be high, but after 2020, *Salmonella*, *Campylobacter*, *Clostridium perfringens*, and EHEC increased more than twofold. In 2022, a group outbreak of hepatitis E was confirmed, and major bacterial pathogens such as norovirus, *Salmonella*, and *Campylobacter* bacteria took center stage. Each year, the causative pathogen could not be identified in >40% of cases.

In 2020, the unknown pathogen rate continued due to delays in reporting (the staffing for epidemiological investigations was focused on responding to COVID-19) and uncollected specimens (due to the nature of WFID epidemiological investigations) during the pandemic. Problems with rapid acquisition and poor cooperation may have had an impact; therefore, it is necessary to strengthen outbreak management and increase the pathogen identification rate during a pandemic.

The identification rate of the presumptive source of infection in group outbreaks has been approximately 20% over the past 5 years. Before and during the COVID-19 pandemic, the unknown source infection rate was 75% to 80%.

The highest proportion of fish and shellfish cases was observed in 2018, which may have been linked to the highest average temperature that year, reflecting climate change. Environmental factors, such as climate change, are directly associated with changes in the distribution of WFID causative pathogens [4]. Several domestic and international studies have reported associations between WFID and meteorological factors, including temperature, humidity, wind speed, and precipitation [20].

In the Republic of Korea, bacterial gastroenteritis primarily occurs during seasons characterized by high temperature and humidity. During 2010–2019, the incidence of enteric infections increased, especially in 2015–2019, where norovirus, *Campylobacter*, and *Salmonella* were the predominant pathogens

[21]. In 2020, the most commonly reported foodborne pathogens were norovirus, *Salmonella*, *Campylobacter*, and *C. perfringens*. The incidence of campylobacteriosis and salmonellosis outbreaks decreased from the 11th week of 2020 to a record low in monthly incidence. However, the incidence rate of both diseases increased after the summer of 2020 [22,23]. Salmonellosis outbreaks in the Republic of Korea in 2020 and 2021 either maintained the 5-year average level or increased, with a gradually earlier onset.

Precipitation amounts are associated with an increased risk of viral diarrhea. Intensive rainfall in summer may not provide a favorable environment for viruses [8]. In a country with a temperate climate like the Republic of Korea, lower temperatures are associated with an increased risk of viral gastroenteritis, including norovirus, rotavirus, and adenovirus infections, with a relatively long lag effect. Conversely, higher temperatures are associated with an increased risk of bacterial gastroenteritis, including salmonellosis and campylobacteriosis, with a relatively short lag effect [20].

When monitoring and surveilling WFID outbreaks, it is necessary to comprehensively analyze climatic factors and consider the seasonal characteristics of major infectious diseases as predictive factors. In this study, we described the current status of domestic meteorological factors and outbreaks over the past 5 years. However, there were limitations in interpretation, and follow-up research is needed to study the relationship with climate factors.

In Germany and Denmark, during the COVID-19 pandemic, a decrease in shopping frequency was associated with an increase in frozen and canned food consumption, indicating that people substituted fresh foods with frozen or canned alternatives. The decreased consumption of fresh fruits, vegetables, meat, and dairy products contributed to the reduction in foodborne illnesses [18]. Furthermore, consumer purchasing patterns rapidly shifted to online purchases, accelerating the transition to digital services and influencing food purchasing preferences. Dining at home replaced dining out; hence, a significant increase in food delivery expenditures was observed [6].

The closure of institutional food service facilities in the Republic of Korea led to an explosive increase in food delivery services. However, WFID outbreaks have consistently occurred in relation to unlicensed or small-scale food delivery businesses, which highlights the potential risks associated with the use of such services. Changes in demand and food consumption patterns during the pandemic indicate the need to strengthen the management and supervision of unlicensed businesses, including hygiene inspections and the provision of employee education.

The decrease in WFID outbreaks can be attributed to

infection control measures implemented during the pandemic and to a combination of environmental, behavioral, political, economic, and social factors, including changes in dietary consumption patterns. This study did not analyze social and economic factors; therefore, further research is necessary to evaluate the impact of these factors.

Conclusion

During the COVID-19 pandemic, a significant decrease in overall WFID outbreaks was observed in the Republic of Korea. However, outbreaks of certain bacteria, such as *Salmonella*, continued to increase (with seasonal fluctuations). The patterns of WFID outbreaks before and during the pandemic exhibited distinctive characteristics, influenced by seasonal factors and infection control measures. Therefore, enhanced monitoring for major infectious diseases should also consider changes in dietary consumption patterns and seasonal factors. Long-term changes in post-pandemic surveillance data must be contextualized to develop specific pathogen-targeted preventive measures and strategies.

Notes

Ethics Approval

This study was approved by the Institutional Review Board of the KDCA (IRB No: 2023-03-01-PE-A). The requirement for informed consent was waived by the IRB.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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None.

Availability of Data

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

Authors' Contributions

Conceptualization: all authors; Data curation: EK; Formal analysis: EK; Investigation: EK; Methodology: all authors; Project administration: all authors; Resources: EK; Software: EK; Supervision: BIK; Validation: EK; Visualization: EK; Writing—original draft: EK; Writing—review & editing: all authors. All authors read and approved the final manuscript.

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Genetic diversity and evolutionary patterns of SARS-CoV-2 among the Bhutanese population during the pandemic

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ABSTRACT

Objectives: The coronavirus disease 2019 (COVID-19) pandemic, caused by a dynamic virus, has had a profound global impact. Despite declining global COVID-19 cases and mortality rates, the emergence of new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants remains a major concern. This study provides a comprehensive analysis of the genomic sequences of SARS-CoV-2 within the Bhutanese population during the pandemic. The primary aim was to elucidate the molecular epidemiology and evolutionary patterns of SARS-CoV-2 in Bhutan, with a particular focus on genetic variations and lineage dynamics.

Methods: Whole-genome sequences of SARS-CoV-2 collected from Bhutan between May 2020 and February 2023 ($n=135$) were retrieved from the Global Initiative on Sharing All Influenza Database.

Results: The SARS-CoV-2 variants in Bhutan were predominantly classified within the Nextstrain clade 20A (31.1%), followed by clade 21L (20%) and clade 22D (15.6%). We identified 26 Pangolin lineages with variations in their spatial and temporal distribution. Bayesian time-scaled phylogenetic analysis estimated the time to the most recent common ancestor as February 15, 2020, with a substitution rate of 0.97×10^{-3} substitutions per site per year. Notably, the spike glycoprotein displayed the highest mutation frequency among major viral proteins, with 116 distinct mutations, including D614G. The Bhutanese isolates also featured mutations such as E484K, K417N, and S477N in the spike protein, which have implications for altered viral properties.

Conclusion: This is the first study to describe the genetic diversity of SARS-CoV-2 circulating in Bhutan during the pandemic, and this data can inform public health policies and strategies for preventing future outbreaks in Bhutan.

Keywords: Coronavirus spike glycoprotein; Molecular epidemiology; Mutation; SARS-CoV-2

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory

syndrome coronavirus 2 (SARS-CoV-2), has had a profound global impact. It has resulted in a significant loss of human life and has posed unprecedented threats to public health systems, food security, economies, and education [1]. On May 5, 2023, the World Health Organization (WHO) declared that COVID-19 no longer constituted a Public Health Emergency of International Concern. Instead, it was recognized as an established and ongoing health challenge [2]. As of May 18, 2023, there have been over 766 million confirmed cases of COVID-19, leading to 6.9 million reported deaths worldwide. In Bhutan, there have been 62,668 confirmed cases of COVID-19, resulting in 21 deaths [3].

The SARS-CoV-2 virus is subject to frequent mutations, which result in the emergence of various new variants. The complete genomic sequence of SARS-CoV-2 was first published on January 3, 2020 [4]. Since that time, over 151 thousand genomic sequences have been submitted to the Global Initiative on Sharing All Influenza Database (GISAID) [5]. The SARS-CoV-2 genome is composed of a positive-sense, single-stranded RNA of approximately 30,000 nucleotides, with a guanine-cytosine (GC) content of 38% [6]. It encodes and expresses a total of 12 proteins. The genome includes 13 to 15 open reading frames (ORFs), of which 12 are functional. Two-thirds of the genome is made up of 2 ORFs, ORF1a and ORF1b, which encode 2 polyproteins, pp1a and pp1ab, through a ribosomal frameshifting mechanism. These polyproteins are subsequently processed by viral proteases into 16 non-structural proteins (NSPs), which facilitate the replication and transcription of the viral RNA. The virus forms a membrane-associated replication and transcription complex that shields the viral RNA from the host's innate immune sensors, thereby enhancing viral replication efficiency [7,8]. Notably, the ORF1ab and 4 structural proteins (spike, envelope, membrane, and nucleocapsid) are arranged in a 5'-3' order and are potential targets for drugs and vaccines. In addition, the genome encodes accessory proteins and other NSPs via the ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10 genes [8].

Nucleotide variations in genes can alter the structure and function of viral proteins. These mutations can influence viral characteristics, potentially triggering new outbreaks, reducing vaccine effectiveness, and impeding the development of new drugs and diagnostic kits [9]. Initially, genetic lineages were identified based on the phylogenetic framework of GISAID [5], Nextstrain [10], or Pangolin [11]. In response to the emergence of new variants and to avoid stigmatizing the countries where these key variants originated, the WHO established a working group of experts. This group is responsible for monitoring the virus's evolution and creating a nomenclature to differentiate these variants. The working group assigned these variants

HIGHLIGHTS

- A phylogenetic analysis of severe acute respiratory syndrome coronavirus 2 genomes from Bhutan revealed rapid diversification across 14 Nextstrain clades and 29 Pangolin lineages with temporal variations.
- The time to the most recent common ancestor of the Bhutanese sequences was February 15, 2020 (95% highest posterior density [HPD], January 3, 2020 to September 13, 2020), with a substitution rate of 0.97×10^{-3} substitutions per site per year (95% HPD, 0.85×10^{-3} to 1.10×10^{-3}).
- The spike glycoprotein (S) exhibited the highest frequency of mutations among major viral proteins, with 116 distinct types, while NSP7 and NSP11 were highly conserved, with no mutations observed.

with letters from the Greek alphabet and classified them as either variants of interest (VOIs) or variants of concern (VOCs), depending on their transmissibility, severity, and ability to evade the immune system [12].

While the overall number of COVID-19 cases is on the decline, thanks to increasing population immunity and decreasing mortality rates, the ongoing evolution of SARS-CoV-2 and the emergence of VOIs and VOCs remain major global concerns [13]. These variants, which include Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Omicron BA.2 (B.1.1.529.2), BQ.1 (B.1.1.529.3), and XBB (B.1.1.529.5), have demonstrated increased transmissibility, immune evasion, or diminished vaccine efficacy in comparison to the original strain [14]. It is essential to monitor the circulation and impact of these variants in order to adjust public health measures as necessary.

The primary objective of this study was to identify the variants and amino acid mutations of SARS-CoV-2 strains that circulated in Bhutan during the pandemic. We conducted a comparative analysis of the nucleotide and amino acid sequences of Bhutanese SARS-CoV-2 strains, using the Wuhan-Hu-1 strain as a reference. In addition, we explored the phylogenetic relationship among these strains to gain insight into their evolutionary links. Our focus was on pinpointing specific mutations and alterations in the amino acid sequence of functional proteins within the SARS-CoV-2 strains from Bhutan, including the spike, envelope, membrane, and nucleocapsid proteins. We analyzed samples collected from Bhutan over an extended period, from May 2020 to February 2023 (i.e., nearly 3 years), to track the

evolutionary changes and trends of SARS-CoV-2 within the Bhutanese population. This extensive temporal distribution enabled us to observe changes over time in the virus's genetic composition and its potential influence on disease dynamics. This study represents the first report on the genomic characterization of SARS-CoV-2 in Bhutan.

Materials and Methods

Sequence Data Acquisition

Full-genome sequences of SARS-CoV-2 from Bhutan were retrieved from the GISAID database (<https://gisaid.org>) for samples collected between May 2020 and February 2023. We only included high-quality whole-genome sequences that exceeded 29,000 base pairs (bp). All sequences were obtained in a multi-sequence FASTA format, and each was accompanied by metadata detailing patient status and sequencing technology. Our dataset consisted of 135 whole-genome sequences, distributed across the years as follows: 40 from 2020, 38 from 2021, 46 from 2022, and 11 from 2023. We downloaded the reference sequence NC_045512.2 as a separate FASTA file. [Supplementary Material 1](#) includes the EPI_SET ID and the respective digital object identifier (DOI) for each SARS-CoV-2 sequence used in this study.

Phylogenetic Analysis

Nextstrain (<https://nextstrain.org>), an open-source bioinformatics visualization platform designed for the phylogenetic analysis of complete SARS-CoV-2 genomic sequences, was used. Nextclade ver. 2.13.0 [15], a web-based tool integrated within Nextstrain, was employed for sequence quality assessment and clade assignment. GISAID clades and WHO labels were determined using the GISAID CoVsurver tool (<https://gisaid.org/>) [16] and the coronavirus typing tool [17], respectively. The Pangolin webserver data ver. 1.19 (<https://pangolin.cog-uk.io/>) was used to further classify lineage typology. The geographical distribution of clades was visualized using Microreact (<https://microreact.org>).

Sequence Alignment and Recombination Analysis

To align the sequences, we obtained pairwise sequence alignment data in FASTA format from Nextclade and conducted multiple sequence alignment (MSA) using MAFFT ver. 7 (<https://mafft.cbrc.jp/alignment/software/>) [18]. This ensured optimal sequence congruence for the following procedures. To explore the presence of recombination events and pinpoint breakpoints within the aligned sequences, we utilized the Recombinant Detection Program ver. 4.5 (RDP4, <http://web.cbio.uct.ac.za/~darren/rdp.html>) [19]. This program

incorporates various algorithms, including RDP, GENECONV, Maxchi, Chimaera, SiScan, PhylPro, and 3Seq. We constructed a maximum likelihood phylogenetic tree directly using RDP4, in conjunction with the RAXML8 [20] algorithm, which takes recombination events into consideration. The final tree annotation, which depicts the evolutionary relationships among the SARS-CoV-2 sequences, was produced using iTOL ver. 6.7.4 (<https://itol.embl.de/>) [21].

Estimation of Time to the Most Recent Common Ancestor

To calculate the time to the most recent common ancestor (TMRCA) and the 95% highest posterior density (HPD) of SARS-CoV-2 genomes from Bhutan, we performed a Bayesian Markov chain Monte Carlo (MCMC) [22] analysis using BEAST ver. 1.10.4 (<https://beast.community/>) [23]. The MSA created by MAFFT was imported into BEAST. We used a Hasegawa-Kishino-Yano substitution model [24] for nucleotide frequencies and kappa, along with a strict molecular clock with uniform rates across branches (rate set to 1.0). We also used a coalescent exponential growth model with a Laplace prior (scale 100), and a coalescent population size prior distribution uniform between 0 and 10. The analysis started with a random starting tree. We ran a total of 100 million MCMC steps, sampling and logging parameters every 10,000 generations. We verified the effective sample sizes using Tracer ver. 1.7.2 (<https://beast.community/tracer>) [25] to ensure a sample size greater than 2,000. We extracted the TMRCA and the 95% HPD from the BEAST output log files using Tracer ver. 1.7.2. The maximum clade credibility (MCC) tree was generated using TreeAnnotator ver. 1.10.4 (<https://beast.community/treeannotator>), discarding the first 10% as burn-in. Finally, we visualized the final tree using FigTree ver. 1.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Mutation Analysis

Both nucleotide and amino acid mutations were identified with the SARS-CoV-2 isolate Wuhan-Hu-1 (NC_045512.2) as a reference, using the GISAID CoVsurver tool and the coronavirus typing tool. We analyzed the frequency of mutations for all 135 sequences, and the frequency of mutations for each protein was calculated based on the total number of samples with point mutations.

IRB Approval

Ethical clearance and approval were not applicable since the data used in this study was obtained from a publicly available database, GISAID. No additional demographic or clinical information was used in this study.

Results

SARS-CoV-2 Genome Analysis

We conducted a comprehensive analysis of the genetic diversity in 135 SARS-CoV-2 genomes, which were gathered from Bhutan between May 2020 and February 2023. These genomes were classified using various nomenclature systems, such as GISAID, Nextstrain, WHO, and Pangolin, through phylogenetic analyses. Furthermore, we offered in-depth insights into the demographic characteristics of the sample set, the quality of the genomes, and their temporal distribution.

The distribution of the SARS-CoV-2 genome across Bhutan was as follows: both Paro and Thimphu had 38 genomes each, while Phuentsholing had 35 genomes. Other locations represented in the dataset included Dagana with 4 genomes, and Punakha, Tsirang, and Mongar, each with 3 genomes. Furthermore, both Samdrupjongkhar and Gelephu had 2 genomes each, while Trongsa and Haa each had a single genome. Our sample set comprised 80 males and 55 females, with ages ranging from 10 to 85 years and a median age of 36 years. The average length of the viral genome was 29,800 bp, with an average genome coverage of 98.8%. The average length of amino acids was found to be 9,691. According to Nextclade's overall quality scoring system, of the 135 sequences analyzed, 131 were classified as good quality, while

4 were deemed mediocre quality. None of the sequences were flagged as bad quality.

Phylogenetic Relationships among Bhutanese SARS-CoV-2 Viral Strains

After conducting a phylogenetic analysis, we identified 6 distinct GISAID clades (G, GH, GK, GR, GRA, and GV) to which the Bhutanese SARS-CoV-2 strains were assigned. The GH clade was predominant in 2020, succeeded by the G clade in 2021, and then the GRA clade in 2022 and early 2023. We also identified 4 distinct WHO labels within the Bhutanese SARS-CoV-2 strains: Alpha, Beta, Delta, and Omicron. Notably, approximately 35.6% of the sequences did not match any WHO label and were instead assigned to international A_B diversity by the coronavirus typing tool. From May 2020 to February 2023, we detected a total of 29 Pangolin lineages among the strains. The B.1 variant was the most prevalent in 2020, accounting for 37.7% of the cases. In 2021, the dominant variant was B.1.617, representing 65% of the cases. By 2022, the BA.2.10 lineage became prominent, later transitioning to EP1, which accounted for 81.8% of the cases in early 2023. The distribution of Pangolin lineages within the samples showed temporal variations, reflecting the dynamic nature of the viral strains circulating in Bhutan (Figure 1). Table 1 provides a detailed frequency distribution of each nomenclature system. We also identified 2 recombinant variants, classified

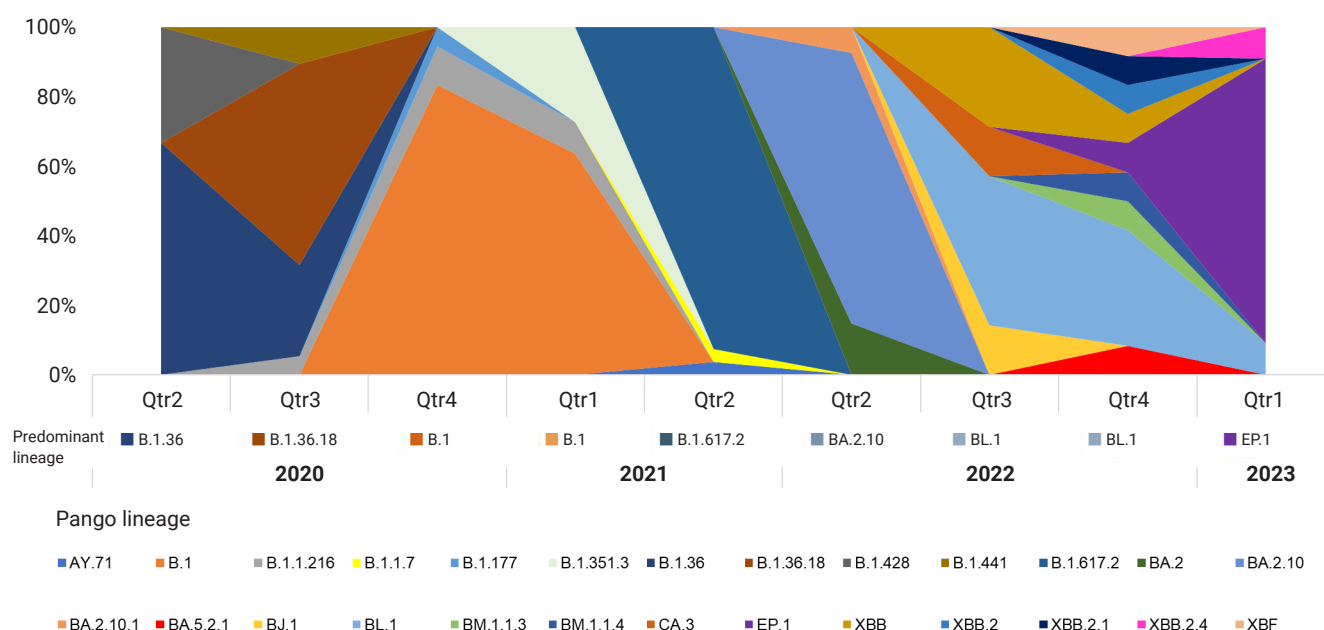


Figure 1. Temporal variations in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral strains. The figure shows the distribution of Pangolin lineages among SARS-CoV-2 genomes from samples collected between May 2020 and February 2023 in Bhutan.

Table 1. SARS-CoV-2 variants in Bhutan

WHO label	Month & year (detection)	GISAID clade	Nextstrain clade	Pangolin lineage	No. of samples
Alpha (B.1.1.7 I20)	April 2021	GRY	20I	B.1.1.7	1
Beta (B.1.351 20H)	March 2021	GH	20H	B.1.351.3	3
Delta (B.1.617.2 21A,21I,21J)	March 2021 to April 2021	GK	21A	B.1.617.2	6
			21I	AY.71	1
				B.1.617.2	2
			21J	B.1.617.2	17
International A_B Diversity	May 2020 to January 2021	G	20A	B.1	22
		GH	20A	B.1.36	7
				B.1.36.18	11
				B.1.441	2
				B.1.428	1
		GR	20B	B.1.1.216	4
		GV	20E	B.1.177	1
Omicron (BA.1/BA.2)	April 2020	GRA	Recombinant	BA.2	1
Omicron (BA.2)	April 2022 to January 2023	GRA	21L	BA.2	3
				BA.2.10	21
				BA.2.10.1	2
				BJ.1	1
			22D	BL.1	8
				BM.1.1.3	1
				BM.1.1.4	1
				CA.3	1
			22F	EP.1	10
				XBB	3
				XBB.2	1
				XBB.2.1	1
				XBB.2.4	1
				XBF	1
Omicron (BA.5 22B)	October 2022	GRA	Recombinant	BA.5.2.1	1
Total					135

The table shows the variants of SARS-CoV-2 from 135 Bhutanese sequences. The Pangolin variants are grouped according to 3 nomenclature systems (WHO, GISAID, and Nextstrain clades) along with the Pangolin lineages.

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization; GISAID, Global Initiative on Sharing All Influenza Database.

as Pangolin lineages BA.2 and XBF, using Nextclade and the coronavirus typing tool. To further explore recombination events, we employed RDP ver. 4.5 software, which confirmed the occurrence of recombination in at least 3 Bhutanese SARS-CoV-2 strains. These findings strongly suggest genetic recombination, highlighting the significant genetic diversity of SARS-CoV-2 in Bhutan. Detailed information on the recombination events in Bhutanese SARS-CoV-2 genomes can be found in [Supplementary Material 2](#).

To infer the origin and evaluate potential divergence events of SARS-CoV-2, we created a maximum likelihood phylogenetic tree. This was based on 135 complete genomes from isolates in Bhutan and the reference virus from Wuhan, China (NC_045512.2), using RDP4 (with RAXML8). The tree demonstrated a swift diversification of SARS-CoV-2, spread across 14 distinct Nextstrain clades. These included 20A,

20B, 20C, 20E, 20H, 20I, 21A, 21I, 21J, 21L, 22B, 22D, and 22F ([Figures 2, 3](#)).

Time to the Most Recent Common Ancestor of SARS-CoV-2 in Bhutan

The TMRCA for the SARS-CoV-2 strains circulating in Bhutan was estimated to be around February 15, 2020, with a 95% HPD interval ranging from January 3, 2020 to September 13, 2020. The mean substitution rate was calculated to be 0.97×10^{-3} substitutions per site per year, with a 95% HPD of 0.85×10^{-3} to 1.10×10^{-3} . [Figure 4](#) illustrates the MCC phylogenetic relationships among the SARS-CoV-2 genomes, as determined from the Bayesian coalescent framework using the coalescent exponential growth and tip-dating method.

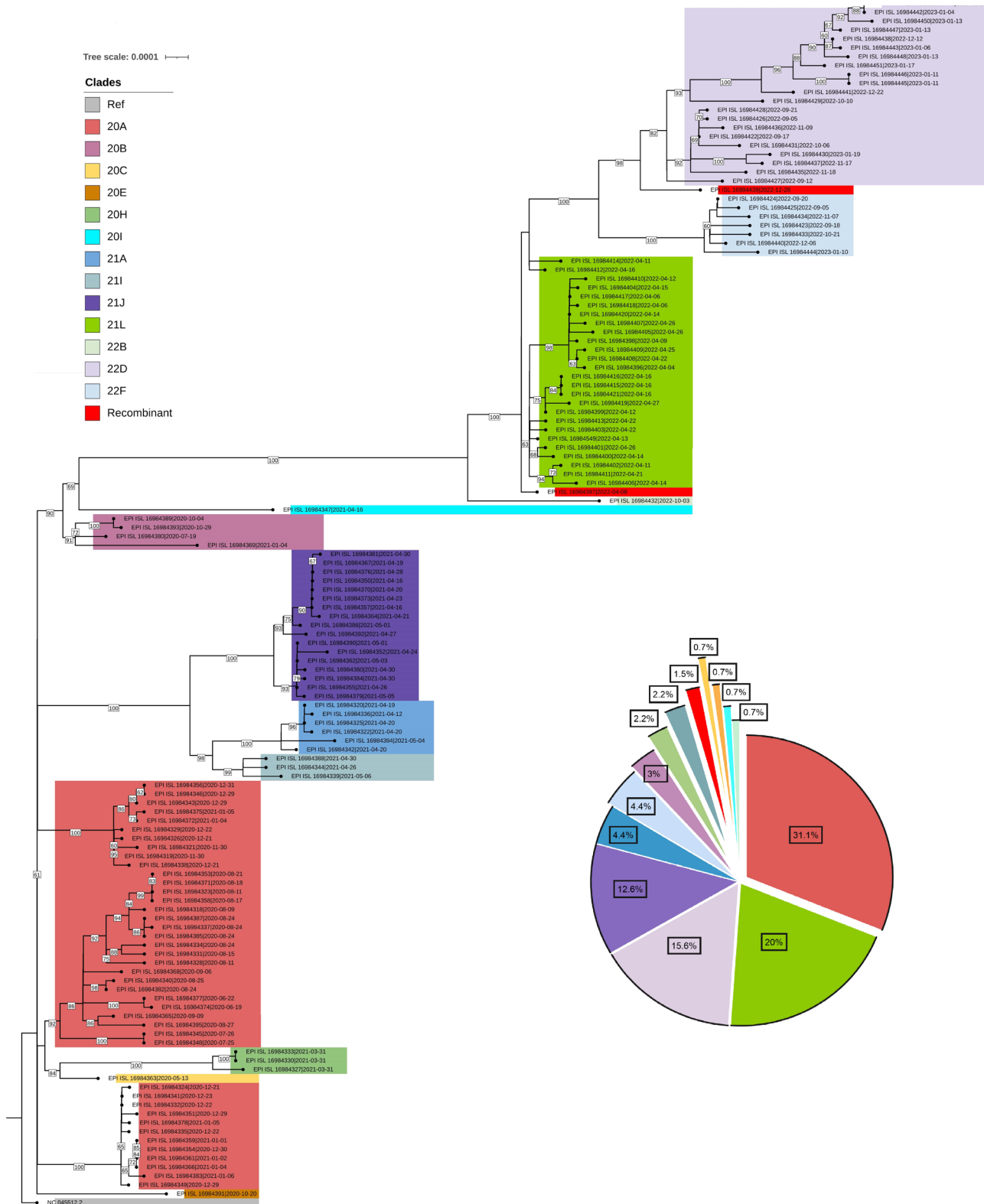


Figure 2. Maximum likelihood phylogenetic tree of severe acute respiratory syndrome coronavirus 2 genomes from Bhutan. The maximum likelihood tree was inferred using RAXML8. The tree is rooted with the reference sequence NC_045512.2. Bootstrap values more than 70 are indicated at major nodes in the fully resolved tree. The scale bar at the top of the tree indicates the number of nucleotide substitutions per site.

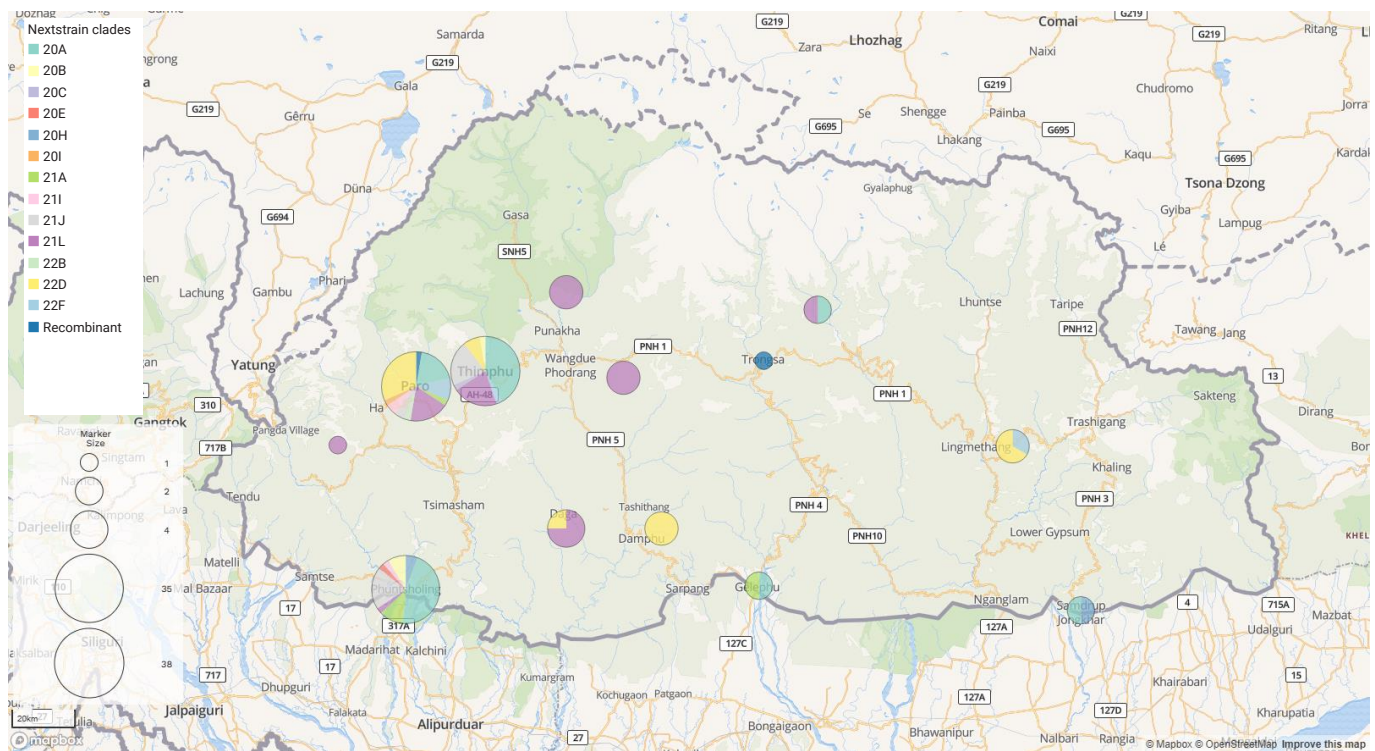


Figure 3. Distribution of severe acute respiratory syndrome coronavirus 2 clades in Bhutan. Map of Bhutan showing the geographical distribution of Nextstrain clades.

Mutation Analysis

Single-nucleotide variations

The mutational revealed 9,413 single-nucleotide variations (SNVs) within the SARS-CoV-2 genomes. These SNVs comprised 6,568 substitutions and 2,845 deletions. As a result of these SNVs, 5,613 amino acid changes were observed, which included 4,762 amino acid substitutions and 851 deletions. Figure 5 illustrates the locations and frequencies of the nucleotide variations plotted along the genomic sequence of the reference SARS-CoV-2 (NC_045512.2). Among the SNVs, the most common alteration was C>T, which accounted for 40% of the observed SNVs, followed by G>A, making up 12%. Importantly, no large deletions or insertions exceeding 50 nucleotides were detected. The longest deletion identified extended from position 29734 to 29759, resulting in the removal of a 26-nucleotide sequence (GAGGCCACGCGGAGTACGATCGAGTG).

Frequency of Mutations among Major Viral Proteins

The spike glycoprotein (S) displayed the highest mutation frequency among the major viral proteins, with 116 unique types identified. The most common amino acid substitutions in S were D614G (135/135), T478K (83/135), G142D (81/135),

and N501Y (61/135). In contrast, NSP7 and NSP11 were highly conserved, with no mutations detected. NSP10 (2/135), NSP9 (1/135), NS6 (1/135), and NS7a (1/135) also exhibited low mutation rates. The specific frequency of amino acid mutations in the major proteins of Bhutanese SARS-CoV-2 viral strains is illustrated in Figure 6 and Table 2.

Discussion

In this study, we conducted a comprehensive analysis of 135 whole-genome sequences of SARS-CoV-2, collected from Bhutan between May 2020 and February 2023. These sequences, obtained from the GISAID database, were compared with the Wuhan-Hu-1 (NC_045512) reference strain. We characterized the genomic variation, as well as the phylogenetic and evolutionary dynamics, of SARS-CoV-2 from Bhutan. Our phylogenetic analysis revealed that multiple SARS-CoV-2 lineages were circulating in Bhutan in 2020, 2021, 2022, and early 2023. We discovered that the virus in Bhutan was distributed across 14 Nextstrain clades and 26 Pangolin lineages. In 2020, the predominant variant was B.1, accounting for 37.7% of the sequenced samples. This variant, which exhibited a global distribution in 2020, gave

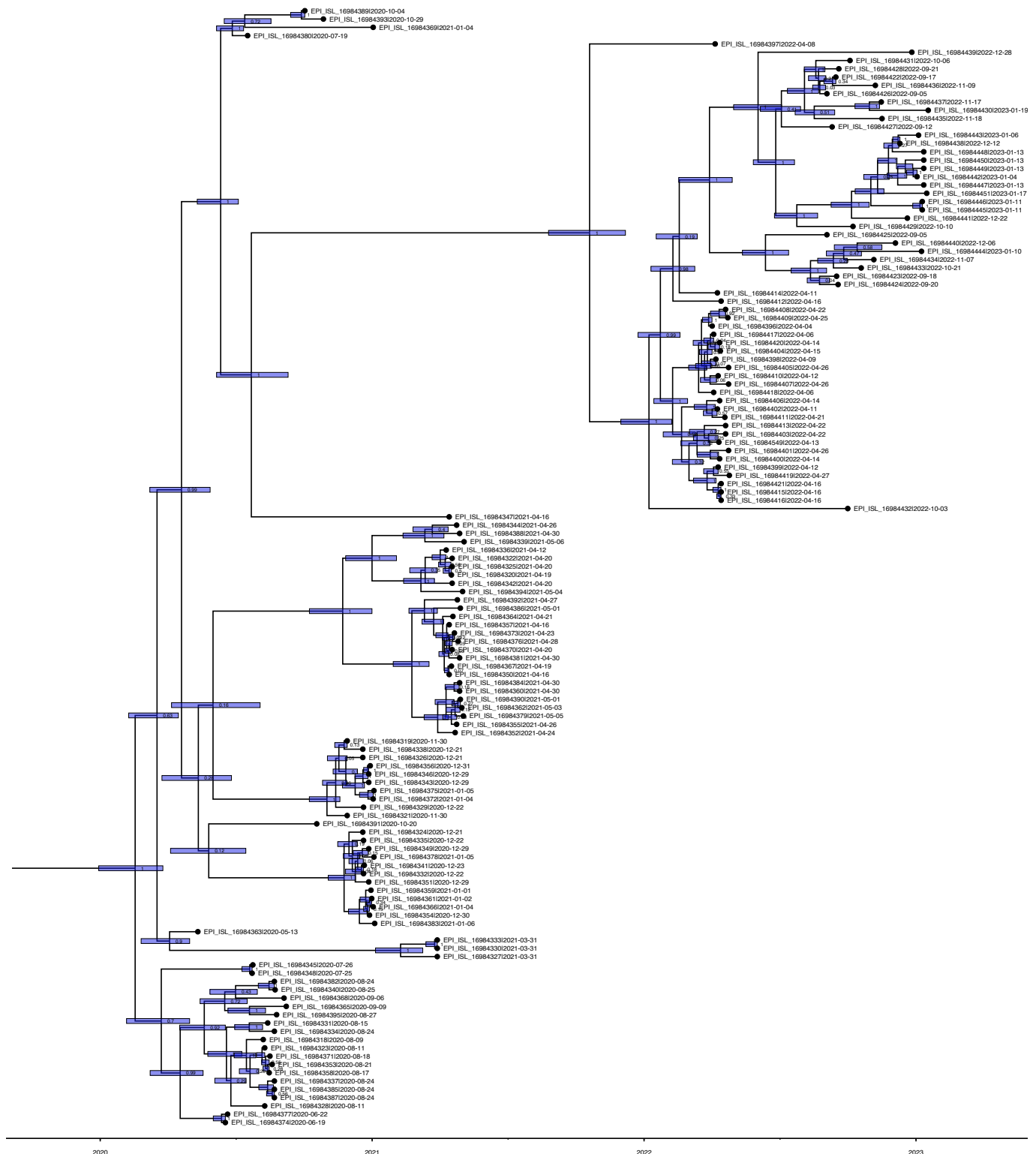


Figure 4. Maximum clade credibility (MCC) tree of Bhutanese severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genomes. Estimated MCC tree of SARS-CoV-2 genomes using a Hasegawa-Kishino-Yano substitution model and a strict molecular clock with uniform rates across branches. Nodes are labeled with posterior probability values and the 95% highest posterior density of the node heights.

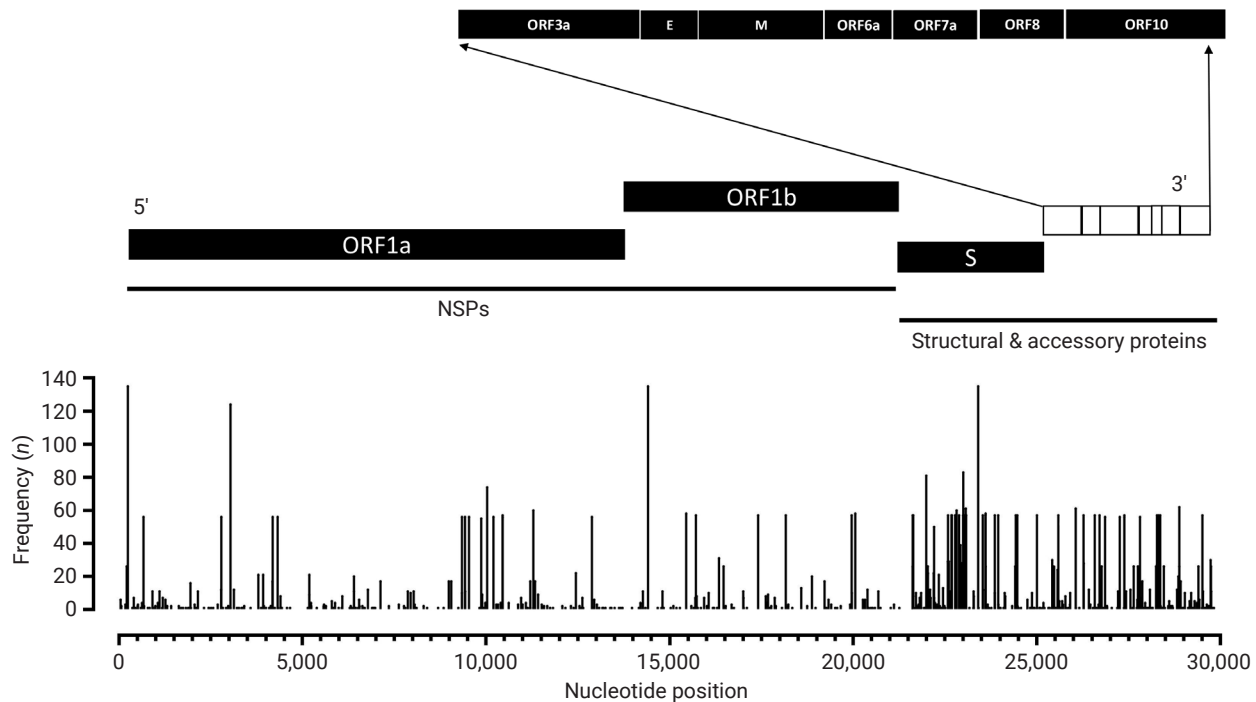


Figure 5. Frequency of single-nucleotide variations (SNVs). The figure shows the frequency of SNVs in Bhutanese severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) isolates. The locations and frequencies of the nucleotide variations were plotted along genomic sequence of NC_045512.2. The open reading frames (ORFs) of SARS-CoV-2 were shown as rectangles that were aligned with nucleotide positions of SARS-CoV-2. Frequency (n) is the number of samples/sequences with nucleotide variation at the nucleotide position. NSP, non-structural protein.

rise to more than 70 sub-lineages, indicating its extensive diversification [11]. In 2021, the dominant lineage in Bhutan shifted to B.1.617, prevalent in 65% of the sequenced samples. This variant, B.1.617, carried 3 key mutations (L452R, E484Q, and P681R) in the viral spike protein and was first detected in India in December 2020 [26]. Lineage B.1.617.2, a sub-lineage of B.1.617 also known as the Delta variant, gradually replaced the B.1.1.7 variant as the dominant global strain by March 2021 [27].

In 2022, the most prevalent lineage of SARS-CoV-2 in Bhutan was BA.2.10 (Omicron), accounting for 48% of the sequenced samples. This variant first appeared in South Africa in November 2021 and led to a worldwide surge in COVID-19 cases due to its increased transmissibility compared to BA.1 [28]. Bhutan also experienced a significant increase in COVID-19 cases that year, resulting in a series of lockdowns and widespread testing [29]. Interestingly, a similar trend was observed in North India, where the Omicron BA.2 lineage became prominent during the third wave of COVID-19 cases, as documented by Zaman et al. [30]. This concurrent emergence of the Omicron variant in both Bhutan and North India prompts inquiries and hypotheses about potential links between these events and the dynamics of the cross-border

transmission of the variant.

The mutations observed in Bhutanese strains offer significant insights into the country's viral dynamics and their potential impact on global trends. Like many other regions, Bhutan has seen dynamic changes in the dominant SARS-CoV-2 lineages throughout the study period. This mirrors global trends and highlights the role of international travel and viral transmission networks. It further emphasizes the interconnectedness of Bhutan's SARS-CoV-2 dynamics with the wider global landscape.

SARS-CoV-2 exhibits a high rate of genomic mutations, and our study identified both SNVs and the distribution of point mutations across the major viral proteins. The most frequently detected SNVs in SARS-CoV-2 genomes from Bhutan were C > T, followed by G > A. This aligns with previous studies, which have reported similar findings and suggested a potential explanation. This explanation posits that there is a selective mutation pressure to decrease the number of CpG sites in the virus's genome. This is due to the abundance of human antiviral proteins, such as APOBEC3 and ZAP [31,32]. Among the proteins we analyzed, the spike glycoprotein exhibited the highest number of point mutations. This was followed by N, NSP3, NSP12, and

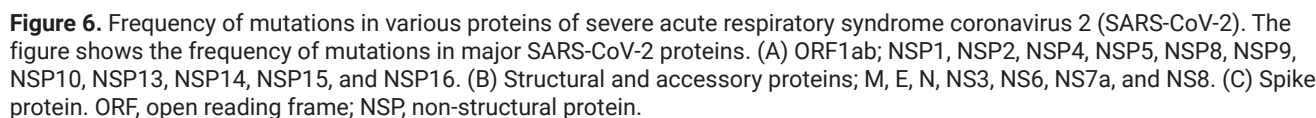


Table 2. The total number of amino acid changes and mutation types in different proteins of SARS-CoV-2

Protein		Total amino acid changes	No. of mutation types
Structural and accessory proteins	Spike (S)	2,503	116
	Envelope (E)	88	3
	Membrane (M)	143	6
	Nucleocapsid (N)	652	36
	NS3	319	22
	NS6	1	1
	NS7a	71	6
	NS7b	17	1
	NS8	23	9
ORF1a	NSP1	101	20
	NSP2	65	21
	NSP3	319	54
	NSP4	274	10
	NSP5	70	4
	NSP6	223	14
	NSP7	0	0
	NSP8	35	6
	NSP9	1	1
	NSP10	2	2
	NSP11	0	0
ORF1b	NSP12	220	13
	NSP13	143	11
	NSP14	94	7
	NSP15	79	7
	NSP16	13	3

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ORF, open reading frame; NSP, non-structural protein.

The proteins are grouped into structural and accessory proteins, ORF1a, and ORF1b. An amino acid change is a substitution, insertion, or deletion of 1 or more amino acids in the protein sequence. A mutation type is a classification of amino acid changes based on their effect on the protein structure and function. The table indicates the total number of amino acid changes and mutation types observed in each protein across all the sequenced samples.

NSP4, in that order. These proteins play crucial roles in the viral life cycle and are promising targets for vaccines and therapeutic interventions [33]. The high frequency of point mutations in these proteins raises questions about the effectiveness of drug candidates being investigated as potential therapies or diagnostics that target these proteins. High mutation rates can undermine the efficacy of targeted treatments, potentially weakening their ability to disrupt viral replication or inhibit viral protein function.

One of the key findings of our study is the abundance of mutations in the spike glycoprotein, a vital component in viral infection and pathogenesis. We observed several noteworthy mutations in the spike protein of Bhutanese strains, including D614G, T478K, G142D, N501Y, K417N, P681H, A27S, D405N, D796Y, E484A, H655Y, L24del, N440K, N679K, N969K, P25del, P26del, Q498R, Q954H, R408S, S371F, S373P, S375F, S477N, T19I, T376A, Y505H, N764K, and V213G. These mutations were identified in over 50 samples. Among the functional proteins of the SARS-CoV-2 virus, the spike protein shows the highest mutation rate. Many studies have underscored the significance of mutated residues on

the S-receptor binding domain (S-RBD) of the Omicron variant, as they enhance its binding affinity with ACE2 [34].

The mutations observed in Bhutanese strains are of significant importance due to their potential impact on various aspects of the virus's behavior. For instance, the D614G mutation, which is commonly observed, has been reported in 99.03% of samples from 212 countries worldwide [5]. Strains carrying this mutation have been found to display lower reverse transcription-polymerase chain reaction cycle thresholds, higher upper respiratory tract viral loads without an increase in disease severity, and enhanced infectivity [14]. Another mutation, N501Y, found in Bhutanese strains, is known to modify the shape of the RBD, thereby increasing its binding affinity with human ACE2 receptors. This mutation is present in VOCs, such as those first identified in the UK (B.1.1.7), South Africa (B.1.351), and Brazil (P.1) [35,36]. These variants have been demonstrated to spread more rapidly than other lineages and may also affect vaccine efficacy to some extent. However, there is no evidence to suggest that they cause more severe disease or increase the risk of death. Other mutations, such as E484K, K417N, and S477N

in the spike protein, have also been associated with changes in viral properties [37] and were frequently found among strains from Bhutan. These findings highlight the potential implications of these mutations for viral pathogenesis, transmission, and vaccine effectiveness. Further research is required to gain a better understanding of their specific roles in disease dynamics.

In addition to the mutations in the spike protein, our analysis also investigated mutations in other key viral proteins, providing insights into potential therapeutic targets and diagnostic challenges. The discovery of mutations in N, NSP3, NSP12, and NSP4 is especially significant, as these proteins play crucial roles in the viral life cycle and are promising targets for vaccine development and therapeutic interventions. However, the high mutation rates observed in these proteins raise questions about the efficacy of drug candidates being considered for potential therapies or diagnostics. The ever-changing nature of the virus highlights the need for ongoing research and development of antiviral therapies that can effectively combat these mutations.

This study also analyzed non-mutated proteins, such as NSP7 and NSP11, emphasizing their conservation. Proteins that are highly conserved, including NSP10, NSP9, NS6, and NS7a, have crucial roles in the viral life cycle and interactions with the host. Previous studies have identified NSP10, NSP7, NSP8, NSP11, NSP16, ORF6, and NSP9 as highly conserved proteins in SARS-CoV-2 [38,39]. Mutations in these proteins could potentially impair their functions and decrease viral fitness. For example, NSP7 and NSP11 are co-factors of the viral polymerase NSP12 and are vital for its activity [38]. NSP10 enhances the processivity and fidelity of NSP12, acting as its co-factor [40]. NSP9, an RNA-binding protein, may aid in viral RNA synthesis. NS6 and NSP7a are accessory proteins that adjust the host immune response and disrupt interferon signaling [7]. The preservation of these proteins underscores their significance in sustaining viral functions. Approaches that target conserved regions or utilize combination therapies may offer potential solutions to the problems created by the virus's mutational landscape, thereby enhancing the effectiveness of therapeutic interventions.

The TMRCA of SARS-CoV-2 strains circulating in Bhutan was estimated in this study to have occurred around February 15, 2020, with a 95% HPD interval ranging from January 3, 2020 to September 13, 2020. This estimated TMRCA aligns with the timing of Bhutan's first confirmed COVID-19 case, which was reported on March 5, 2020 [41]. Additionally, we calculated a substitution rate of 0.97×10^{-3} substitutions per site per year, consistent with findings from other studies [42–45].

In response to the initial detection of its first COVID-19 case, Bhutan implemented swift and comprehensive measures, including the closure of all international borders, to prevent the local transmission of SARS-CoV-2. Of particular note is the remarkable success of Bhutan's vaccination efforts, which underscores the effectiveness of the country's vaccination strategy and the readiness of its citizens to accept the vaccine. Bhutan procured and administered vaccines through a mix of bilateral and multilateral donations from countries such as India, the United States, Denmark, China, Croatia, among others. By April 9, 2021, a total of 472,139 individuals (consisting of 250,362 men and 221,777 women) had been successfully vaccinated with the first dose of COVISHIELD (Oxford-AstraZeneca, produced by the Serum Institute of India), achieving an impressive coverage of 94% among the eligible population [46]. This success can be credited to effective mass communication and social engagement initiatives led by religious figures, De-Suup volunteers, and local leaders. These efforts were instrumental in dispelling myths and misinformation about the vaccine and motivating people to get vaccinated [47].

The high rate of vaccination in Bhutan has not only significantly decreased the risk of severe illness, but it has also maintained COVID-19 morbidity at impressively low levels compared to neighboring countries in the region, such as India, Nepal, Bangladesh, and China. Throughout the entire pandemic, Bhutan has only reported 21 deaths attributed to SARS-CoV-2 [41]. This highlights the effectiveness of Bhutan's proactive and well-organized public health response in protecting the health and well-being of its citizens.

Conclusion

This study offers a comprehensive overview of the genomic variation and evolutionary dynamics of SARS-CoV-2 in Bhutan throughout the course of the pandemic. We found that the SARS-CoV-2 strains present in Bhutan share common mutations with strains from other countries, but they are not identical. Our research revealed that multiple lineages have circulated in Bhutan and that the virus has adapted to environmental and selective pressures. These discoveries illuminate the transmission patterns, disease severity, and vaccine efficacy of SARS-CoV-2. The emergence of new variants with enhanced transmissibility and potential for immune evasion underscores the necessity for ongoing genomic surveillance and effective public health strategies, such as vaccine development and implementation. This study underscores the importance of genomic data in shaping the design and assessment of preventive and control measures against SARS-CoV-2 and its variants. Despite a

decrease in overall cases, the virus continues to evolve, necessitating continued vigilance in our efforts to control the disease.

Limitations

A potential limitation of this study is sample representativeness, as it only included 135 whole-genome sequences of SARS-CoV-2 obtained from Bhutan between May 2020 and February 2023. This sample size is relatively small and may not accurately reflect the genetic diversity and distribution of SARS-CoV-2 throughout the country. Additionally, the dynamic evolution of SARS-CoV-2 could have given rise to new variants that were not detected or analyzed in this study, potentially impacting the relevance of the findings. Moreover, this study did not take into account other factors that could influence the transmission and outcome of SARS-CoV-2 infections.

Supplementary Material

Supplementary Material 1. EPI_SET Identifier for all severe acute respiratory syndrome coronavirus 2 genome sequences and metadata used in the study; **Supplementary Material 2.** Recombination events detected in Bhutanese severe acute respiratory syndrome coronavirus 2 genomes. Supplementary data are available at <https://doi.org/10.24171/j.phrp.2023.0209>.

Notes

Ethics Approval

Ethical clearance and approval were not applicable since the data used in this study were obtained from publicly available database, the Global Initiative on Sharing All Influenza Data (GISAID).

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

The findings of this study are based on metadata associated with 135 sequences available on GISAID, and accessible at DOI: <https://doi.org/10.55876/gis8.230622fy>

Authors' Contributions

Conceptualization: all authors; Data curation: all authors; Formal analysis: TD; Investigation: all authors; Methodology: all authors; Resources: all authors; Software: TD; Validation: all authors; Visualization: all authors; Writing—original draft: TD; Writing—review & editing: all authors. All authors read and approved the final manuscript.

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Estimation of the onset time of diabetic complications in type 2 diabetes patients in Thailand: a survival analysis

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ABSTRACT

Objectives: This study aimed to identify factors associated with the onset time of diabetic complications in patients with type 2 diabetes mellitus (T2DM) and determine the best-fitted survival model.

Methods: A retrospective cohort study was conducted among T2DM patients enrolled from October 1, 2016 to July 15, 2020 at the National Health Security Office (NHSO). In total, 388 T2DM patients were included. Cox proportional-hazard and parametric models were used to identify factors related to the onset time of diabetic complications. The Akaike information criterion, Bayesian information criterion, and Cox-Snell residual were compared to determine the best-fitted survival model.

Results: Thirty diabetic complication events were detected among the 388 patients (7.7%). A 90% survival rate for the onset time of diabetic complications was found at 33 months after the first T2DM diagnosis. According to multivariate analysis, a duration of T2DM ≥ 42 months (time ratio [TR], 0.56; 95% confidence interval [CI], 0.33–0.96; $p = 0.034$), comorbid hypertension (TR, 0.30; 95% CI, 0.15–0.60; $p = 0.001$), mildly to moderately reduced levels of the estimated glomerular filtration rate (eGFR) (TR, 0.43; 95% CI, 0.24–0.75; $p = 0.003$) and an eGFR that was severely reduced or indicative of kidney failure (TR, 0.38; 95% CI, 0.16–0.88; $p = 0.025$) were significantly associated with the onset time of diabetic complications ($p < 0.05$).

Conclusion: Patients with T2DM durations of more than 42 months, comorbid hypertension, and decreased eGFR were at risk of developing diabetic complications. The NHSO should be aware of these factors to establish a policy to prevent diabetic complications after the diagnosis of T2DM.

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Keywords: Diabetes complications; Survival analysis; Type 2 diabetes mellitus

Introduction

Diabetes refers to metabolic diseases characterized by hyperglycemia, which results from

defects in insulin secretion, insulin action, or both [1]. As of 2021, the global prevalence of diabetes among individuals aged 20 to 79 was estimated to be 10.5% (95% confidence interval [CI], 8.3%–12.0%), and this figure is projected to rise to 12.2% (95% CI, 9.5%–14.0%) by 2045. In the Western Pacific region, the prevalence of diabetes was 11.9% (95% CI, 10.1%–13.5%), and it is expected to increase to 14.4% (95% CI, 12.1%–16.4%) by 2045 [2]. In Thailand, the prevalence of diabetes was estimated to be 9.7% among individuals aged 20 to 79 years in 2021 [3]. In 2019, the International Diabetes Federation Atlas reported that 4.2 million people died from diabetes, corresponding to approximately 11.3% of deaths from all causes [4].

Type 2 diabetes mellitus (T2DM) accounts for over 90% of all diabetes mellitus cases and is characterized by impaired insulin secretion from pancreatic islet β -cells, tissue insulin resistance, and an insufficient compensatory insulin secretion response [5]. In 2017, T2DM was prevalent in approximately 6.28% of the global population, corresponding to a rate of 6,059 cases per 100,000 individuals. This figure is projected to increase to 7,079 cases per 100,000 individuals by 2030 [6]. The incidence and prevalence of T2DM continue to rise, and uncontrolled blood glucose levels in patients can result in long-term complications.

An observational study conducted across 28 countries in Asia, Africa, South America, and Europe found that 50% of patients with T2DM experienced microvascular complications, while 27% experienced macrovascular complications [7]. A prospective study of Thai T2DM patients revealed a prevalence of 38.3% for diabetic nephropathy, 23.7% for retinopathy, and over 15% for foot problems [8]. Numerous studies have highlighted significant factors contributing to diabetic complications in T2DM patients. These include age, sex, body mass index (BMI), smoking habits, family history of diabetes, duration of diabetes, ethnic group, hyperglycemia, hypertension, and hypercholesterolemia [9–12]. Additionally, several studies have found that screening for foot and eye issues could delay the onset of diabetes-related complications [13–15].

Survival analysis is a statistical method employed to examine the time leading up to a failure event or the conclusion of a study, and it can be instrumental in identifying risk factors [16]. Regression models, such as Cox proportional hazard (PH) and parametric models, can be used to estimate the time until the occurrence of a failure event [17].

The primary assumption of the PH model is that the hazard ratio is constant over time. In contrast, parametric models are capable of modeling survival times, even when the PH assumption is not met, due to their assumption of a specific distribution for the outcome variable [18,19]. This study

HIGHLIGHTS

- Within 33 months, the survival rate for the onset of diabetic complications in patients with type 2 diabetes mellitus (T2DM) was 90%.
- The duration of T2DM, comorbid hypertension, and estimated glomerular filtration rate were identified as factors associated with the onset time of diabetic complications in patients with T2DM.
- A log-normal accelerated failure time (AFT) model was more effective and appropriate to our type 2 diabetes dataset than a Cox proportional-hazard model.
- The log-normal AFT model was the best-fitted survival model to explain the onset time of diabetic complications in patients with T2DM.

focused on parametric models, including Weibull and log-normal accelerated failure time (AFT). The characteristics of the hazard function for these 2 models are as follows: (1) the Weibull model can either increase or decrease with increasing survival time, and (2) the log-normal model can increase, decrease, or invert [20]. These models are also suitable for fitting diabetes data.

This study compared the performance of Cox PH, Weibull, and log-normal AFT models to identify factors associated with the onset time of diabetic complications in patients with T2DM. The best-fitted survival model was also determined.

Materials and Methods

This study was structured as a retrospective cohort study. We collected secondary data from diabetes patients, spanning from October 1, 2016 to July 15, 2020, from the National Health Security Office (NHSO) database. We selected patients who were first diagnosed with T2DM between October 1, 2016, and September 30, 2017. Patients with incomplete laboratory data, such as fasting blood glucose (FBG), hemoglobin A1c (HbA1c), total triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and estimated glomerular filtration rate (eGFR), were excluded. Additionally, we excluded patients who had a history of health issues related to complications. The final dataset for analysis consisted of 388 records from T2DM patients with complete data.

Using the survival analysis formula [21], it was established that the data collected in this study constituted a suitable sample size.

With the alpha error set to 95% and a power of 90%, a

probability of 0.32 for diabetic neuropathy in T2DM patients, and a hazard ratio of 0.726 for developing neuropathy for patients with and without hypertension [22], the minimum sample size was determined to be 329.

Data from the NHSO database, comprising 388 entries, were analyzed. The variables considered included sex, age, age at T2DM diagnosis, duration of T2DM, BMI, comorbidity with hypertension, foot and retina examinations, FBG, HbA1c, TG, LDL-C, eGFR, and diabetic complications such as nephropathy, retinopathy, neuropathy, and peripheral vascular disease.

The onset time of diabetic complications was defined as the duration, in months, between the diagnosis of T2DM and the development of complications. Clinical complications such as nephropathy, retinopathy, neuropathy, and peripheral vascular disease were diagnosed by a physician and documented in the NHSO database. Patients were categorized into 2 groups: those with diabetic complication events and those with censored data.

The other variables were defined as independent variables. Sex was categorized into 2 groups: male and female. Age was categorized into 3 groups: ≤ 49 years, 50 to 59 years, and ≥ 60 years [23]. Age at the diagnosis of T2DM was categorized into 2 groups: < 35 years and ≥ 35 years. The duration of T2DM was categorized into 2 groups: < 42 months and ≥ 42 months. BMI was categorized into 4 groups according to the Asian criteria: underweight ($< 18.5 \text{ kg/m}^2$), normal ($18.5\text{--}22.9 \text{ kg/m}^2$), overweight ($23\text{--}24.9 \text{ kg/m}^2$), and obese ($\geq 25 \text{ kg/m}^2$) [24]. Comorbid hypertension was dichotomized as yes and no. Whether foot examinations or retina examinations had been performed was also categorized into 2 groups: yes and no. FBG was categorized into 2 groups: $< 130 \text{ mg/dL}$ and $\geq 130 \text{ mg/dL}$. HbA1c was categorized into 2 groups: $< 7\%$ and $\geq 7\%$. TG was also dichotomized as $< 150 \text{ mg/dL}$ and $\geq 150 \text{ mg/dL}$. LDL-C was categorized into 2 groups: $< 100 \text{ mg/dL}$ and $\geq 100 \text{ mg/dL}$. eGFR was categorized into 3 groups: normal to mild decline ($> 60 \text{ mL/min/1.73 m}^2$), mild to moderate decline ($30\text{--}59 \text{ mL/min/1.73 m}^2$), and severe decline to kidney failure ($< 30 \text{ mL/min/1.73 m}^2$) [25–27].

Model Estimation

The Kaplan-Meier (KM) technique is a nonparametric survival probability estimator for observations of both censored data and events of interest [28]. The KM estimator of a survival function $S(t) = P(T \geq t)$ is given by

$$\hat{S}(t) = \prod_{i=1}^k \left(1 - \frac{d_i}{n_i} \right)$$

Where d_i is the observed number of events at time t_i and n_i is number of individuals at risk at time t_i .

The Cox PH model is the most widely used multivariate statistical model for survival analysis [29]. The Cox PH is a semi-parametric model, where the baseline hazard can be described as follows:

$$h_i(t|\chi) = h_0(t)\exp(\beta\chi)$$

Where $h_0(t)$ is the baseline hazard function and X_i is a vector of covariates and β is a vector of parameters for effect of the predictors.

Two types of graphical techniques are used to evaluate the PH assumption. The first is the $\ln(-\ln(s(t)))$ plot, where parallel curves indicate that the PH hypothesis has not been violated. The second technique involves the KM and predicted survival plot. If the observed and predicted survival curves are closely aligned, it suggests that the PH assumption has not been violated. After fitting a Cox model, we can also test using Schoenfeld residuals. In this case, there was no evidence to suggest that the PH assumption was violated ($p > 0.05$) [30].

An alternative to the Cox model is a parametric survival model, which assumes a specific form for the survival distribution. The models most frequently used are the Weibull and log-normal models. The AFT model posits that the effect of covariates multiplies with survival time [31]. In this study, we used the Weibull and log-normal AFT models for evaluation within the parametric model. The distribution of time to event, represented as T , as a function of a single covariate, is expressed as follows:

$$\log(T) = \beta_0 + \beta_1\chi + \sigma\varepsilon$$

Where β_1 is the coefficient for corresponding covariate, ε follows the extreme minimum value distribution $G(0, \sigma)$, and σ is the shape parameter [32].

Data Analysis

All data were cleaned to ensure completeness and consistency. The variables were categorized based on the criteria used to determine the reference group, as derived from the literature review, and were then exported to STATA statistical software ver. 17.0 (STATA Corp.). Descriptive statistics were employed to outline the characteristics of the sample. The KM method was used to estimate the survival experience of patients across different groups, represent median time, and estimate percentiles across various time scales [33]. The log-rank test was utilized to compare survival times between subgroups within each variable. Factors associated with diabetic complication events were examined using univariate and multivariate analysis, based on the Cox PH,

Weibull, and log-normal AFT models, in order to identify the most fitting model. The best-fitted survival model was determined by comparing the Akaike information criterion (AIC) and Bayesian information criterion (BIC). The model with the lowest AIC and BIC values was selected. The Cox-Snell residuals were used to assess the overall fit of the model.

Ethics Approval

All procedures conducted in the studies received ethical approval from the Faculty of Public Health, Mahidol University, Thailand (No: MUPH 45/2021). The Committee for Human Research Ethics granted an exemption for this research under protocol number MUPH 45/2021. The research complied with the non-disclosure agreement with

Table 1. Characteristics of T2DM patients in 2020

Characteristic	No. of diabetic complications (<i>n</i> = 30)	No. of censored data points (<i>n</i> = 358)	Total (<i>n</i> = 388)
Sex			
Male	11	127	138 (35.6)
Female	19	231	250 (64.4)
Age (y)			
≤ 49	2	56	58 (14.9)
50–59	10	92	102 (26.3)
≥ 60	18	210	228 (58.8)
Age at the diagnosis of T2DM (y)			
< 35	1	11	12 (3.1)
≥ 35	29	347	376 (96.9)
Duration of T2DM (mo)			
< 42	17	302	319 (82.2)
≥ 42	13	56	69 (17.8)
Body mass index			
Underweight	1	12	13 (3.4)
Normal	11	90	101 (26.0)
Overweight	4	75	79 (20.4)
Obese	14	181	195 (50.3)
Comorbid hypertension			
No	23	334	357 (92.0)
Yes	7	24	31 (8.0)
Foot examination			
No	21	275	296 (76.3)
Yes	9	83	92 (23.7)
Retina examination			
No	25	298	323 (83.2)
Yes	5	60	65 (16.8)
FBG (mg/dL)			
< 130	9	143	152 (39.2)
≥ 130	21	215	236 (60.8)
HbA1c (%)			
< 7	14	130	144 (37.1)
≥ 7	16	228	244 (62.9)
TG (mg/dL)			
< 150	16	167	183 (47.2)
≥ 150	14	191	205 (52.8)
LDL-C (mg/dL)			
< 100	6	90	96 (24.7)
≥ 100	24	268	292 (75.3)
eGFR (mL/min/1.73 m ²)			
Normal to mild decrease (> 60)	16	274	290 (74.7)
Mild to moderate decrease (30–59)	10	68	78 (20.1)
Severe decrease to kidney failure (< 30)	4	16	20 (5.2)

Data are presented as *n* or *n* (%).

T2DM, type 2 diabetes mellitus; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate.

the National Health Security Office.

Results

Characteristics of Study Participants

Of the 388 newly diagnosed T2DM patients, 250 (64.4%) were women. The average age of the patients was 61 ± 12 years, with ages ranging from 18 to 94 years. The majority of patients ($n=376$, 96.9%), were diagnosed with T2DM at or after the age of 35 and had been living with T2DM for less than 42 months, accounting for 319 (82.2%) of the patients. Half of the patients ($n=195$, 50.3%), were classified as obese. Most patients ($n=357$, 92.0%), did not have comorbid hypertension. The number of patients who had not undergone foot and retinal examinations was 296 (76.3%) and 323 (83.2%), respectively. More than half of the patients ($n=236$, 60.8%), had an FBG level of 130 mg/dL or higher, and 244 (62.9%) had an HbA1c level of 7% or higher. Furthermore, 205 (52.8%) had TG levels of 150 mg/dL or higher. Three-quarters of the patients ($n=292$, 75.3%), had LDL-C levels of 100 mg/dL or higher. The majority of patients ($n=290$, 74.7%), had normal to mildly decreased eGFR (> 60 mL/min/1.73 m²) (Table 1).

Overall Survival to the Onset of Diabetic Complications in T2DM Patients by the KM Method

Patients with T2DM were followed for a median period of 37 months, ranging from 4 to 43 months. Throughout the

study, the incidence rate of diabetic complications was 2.5 cases (95% CI, 0.17–0.36) per 1,000 person-months. The incidence rates for specific complications such as retinopathy, peripheral vascular disease, nephropathy, and neuropathy were 6.35 (95% CI, 3.17–12.70), 4.76 (95% CI, 0.67–33.81), 4.19 (95% CI, 2.71–6.50), and 3.13 (95% CI, 0.44–22.18) cases per 100 person-months, respectively.

By the conclusion of the study, the number of patients experiencing diabetic complication events was below the 50th percentile—that is, the median onset time for diabetic complications could not be determined. It was observed that after 33 months, patients with T2DM had a survival probability of 0.90, indicating a survival rate of 90% until the study's end (Figure 1). The cumulative probabilities of experiencing diabetic complications among T2DM patients at 12, 24, and 36 months were 0.0131, 0.0492, and 0.1010, respectively.

Predictors of Diabetic Complications in T2DM Patients

The log-rank test revealed factors significantly related to the onset time of diabetic complications in patients with T2DM. These factors included the duration of T2DM, comorbidity with hypertension, and eGFR ($p < 0.05$). Other factors, however, were found to be insignificant (Table 2).

The results of the univariate analysis for the Cox PH, Weibull, and log-normal AFT models are presented in Table 3. In all 3 models, variables such as age, duration of T2DM,

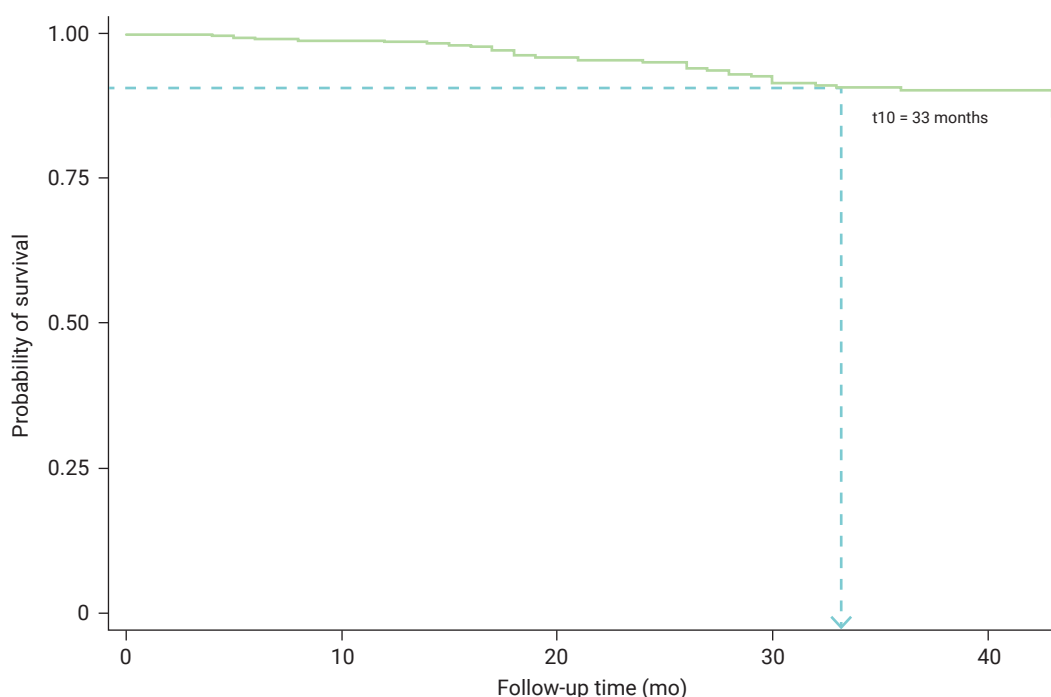


Figure 1. Overall Kaplan-Meier survival curve of 388 type 2 diabetes patients.

Table 2. Comparison of the survival probability for the onset time of diabetic complications in T2DM patients using the Kaplan-Meier and log-rank tests

Variable	Survival probability at 36 months (95% CI)	Log-rank test	
		χ^2	<i>p</i>
Sex		0.03	0.868
Male	0.90 (0.83–0.94)		
Female	0.91 (0.85–0.94)		
Age (y)		2.10	0.349
≤ 49	0.96 (0.84–0.99)		
50–59	0.88 (0.79–0.94)		
≥ 60	0.90 (0.84–0.94)		
Age at the diagnosis of T2DM (y)		0.00	0.952
< 35	0.92 (0.54–0.99)		
≥ 35	0.90 (0.86–0.93)		
Duration of T2DM (mo)		6.81	0.009*
< 42	0.92 (0.88–0.95)		
≥ 42	0.82 (0.71–0.90)		
Body mass index		2.25	0.523
Underweight	0.90 (0.47–0.99)		
Normal	0.86 (0.76–0.92)		
Overweight	0.94 (0.83–0.98)		
Obese	0.91 (0.85–0.95)		
Comorbid hypertension		13.19	<0.001*
No	0.92 (0.88–0.95)		
Yes	0.70 (0.46–0.85)		
Foot examination		0.39	0.535
No	0.90 (0.86–0.97)		
Yes	0.90 (0.81–0.95)		
Retina examination		0.01	0.904
No	0.90 (0.86–0.93)		
Yes	0.91 (0.79–0.96)		
FBG (mg/dL)		1.31	0.253
< 130	0.93 (0.87–0.97)		
≥ 130	0.88 (0.83–0.92)		
HbA1c (%)		1.42	0.234
< 7	0.88 (0.81–0.93)		
≥ 7	0.92 (0.87–0.95)		
TG (mg/dL)		0.89	0.345
< 150	0.89 (0.82–0.93)		
≥ 150	0.92 (0.86–0.95)		
LDL-C (mg/dL)		0.12	0.734
< 100	0.92 (0.82–0.96)		
≥ 100	0.90 (0.85–0.93)		
eGFR (mL/min/1.73 m ²)		10.18	0.006*
Normal to mild decrease (>60)	0.93 (0.89–0.96)		
Mild to moderate decrease (30–59)	0.83 (0.70–0.91)		
Severe decrease to kidney failure (<30)	0.77 (0.49–0.91)		

T2DM, type 2 diabetes mellitus; CI, confidence interval; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate.

**p* < 0.05.

comorbid hypertension, HbA1c, LDL-C, and eGFR were found to be significant (*p* < 0.10).

Multivariate analysis utilizing the Cox PH, Weibull, and log-normal AFT models revealed that the duration of T2DM, the presence of hypertension, and eGFR values

were significant predictors of the onset time for diabetic complications in T2DM patients (*p* < 0.05) (Table 4).

The results of the univariate and multivariate analyses did not differ between the Cox PH, Weibull, and log-normal AFT models. However, the AIC and BIC values of these

Table 3. Comparison of the results of Cox PH, Weibull AFT and log-normal AFT models in the univariate analysis for the onset time of diabetic complications in T2DM patients

Characteristic	Cox PH	Weibull AFT	Log-normal AFT
Sex			
Male	Ref.		
Female	0.959	0.907	0.946
Age (y)			
≤ 49	Ref.		
50–59	0.052*	0.065*	0.045*
≥ 60	0.121	0.132	0.092*
Age at the diagnosis of T2DM (y)			
< 35	Ref.		
≥ 35	0.134	0.153	0.166
Duration of T2DM (mo)			
< 42	Ref.		
≥ 42	0.003*	0.010*	0.029*
Body mass index			
Underweight	Ref.		
Normal	0.666	0.655	0.959
Overweight	0.725	0.736	0.414
Obese	0.599	0.595	0.445
Comorbid hypertension			
No	Ref.		
Yes	<0.001*	0.001*	<0.001*
Foot examination			
No	Ref.		
Yes	0.101	0.114	0.363
Retina examination			
No	Ref.		
Yes	0.128	0.131	0.391
FBG (mg/dL)			
< 130	Ref.		
≥ 130	0.382	0.394	0.627
HbA1c (%)			
< 7	Ref.		
≥ 7	0.091*	0.099*	0.182
TG (mg/dL)			
< 150	Ref.		
≥ 150	0.458	0.493	0.691
LDL-C (mg/dL)			
< 100	Ref.		
≥ 100	0.098*	0.103	0.215
eGFR (mL/min/1.73 m ²)			
Normal to mild decrease (> 60)	Ref.		
Mild to moderate decrease (30–59)	0.002*	0.005*	0.002*
Severe decrease to kidney failure (< 30)	0.007*	0.014*	0.031*

PH, proportional hazards; AFT, accelerated failure time; ref., reference group; T2DM, type 2 diabetes mellitus; ref., reference; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate.

* $p < 0.10$.

models suggested that the log-normal AFT model was the most suitable for explaining the onset time of diabetic complications in patients with T2DM (Table 5). Similarly, the Cox-Snell residuals from the 3 models indicated that the log-normal AFT model closely aligned, with a straight line at a 45° angle (Figure 2).

Discussion

This study's findings revealed that the KM method could not estimate the median time of diabetic complications.

The 90% survival rate for the onset time of diabetic complications in patients with T2DM was observed at 33

Table 4. Comparison of the final fitted Cox PH, Weibull AFT and log-normal AFT models in the multivariate analysis for the onset time of diabetic complications in T2DM patients ($p < 0.05$)

Variable	Model								
	Cox PH			Weibull AFT			Log-normal AFT		
	B	HR	<i>p</i>	B	TR	<i>p</i>	B	TR	<i>p</i>
Intercept				7.3	1,480.53	<0.001	7.564	1,926.99	<0.001
Duration of T2DM (mo)									
< 42	Ref.								
≥ 42	0.961	2.61	0.012	−0.575	0.56	0.030	−0.575	0.56	0.034
Comorbid hypertension									
No	Ref.								
Yes	1.619	5.05	<0.001	−1.014	0.36	0.002	−1.192	0.30	0.001
eGFR									
Normal to mild decrease	Ref.								
Mild to moderate	1.266	3.55	0.003	−0.798	0.45	0.006	−0.850	0.43	0.003
Severe to kidney failure	1.468	4.34	0.009	−0.895	0.41	0.021	−0.962	0.38	0.025

PH, proportional hazards; AFT, accelerated failure time; T2DM, type 2 diabetes mellitus; HR, hazard ratio; TR, time ratio; ref., reference; eGFR, estimated glomerular filtration rate.

Table 5. Comparison of model fit based on the AIC and BIC

Model	df	LL	AIC	BIC
Cox PH	4	−155.25	318.51	334.35
Weibull AFT	6	−105.87	223.79	247.48
Log-normal AFT	6	−103.72	219.44	243.21

AIC, Akaike information criterion; BIC, Bayesian information criterion; df, degrees of freedom; LL, log-likelihood; PH, proportional hazards; AFT, accelerated failure time.

months of follow-up. This is consistent with a study conducted in southern Lithuania, which reported a survival rate of 93% at 2 years, decreasing to 41% over 13 years of living with diabetes [34]. A study in Northwest Ethiopia [35] found that the median time to develop microvascular complications was 30 months, and in Iran [36], the median time until retinopathy was 58 months. A multivariate analysis of the model for the onset time of diabetic complications in T2DM patients revealed that a duration of T2DM exceeding 42 months, the presence of comorbid hypertension, and eGFR levels (ranging from mildly to moderately decreased and severely decreased to kidney failure) were identified as factors associated with diabetic complications ($p < 0.05$).

The duration of T2DM was significantly associated with diabetic complications ($p = 0.001$), a finding that aligns with other studies [37–40]. Among patients with diabetic complications, the median duration of T2DM was approximately 3 years, a figure lower than that reported by Zoungas et al. [41]. They discovered that a diabetes duration of 5 years or longer was linked to an increased risk of both macrovascular and microvascular complications.

Our results indicated a shorter survival time for diabetic complications in patients with a T2DM duration of 42 months or longer, compared to those with a duration of less than 42 months, by about 44%. A similar study conducted in Northwest Ethiopia [42] found that a duration of less than 4 years was a significant predictor of diabetic neuropathy in patients with T2DM.

The findings suggest that patients with comorbid T2DM and hypertension are at a heightened risk of diabetic complications. This aligns with research conducted in Ethiopia [43,44], Indonesia [45], and Taiwan, which also found an increased risk of major diabetic foot complications and cardiovascular events in T2DM patients with hypertension [46]. The study revealed that the time from onset to diabetic complications in T2DM patients with hypertension is estimated to be 70% shorter than in patients without hypertension. This could be due to hyperglycemia causing a systemic increase in blood pressure by expanding the volume of circulating fluid. Additionally, the progression of vascular remodeling can increase peripheral artery resistance, contributing to hypertension [47]. Therefore, T2DM patients with hypertension tend to develop diabetic complications more rapidly than those without hypertension ($p < 0.001$).

A reduction in the GFR signifies a decrease in hyperfiltration, a process that initiates diabetic nephropathy [48]. This study found that the level of eGFR is a significant factor in diabetic complications ($p < 0.05$), a result that aligns with other studies conducted in China and Japan. The influence of undiagnosed diabetes mellitus was also assessed, identifying the eGFR as a risk factor for the development of diabetic kidney disease [49–51]. The estimated survival time for patients with T2DM

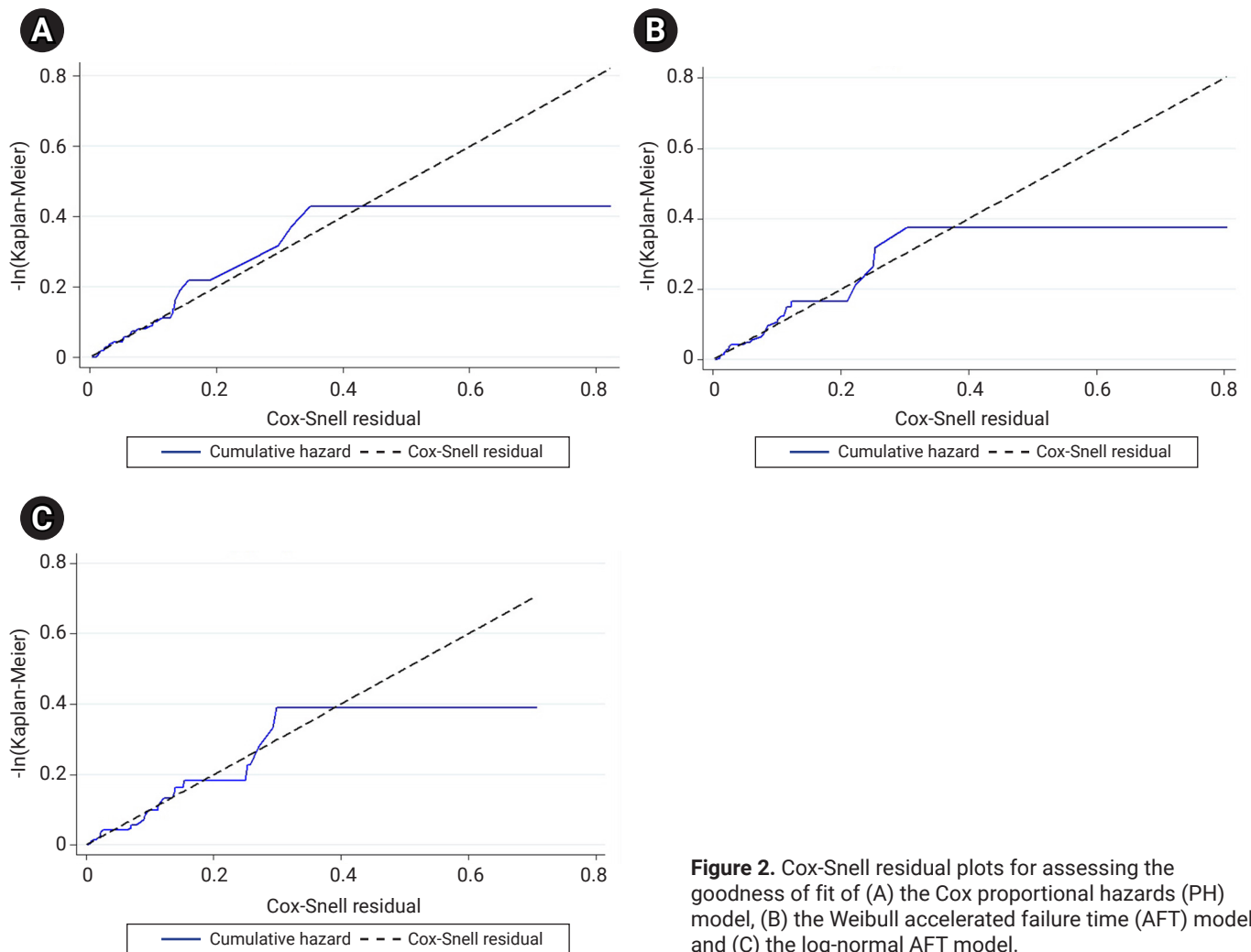


Figure 2. Cox-Snell residual plots for assessing the goodness of fit of (A) the Cox proportional hazards (PH) model, (B) the Weibull accelerated failure time (AFT) model, and (C) the log-normal AFT model.

and eGFR levels ranging from mildly to moderately decreased to kidney failure (G3a to G5) is shorter than that of patients with a normal eGFR and those with a mildly decreased eGFR, at 57% and 62% respectively. Consequently, a decreased eGFR can lead to the rapid progression of diabetic nephropathy and cardiovascular diseases [52,53].

The AIC and BIC values were utilized to compare models and identify the best-fitted survival model [36,54–56], and a Cox-Snell residual plot was used to assess whether the overall model fit the data [20,57,58] for semi-parametric and parametric models. This study found that the log-normal AFT model had the lowest AIC and BIC values. Additionally, in the Cox-Snell residual plot, the lines were closely aligned with a straight line at a 45° angle. Consequently, the log-normal AFT model was determined to be the best-fitted survival model for explaining the onset time of diabetic complications in patients with T2DM, compared to other models. Parametric models provide more informative and precise estimates

when the distribution is accurately specified, as compared to the Cox model [32,59].

This study adhered to recommendations for data handling to estimate and reduce right-censored data in a precise manner. Diabetes mellitus is a chronic disease influenced by individuals' behavior. Therefore, behavioral factors (smoking and alcohol drinking) and other comorbid factors should be added in future studies to identify risk factors for the onset time of diabetic complications.

Conclusion

This study found that the duration of T2DM, the presence of hypertension, and the eGFR were factors associated with the onset of diabetic complications in patients with T2DM. It is crucial for the NHSO to consider these factors, as identified by the most suitable survival model, in order to formulate and implement policies aimed at preventing the

development of diabetic complications.

The log-normal AFT model proved to be more effective and suitable for our type 2 diabetes dataset than the Cox PH model. Based on the AIC and BIC values, as well as the Cox-Snell residual plot, the log-normal AFT parametric model was identified as the best-fitting survival model. This model most accurately explains the onset time of diabetic complications in patients with T2DM.

Notes

Ethics Approval

This study was approved by the Institutional Review Board of the Faculty of Public Health, Mahidol University, Thailand (No: MUPH 45/2021) and performed in accordance with the principles of the Declaration of Helsinki. The informed consent was waived because of the retrospective nature of this study.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

The datasets are not publicly available due to privacy or ethical restrictions according to the data sharing agreement with the National Health Security Office.

Authors' Contributions

Conceptualization: all authors; Data curation: NS, JS; Formal analysis: NS, JS; Investigation: NS, JS; Methodology: all authors; Project administration: NS, JS; Supervision: JS, CV, PS; Validation: JS, CV, PS; Visualization: NS; Writing—original draft: NS, JS; Writing—review & editing: all authors. All authors read and approved the final manuscript.

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Factors affecting depression and health-related quality of life in the elderly during the COVID-19 pandemic

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ABSTRACT

Objectives: This study investigated changes in the health behaviors of the elderly due to coronavirus disease 2019 (COVID-19), concerns due to COVID-19, depression, and health-related quality of life (HRQOL), and aimed to identify factors that affect depression and HRQOL in the elderly.

Methods: This study was conducted using data from the 2021 Community Health Survey of the Korea Disease Control and Prevention Agency. From a total sample size of 229,242 individuals, 74,376 elderly people aged 65 or older were selected as subjects, and changes in health behaviors, concerns due to COVID-19, depression, and HRQOL were measured and analyzed.

Results: The level of depression associated with sleep and fatigue was high. The lowest HRQOL was related to physical pain and discomfort, while the most common concerns were related to economic difficulties. Factors influencing depression included worries about infection and economic harm, while factors impacting HRQOL encompassed concerns about infection, economic harm, and criticism from others.

Conclusion: If an infectious disease situation such as COVID-19 reoccurs in the future, it will be necessary to encourage participation in hybrid online and offline programs at senior welfare centers. This should also extend to community counseling institutions like mental health welfare centers. Additionally, establishing connections with stable senior job projects can help to mitigate the effects of social interaction restrictions, physical and psychological health issues, and economic difficulties experienced by the elderly.

Keywords: Aged; Changes in health behaviors; Concern; COVID-19; Depression; Health-related quality of life

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Introduction

The coronavirus disease 2019 (COVID-19) spread rapidly worldwide, leading the World Health Organization to declare a pandemic in March 2020 [1]. This disease has significantly impacted various aspects of our lives, including the economy, society, and culture. One of the most notable changes following the outbreak of COVID-19 has been the transformation of our social

networks [2]. COVID-19 is primarily transmitted through interpersonal contact and droplets, making limiting face-to-face interactions a key strategy in reducing its spread. Consequently, governments have developed and implemented various social distancing measures [3]. While social distancing is effective in preventing the spread of COVID-19, it also carries the risk of social isolation. Many individuals have faced significant restrictions in their relationships with family and friends, as well as in their social interactions, due to sudden isolation from their external environment and limitations on their daily activities. This is particularly true for the elderly, who often struggle with adapting to environmental changes and may find non-face-to-face communication methods, such as social networking services, challenging. This has often resulted in a more isolated lifestyle, leading to an increase in depression [4]. Furthermore, the spread of inaccurate information through various media outlets has fueled anxiety and fear about the spread of infectious diseases. As concerns about infection rise, it is anticipated that this will negatively impact not only the mental health of the elderly, but also their health-related quality of life (HRQOL).

Depression is a common mental illness in old age, and it significantly impacts life satisfaction among the elderly [5]. The elderly population is particularly susceptible to depression due to the physical decline associated with aging, as well as social factors such as isolation and the loss of relationships [6]. For instance, during the severe acute respiratory syndrome outbreak in Hong Kong in 2003, the elderly experienced heightened levels of loneliness and isolation due to the cessation of social and family gatherings, medical treatments, and participation in senior-related institutions. Consequently, the suicide rate among the elderly in Hong Kong surged by approximately 32% the following year [7]. This serves as a reminder of the critical importance of addressing the psychological needs of the elderly, particularly in the context of a pandemic such as COVID-19.

In the realm of physical function, health behaviors frequently serve as indicators for assessing the level of care required and the success of aging [8]. It has been observed that elderly individuals who exhibit high performance in health behaviors are often able to maintain good cognitive function and enjoy a high HRQOL [9]. However, research has indicated that social distancing has led to decreased physical activity and altered exercise behaviors among the elderly, which in turn has negatively impacted their overall performance in activities of daily living [4,10].

Research is currently being conducted on the issues faced by the elderly due to COVID-19, with a primary focus

HIGHLIGHTS

This study sought to determine factors affecting depression and health-related quality of life (HRQOL) in the elderly in the COVID-19 pandemic situation. Factors influencing depression and HRQOL included worries about infection, economic damage, and criticism from others.

on the epidemiological characteristics and current status of this demographic. However, there is a noticeable lack of comprehensive research that also considers psychological problems and aspects of HRQOL. COVID-19 has been reclassified from a first-degree infectious disease to a second-degree infectious disease, and most social distancing measures have been lifted. Nevertheless, given the uncertainty of when another infectious disease outbreak might occur, it is crucial to understand the impact of COVID-19 on the elderly and to prepare for future infectious disease scenarios. This study is a secondary analysis using data from the 2021 Community Health Survey. The aim is to understand the current changes in health behaviors among the elderly due to COVID-19, their concerns related to the disease, depression, and HRQOL. Ultimately, the goal is to identify factors that influence depression and HRQOL in the elderly population.

Materials and Methods

Participants

This study utilized raw data from the 2021 Community Health Survey conducted by the Korea Disease Control and Prevention Agency. The purpose of the Community Health Survey is to generate community health statistics that can be used to establish and evaluate community health care plans, as mandated by the Community Health Act. The 2021 dataset included new items related to COVID-19, in addition to the existing data. The data collection period spanned from August 16, 2021, to October 31, 2021. The Community Health Survey is executed through the collaborative efforts of 3 committees and management offices, in conjunction with the Korea Disease Control and Prevention Agency, various cities and provinces, public health centers, and responsible universities. The survey targeted adults aged 19 years or older who resided in the sample households. The survey was conducted as a one-on-one electronic survey, comprising 18 areas and 163 questions. Trained surveyors visited the sample households to administer the survey [11]. The Korea Disease Control and Prevention Agency appointed a third-

party agency to verify the survey data and ensure quality control. This agency re-extracted 13% of the completed surveys and conducted a telephone inspection. The survey was administered to 229,242 adults aged 19 years or older. Of these, 74,492 were aged 65 years or older. After excluding 116 patients with missing values in general characteristics, health behavior, and items of concern, 74,376 patients were included in the final analysis.

Research Model

This study explored the demographic, sociological, and economic traits of the elderly population, as well as the current shifts in health behavior and concerns brought about by COVID-19. It also aimed to identify the factors influencing depression and HRQOL. The research model is depicted in Figure 1.

Measurements

Changes in health behaviors due to COVID-19

Four items were utilized to inquire about changes in health behaviors due to COVID-19: physical activity (such as walking and exercise), consumption of instant food or soda, drinking, and smoking. For each question, participants were asked, "How has this changed compared to before COVID-19?" Responses were scored as follows: 1 point for "increased," 2 points for "similar," 3 points for "decreased," and 4 points for "not applicable."

Concerns due to COVID-19

In relation to concerns arising from COVID-19, 3 specific

items were addressed: concern about infection, concern about criticism from others due to infection, and concern about economic damage due to COVID-19. For each item, respondents were asked the question, "How concerned are you about the following due to COVID-19?" They were then instructed to rate their level of concern on a scale of 1 to 5, with 1 point for "very concerned," 2 points for "concerned," 3 points for "somewhat concerned," 4 points for "not very concerned," and 5 points for "not concerned at all." A higher score indicates a lower level of concern.

Depression

In this study, depression was measured using the patient health questionnaire-9 (PHQ-9), a depression screening tool. This self-report test is designed to gauge the severity of depression, and it comprises 9 sub-items. The scores are summed to determine the level of depression: 0-4 points indicate normal levels, 5 to 9 points suggest mild depression, 10 to 19 points denote moderate depression, and a score of 20 or more signifies severe depression. Essentially, a higher score corresponds to a higher level of depression. The test-retest reliability of the PHQ-9, as measured by Cronbach alpha, was found to be 0.89, while its concurrent validity was determined to be 0.81 [12].

HRQOL

In this study, we utilized the EuroQoL-5 dimensions (EQ-5D) index to measure HRQOL. This index was specifically designed to measure HRQOL based on a variety of dimensions. The EQ-5D index comprises questions that assess the current health status in 5 key areas: mobility, self-care, usual activities, pain/

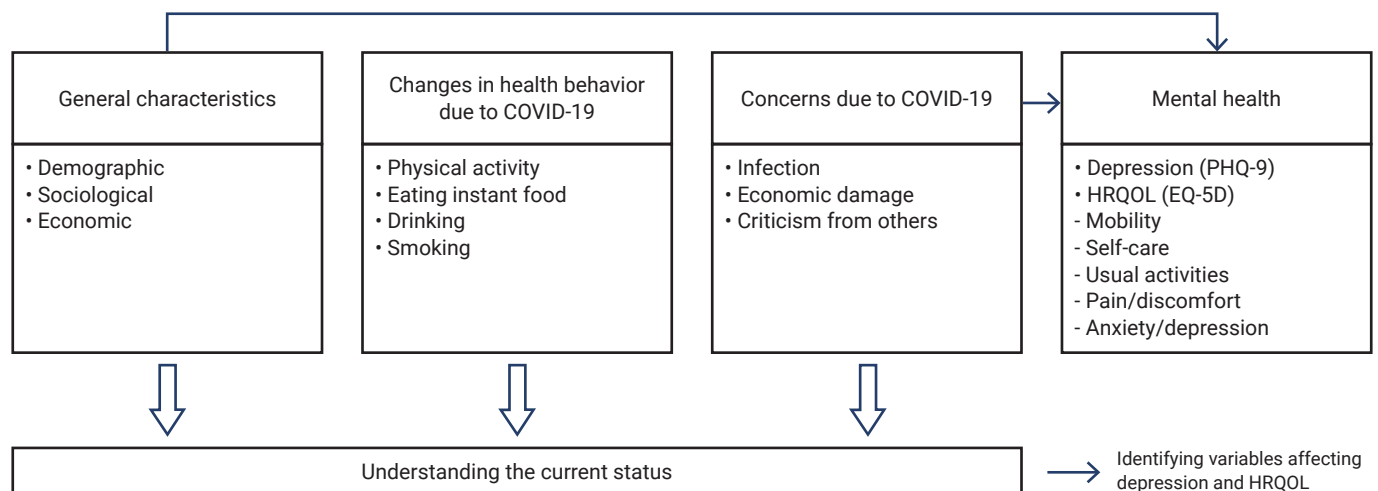


Figure 1. Research model.

PHQ-9, patient health questionnaire-9; HRQOL, health-related quality of life; EQ-5D, EuroQoL-5 dimensions.

discomfort, and anxiety/depression. Each item is scored as follows: “no problem” earns 1 point, “slight problem” earns 2 points, and “severe problem” earns 3 points. The resulting score provides a comprehensive indication of HRQOL, with a lower score signifying a higher HRQOL [13]. The test-retest reliability of this tool, as measured by Cronbach alpha, was found to be 0.93, and its convergent validity was determined to be 0.85 [14].

Statistical Analysis

The data collected were analyzed using the IBM SPSS ver. 27.0 (IBM Corp.). We calculated the general characteristics, changes in health behaviors due to COVID-19, and the current state of concerns related to COVID-19 using frequencies and percentages. The results for depression and HRQOL were calculated as means and standard deviations. To determine the impact of general characteristics and COVID-19-related concerns on depression and HRQOL, we used the independent t-test and analysis of variance, as well as multiple regression analysis. A *p*-value of less than 0.05 was considered statistically significant.

Results

Participant Characteristics

Table 1 presents the general characteristics of the study participants. The gender distribution was 42.3% male and 57.7% female. In terms of age, 40,288 participants (54.2%) were between 65 and 74 years old, 27,345 (36.8%) were between 75 and 84 years old, and 6,743 (9.1%) were 85 years or older. The majority of participants, 46,470 (62.5%), lived with a spouse. A significant portion, 43,904 (59.0%), were not engaged in economic activities, outnumbering those who were. Regarding changes in total income due to COVID-19, the majority, 54,334 (73.1%), reported no change. Similarly, 54,803 (73.7%) reported no change in spending on major items related to COVID-19.

Changes in Health Behaviors due to the COVID-19

Table 2 shows the results for changes in health behaviors

due to COVID-19. For physical activities such as walking and exercise, the most common response was “similar to before COVID-19,” with 40,046 respondents (53.8%). However, the second most common response was “decreased,” reported by 24,240 respondents (32.6%). Regarding the consumption of instant food or soda, the most common response was “similar,” reported by 33,047 respondents (44.4%). For drinking and smoking habits, “not applicable” was the most common response, but there were more reports of a decrease than an increase. The average score for each item was as follows: physical activity scored 2.45 ± 0.72 and eating instant food scored 2.96 ± 0.99 , indicating a score distribution close to “similar to before COVID-19.” Drinking scored 3.40 ± 0.85 and smoking scored 3.72 ± 0.67 , suggesting an

Table 1. General characteristics of the subjects (*n* = 74,376)

Characteristic	<i>n</i> (%)
Sex	
Male	31,432 (42.3)
Female	42,944 (57.7)
Age (y)	
65–74	40,288 (54.2)
75–84	27,345 (36.8)
≥85	6,743 (9.1)
Marital status	
Married, living together	46,470 (62.5)
Married, separated	1,941 (2.6)
Widowed	23,153 (31.1)
Divorced	2,331 (3.1)
Single	481 (0.6)
Economic activity	
Yes	30,472 (41.0)
No	43,904 (59.0)
Changes in total income related to COVID-19	
Decreased	19,049 (25.6)
Similar	54,334 (73.1)
Increased	993 (1.3)
Changes in spending on major items related to COVID-19	
Decreased	9,221 (12.4)
Similar	54,803 (73.7)
Increased	10,352 (13.9)

Table 2. Changes in health behavior due to COVID-19 (*n* = 74,376)

Category	Increased	Similar	Decreased	Not applicable	Mean ± SD
Physical activity	3,620 (4.9)	40,046 (53.8)	24,240 (32.6)	6,470 (8.7)	2.45 ± 0.72
Eating instant food	1,611 (2.2)	33,047 (44.4)	6,764 (9.1)	32,954 (44.3)	2.96 ± 0.99
Drinking	767 (1.0)	15,574 (20.9)	11,205 (15.1)	46,830 (63.0)	3.40 ± 0.85
Smoking	460 (0.6)	7,655 (10.3)	3,833 (5.2)	62,428 (83.9)	3.72 ± 0.67

Data are presented as *n* (%).

SD, standard deviation.

overall score distribution close to “not applicable.”

Concerns due to COVID-19

Table 3 presents the results for concerns due to COVID-19. The most common response was “very much so” for the following concerns: “concerns about infection” with 27,507 (37.0%), “criticism from others” with 32,099 (43.2%), and “economic damage” with 37,701 (50.7%). This suggests that elderly individuals were highly concerned about COVID-19. When examining the average score for each item, the concern due to infection was 2.18 ± 1.16 , the concern due to economic damage was 1.94 ± 1.06 , and the concern due to criticism from others was 1.82 ± 1.04 . This indicates that the average distribution of scores ranged from “yes” to “very much so.”

Depression and HRQOL

In the depression assessment (PHQ-9), the item “difficulty falling asleep or sleeping too much” scored 0.63 ± 0.96 points, while “tiredness and low energy” scored 0.59 ± 0.79 points. These 2 items had the highest scores. In the HRQOL evaluation (EQ-5D), “self-care” had the highest score

(1.15 ± 0.39 points), while “pain/discomfort” had the lowest score (1.59 ± 0.61 points) (Table 4).

Depression and HRQOL according to General Characteristics

Table 5 presents the results for depression and HRQOL in relation to general characteristics. Higher levels of depression were observed in women, individuals aged 85 years or older, those living alone due to the loss of a spouse, those not participating in economic activities, and those experiencing an increase in income and expenditure related to COVID-19 ($p < 0.001$). In contrast, men aged between 65 and 75 years, those living separately from their spouses, those engaged in economic activities, and those experiencing a decrease in income and expenditure due to COVID-19 generally exhibited higher HRQOL ($p < 0.001$). Among the subcategories of HRQOL, the “anxiety/depression” component did not appear to be associated with changes in income due to COVID-19 ($p > 0.05$).

Factors Affecting Depression and HRQOL

A multiple regression analysis was performed to explore

Table 3. Concerns due to COVID-19 ($n = 74,376$)

Category	Very yes	Yes	Normal	No	Not at all	Mean \pm SD
Infection	27,507 (37.0)	21,149 (28.4)	13,086 (17.6)	10,260 (13.8)	2,374 (3.2)	2.18 ± 1.16
Economic damage	37,701 (50.7)	21,684 (29.2)	7,445 (10.0)	5,918 (8.0)	1,628 (2.2)	1.94 ± 1.06
Criticism from others	32,099 (43.2)	24,870 (33.4)	8,759 (11.8)	7,098 (9.5)	1,550 (2.1)	1.82 ± 1.04

Data are presented as n (%).
SD, standard deviation.

Table 4. Depression (PHQ-9 score) and HRQOL (EQ-5D score)

Variable	Category	Mean \pm SD	Minimum	Maximum
PHQ-9	No interest or fun in work	0.28 ± 0.63	0	3
	Feeling subdued, depressed, or hopeless	0.28 ± 0.59	0	3
	Difficulty falling asleep or sleeping too much	0.63 ± 0.96	0	3
	Tiredness, low energy	0.59 ± 0.79	0	3
	Loss of appetite or overeating	0.30 ± 0.66	0	3
	I feel like I'm a bad person	0.09 ± 0.37	0	3
	Difficulty concentrating on newspapers or TV	0.14 ± 0.47	0	3
	Slow movement or speech	0.07 ± 0.37	0	3
	Thought it would be better to die	0.09 ± 0.37	0	3
	Total PHQ-9 score	2.49 ± 3.43	0	27
EQ-5D	Mobility	1.38 ± 0.51	1	3
	Self-care	1.15 ± 0.39	1	3
	Usual activities	1.29 ± 0.50	1	3
	Pain/discomfort	1.59 ± 0.61	1	3
	Anxiety/depression	1.20 ± 0.43	1	3
	Total EQ-5D score	6.61 ± 1.86	5	15

PHQ-9, patient health questionnaire-9; HRQOL, health-related quality of life; EQ-5D, EuroQoL-5 dimensions; SD, standard deviation.

Table 5. Depression and HRQOL according to general characteristics

Characteristic	Depression		Mobility		Self-care		Usual activities		Pain/discomfort		Anxiety/depression		Total	
	Mean±SD	p	Mean±SD	p	Mean±SD	p	Mean±SD	p	Mean±SD	p	Mean±SD	p	Mean±SD	p
Sex		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001
Male	1.89±2.97		1.26±0.46		1.10±0.35		1.20±0.45		1.43±0.57		1.14±0.37		6.13±1.67	
Female	2.92±3.67		1.47±0.53		1.18±0.42		1.36±0.53		1.72±0.61		1.24±0.47		6.97±1.91	
Age (y)		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001
65–74	2.08±3.00		1.23±0.43		1.06±0.26		1.16±0.37		1.47±0.57		1.16±0.39		6.09±1.50	
75–84	2.80±3.66		1.51±0.53		1.19±0.44		1.39±0.54		1.70±0.62		1.22±0.46		7.02±1.95	
≥85	3.61±4.37		1.78±0.52		1.45±0.61		1.69±0.62		1.88±0.61		1.30±0.51		8.09±2.19	
Marital status		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001
Married, living together	2.11±3.06		1.30±0.48		1.10±0.34		1.22±0.46		1.51±0.58		1.16±0.39		6.29±1.70	
Married, separated	2.34±3.34		1.27±0.46		1.09±0.31		1.21±0.43		1.47±0.58		1.21±0.44		6.24±1.68	
Widowed	3.19±3.91		1.57±0.53		1.24±0.48		1.45±0.56		1.78±0.61		1.27±0.49		7.31±1.99	
Divorced	3.23±4.09		1.28±0.47		1.09±0.31		1.21±0.44		1.55±0.60		1.30±0.51		6.44±1.73	
Single	3.02±3.77		1.36±0.51		1.15±0.39		1.29±0.50		1.60±0.65		1.32±0.50		6.72±1.94	
Economic activity		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001
Yes	1.86±2.63		1.25±0.44		1.05±0.22		1.16±0.37		1.48±0.56		1.12±0.35		6.06±1.38	
No	2.93±3.84		1.47±0.54		1.21±0.47		1.39±0.55		1.68±0.63		1.25±0.48		6.99±2.05	
Changes in total income related to COVID-19		<0.001		<0.001		<0.001		<0.001		<0.001		0.652		<0.001
Decreased	2.47±3.29		1.32±0.49		1.12±0.36		1.24±0.47		1.55±0.59		1.20±0.43		6.42±1.77	
Similar	2.49±3.47		1.40±0.52		1.15±0.40		1.31±0.51		1.61±0.61		1.20±0.44		6.68±1.88	
Increased	3.00±3.79		1.44±0.53		1.17±0.43		1.34±0.52		1.65±0.60		1.21±0.46		6.81±1.94	
Changes in spending related to COVID-19		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001
Decreased	2.59±3.48		1.33±0.49		1.12±0.37		1.25±0.47		1.55±0.59		1.21±0.45		6.47±1.81	
Similar	2.38±3.35		1.39±0.51		1.15±0.39		1.30±0.50		1.59±0.61		1.19±0.42		6.61±0.85	
Increased	3.02±3.79		1.39±0.52		1.15±0.41		1.31±0.52		1.64±0.62		1.24±0.46		6.73±1.94	

HRQOL, health-related quality of life; SD, standard deviation.

the impact of general characteristics and COVID-19-related concerns on depression and HRQOL in the elderly population. The independent variables were general characteristics and COVID-19-related concerns, while the dependent variables were depression and HRQOL. Upon examining the multi-collinearity among the variables influencing each variable, it was found that the variance inflation factor value was less than 10 for all variables, indicating no multi-collinearity issue. In terms of depression, the F value, which signifies the model's suitability, was 361.983 ($p < 0.001$), with $R^2 = 0.064$ and an adjusted $R^2 = 0.064$. For HRQOL, the F value indicating the model's suitability was 1,212.218 ($p < 0.001$), with $R^2 = 0.186$ and an adjusted $R^2 = 0.186$. All variables, except for those pertaining to individuals who were divorced and living alone, had an impact on depression. Concerns related to COVID-19, specifically worries about infection and economic damage, were also found to influence depression ($p < 0.05$, $p < 0.01$, $p < 0.001$). All variables, except for those related to individuals living alone due to bereavement, had an impact on HRQOL. Concerns related to COVID-19 were found to influence HRQOL ($p < 0.05$, $p < 0.01$, $p < 0.001$) (Table 6).

Discussion

This study utilized data from the 2021 Community Health Survey to examine the changes in health behaviors among the elderly population due to COVID-19. It also explored their concerns related to COVID-19, depression, and HRQOL. The ultimate goal was to identify factors that influence depression and HRQOL among the elderly.

Upon examining the changes in health behaviors among subjects due to COVID-19, it was found that many reported their physical activity levels to be similar to or less than those prior to the pandemic. When asked about their concerns related to COVID-19, the majority responded with "very much so" to all queries about infection fears, criticism from others, and economic damage. Notably, concerns about economic damage were the most prevalent. These growing fears about infection can cause older individuals to avoid social interactions, leading to increased social isolation [15]. The elderly are at a higher risk of disease exposure due to decreased immunity, and many also have chronic conditions. COVID-19, in particular, has a high fatality rate among older individuals with underlying health conditions [16]. This

Table 6. Factors affecting depression and HRQOL

Variable	Depression		HRQOL	
	Beta (β)	p	Beta (β)	p
General characteristic				
Sex (ref.: female)				
Male	-0.104	<0.001	-0.143	<0.001
Age	0.105	<0.001	0.273	<0.001
Spouse (ref.: single)				
Married, living together	-0.125	<0.001	-0.123	<0.001
Married, separated	-0.028	<0.001	-0.039	<0.001
Widowed	-0.065	0.002	-0.036	0.064
Divorced	0.016	0.056	-0.016	0.036
Changes in total income related to COVID-19 (ref.: increased)				
Decreased	-0.040	0.004	-0.035	0.007
Similar	-0.051	<0.001	-0.036	0.006
Changes in spending on major items related to COVID-19 (ref.: increased)				
Decreased	-0.035	<0.001	-0.031	<0.001
Similar	-0.089	<0.001	-0.052	<0.001
Economic activity (ref.: yes)				
No	0.108	<0.001	0.151	<0.001
Concerns due to COVID-19				
Infection	-0.029	<0.001	-0.036	<0.001
Economic damage	-0.027	<0.001	-0.033	<0.001
Criticism from others	0.008	0.130	0.013	0.006
R^2 (adjusted R^2)	0.064 (0.064)		0.186 (0.186)	
F(p)	361.983 (<0.001)		1,212.218 (<0.001)	

HRQOL, health-related quality of life; ref., reference.

reality caused many elderly individuals to increasingly avoid outdoor activities due to infection fears, creating a vicious cycle that further deteriorated their physical functions.

The number of responses indicating a decrease in income following the COVID-19 pandemic was approximately 20 times greater than those reporting an increase. There was a high level of concern about economic damage due to COVID-19, suggesting that the elderly may face financial hardship in the event of an infectious disease outbreak. A previous study found that 73.1% of respondents reported economic difficulties due to the impact of COVID-19. The majority of these respondents experienced financial hardship, with self-employed individuals, freelancers, day laborers, and the elderly from vulnerable groups suffering particularly severe difficulties [17]. The job project for the elderly, a component of the social participation program for this demographic, provided partial financial support to the elderly by disbursing a small amount of activity-related expenses each month. However, due to the suspension of this project amid the COVID-19 pandemic, many elderly individuals reported financial difficulties resulting from the loss of this income [4]. In 2020, the Seoul Welfare Foundation conducted a survey on lifestyle changes among Seoul citizens. Respondents reported difficulties related to changes in their income, including an inability to pay rent or utility bills, foregoing hospital visits due to costs, and issues that directly impact daily life, such as not being able to maintain a balanced diet [18]. The economic difficulties stemming from a decrease in children's income and the suspension of their own income are considered among the most significant concerns arising from the COVID-19 pandemic.

The depression test results indicated high scores for sleep or fatigue-related issues, such as "difficulty falling asleep or sleeping too much" and "tiredness, low energy." The highest HRQOL item was "self-care," while the lowest was "pain/discomfort." This suggests that while there was little difficulty in self-care, the HRQOL was negatively impacted by bodily pain and discomfort. Kim [19] conducted an analysis of the interactions among risk factors in elderly individuals whose physical activity had decreased due to COVID-19. The findings revealed that sleep disorders significantly influenced depression in the elderly, indicating a strong correlation between sleep disorders and depression. The diagnostic and statistical manual of mental disorders-5 has already established that depression and sleep disorders are closely linked, to the extent that sleep disorders are included in the diagnostic criteria for major depressive disorders. A reduction in physical activity can lead to a deterioration in health status, and subsequent fatigue, loss of energy,

physical pain, and difficulty sleeping can create a vicious cycle. Therefore, maintaining a positive perception of one's health is crucial.

Depression rates were higher among women, older individuals, those living alone due to the loss of a spouse, those with no economic activity, and those experiencing significant changes in income and expenditure due to COVID-19. The analysis revealed that all these factors influenced depression. Generally, it is recognized that women have a higher incidence of depression than men, a fact that was also reflected in a study conducted post-COVID-19, which showed a higher risk of depression among elderly women compared to men [20]. Elderly individuals living alone have been found to experience higher levels of depression compared to those living with family, reinforcing the findings of this study and underscoring the importance of familial cohabitation in old age [21]. The respondents in this study cited economic difficulties as their primary concern due to COVID-19, suggesting a correlation between the increased expenditure brought on by the pandemic and depression.

HRQOL was higher in older men, those living separately from a spouse, those engaged in economic activity, and those experiencing minimal changes in income and expenditure due to COVID-19. Upon analyzing the factors influencing HRQOL, it was found that all the aforementioned factors had a significant impact. Previous studies have suggested that having a spouse positively affects the mental health of the elderly. However, this study's results indicate that living separately from a spouse can lead to a higher HRQOL, warranting further discussion on the topic.

In Republic of Korea, the rate of divorce among couples married for over 20 years is rapidly increasing, particularly among those aged 65 or older, a phenomenon referred to as "twilight divorce" [22]. These couples, who may have endured an incompatible marriage for the sake of their children, often seek divorce in their later years, dreaming of a second life. According to 2021 data, only 12.8% of elderly individuals expressed a desire to live with their grown children, a significant decrease from 32.5% in 2008 [23]. This suggests a shift towards a more independent lifestyle among the elderly. The higher HRQOL observed in those living alone without a spouse, maintaining steady economic activity, and experiencing minimal changes in spending due to COVID-19, can be seen as a reflection of this changing perception.

The COVID-19 pandemic has also led to an increase in home-based work and study, resulting in families spending more time together, which has been associated with increased conflicts [24]. This suggests that while humans are social creatures, HRQOL may be higher when individuals

have time to themselves. Factors influencing depression included concerns about infection and economic damage, while factors affecting HRQOL included concerns about infection, economic damage, and criticism from others. Fear of infection leads individuals to isolate themselves, potentially leading to a vicious cycle of continued isolation and deteriorating physical health. However, it is important to prioritize personal comfort and health maintenance, rather than forcing social interactions. Activities such as moderate-intensity outdoor walking at least 3 times a week and home-based stretching exercises are recommended for this purpose [25]. Regular walking, even without a specific exercise program, can effectively reduce depression in the elderly and improve HRQOL. For the elderly, who are more vulnerable to infection, concern about this issue is inevitable. Therefore, national and community-level interventions are needed to alleviate the concerns and stress of the elderly in the event of future infectious disease outbreaks. It is crucial to identify and provide tailored support to vulnerable elderly groups who may experience unstable emotions such as depression. Particularly, those over 75 years of age or those living alone, who are at high risk of emotional difficulties and social isolation, may benefit from support services such as home visits. Some dementia care centers and mental health welfare centers currently offer home visits once or twice a month to check on the health of the elderly and organize their home environment [26]. Additionally, many community organizations are planning to implement a service to check on the health of the elderly via phone calls on a weekly basis. If expanded, this system could greatly assist in managing the emotional health of the elderly. Therefore, services that monitor the elderly through home visits or phone calls should be expanded. As previously mentioned, providing information on affordable physical activities and emotional support services can help improve the HRQOL of the elderly.

This study has certain limitations, primarily due to its reliance on raw data from the Community Health Survey, which restricted its ability to identify changes in all aspects of life. Additionally, it was not possible to consider more nuanced sociodemographic characteristics. Despite these constraints, this study successfully identified changes in the health behavior and concerns of the elderly population in Korea due to COVID-19. It also investigated the factors influencing depression and HRQOL. It also makes a significant contribution by proposing a strategy to support the mental health of the elderly.

Conclusion

This study investigated changes in the health behaviors of

the elderly due to COVID-19, the current status of concerns due to COVID-19, depression, and HRQOL, and aimed to identify factors affecting depression and HRQOL in the elderly.

The main results of the study were as follows: There was a high level of depression related to sleep and fatigue. In terms of HRQOL, the lowest scores were associated with physical pain and discomfort. Upon examining the impact of COVID-19-related concerns on depression and HRQOL among the elderly, it was discovered that increased concerns led to heightened depression and negatively affected HRQOL. In the event of future infectious disease outbreaks like COVID-19, priority should be given to early screening and support for those who are emotionally vulnerable. Participation in hybrid online and offline programs at senior welfare centers, community counseling institutions such as mental health welfare centers, and involvement in stable senior job projects should be encouraged to mitigate the effects of social interaction restrictions, physical and psychological health issues, and economic difficulties faced by the elderly.

Notes

Ethics Approval

This data utilized the data from the Korea Disease Control and Prevention Agency's 2021 Community Health Survey, and the Community Health Survey was conducted directly by the state for the public. Therefore, this research could be conducted without deliberation by the Research Ethics Committee.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

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

Additional Contributions

The author appreciates the help from the Korea Disease Control and Prevention Agency, to conduct the study using the 2021 Community Health Survey.

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Correction to “The risk associated with psychiatric disturbances in patients with diabetes in Indonesia (2018): a cross-sectional observational study” [Osong Public Health Res Perspect 2023;14(5):368–78]

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In the article entitled “The risk associated with psychiatric disturbances in patients with diabetes in Indonesia (2018): a cross-sectional observational study” [1], the authors want to update the errors in the author byline, Highlights, Results, and [Figure 1](#).

The city name of the affiliation has been published improperly. The correct affiliation is as follows:

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In the Highlights section, the sentence, “All participants had high COVID-19 vaccine confidence.”, should be deleted.

In the Results section, “single individuals (cOR, 1.65)” should be changed to “single individuals (cOR, 1.66).”

[Figure 1](#) should be changed as follows.

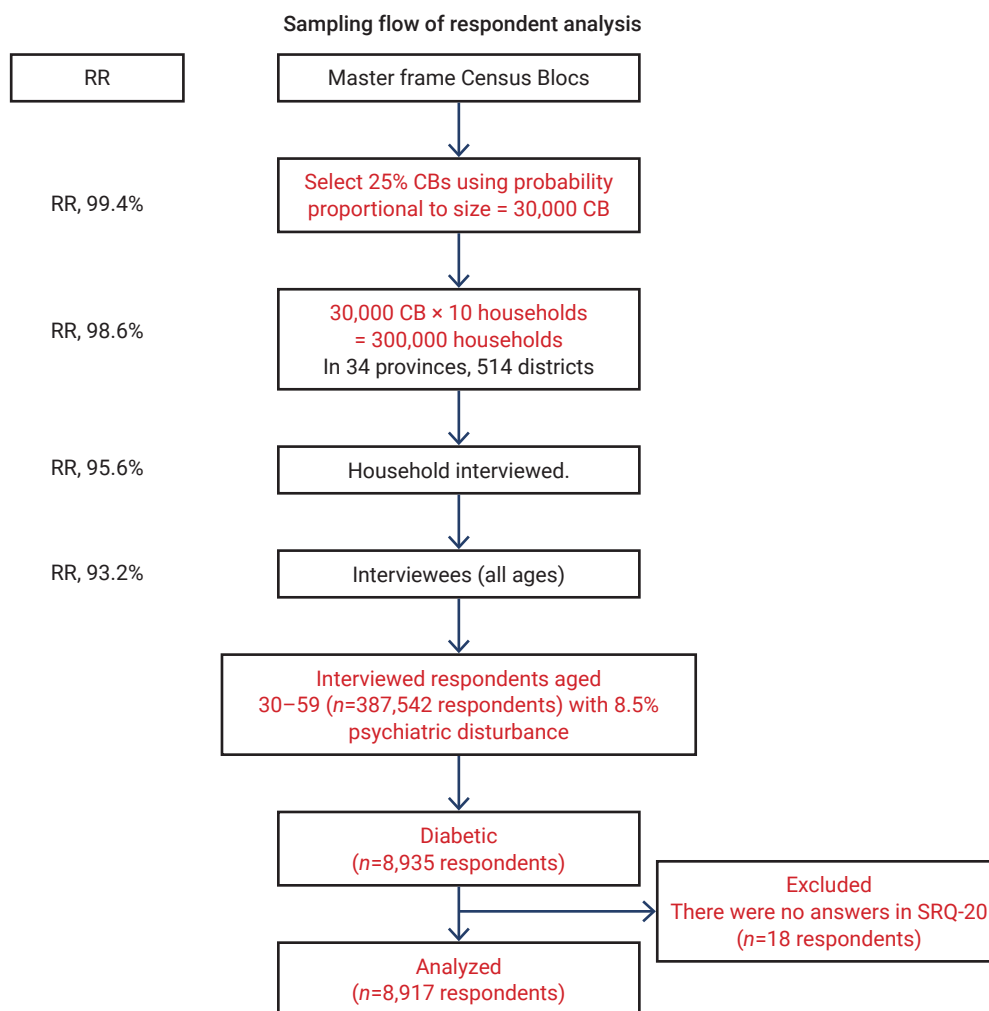


Figure 1. Flow chart of data selection. Modified from Report of national community health survey 2018 [11] and Idaiani and Indrawati. BMC Public Health 2021;21:2332, according to the Creative Commons license [12]. RR, response rate; CB, census blocks; SRQ-20, 20-question self-reporting questionnaire.

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ARTICLE PROCESSING CHARGES

The author does not have pay publication charges for open

access. The KDCA will pay to make the article open access.

RESEARCH AND PUBLICATION ETHICS

The journal adheres to the guidelines and best practices published by professional organizations, including the ICMJE Recommendations and the Principles of Transparency and Best Practice in Scholarly Publishing (joint statement by the Committee on Publication Ethics [COPE], Directory of Open Access Journals [DOAJ], World Association of Medical Editors [WAME], and Open Access Scholarly Publishers Association [OASPA]; <https://doaj.org/bestpractice>). Furthermore, all processes of handling research and publication misconduct shall follow the applicable COPE flowchart (<https://publicationethics.org/resources/flowcharts>).

Human and Animal Rights

Clinical research should be conducted in accordance with the World Medical Association's Declaration of Helsinki (<https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/>) and approved by the Institutional Review Board (IRB) of the institution where the experiment was performed. Animal experiments should also be reviewed by an appropriate committee (Institutional Animal Care and Use Committee [IACUC]) for the care and use of animals. Studies involving pathogens requiring a high degree of biosafety should pass review of a relevant committee (Institutional Biosafety Committee [IBC]). Clinical studies that do not meet the Helsinki Declaration will not be considered for publication.

Statement of Informed Consent and Institutional Approval

The editor of PHRP may request submission of copies of informed consent forms from human subjects in all studies and IRB approval documents. Articles where human subjects can be identified in descriptions, photographs, or pedigrees must be accompanied by a signed statement of informed consent to publish (in print and online) the descriptions, photographs, and pedigrees of each subject who can be identified. Articles describing the use of human samples in research and human experiments must be approved by the relevant review committee. Articles describing the use of animals in experiments must be approved by the relevant authorities.

Originality

Manuscripts are considered with the understanding that no part of the work has been published previously in print or electronic format and the paper is not under consideration by another publication or electronic medium.

Secondary Publication

It is possible to republish manuscripts if the manuscripts satisfy the conditions for secondary publication as described in the ICMJE Recommendations (<http://www.icmje.org/icmje-recommendations.pdf>).

Plagiarism and Duplicate Publication

Attempting to publish substantially similar work more than once without attribution of the original source(s) is considered a redundant publication. The definition of "substantially similar" is as follows: (1) at least one of the authors is common to all reports (it is likely to be plagiarism if there are no common authors); (2) the subject or study populations are the same or similar; (3) the methodology is typically identical or nearly so and; (4) the results and interpretation vary little or not at all.

If all or part of the subject population has been reported previously, it should be declared in the Materials and Methods and must be appropriately referenced. In cases where authors are concerned with any potential overlap with published manuscripts or manuscripts being reviewed, the authors must include a letter explaining how the manuscript submitted to PHRP significantly differs from other materials. For more information, please refer to ICMJE Recommendation (available at: <http://www.icmje.org/recommendations/>).

Authorship and the Author's Responsibilities

Authorship credit must be based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; (3) final approval of the version to be published; and (4) agreeing to be accountable for all aspects of the work in ensuring that the questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The authors should meet these 4 conditions. If the number of authors exceeds 3, the specific role(s) of authors should be described at the end of the main text.

- **Correction of authorship:** Any requests for changes in authorship (adding author(s), removing author(s), or re-arranging the order of authors) after the initial manuscript submission and before publication should be explained in writing to the editor in a letter or e-mail from all authors. This letter must be signed by all authors of the paper. A copyright assignment must be completed by every author.
- **Role of the corresponding author:** The corresponding author takes primary responsibility for communication with the journal during the manuscript submission, peer review, and publication process. The corresponding author typically ensures that all of the journal's administrative requirements, such as providing the details of authorship, ethics committee approval, clinical trial registration documentation, and conflict of interest forms and statements, are properly completed, although these duties may be delegated to one or more coauthors. The corresponding author should be available throughout the submission and peer review process to respond to editorial queries in a timely manner, and after publication, should be available to respond to critiques of the work and cooperate with any requests from the journal for data or additional information or questions about the article.
- **Contributors:** Any researcher who does not meet all 4 ICMJE criteria for authorship discussed above but contributes substantively to the study in terms of idea development, manuscript writing, conducting research, data analysis, and financial support should have their contributions listed in the Notes section of the article.
- **Recommendations for working with people with personal connections:** Authors who intend to include minors (under the age of 19) or their family members (such as spouse, children, and relatives) in their research, including when publishing or presenting papers jointly with them, should clearly indicate this in the cover letter. For further information, please refer to the "Guidelines for Preventing Illegitimate Authorship" by the National Research Foundation of Korea (<https://www.cre.re.kr/>).

Conflict of Interest Statement

The corresponding author must inform the editor of any potential conflicts of interest that could influence the authors' interpretation of the data. Examples of potential conflicts of interest are financial support from or connections to companies, political pressure from interest groups, and academically related issues. In particular, all sources of funding applicable to the study should be explicitly stated.

Role of the Funding Source

The author is requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor (s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement, then this should be stated.

Process for Managing Research and Publication Misconduct

When the journal faces suspected cases of research and publication misconduct such as redundant (duplicate) publication, plagiarism, fraudulent or fabricated data, changes in authorship, an undisclosed conflict of interest, ethical problems with a submitted manuscript, a reviewer who has appropriated an author's idea or data, complaints against editors, and so on, the resolution process will follow the flowchart provided by the COPE (<http://publicationethics.org/resources/flowcharts>). The editorial boards of PHRP will carry out the discussion and decision for suspected cases. We will not hesitate to publish errata, corrigenda, clarifications, retractions, and apologies when needed.

Complaints and Appeals Policy

The policies of PHRP are principally intended to protect the authors, reviewers, editors, and the publisher of the journal. The process of handling complaints and appeals follows the guidelines of the COPE (<https://publicationethics.org/guidance/Guidelines>).

Editorial Responsibilities

The Editorial Board will continuously work to monitor and safeguard publication ethics, including guidelines for retracting articles; maintenance of the integrity of the academic record; preclusion of business needs from compromising intellectual and ethical standards; publishing corrections, clarifications, retractions, and apologies when needed; and excluding plagiarism and fraudulent data. The editors maintain the following responsibilities: responsibility and authority to reject and accept articles; avoiding any conflict of interest with respect to articles they reject or accept; promoting publication of corrections or retractions when errors are found; and preservation of the anonymity of reviewers.

EDITORIAL POLICY

Copyright

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Every article appearing in this journal will be published as open-access. Articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derives (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. Author(s) do not need to permission to use tables or figures published in PHRP in other journals, books, or media for scholarly and educational purposes.

Article Sharing (Author Self-Archiving) Policy

PHRP is an open access journal, and authors who submit manuscripts to PHRP can share their research in several ways, including on preprint servers, social media platforms, at conferences, and in educational materials, in accordance with our open access policy. However, it should be noted that submitting the same manuscript to multiple journals is strictly prohibited.

Data Sharing Policy

To foster transparency, we encourage authors to state the availability of their data in your submission. This may be a requirement of your funding body or institution. If the data are unavailable to access or unsuitable to post, authors will have the opportunity to indicate why during the submission process, for example by stating that the research data are confidential.

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

Archiving Policy

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

Preprint Policy

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

Peer Review Policy

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

SUBMISSION & PEER REVIEW PROCESS

Online Submission

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

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

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

Peer Review Process

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

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

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

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

MANUSCRIPT PREPARATION

General Requirements

- All manuscripts must be in grammatically correct English and should be created using MS Word. The manuscript must be double-spaced and written in an A4 page format. Do not leave a space between paragraphs. Only a single font (preferably Times New Roman) should be used in 11 point with margins of 2.5 cm.
- All pages should be paginated consecutively.
- All numbers should be written in Arabic numerals throughout the manuscript except for the first word of the sentence. Texts should be justified on both sides and not hyphenated and headings should be in bold letters, aligned in the center. If possible, avoid using abbreviated words at the beginning of sentences.
- Abbreviations: Where a term/definition is repeatedly referred to (i.e., 3 times in the text), it is written in full when it first appears, followed by the subsequent abbreviation in parentheses (even if it was previously defined in the abstract); thereafter, the abbreviation is used.
- Gene nomenclature: Current standard international nomenclature for genes should be adhered to. Genes should be typed in italic font and include the accession number. For human genes, use the genetic notation and symbols approved by the HUGO Gene Nomenclature Committee (<http://www.genenames.org/>) or refer to PubMed (<http://www.ncbi.nlm.nih.gov/sites/entrez>).

www.ncbi.nlm.nih.gov/sites/entrez).

- Units: Système International (SI) units must be used, with the exception of blood pressure values, which are to be reported in mmHg. Please use the metric system for expressions of length, area, mass, and volume. There should be a space between the numerals and the unit symbol. When indicating time, the 24-hour system is to be used.
- Math formulae: Present simple formulae in the line of normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by “exp.” Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Reporting Guidelines for Specific Study Designs

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

Manuscript Types

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

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

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

- **Guidelines** are similar to original articles, but provide evidence-based recommendations expected to impact clinical research and practice. This category can include consensus-based statements of reporting standards or clinical practice guidelines.
- **Data Profiles (including Cohort Profiles)** present large data sets from specific populations that could be analyzed in epidemiological studies. Data Profiles should be structured with the following headings in the main text: Introduction, Collection, Data Resource Use, Strengths and Weaknesses, and Access. Cohort Profiles present up-to-date information about large population-based cohorts for which long-term data collection is planned. Data Profiles should be structured with the following headings in the main text: Introduction, Study Participants, Measurements, Key Findings, Strengths and Weaknesses, and Access. The main text of Data and Cohort Profiles is limited to 4,000 words, with an unstructured abstract of up to 200 words, a maximum of 7 tables and figures, and no more than 40 references.
- **Special Articles** deal with topics or issues that are relevant to public health, but without following a traditional study format. For example, articles in this category may address scientific methodology, wide-ranging ethical and social issues, scientific methodology, or other scholarly topics. Reports from consensus committees and working groups can be published as Special Articles. This category has a main text limit of 3,500 words, with an unstructured abstract of no more than 200 words, a maximum of 7 tables and figures, and no more than 40 references.
- **Brief reports** deal with issues of importance to biomedical researchers. The maximum length of the manuscript should be 2,000 words, including tables and figures.
- **Short communications** follow the general rules of the original article. The maximum length of the manuscript should be 3,000 words, including tables and figures.
- **Viewpoints** may deal with almost any topic deemed to be important in the fields of public health, ethics, health law, prevention, or health policy, and are not typically written in response to a specific article. Viewpoints should have a clear focus and present material in a well-organized and scholarly manner, but should not contain novel research findings or previously unpublished data. Although we welcome unsolicited viewpoint contributions, we request that authors contact the Editorial Office (ophrp@korea.kr) prior to submission to confirm that the proposed topic

is suitable for the journal. The main text of Viewpoints is limited to 3,000 words, with an unstructured abstract of up to 150 words, a maximum of 4 tables and figures, and no more than 30 references.

- **Editorials** provide invited perspective on an area of PHRP, dealing with very active fields of research, current interests, fresh insights, and debates. An abstract is not required and a brief unstructured text should be prepared. Although editorials are normally invited or written by an editor, unsolicited editorials may be submitted. Manuscript limitations are 1,000 words and 20 references.
- **Commentaries** are brief articles with a narrow focus. The journal commissions most commentaries, but unsolicited commentaries will also be considered. Commentaries may undergo peer review. The length of commentaries should be limited to 1,000 words, 10 references, and 1 figure or small table.
- **Correspondence** is a comment from readers regarding a published article with a reply from the authors of the article. Manuscript limitations are 500 words, 2 tables/figures, and 5 references.
- **Book reviews** may be published. Please dispatch a book to the editorial office if you think the book is essential to public health personnel.

Title Page

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

Abstract and Keywords

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

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

Highlights

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

Main Body

- **Introduction** should provide concise yet sufficient background information about the study to provide the readers with a better understanding of the study, avoiding a detailed literature survey or a summary of the results.
- **Materials and methods** should contain detailed procedures of the study or experiment including investigation period, methods of subject selection, and information on subjects such as age, sex or gender, and other significant features, in order to enable the experiment to be repeated. A procedure that has been already published or standardized should be described only briefly using literature citations. Clinical trials or experiments involving laboratory animals or pathogens must elaborate on the animal care and use and experimental protocols, in addition to mentioning approval from the relevant committees. The sources of special equipment and chemicals must be stated with the name of the manufacturer. All statistical procedures used in the study and criteria for determining significance levels must be described. Ensure correct use of the terms “sex” (when reporting biological factors) and “gender” (identity, psychosocial or cultural factors). Unless inappropriate, report the sex and/or gender of study participants, the sex of animals or cells, and describe the methods used to determine sex or gender. If the study involved an exclusive population (only one sex, for example), authors should justify why, except in obvious cases (e.g., prostate cancer). Authors should define how they determined race or ethnicity, and justify its relevance. Institutional Review Board approval and informed consent procedures can be described as follows: The study protocol was approved by the Institutional Review Board of OOO (IRB No: OO-OO-OO). Informed consent was confirmed (or waived) by the IRB.
- **Results** should be presented in logical sequence. Only the most important observations should be emphasized

or summarized, and the main or the most important findings should be mentioned first. Tables and figures must be numbered in the order they are cited in the text, kept to a minimum, and should not be repeated. Supplementary materials and other details can be separately presented in an appendix. The authors should state the statistical method used to analyze the results (statistical significance of differences) with the probability values given in parentheses.

- **Discussion** should contain an interpretation and explanation of the results and important aspects of the study, followed by the conclusions drawn from them. Information already mentioned in the Introduction or Results sections should not be repeated and the main conclusions of the study may be presented in the discussion.
- **Conclusion** (if any) must be linked with the purpose of the study stated in the abstract, and clearly supported by the data produced in the study. New hypotheses may be stated when warranted, but must be clearly labeled.

References

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

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

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

Please refer to the following examples.

• **Journal articles**

1. Park AK, Kim IH, Kim J, et al. Genomic surveillance of SARS-CoV-2: distribution of clades in the Republic of Korea in 2020. *Osong Public Health Res Perspect* 2021; 12:37-43.
2. Hyun J, Lee JH, Park Y, et al. Interim epidemiological and clinical characteristic of COVID-19 28 cases in South Korea. *Public Health Wkly Rep* 2020;13:464-74. Korean.
3. Gultekin V, Allmer J. Novel perspectives for SARS-CoV-2 genome browsing. *J Integr Bioinform* 2021 Mar 15 [Epub]. <https://doi.org/10.1515/jib-2021-0001>.

• **Books**

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

• **Websites**

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

• **Conference papers**

1. Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming*; 2002 Apr 3-5; Kinsdale, IE. Springer; 2002. p. 182-91.

• **Dissertation**

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

Tables and Figures

Tables should be simple, self-explanatory, and supplemental, and should not duplicate the text or figures. Each table must be on a separate page, not exceeding 1 page when printed, and have a concise and informative title. The tables should be numbered with Arabic numerals in consecutive order.

Each column should be appropriately headed with units in parentheses if numerical measures are given. All units of measurements and concentrations must be indicated. Footnotes are followed by the source notes, other general notes, abbreviation, notes on specific parts of the table (^a, ^b, ^c, ^d...), and notes on level of probability (*, **, *** for *p*).

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

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

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

Appendix and Supplemental Data

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

FINAL PREPARATION FOR PUBLICATION

Final Version

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

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

Manuscript Corrections

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

Proofs and Reprints

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

Errata and Corrigenda

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

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

General Requirements

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

Title Page

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

Abstract

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

Main Text

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

Tables and Figures

- All tables and figures are numbered in the order of their appearance in a main text.
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