OSONG PUBLIC HEALTH
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Vol. 12, No. 5, October 2021

Pages 275-341

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OSONG PUBLIC HEALTH
AND RESEARCH PERSPECTIVES

Vol. 12, No. 5, October 2021

pISSN 2210-9099
eISSN 2233-6052

Vol. 12, No. 5, October 2021

Korea Disease Control and
Prevention Agency

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

https://ophrp.org

pISSN 2210-9099
eISSN 2233-6052

Vol. 12, No. 5, October 2021

Published on 31 October 2021

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, brief reports, short communications, editorials, correspondence, and book reviews, with a focus on the following areas of expertise: emerging infectious diseases, vaccinology, zoonotic diseases, non-communicable diseases, intractable and rare diseases, and human genomics.

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Publisher: The Korea Disease Control and Prevention Agency

Editor-in-Chief: Jong-Koo Lee, MD, PhD
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♾ This paper meets the requirements of KS X ISO 9706, ISO 9706-1994 & ANSI/NISO Z39.48-1992 (Permanence of Paper).

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2021.0273
Osong Public Health Res Perspect 2021;12(5):275-277

Editorial

How can we prepare for this autumn and winter?

Jong-Koo Lee

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In the first week of October 2021, the fourth wave of the coronavirus disease 2019 (COVID-19) pandemic in the Republic of Korea continued, with a daily average of 1,806.4 cases, although the number of confirmed cases of COVID-19 has recently begun to subside [1]. In this situation, the Korean government is preparing to switch the public health emergency preparedness and response plan to "living with coronavirus," which means having a non-restricted, safe, and responsible society in daily life, despite the current lack of a consensus. In order to achieve this goal, given the considerable diversity of opinions regarding the proper steps to take, guidance will be developed by the Covid-19 Normal Life Recovery Committee under the Prime Minister's Office, which consists of 4 divisions handling livelihood and the economy, education and culture, internal affairs and safety, and public health and medical care. However, in terms of the relationship between civil liberties, economic loss, the stringency of public health measures, and deaths from COVID-19, the Republic of Korea has achieved very good results relative to other Organization for Economic Co-operation and Development countries even without a lockdown policy. The increasing vaccine uptake rate has been accompanied by decreases in the incidence of severe COVID-19, the hospitalization rate, and the death rate, and plans are being made to lift social distancing requirements for small businesses and events. However, unless the virus fully disappears through natural selection, there will be no way to completely return to the pre-COVID-19 daily lifestyle in a strict sense.

First, we must ask: what are the prerequisites for implementing these mitigation strategies? Increasing the vaccination rate is key. A report from a German institute showed that the death rate and the number of patients will decrease significantly when the COVID-19 vaccination rate reaches 90% of those over 60 and 80% to 85% of those aged 18 to 59 [2]. This is not an easy goal to achieve because approximately 5.6 million adults who are eligible for vaccination still refuse to be vaccinated for various reasons, and vaccination is not recommended for 5.2 million children under the age of 12, leaving about 20% of the population unvaccinated. According to recent data, although the protection provided by the Pfizer vaccine against COVID-19 infection wanes considerably—with an estimated effectiveness of roughly 20% for preventing infection by 6 months after vaccination—the efficacy of the vaccine for preventing severe cases and hospitalizations is preserved [3]. Despite the waning of vaccine efficacy and the occurrence of breakthrough infections, the vaccine can increase the speed of recovery, meaning that vaccine uptake should continue to be promoted. The Korea Disease Control and Prevention Agency recently reported that

Received: October 12, 2021
Accepted: October 18, 2021

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unvaccinated individuals accounted for 89.8% of cases in patients over the age of 18 [4]. First of all, the vaccine uptake rate should be raised to 80% or higher through voluntary participation rather than legal regulations and coercion. Furthermore, mask use (which has increased by 95% compared to pre-pandemic levels) in public places, subways, and buses; hand-washing; ventilation; refraining from holding or attending events; and social distancing [5] are behavioral changes that should be maintained through voluntary responsibility until the end of the pandemic.

A second question is whether relaxation of these mitigation policies will lead to an increase in the number of confirmed cases. On September 25, 2021, the number of new confirmed cases exceeded 3,000 in a single day, but the daily case load is decreasing again due to the strengthening of social distancing measures. However, the proportion of patients managed within the quarantine network is gradually falling. It is now down to 29.9%, whereas just 3 or 4 weeks ago, that percentage was around 35%. This means that ability of the quarantine network to conduct follow-up of confirmed cases has reached its limit [4]. Therefore, as the number of contact tracing-testing-isolation-quarantine (TTIQ) cases and the workload of health workers are increasing without recruiting new personnel, it is necessary to use an application APP that can reverse-check whether a person has overlapped with a confirmed patient so that people exposed to risk can receive prompt testing. The number of confirmed cases should decrease, thereby reducing the burden on the medical care system.

As a third question, if the vaccine uptake rate rises, will we be able to return to our daily lives? It is not an easy question to answer, due to the possibility of emerging new variants and waning vaccine efficacy; however, for the time being, freedom day such as in the UK can not be suggested. Singapore lifted many social distancing measures at the end of June 2021. However, as the number of patients has increased recently, people are again severely restricting their activities, as follows: gatherings of up to 5 people have recently been reduced to 2, all workplaces are telecommuting and schools have switched to distance learning, and booster vaccinations have been started. However, if social distancing is strengthened again, as was the case before June, small business owners will also suffer considerably. Therefore, Singapore created a disaster relief fund worth $650 million (about 569.8 billion Korean won) to support small business owners [6]. In contrast, in the United Kingdom (UK), the number of patients is increasing to a level similar to that observed during the second wave of COVID-19, but the hospitalization rate and death rate are 1 to 2 per 100,000 people by age group. The UK has implemented the following measures: building defenses through pharmaceutical interventions, identifying and isolating positive cases to limit transmission, supporting the National Health Service and social care, advising people on how to protect themselves and others, and pursuing an international approach [7].

In summary, how can we prepare for the fourth wave in the Republic of Korea? Even if the number of patients increases, high vaccine coverage will enable our medical care system to cope well with a surge in cases, making it unlikely that deaths will occur without medical intervention if booster vaccinations are given to high-risk groups. Although we had issues last year with the seasonal influenza vaccination, it will be given safely this year. While the United States and the UK, where outbreaks occurred in nursing homes or residential facilities, those facilities were closed and patients and residents were sent home and managed through home care, the Republic of Korea had also several outbreaks in nursing facilities in the early days of the pandemic, but the paralysis of medical institutions was prevented by managing mild cases in residential treatment facilities. In 2021, we will strengthen home care if a large surge occurs, exceeding 3,000 cases per day and continuing for more than several days. In addition, TTIQ plays a major role in interrupting transmission and reducing the number of patients, antiviral drug will support the home care policy if it can be supplied. Furthermore, opening the border requires global cooperation with many countries throughout the world, and vaccine coverage is an important indicator for permitting travel and opening the border. The Korean government will actively contribute to scaling up vaccination production and take steps to ensure the fair distribution and donation of vaccines to control this pandemic.

Notes

Ethics Approval
Not applicable.

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

Funding
None.

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2021.0153
Osong Public Health Res Perspect 2021;12(5):278-285

Review Article

The role of lipids in the pathophysiology of coronavirus infections

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ABSTRACT

Coronaviruses, which have been known to cause diseases in animals since the 1930s, utilize cellular components during their replication cycle. Lipids play important roles in viral infection, as coronaviruses target cellular lipids and lipid metabolism to modify their host cells to become an optimal environment for viral replication. Therefore, lipids can be considered as potential targets for the development of antiviral agents. This review provides an overview of the roles of cellular lipids in different stages of the life cycle of coronaviruses.

Keywords: Human coronavirus; Lipids; Metabolism; SARS-CoV-2

Received: June 18, 2021
Revised: September 29, 2021
Accepted: October 6, 2021

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Introduction

Coronaviruses are a group of viruses that belong to the *Coronaviridae* family. This viral family is subdivided into 4 genera, including alpha-coronaviruses, beta-coronaviruses, gamma-coronaviruses, and delta-coronaviruses [1]. Human coronaviruses (HCoVs) belong to the alpha-coronavirus or beta-coronavirus genera. Although HCoVs generally cause mild to severe respiratory diseases [2], some coronaviruses have evolved to cross the species barrier [3], giving rise to diseases such as severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), which caused viral outbreaks in 2003 and 2012, respectively [4,5].

In late December 2019, a new member of *Coronaviridae* family, named SARS-CoV-2, was discovered in China [6]. SARS-CoV-2, like SARS-CoV and MERS-CoV, is a zoonotic virus that has crossed the species barrier. Thus, SARS-CoV-2 is an emerging virus, and it causes coronavirus disease 2019 (COVID-19) [7,8]. As of September 14, 2021, the World Health Organization has

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reported more than 200 million confirmed cases of COVID-19, including more than 4 million deaths [9].

Coronaviruses have a positive-strand RNA enclosed in a protein shell that is surrounded by a host cell-derived membrane [10]. Coronaviruses cross the host cell membrane at least twice in the cell entry and exit stages of viral particles during the replication cycle. Replication of coronaviruses depends on their interactions with cellular lipids, which are essential for the successful life cycle of coronaviruses [7,11].

Cellular lipids play key roles in viral infections, particularly during the entry process and virion maturation. Lipid signaling and its synthesis machinery in host cells can be remodeled via proteins encoded by viruses [12]. Moreover, viruses alter cellular metabolism to promote proficient viral replication [13]. In this paper, we review the interactions of coronaviruses with the cellular lipids required for an effective viral life cycle.

Lipids and Viral Infections

Viruses have complex interactions with cells. According to recent studies, cellular lipids play important roles in the viral life cycle, such as viral-to-host cell membrane fusion, viral replication, and endocytosis and exocytosis [12]. Viral entry involves specific lipids, which vary among viruses [14]. The combination of lipids and proteins in the host cell membrane and the viral envelope plays a key role in viral infections [10]. In fact, viruses can alter the metabolism and signaling of lipids in host cells in order to facilitate their replication, and such viral interactions with cellular lipids have shown to be different among viruses [15]. The pathways of cellular lipid biogenesis are among the most important cellular pathways hijacked by viruses. Lipids play a crucial role in the formation of viral replication organelles, as well as energy production for viral replication. Lipids are also important for regulating the proper cellular arrangement of viral proteins and the assembly, trafficking, and release of viral particles [16–18].

Lipid rafts are specialized microdomains (10 to 200 nm) of the cell membrane that are found on the membranes of endosomes and exosomes in the endoplasmic reticulum (ER) and the Golgi complex [19]. These microdomains contain sphingolipids, cholesterol, various receptors, and other proteins [20]. Lipid rafts play important roles in viral infection, for instance in endocytosis or during different stages of pathogenesis [21]. A vital component of lipid rafts is cholesterol, which plays a major role in viral entry and release for enveloped viruses such as coronaviruses and influenza virus [22]. The major surface glycoproteins of influenza virus, hemagglutinin (HA) and neuraminidase, are associated with lipid rafts, and depletion of cholesterol by methyl-β-cyclodextrin (MβCD) can reduce the transport of HA from the trans-Golgi network to the cell membrane [23]. The requirement of cholesterol for viral pathogenesis may differ in respiratory RNA viruses, and the depletion of cholesterol by MβCD is involved in the increased budding of influenza A virus (IAV) from the host cells during pathogenesis [24]. In fact, cholesterol is essential as a vital component for sustaining IAV and respiratory syncytial virus (RSV) infectivity [25].

In a previous study, pretreatment of influenza virions with MβCD efficiently depleted cholesterol in the envelope and considerably reduced the infectivity of the virus in a dose-dependent manner [15]. In addition, the depletion of cholesterol by MβCD decreased RSV infection and interrupted lipid raft microdomains, implying that cholesterol in lipid rafts is essential for the interactions of viral proteins during infection [25]. According to prior research, human rhinovirus serotype 2 can enter into the cell through clathrin-mediated endocytosis, and the depletion of cholesterol by MβCD can prevent clathrin-mediated endocytosis and decrease viral entry [26]. Some studies showed that ceramide-rich platforms play key roles in rhinovirus infections. This suggests a novel target to treat rhinovirus infections [27]. For hepatitis C virus (HCV), it is clear that the utilization of very low-density lipoprotein secretion machinery during HCV infection supports the exit of the virus from its cellular host [28].

The Genome of Coronaviruses

Coronaviruses are enveloped positive-stranded RNA viruses with a genome size of about 27 to 30 kb, 5′-cap structure, and 3′-poly-A tail [29]. The genome of coronaviruses contains several segments—including untranslated regions (UTRs), the spike (S) protein, the envelope (E) protein, the membrane (M) protein, and the nucleocapsid (N) protein—organized as follows: 5′-leader-UTR-replicase-S-E-M-N-3′UTR-poly(A) tail [30,31]. There are 2 overlapping open reading frames (ORFs), referred to as ORF1a and ORF1b, in the replicase gene. These ORFs encode 2 polypeptides (pp1a and pp1ab), which are processed into 16 nonstructural proteins by viral-encoded enzymes including 3-chymotrypsin-like protease or main protease and 1 or 2 papain-like proteases (Figure 1) [31,32].

Structural Proteins of Coronaviruses

The surface spike proteins of coronaviruses are formed by

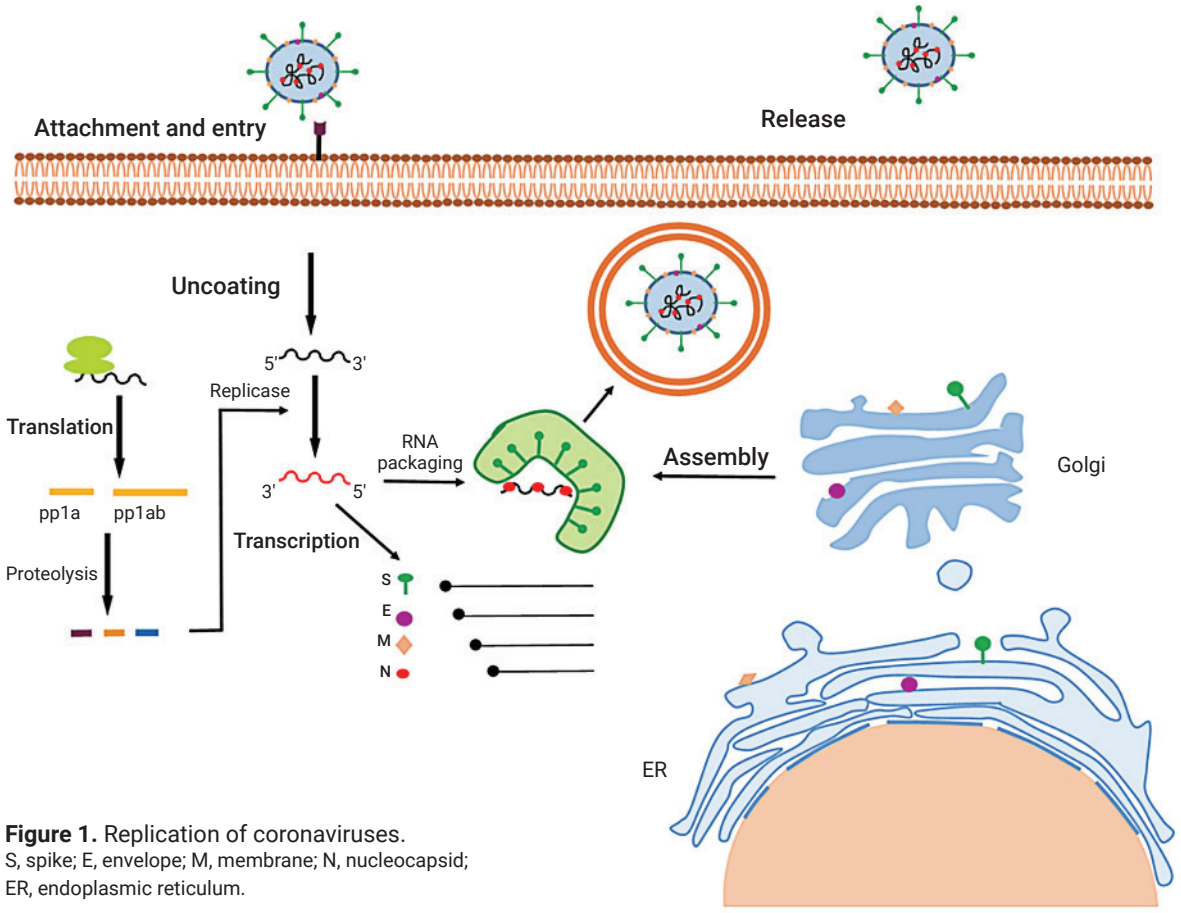

Figure 1. Replication of coronaviruses.
S, spike; E, envelope; M, membrane; N, nucleocapsid; ER, endoplasmic reticulum.

trimers of S molecules. The S protein is a class I viral fusion protein that plays a major role in viral binding to cellular receptors in order to enter the host cell [33]. The S protein undergoes modification by N-linked glycosylation in the ER [34]. The S glycoprotein contains 2 subunits (S1 and S2). The S1 subunit is variable, whereas the S2 subunit is conserved among diverse isolates of a single coronavirus. The S1 subunit is responsible for the binding of the virus to the cell receptor, whereas S2 mediates the fusion of the viral envelope and cellular membranes [35]. The M protein, which is considered to be the most abundant structural protein in coronaviruses, is N-glycosylated [36], while some beta-coronaviruses are modified by O-linked glycosylation [37]. The glycosylation of the M protein influences the interferon-inducing ability of some coronaviruses and also shapes the virion envelope [36]. The E protein is the smallest protein and is found in virions in limited amounts. Furthermore, the E protein is essential for viral infectivity and also plays a key role in virion assembly [38]. Some studies have shown that the E protein plays a role in viral pathogenesis [39]. The N protein, which is the only protein located in the ribonucleoprotein core, is made up of 3 domains, including the N-terminal domain and C-terminal domain, which are separated by an intrinsically disordered central region. The N protein is a phosphoprotein that binds to the RNA genome and is involved in the formation of the nucleocapsid [40]. In addition, HA esterase as a structural protein is encoded by beta-coronaviruses of lineage A, such as OC43-CoV. During viral infection, viruses can alter host cell metabolism and reprogram it to use cellular factors [13].

Lipids and Coronavirus Entry

To initiate infection, coronaviruses need to enter into the cell through interactions between the viral spike proteins and the cellular receptors located on the surface of the host cell [33]. The cellular plasma membrane contains subdomains of lipid rafts composed of cholesterol and glycosphingolipids [41]. SARS-CoV-2 and SARS-CoV use the angiotensin-converting enzyme-2 (ACE2) receptor for cellular entry [42]. Interactions between the ACE2 receptor and the spike protein are facilitated by cholesterol-rich

https://doi.org/10.24171/j.phrp.2021.0153

microdomains. Studies have shown that the depletion of cholesterol by MβCD in cells expressing ACE2 leads to a decrease in binding viral S glycoproteins in 50% of SARS-CoV infections. Therefore, MβCD affects cholesterol levels and ACE2 receptor expression. The depletion of cholesterol by MβCD also prevents the attachment of coronaviruses to the cell membrane [43]. Human coronavirus 229E (HCoV-229E) binds to the cellular receptor (aminopeptidase N or cluster of differentiation 13 [CD13]) for cellular infection, CD13 localized in lipid rafts, so the depletion of cholesterol by MβCD decreases the likelihood of HCoV-229E infection and prevents viral entry into host cells [44]. According to *in vitro* experiments, cholesterol supplementation enhances the propensity of the virus to fuse with the cell membrane. Clathrins, caveolins, and dynamin in lipid rafts play an important role in viral entry [43]. Some coronaviruses utilize lipid rafts for the cellular entry process. It has been reported that lipid rafts are required in the attachment process during infectious bronchitis virus infection [45], and another study also showed that lipid rafts are required for the entry of SARS-CoV into Vero E6 cells [46].

Cholesterol in the plasma membrane of target cells is also important for SARS-CoV infection [47]. It has been reported that drugs causing cholesterol depletion can inhibit the entry of murine hepatitis virus and HCoV-229E into host cells [48]. Since coronaviruses are enveloped particles, fusion with the host cell membrane is necessary before the internalization of viral particles into cells [49], SARS-CoV uses various endocytic routes including clathrin-mediated dependent, lipid raft-dependent, and clathrin- and caveolae-independent endocytosis [50]. However, HCoVs such as human coronavirus OC43 (HCoV-OC43) use caveolae-dependent endocytosis as the entry pathway [51] and feline infectious peritonitis virus uses clathrin-independent and caveolin-independent endocytosis to enter the host cell (Table 1) [33,44–46,48–60]. Therefore, it is necessary to investigate the entry pathways of coronaviruses and the mechanisms of those pathways in order to design selective inhibitors for the entry stage of coronaviruses.

Table 1. Lipid interactions in the coronavirus life cycle

Virus	Receptor	Steps of the coronavirus life cycle	Lipid interactions	Endocytic pathway	References
CCoV serotype 2	APN	Entry	Plasma membrane cholesterol	Unknown	[54,55]
MHV	CEACAM1	Entry	Lipid rafts	Clathrin-mediated endocytosis	[33,48,49]
FCoV serotype 2	APN	Entry	Cholesterol	Clathrin- and caveolae-independent pathway that depends strongly on dynamin	[33,54]
PEDV	APN	Entry	Cholesterol, lipid rafts	Clathrin- and caveolae-mediated endocytosis pathways	[33,56]
TGEV	APN	Entry	Cholesterol	The route of entry is not yet known in terms of which endocytosis pathway occurs (clathrin- or non-clathrin-dependent mechanism or both)	[57,58]
IBV	Not recognized	Entry	Lipid rafts	Macropinocytosis	[45]
HCoV-229E	APN	1. Entry 2. Replication	1. Cholesterol 2. LA and AA	Caveolae-dependent endocytic	[44,49,53,59]
HCoV-OC43	HLA class I, sialic acids, Neu5,9	Entry	Cholesterol	Caveolae- dependent endocytosis	[33,51]
MERS-CoV	DPP4 or CD26	Replication	LA and AA	Clathrin-mediated endocytosis	[51,53,60]
SARS-CoV	ACE2	Entry	Lipid rafts	Clathrin- and caveolae-independent mechanism; may involve a clathrin-mediated or clathrin-dependent mechanism	[46,50]

CCoV, canine coronavirus; APN, aminopeptidase N; MHV, murine hepatitis virus; CEACAM1, carcinoembryonic antigen-related cell adhesion molecule 1; FCoV, feline coronavirus; PEDV, porcine epidemic diarrhea virus; TGEV, transmissible gastroenteritis virus; IBV, infectious bronchitis virus; HCoV-229E, human coronavirus 229E; LA, linoleic acid; AA, arachidonic acid; HCoV-OC43, human coronavirus OC43; HLA, human leukocyte antigen; MERS-CoV, Middle East respiratory syndrome coronavirus; DPP4, dipeptidyl peptidase 4; SARS-CoV, severe acute respiratory syndrome coronavirus; ACE2, angiotensin-converting enzyme-2.

Lipids and Proliferation of Coronaviruses

The replication of most positive-strand RNA viruses occurs in the cytoplasm of the host cell. These viruses induce the formation of subcellular membranes known as virus factories or viroplasm, where they can efficiently replicate, recruit host components, and escape from the defense mechanisms of host cells [61,62].

Depending on the viral family and genus, these remodeled intracellular membranes may originate from various organelles, including the ER, late endosomes/lysosomes, or the mitochondrial outer membrane. Positive-sense RNA viruses induce the formation of 2 types of vesicles: (1) spherules, which are generated by viruses in the *Togaviridae, Bromoviridae*, and *Nodaviridae* families; and (2) double-membrane vesicles (DMVs), generated by the *Coronaviridae, Arteriviridae*, and *Picornaviridae* families [63,64].

Although the role of DMVs has not been fully clarified, they may act as autophagosomes in autophagy. The virus uses DMVs to protect against host antiviral responses or in viral replication [65]. DMV formation requires both viral and host factors. In coronaviruses, the nonstructural proteins nsp3, nsp4, and nsp6, which contain predicted transmembrane domains, play an important role in DMV biogenesis [66].

The replication process of coronaviruses that occurs in the cytoplasm of the infected host cells is similar to that of other positive-strand RNA viruses and is associated with intracellular lipid membranes derived from various organelles. Moreover, coronaviruses can also utilize cellular lipids for their replication, and thus the replication of viruses induces cellular membrane remodeling [67]. HCoV-229E infection can rearrange the cellular lipid profile, and lysophosphatidylcholine, lysophosphatidylethanolamine, and fatty acids are upregulated after infection with HCoV-229E. However, HCoV-229E replication is suppressed by exogenous supplementation of linoleic acid (LA) or arachidonic acid (AA) in infected cells. LA and AA have potent modulatory effects on MERS-CoV infection and replication of HCoV-229E [53].

The nonstructural proteins nsp3, nsp4, and nsp6 facilitate the formation of replication/transcription complexes by inducing the formation of DMVs [68]. Some cellular enzymes such as cytosolic phospholipase A2α are involved in the formation of DMVs, which take part in the replication of coronaviruses [69]. As described above, the development of new inhibitors could play a strategic role in preventing virus transmission from infected individuals to the healthy population.

Lipid Pathways as Potential Therapeutic Targets in HCoV Infections

Since lipids play vital roles in the viral life cycle, using drugs that can target lipid metabolism may therefore interfere with infections of SARS-CoV-2 and other coronaviruses [70]. Phytosterols can affect viral infection by decreasing the levels of cholesterol in cell membrane [71]. In this regard, umifenovir and chloroquine are antiviral drugs that inhibit the process of endocytosis [72]. The mechanism of actions of these antiviral drugs suggests the significance of the viral membrane for developing potent drugs. Statins can reduce cholesterol levels and disrupt lipid rafts, thereby inhibiting coronavirus infection [73].

Conclusion

Overall, the replication process of coronaviruses relies on cellular lipids, and these viruses alter the cellular lipid profile. Coronaviruses can stimulate membrane lipid remodeling in host cells and utilize cellular lipids to form viral particles or viral replication complexes, which are involved in the replication and infection process of coronaviruses. In conclusion, the study of cellular lipids and remodeling of lipid metabolism in coronavirus infections provides a good background for the development of antiviral drugs and vaccines.

Notes

Ethics Approval
Not applicable.

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

Funding
None.

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

Authors' contributions
Conceptualization: MZ; Data curation: PH, SS, AR; Investigation: PH, SS; Supervision: MZ; Writing–original draft: SN, MM, FB; Writing–review & editing: MZ, PH.

Additional Contributions
The images that constitute Figure 1 were provided by Milad Zandi (Tehran University of Medical Sciences, Tehran, Iran).

https://doi.org/10.24171/j.phrp.2021.0153

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2021.0140
Osong Public Health Res Perspect 2021;12(5):286-292

Review Article

Severe acute respiratory syndrome coronavirus 2 and respiratory syncytial virus coinfection in children

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has infected many people around the world. Children are considered an important target group for SARS-CoV-2, as well as other viral infections such as respiratory syncytial virus infection. Both SARS-CoV-2 and respiratory syncytial virus can affect the respiratory tract. Coinfection of SARS-CoV-2 and respiratory syncytial virus can pose significant challenges in terms of diagnosis and treatment in children. This review compares the symptoms, diagnostic methods, and treatment of COVID-19 and respiratory syncytial virus infection in children.

Keywords: Child; COVID-19; Respiratory syncytial viruses; SARS-CoV-2

Received: June 2, 2021
Revised: September 6, 2021
Accepted: September 8, 2021

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Introduction

Coronavirus disease 2019 (COVID-19), which causes viral pneumonia, has recently challenged the global healthcare system. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which belongs to the *Coronaviridae* family [1]. As it spread rapidly to more than 100 countries all over the world, the World Health Organization declared COVID-19 as a pandemic on March 11, 2020 [2]. Meanwhile, acute lower respiratory tract infections are the cause of death for about 160,000 neonates and more than 760,000 infants annually [3]. Studies have shown that *Streptococcus pneumoniae* and *Haemophilus influenzae* type b are common causes of bacterial pneumonia in children [4,5]. Furthermore, postmortem studies of SARS-CoV-2-positive patients have also reported coinfections of SARS-CoV-2 with

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respiratory viruses such as respiratory syncytial virus (RSV), influenza virus, human bocavirus, parainfluenza virus, and human metapneumovirus [6].

It has been estimated that 22% of episodes of acute lower respiratory infections in children younger than 5 years are associated with RSV [7]. RSV can affect the bronchioles of the lungs. Coinfection of RSV with SARS-CoV-2 can be a key factor in the development, diagnosis, and treatment of COVID-19 in children.

Severe Acute Respiratory Syndrome Coronavirus-2

SARS-CoV-2 affects both the upper and lower respiratory tracts, as well as involving other organs such as the liver, heart, kidney, and brain [8]. Acute lung injury is observed in COVID-19 patients, as well as uncontrolled production of various cytokines and changes in the balance of immune cells, which eventually lead to acute respiratory distress syndrome (ARDS) [9]. Both sexes and all ages are affected by COVID-19, and older individuals and people with underlying diseases are classified as high-risk groups [10]. SARS-CoV-2 can also infect children and infants; although COVID-19 tends to be milder in children than in adults, children can carry and transmit SARS-CoV-2 [11]. Coinfection of SARS-CoV-2 with other respiratory agents can complicate the diagnosis, treatment, and prognosis of COVID-19, as well as increasing patients' susceptibility (morbidity, symptom severity, and mortality) to COVID-19 [12]. Netea et al. [13] reported that COVID-19 is mild in most cases, although coinfections with other respiratory agents can increase the susceptibility of patients to severe disease.

Respiratory Syncytial Virus

RSV was first identified in chimpanzees in 1955; after a short time, it was confirmed that RSV could be pathogenic in humans. Human RSV is a single-stranded, negative-sense RNA virus that belongs to the genus *Pneumovirus* in the *Pneumoviridae* family [14,15]. RSV, which is recognized as one of the most common respiratory viruses that infect children worldwide [16], usually causes bronchiolitis in young children; however, it sometimes can develop into pneumonia. Studies have shown that RSV can infect 90% of children in the first 2 years of life [17]. It has been estimated that RSV annually causes 33 million lower respiratory tract infections, as well as 199,000 deaths in children under 5 years of age in the world [18]. Studies have reported that underlying lung or heart diseases may increase the severity of RSV infection. Premature infants, young children, adults, and older adults with weakened immune systems are at especially high risk for severe illness resulting from RSV [19]. Exposure to smoke is an important environmental risk factor for infant respiratory infections, such as RSV infection [20]. McConnochie and Roghmann [21] reported that exposure to passive smoke may be an important risk factor for bronchiolitis in infants. In RSV infection, host immune responses including cellular immunity and humoral immunity are involved. Cellular immunity plays a key role in the clearance of RSV infection, and the immunopathogenesis of RSV bronchiolitis is affected by cellular immunity. However, neutralizing antibodies against RSV are not stable, and their levels decrease over time [22].

Incidence of COVID-19 and RSV Infection in Children

COVID-19 and RSV infection are both infectious respiratory diseases, but they are caused by different viruses. It has been reported that children have a lower COVID-19 incidence rate than adults [23]. However, the real incidence of COVID-19 in children is unclear because of the lack of widespread diagnostic testing and the prioritization of testing for those with severe disease and adults. Studies showed that hospitalization for COVID-19 is more common among adults than among children, reflecting the higher severe illness rate in adults [24]. The susceptibility rate of children and adolescents to SARS-CoV-2 is lower than that of adults [25].

RSV affects nearly all children in the first 2 years of life. It has been reported that about 0.5% to 2.0% of all children are hospitalized with lower respiratory tract illnesses, of whom 50% to 90% have lung infections and 5% to 40% show pneumonia [26]. RSV can infect people of all age groups, but infants, children under 2 years of age, and elderly people are at particularly high risk [27]. The mortality rate of RSV is higher than that of seasonal influenza infections [28]. In the general population, the frequency of RSV-positive hospitalizations is about 1% to 3%, although the proportion among premature infants is approximately 10% to 25% [29].

Coinfection of RSV and SARS-CoV-2 may have a substantial effect on the treatment and prognosis of the disease. Viral coinfection may be associated with the need for a higher level of care, increased length of stay, and progression to ARDS [30]; furthermore, coinfection can result in more serious damage to the immune system [31]. In other words, patients with COVID-19 and other viruses have a more serious problem, their treatment is more complicated, and their treatment cycle is longer [32]. The progress and outcome of SARS-CoV-2 coinfections with other viruses including RSV are related to the host immune response

[33,34].

Several literature reviews have investigated coinfections of SARS-CoV-2 with other respiratory pathogens, such as RSV. Alvares [35] analysed 32 pediatric patients under 24 months of age hospitalized with COVID-19, and found that 18.7% of cases were coinfected with RSV. These patients had a significantly longer length of stay. However, no significant differences were observed in terms of the need for intensive care, mechanical ventilation, or mortality rate. In a study in Wuhan, among 250 patients diagnosed with COVID-19, RSV was detected in 12 patients (4.8%). That study showed that patients coinfected with viral pathogens had longer hospital stays than patients coinfected with atypical bacterial pathogens [36]. In the study of Hazra et al. [37] in 459 SARS-CoV-2-positive cases, there were no positive cases of RSV infection. In a study in Ontario, Canada that included 7,225 SARS-CoV-2-positive cases, 177 (2.4%) were coinfected with RSV. Their results showed that patients with viral coinfections were more likely to be younger than 65 years of age and male, and patients with SARS-CoV-2 coinfections mostly showed mild respiratory signs, including fever and cough [38].

Causes of Viral Coinfection

The mechanisms of viral coinfection may include virus-induced airway damage, reduced mucociliary clearance, and damage to the immune system [39]. Furthermore, several viruses can destroy the airway epithelium, which might lead to enhanced viral coinfection. Virus-associated immune system disorders can increase the risk of infection with other viruses [40,41].

Symptoms of COVID-19 and RSV in Children

The clinical manifestations of COVID-19 are milder in children than in adults [42]; however, fever, cough, diarrhea, nasal congestion, dyspnea, and abdominal pain are the most common symptoms of COVID-19 in children (Figure 1). The incubation period of SARS-CoV-2 is 2–5 days [43,44].

Multisystem inflammatory syndrome in children (MIS-C) is a hyperinflammatory condition wherein various body parts can become inflamed, such as the heart, lungs, kidneys, brain, skin, eyes, or gastrointestinal organs [25]. MIS-C develops about 2 to 6 weeks after COVID-19 infection. The disease usually affects children with a median age of 8 years [45]. The presenting clinical features in children with MIS-C included persistent fever, abdominal pain, vomiting, diarrhea, cardiac dysfunction, rash, conjunctivitis, headache, and meningismus [46]. Although the specific risk factors for MIS-C are not well understood, it has been hypothesized that lower pre-existing immunity to coronaviruses can elevate individuals' risk [47]. MIS-C is a progressive disease, and patients who initially have mild symptoms can progress to severe disease with multi-organ dysfunction within a few days of symptom/sign onset. Serious symptoms and signs can include hemodynamic instability, tachycardia, left ventricular dysfunction, and respiratory distress, which might be primary or caused by cardiac dysfunction [48]. The laboratory findings of MIS-C include lymphopenia, anemia, and thrombocytopenia; increased levels of liver enzymes, creatinine, pro-brain natriuretic protein, and troponin; and coagulation [49].

RSV infection in young children is often mild in the early phase, but in children under 3 years of age, RSV may cause coughing, wheezing, congestion, rhinorrhea, sneezing, and apnea [49]. RSV is the most common cause of bronchiolitis and pneumonia in children under 1 year of age, and the incubation period of RSV is about 2 to 8 days [50]. In children, RSV can lead to severe respiratory disease requiring hospitalization, and causes death in some rare cases. Other RSV symptoms common among infants include listlessness, poor or diminished appetite, and fever [16–18]. RSV and COVID-19 coinfection may increase the severity of disease in children.

SARS-CoV-2 and RSV Diagnosis in Children

The early and accurate diagnosis of SARS-CoV-2 is an important tool for limiting and controlling COVID-19.

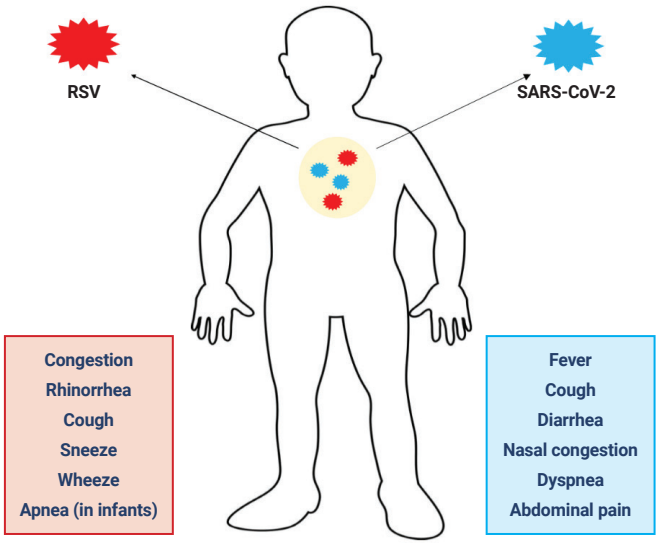

Figure 1. Common symptoms of coronavirus disease 2019 and respiratory syncytial virus (RSV) infection in children.
SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

https://doi.org/10.24171/j.phrp.2021.0140

Contact with infected individuals is a key way to transmit SARS-CoV-2 in both children and adults [51].

Molecular and serological tests are recommended by the Centers for Disease Control and Prevention to diagnose patients with SARS-CoV-2 infection. COVID-19 can result in significant alterations in the white blood cell count, elevated number of neutrophils and decreased number of lymphocytes have been reported in patients with severe COVID-19 [52]. Some laboratory predictors of COVID-19 have been identified in children, including increased or decreased levels of markers such as procalcitonin, D-dimer, and creatine kinase, as well as elevated liver enzymes [41].

The radiological imaging findings of COVID-19 pneumonia include lung changes (e.g., ground-glass opacities), bronchial changes, and pleural changes. However, the symptoms and radiological findings are similar to other respiratory viral infections [53]. Hence, determining the type and number of viruses present in a patient based on imaging findings and the medical history may be difficult, and misdiagnoses of COVID-19 and other respiratory viruses are possible. Radiographic images and clinical signs can only be used as diagnostic auxiliaries, and patients cannot be diagnosed solely on the basis of these 2 modalities [54].

Analyses of laboratory findings have shown that SARS-CoV-2 infection was associated with lymphopenia, prolonged prothrombin time, and elevated levels of lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, D-dimer, neutrophils, eosinopenia, C-reactive protein, and troponin [55]. The most common laboratory data include a decreased lymphocyte count and increased high-sensitivity C-reactive protein levels. When combined with other viral infections, these results might change. Laboratory finding results are often influenced by the degree of disease progression and the pathogens that infect the patient, so they can only be used as a reference for diagnosing the disease [56]. Multiplex reverse transcription-polymerase chain reaction can simultaneously detect respiratory viruses such as SARS-CoV-2 and RSV. Nevertheless, this technique may cause some false-negative results because of certain factors during sample recovery, processing, or transportation. Thus, repeat nasopharyngeal testing is important for confirming the diagnosis [57].

Computed tomography (CT) is used as another diagnostic tool for SARS-CoV-2, but it is less specific in children than in adults. The radiologic results in children with COVID-19 include unilateral or bilateral infiltrates and ground-glass opacities on CT [58].

Two main predictors are important for the diagnosis of RSV infection: the physical examination and patient history. Children typically present with cough, rhinitis, and wheezing. Laboratory testing and chest radiography are not required for a diagnosis. The white blood cell count is not abnormal, but it may be increased in some cases. The results of chest radiography in children with bronchiolitis sometimes are abnormal [59]. Thus, laboratory tests and chest radiography are not routinely recommended for RSV infection.

SARS-CoV-2 and RSV Treatment in Children

Combinations of antiviral agents and immunomodulators (oseltamivir + hydroxychloroquine + Kaletra [lopinavir + ritonavir]) are used to treat COVID-19 pneumonia in children [60], while RSV treatment is based on supportive care; however, palivizumab is recommended to prevent serious complications of RSV infection. Supportive care is the key treatment for RSV bronchiolitis [61]. Most infants can be managed at home, although hospitalization is necessary for children with RSV infection who are ill-appearing, have poor feeding, are dehydrated, have apnea, develop respiratory distress, or require supplemental oxygen [61].

Supportive care includes hydration, using saline nose drops to clear nasal obstruction, suction with a nasal bulb in the hospital, and adequate nutrition for the patient. Oxygen is recommended in some cases to keep oxygen saturation >90% [62]. Mechanical ventilation is needed in some otherwise healthy infants with RSV infection who are hospitalized and infants with underlying diseases such as chronic lung disease, congenital heart disease, or immunosuppression [63].

Conclusion

Both SARS-CoV-2 and RSV are infectious causes of respiratory disease in children. RSV and SARS-COV-2 coinfection in children can be considered a serious problem in the context of the COVID-19 pandemic. The pathogenicity of SARS-CoV-2 in children is unclear, but coinfection of RSV with SARS-CoV-2 may promote severe disease in children, as well as affecting the diagnosis and treatment of disease. Although several studies have investigated viral respiratory coinfections in COVID-19 patients, no study has evaluated the rate of RSV coinfection in SARS-CoV-2-positive samples from children. The clinical data of SARS-CoV-2 coinfection are of considerable value for the treatment of COVID-19. In patients with severe COVID-19, the rate of coinfections (e.g., RSV infection) is significantly higher than in those who have not been severely affected. Therefore, it is very important to prevent coinfection with SARS-CoV-2 and

other viruses, including RSV, as a step towards preventing and controlling the spread of COVID-19. To prevent transmission, social distancing must be encouraged. In the process of diagnosing and treating patients with other viral infections, it is best to provide a separate room for specific individuals in the clinical setting so that they can be isolated and treated after evaluating the risk of transmission. These actions facilitate the accurate prevention and treatment of infectious complications and effective reduction of mortality in patients with COVID-19.

Notes

Ethics Approval
Not applicable.

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

Funding
The current study was financially supported by Abadan University of Medical Sciences (Ethics No: IR.ABADANUMS.REC. 1399.218).

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.

Additional Contributions
Conceptualization: MZ; Data curation: SS, MF, SE; Investigation: SS, MF, AR; Supervision: SA; Writing–original draft: SS, MF, AR; Writing–review & editing: MZ, SA.

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https://doi.org/10.24171/j.phrp.2021.0140

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2021.0063
Osong Public Health Res Perspect 2021;12(5):293-303

Original Article

Delays in the diagnosis and treatment of tuberculosis during the COVID-19 outbreak in the Republic of Korea in 2020

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ABSTRACT

Objectives: We investigated the impact of the coronavirus disease 2019 (COVID-19) pandemic on tuberculosis (TB) "diagnosis and" management in the Republic of Korea (ROK).
Methods: This retrospective cross-sectional study used nationwide ROK TB notification data (98,346 cases) from 2017 to 2020. The median time from the onset of TB symptoms to treatment initiation and the compliance rates with the required timing for notification and individual case investigations were measured and compared across periods and regions affected by the COVID-19 epidemic.
Results: TB diagnosis during the COVID-19 pandemic was delayed. The median time to TB treatment initiation (25 days) in 2020 increased by 3 days compared to that of the previous 3 years (22 days) ($p < 0.0001$). In the outbreak in Seoul, Incheon, and Gyeonggi province during August, the time to TB diagnosis was 4 days longer than in the previous 3 years ($p = 0.0303$). In the outbreak in Daegu and Gyeongbuk province from February to March 2020, the compliance rate with the required timing for individual case investigations was 2.2%p lower than in other areas in 2020 ($p = 0.0148$). For public health centers, the rate was 13%p lower than in other areas (80.3% vs. 93.3%, $p = 0.0003$).
Conclusion: TB diagnoses during the COVID-19 pandemic in the ROK were delayed nationwide, especially for patients notified by public-private mix TB control hospitals. TB individual case investigations were delayed in regional COVID-19 outbreak areas (Daegu and Gyeongbuk province), especially in public health centers. Developing strategies to address this issue will be helpful for sustainable TB management during future outbreaks.

Keywords: COVID-19; Delay in TB diagnosis; Disease outbreaks; Tuberculosis

Received: March 8, 2021
Revised: September 5, 2021
Accepted: September 6, 2021

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Introduction

On January 2, 2020, in Wuhan, China, coronavirus disease 2019 (COVID-19) was first identified, and in March 2020, the World Health Organization (WHO) declared COVID-19 as a global pandemic [1,2]. In the Republic of Korea (ROK), a nationwide COVID-19 epidemic began after the first case was confirmed on January 20, 2020 [3]. In particular, a few large-scale regional outbreaks occurred in 2020. In Daegu and Gyeongbuk province, a COVID-19 outbreak related to religious facilities occurred from February to March 2020. In Seoul, Incheon, and Gyeonggi province, a COVID-19 outbreak related to nightclubs in Itaewon, Seoul took place in May [4]. Another outbreak related to religious facilities occurred in Seoul, Incheon, and Gyeonggi province in August 2020. Dozens of medical institutions, including the Masan National Tuberculosis Hospital, were designated as infectious disease control institutions under the Infectious Disease Control and Prevention Act (Article 37) to accommodate COVID-19 cases. Tuberculosis (TB) patients who were being treated at the Masan National Tuberculosis Hospital were sent to another national TB hospital, and no TB patients could be admitted to the designated Masan National TB Hospital during the corresponding period. COVID-19 patients were also prioritized for negative-pressure units in hospitals.

TB is a major cause of death worldwide and should not be neglected even during the current pandemic [5–7]. However, data collated by the WHO from high TB burden countries showed sharp drops in TB notifications in 2020 [7]. In the ROK, the total number of TB cases in 2020 was 25,350 (49.4 cases per 100,000 population), corresponding to a decrease by 16.3% from 2019 [8]. TB has many similarities to COVID-19 regarding its main site of occurrence (the lungs), treatment resources (medical teams in the pulmonary or infectious disease divisions), and prevention methods (masks, ventilation, etc.). A prior study reported a high mortality rate among COVID-19 patients with TB [9]. In the ROK, a cross-sectional survey was conducted to evaluate the impact of COVID-19 on TB management in public-private mix (PPM) TB control hospitals in Korea based on the WHO's survey tool in August and September, 2020 [10]. Of the 201 survey respondents, 63.2% responded that they experienced a decline in routine TB services during the COVID-19 pandemic and 47.3% responded that the number of newly diagnosed TB patients had decreased. In addition, COVID-19 led to failure to hospitalize some TB patients who needed hospitalization (26.9%) and to the discharge of hospitalized TB patients (16.9%). Kwak et al. [11] found that there was a 24% decrease in TB notifications after the COVID-19 pandemic began. Early diagnosis of TB, as a main indicator of TB management, increases the likelihood of successful treatment and could reduce the risk of community transmission by shortening the duration of exposure to others [12]. Therefore, it is important to understand the impact of the COVID-19 pandemic on TB diagnosis and management.

Diagnostic delay is defined as the time from the first onset of symptoms related to pulmonary TB to the start of TB treatment, and can be subdivided into patient delay and health system delay [13]. Patient delay refers to the duration from the onset of the first symptoms to the date when the patient first visits any type of health care institution for those symptoms. Health system delay refers to the period from the date of the patient's first contact with the health care institution to the date of diagnosis.

The effect of the COVID-19 pandemic on TB is not confined to TB diagnosis. TB management can also be delayed, and it is necessary to assess whether there have been delays in TB management during the COVID-19 pandemic. When physicians diagnose and treat TB or suspected TB patients, they are required to notify the health authorities within 24 hours. As of 2020, an individual case investigation must be performed within 3 days for all notified patients, while until 2019, investigations had to be performed within 3 days for sputum smear-positive patients and within 7 days for sputum smear-negative patients. Delays in initial TB management, such as TB notifications or individual case investigations, based on legal requirements and the guidelines for TB management may be affected by changes in the numbers or roles and responsibilities of the staff who are in charge of those tasks as a result of the COVID-19 pandemic. Therefore, in order to determine whether the COVID-19 pandemic affected TB management, it is necessary to evaluate changes in the compliance rate with the required timing for notification and investigations.

This is the first study to evaluate the potential impact of the COVID-19 pandemic on TB cases and delays in TB diagnosis or individual case investigations during the COVID-19 pandemic in the ROK. We expect that our study findings will serve as baseline data for establishing a TB management policy, which will be necessary to prepare for outbreaks of other respiratory infections such as COVID-19 in the future.

Materials and Methods

Study Design and Population

This was a retrospective cross-sectional study analyzing mandatorily collected Korean TB report data from January

https://doi.org/10.24171/j.phrp.2021.0063

2017 to December 2020 according to the Tuberculosis Prevention Act. Of the data on 125,494 cases that were extracted from the Korean National TB Surveillance System, data on 98,346 cases were included in this study after excluding 27,148 cases that were not new TB cases or duplicates. From 2017 to 2019, there were 78,445 cases, of which 53,231 were symptomatic. The data from 2017 to 2019 were used as a baseline for comparisons with 2020. In 2020, there were 19,933 cases, of which 13,354 were symptomatic. We compared delays in diagnosis and TB management between before and during the pandemic, which occurred in 2020. There were 3 outbreaks in 2020. Outbreak A was the first outbreak, which occurred in Daegu and Gyeongbuk province from February to March 2020. Outbreak B occurred in Seoul, Incheon, and Gyeonggi province in May, and was related to nightclubs in Itaewon, Seoul. Outbreak C occurred in August 2020 in Seoul, Incheon, and Gyeonggi province and was related to religious facilities. Each outbreak corresponded to specific months. We compared the values of variables in the months corresponding to each outbreak between 2017–2019 and 2020 in the outbreak areas and in other areas.

Individual Case Investigations

Based on the Tuberculosis Prevention Act and the national guideline [14], each TB case notified by physicians is mandatorily investigated following the Tuberculosis Case Investigation report form within 3 days after notification. Until 2019, investigations had to be performed within 3 days for sputum smear-positive patients and within 7 days for sputum smear-negative patients. The purpose of individual case investigations is to investigate whether the cases have family members and other contacts in settings where people congregate (e.g., schools or workplaces) or in regular gatherings. TB epidemiologic investigators who belong to the Korea Disease Control and Prevention Agency (KDCA) and TB management staff in local public healthcare centers conduct contact investigations based on the facts identified through the individual case investigations. The transmission of TB to others can be prevented if these investigations are performed rapidly and thoroughly. Another purpose of individual case investigations is to follow up on the results of tests or treatment related to TB. Individual case investigations are performed by dedicated TB nurses in PPM TB control project hospitals or by TB management staff in public health centers. The TB management staff in public health centers are also responsible for individual case investigations for cases notified by non-PPM institutions.

Variables and Statistical Analysis

To determine the TB diagnostic delay and TB management delay, we defined the following variables. The time to TB treatment initiation was defined as the period from the onset of symptoms to the time when TB treatment was started. It was not possible to divide the diagnostic delay into patient versus health system delays since the data of TB notifications and individual case investigations did not contain information on the dates of visits to medical institutions or health care centers. This variable was calculated only for TB patients who had TB symptoms. Among symptomatic cases, 769 cases were excluded because they had no information on the date of TB treatment initiation. In this study, we divided the regions into those where the 3 specific COVID-19 outbreaks (outbreaks A–C) occurred and others and compared variables between those 2 groups for each outbreak using the non-parametric Mann-Whitney test because the data did not follow a normal distribution. The data are presented as median values. The median numbers of days from the onset of symptoms to TB treatment initiation in 2020 were compared with those in the previous 3 years before the COVID-19 pandemic (2017–2019). The median numbers in the outbreak areas during the outbreak period were also compared with the same variables in other areas during the same period. To evaluate the delay in the initial management of TB patients, we defined 2 variables. The time to TB notification was defined as the period from when TB patients' treatment was started to the notification date. The time to individual case investigation was defined as the time from the date of TB notification to the date of submission of individual case investigation reports. For these 2 variables, 1,860 cases and 2,609 cases were excluded, respectively, due to a lack of the relevant information. The national guideline indicates that investigations should not be conducted in certain cases (e.g., prisoners or the dead). We compared the compliance rate with the required timing in 2017–2019 and 2020, between outbreak areas and other areas using the chi-square test. In this study, we applied the previous timing requirement to compare the compliance rates for individual case investigations between before and after the pandemic. For each of the 3 variables, we excluded outliers (data in the upper or lower 1%). A p-value < 0.05 was considered to indicate statistical significance. The statistical analysis was performed using SAS ver. 9.4 (SAS Institute Inc., Cary, NC, USA).

Ethical Approval and Consent to Participate

TB is a nationally notifiable disease according to the Korean Infectious Disease Control and Prevention Act and the Tuberculosis Prevention Act. Thus, the requirement for

written informed consent from patients was waived. This was a retrospective study using existing data, and all data for the study were de-identified before analysis. The study was approved by the KDCA Institutional Review Board (IRB No: 2021-06-14-PE-A).

Results

Time to TB Treatment Initiation

We compared the time from the onset of symptoms to TB treatment initiation between the COVID-19 pandemic of 2020 and the previous 3 years (2017–2019) (Tables 1, 2).

Table 1. Overall time to tuberculosis treatment initiation in 2017–2019 and 2020

Variable	2017–2019		2020		Difference (day)	*p*
	n (%)	Median (IQR), day	*n* (%)	Median (IQR), day		
Total	51,739 (100)	22 (10–49)	13,002 (100)	25 (10–58)	3	<0.0001
Sex						
Male	29,470 (57.0)	22 (10–47)	7,435 (57.2)	24 (10–54)	2	<0.0001
Female	22,269 (43.0)	24 (10–52)	5,567 (42.8)	26 (10–61)	2	<0.0001
Age (y)						
0–9	60 (0.1)	30 (13.5–67)	12 (0.1)	18.5 (11–47.5)	−11.5	0.2798
10–19	909 (1.8)	18 (8–39)	140 (1.1)	22 (12–48.5)	4	0.0077
20–29	4,159 (8.0)	22 (10–49)	936 (7.2)	26 (11–59)	4	0.008
30–39	4,259 (8.2)	24 (10–54)	1,041 (8.0)	27 (11–60)	3	0.0162
40–49	5,459 (10.6)	26 (11–58)	1,238 (9.5)	31 (13–67)	5	<0.0001
50–59	7,873 (15.2)	27 (12–59)	1,848 (14.2)	31 (13–64)	5	<0.0001
60–69	7,581 (14.7)	27 (11–58)	2,015 (15.5)	30 (12–62)	3	0.0045
70–79	10,405 (20.1)	22 (10–46)	2,555 (19.7)	24 (10–57)	2	0.0032
≥80	11,034 (21.3)	17 (8–37)	3,217 (24.7)	17 (8–41)	0	0.3433
Type of medical institution						
PPM	38,363 (74.1)	22 (10–50)	10,442 (80.3)	26 (10–59)	4	<0.0001
Non-PPM	11,644 (22.5)	21 (10–44)	2,429 (18.7)	21 (9–47)	0	0.9607
PHC	1,732 (3.3)	33 (17–67)	131 (1.0)	32 (15–95)	−1	0.418
Result of sputum smear test						
Positive	16,936 (32.7)	24 (10–58)	4,084 (31.4)	27 (10–62)	3	0.0317
Negative	28,639 (55.4)	21 (10–44)	7,358 (56.6)	23 (10–50)	2	<0.0001
Province						
Seoul	11,591 (22.4)	25 (11–57)	2,828 (21.8)	30 (12–64)	5	<0.0001
Busan	4,017 (7.8)	24 (10–56)	905 (7.0)	27 (11–62)	3	0.0421
Daegu	3,892 (7.5)	21 (10–45)	984 (7.6)	27 (11–62)	6	<0.0001
Incheon	2,688 (5.2)	27 (11–56)	683 (5.3)	24 (10–53)	−3	0.1996
Gwangju	1,980 (3.8)	20 (9–43.5)	479 (3.7)	24 (8–55)	4	0.1923
Daejeon	1,704 (3.3)	25 (11–56)	402 (3.1)	25 (10–52)	0	0.5698
Ulsan	1,128 (2.2)	18 (7–40.5)	254 (2.0)	17.5 (8–40)	−0.5	0.4653
Sejong	10 (0.0)	32 (22–73)	16 (0.1)	40 (14.5–104)	8	1
Gyeonggi	10,286 (19.9)	22 (10–49)	2,504 (19.3)	24 (10–54)	2	0.0048
Gangwon	2,178 (4.2)	26 (12–57)	559 (4.3)	22 (9–51)	−4	0.0214
Chungbuk	1,343 (2.6)	21 (10–42)	350 (2.7)	24.5 (10–49)	3.5	0.4261
Chungnam	1,781 (3.4)	21 (10–42)	507 (3.9)	27 (10–54)	6	0.0065
Jeonbuk	1,652 (3.2)	19 (9–42)	517 (4.0)	20 (8–45)	1	0.6256
Jeonnam	1,701 (3.3)	16 (7–35)	461 (3.5)	18 (9–43)	2	0.0052
Gyeongbuk	2,394 (4.6)	18 (8–37)	630 (4.8)	18 (8–39)	0	0.4413
Gyeongnam	2,750 (5.3)	24 (11–49)	779 (6.0)	22 (11–50)	−2	0.7085
Jeju	644 (1.2)	27 (11–50.5)	144 (1.1)	28.5 (9–65)	1.5	0.5756

IQR, interquartile range; PPM, public-private mix (tuberculosis control project hospitals); PHC, public health centers.

https://doi.org/10.24171/j.phrp.2021.0063

Table 2. Time to tuberculosis treatment initiation in each outbreak compared to the same area in the same period in the previous 3 years (2017–2019)

Outbreak	2017–2019		2020		Difference (day)	*p*
	n	Median (IQR), day	*n*	Median (IQR), day		
A	1,047	21 (9–41)	267	24 (10–50)	3	0.1368
B	2,154	24.5 (11–51)	466	27 (12–61)	2.5	0.1203
C	2,099	24 (10–55)	533	28 (11–64)	4	0.0303

IQR, interquartile range.
Outbreak A occurred in Daegu and Gyeongbuk province in February to March 2020. Outbreaks B and C occurred in Seoul, Incheon, and Gyeonggi province in May and August 2020, respectively.

Table 3. Time to tuberculosis treatment initiation in each COVID-19 outbreak area and other areas in 2020

Outbreak	Outbreak areas, 2020		Other areas, 2020		Difference (day)	*p*
	n	Median (IQR), day	*n*	Median (IQR), day		
A	267	24 (10–50)	1,833	25 (10–52)	1	0.6884
B	466	27 (12–61)	599	22 (10–58)	−5	0.1335
C	533	28 (11–64)	590	22 (9–58)	−6	0.0595

IQR, interquartile range.
Outbreak A occurred in Daegu and Gyeongbuk province in February to March 2020. Outbreaks B and C occurred in Seoul, Incheon, and Gyeonggi province in May and August 2020, respectively.

We also evaluated the time to TB treatment initiation in 3 outbreak areas compared to other areas in the same period when outbreaks occurred (Table 3).

The overall findings are shown in Table 1. The median number of days from the onset of symptoms to TB treatment initiation was 22 in 2017–2019 and 25 in 2020. This difference of 3 days was statistically significant ($p < 0.0001$). The time to TB treatment initiation was 2 days longer in 2020 during the COVID-19 pandemic in both men and women ($p < 0.0001$). The time to TB treatment initiation was 2 to 5 days longer in each age group, except for the 0- to 9-year-old group and the over-80 age group. The time was 11.5 days shorter in 2020 in the 0 to 9-year-old group ($p = 0.2798$). In the over-80 group, the time in 2020 was the same as in 2017–2019 ($p = 0.3433$). For PPM hospitals, the time to TB treatment initiation was 4 days longer in 2020, which was a statistically significant difference ($p < 0.0001$). For non-PPM or public health centers, the time in 2020 was similar to that in 2017–2019. In a comparison according to the results of the sputum smear test, the time was 2 days longer for sputum smear-positive cases and 3 days longer for negative cases in 2020. In an analysis by region, longer times to TB treatment initiation in 2020 were found in Seoul (5 days longer), Busan (3 days longer), Daegu (6 days longer), Sejong (8 days longer), Gyeonggi province (2 days longer), Chungnam province (6 days longer), and Jeonnam province (2 days longer) ($p < 0.05$). Gangwon province had the opposite result, with a time that was 4 days shorter in 2020 ($p = 0.0214$) (Figure 1).

We compared the time to TB treatment initiation in the areas where COVID-19 outbreaks occurred between the period during which the outbreak occurred in 2020 and the same months in the previous 3 years (Table 2, Figure 2). For outbreak A (in February to March, 2020), the time was 3 days longer than that of the same months in the previous 3 years in Daegu and Gyeongbuk province. For outbreaks B and C, the time was 2.5 days longer during May and 4 days longer during August 2020 than in the corresponding months in the previous 3 years in Seoul, Incheon, and Gyeonggi province. This difference was only statistically significant for outbreak C ($p = 0.0303$).

The time to TB treatment in the outbreak A areas (24 days) was 1 day shorter than in other areas (25 days), which was not a statistically significant difference ($p = 0.6884$). The time to TB treatment initiation in the outbreak B areas (27 days) was 5 days longer than in other areas (22 days). The time to TB treatment initiation in the outbreak C areas (28 days) was 6 days longer than in other areas (22 days). The differences between the outbreak areas (outbreak B and outbreak C) and other areas in the same month were not statistically significant ($p = 0.1335$, $p = 0.0595$) (Table 3).

Time to TB Notification

We compared the compliance rate with the required timing for notifying TB cases between the COVID-19 pandemic of 2020 and the previous 3 years (2017–2019) and between each outbreak area and other areas in the same period

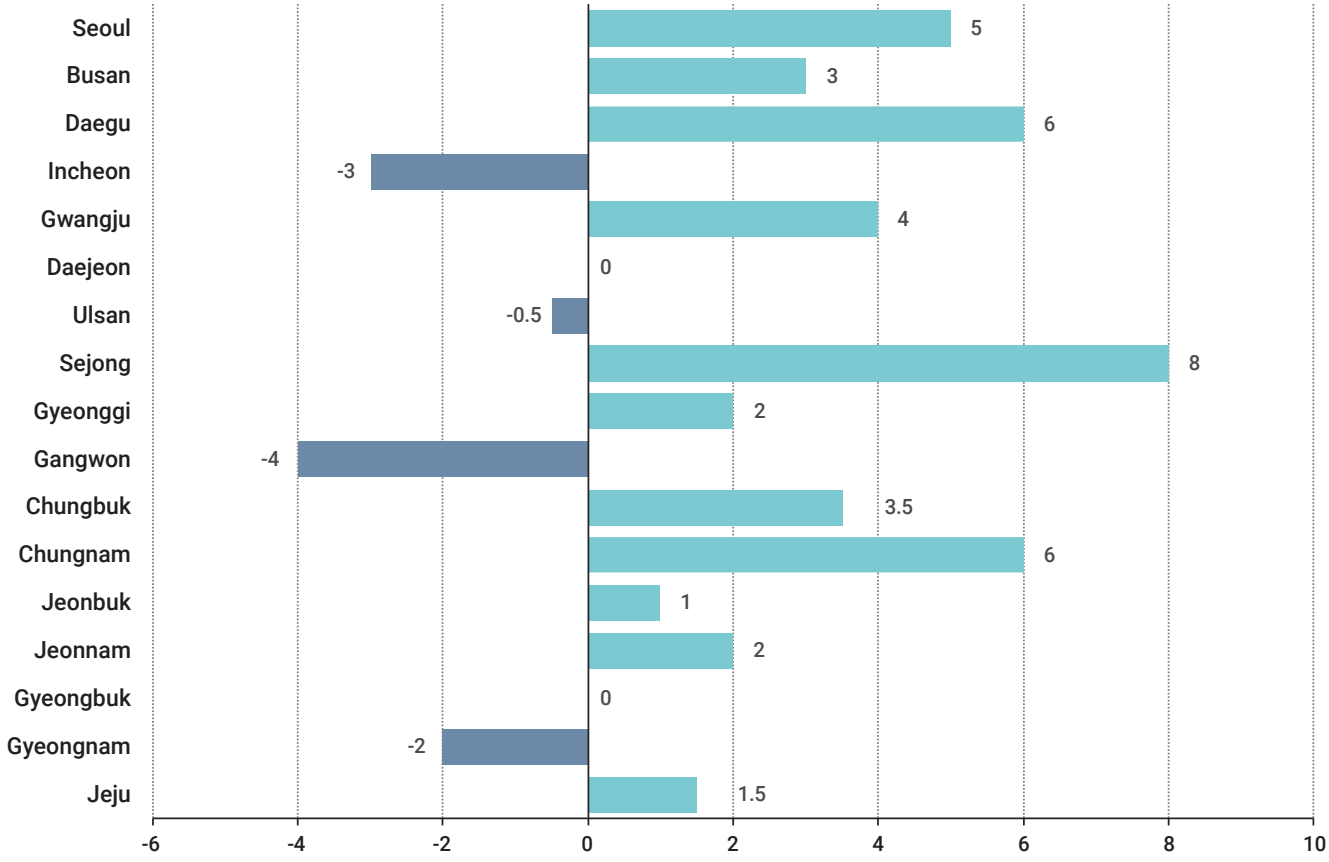

Figure 1. Differences in time to tuberculosis treatment initiation between 2017–2019 and 2020, by province.

in 2020. The compliance rate was 81.8% throughout the nation in 2017–2019 and 88.1% in 2020 ($p < 0.0001$) (Table 4). For the outbreak A areas, the compliance rate was 85.4% in the outbreak period in 2020 and 77.7% in the same months during 2017–2019 ($p = 0.0017$). For the outbreak B and outbreak C areas, the compliance rates were 86.1% and 86.2% in the outbreak periods in 2020 and 79.6% and 83.3% in the same months in 2017–2019, respectively. The rates were 6.5%p higher in 2020 for outbreak B ($p < 0.0001$) and 2.9%p higher in 2020 for outbreak C ($p = 0.0458$).

The compliance rates were 85.4%, 86.1%, and 86.2% in outbreak A, outbreak B, and outbreak C, respectively. The rates of the other areas in the same period as each outbreak were 87.7%, 88.0%, and 88.9%, respectively. The compliance rates in other areas were higher than in the outbreak areas, but none of the differences were statistically significant (Table 5).

Time to Individual Case Investigation

We compared the compliance rate with the required timing for performing individual case investigations between the COVID-19 pandemic in 2020 and the previous 3 years (2017–2019) and between each outbreak area and other areas in the same period in 2020 (Table 6). The compliance rate was 98.4% throughout the nation in 2017–2019 and 98.9% in 2020. The rate in 2020 was 0.5%p higher than that in 2017–2019 ($p < 0.0001$). Public health centers are responsible for conducting individual case investigations of cases notified by non-PPM medical institutions. We compared the compliance rate by the type of medical institution (PPM hospitals or public health centers) with the previous time requirement. For PPM hospitals, the compliance rate improved by 0.4%p, which was statistically significant ($p = 0.0004$). For public health centers, it was 0.6%p higher in 2020, which was not statistically significant. For the outbreak A areas, the compliance rate was 98.4% in the corresponding months in 2017–2019, and the rate in 2020 was similar to before (0.2%p lower, $p = 0.8302$). For the outbreak B and outbreak C areas, the compliance rates were the same (98.4%) in the corresponding periods in 2017–2019 and the same (99.0%) in 2020. For PPM hospitals, the compliance rates improved by 0.2%p to 0.5%p in each outbreak area in 2020, which was not statistically significant. For public health centers, there was a fluctuation of some percentage points

https://doi.org/10.24171/j.phrp.2021.0063

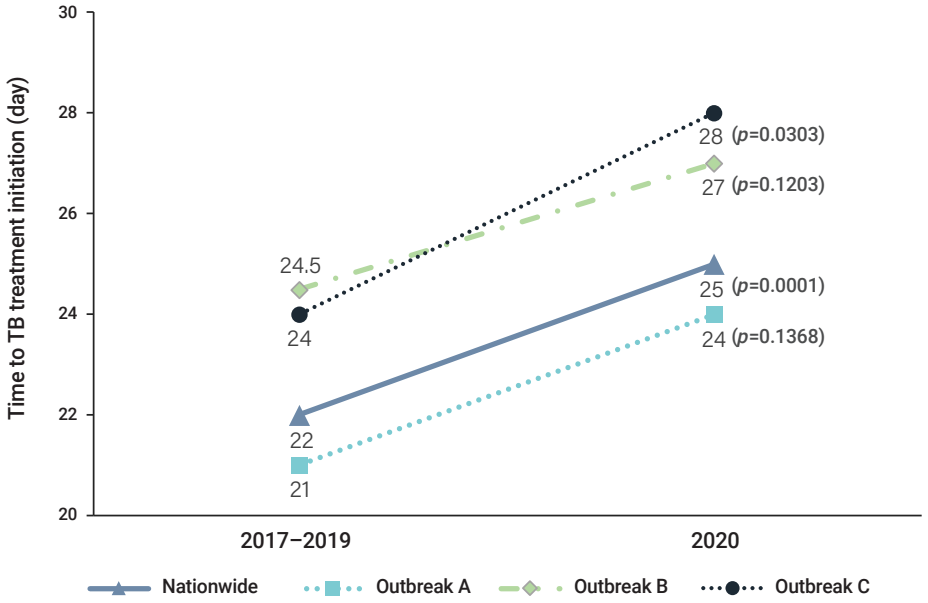

Figure 2. Time to tuberculosis treatment initiation in 2017–2019 and 2020, nationwide and in outbreak areas.
Outbreak A occurred in Daegu and Gyeongbuk province from February to March 2020. Outbreaks B and C occurred in Seoul, Incheon, and Gyeonggi province in May and August 2020, respectively.

Table 4. Compliance rate with the required timing for tuberculosis notification nationwide and in each outbreak compared to the same area in the same period in the previous 3 years (2017–2019)

Variable	2017–2019		2020		*p*
	n	Compliance rate (%)	*n*	Compliance rate (%)	
Nationwide	75,495	61,766 (81.8)	19,224	16,930 (88.1)	< 0.0001
Outbreak A	1,372	1,066 (77.7)	342	292 (85.4)	0.0017
Outbreak B	3,178	2,531 (79.6)	696	599 (86.1)	< 0.0001
Outbreak C	3,270	2,724 (83.3)	804	693 (86.2)	0.0458

Outbreak A occurred in Daegu and Gyeongbuk province from February to March 2020. Outbreaks B and C occurred in Seoul, Incheon, and Gyeonggi province in May and August 2020, respectively.

Table 5. Compliance rate with the required timing for tuberculosis notification in each COVID-19 outbreak area and other areas in 2020

Outbreak	Outbreak areas, 2020		Other areas, 2020		*p*
	n	Compliance rate (%)	*n*	Compliance rate (%)	
A	342	292 (85.4)	2,707	2,375 (87.7)	0.215
B	696	599 (86.1)	820	722 (88.0)	0.2498
C	804	693 (86.2)	849	755 (88.9)	0.0919

Outbreak A occurred in Daegu and Gyeongbuk province from February to March 2020. Outbreaks B and C occurred in Seoul, Incheon, and Gyeonggi province in May and August 2020, respectively.

in 2020 relative to previous values, but these differences were not statistically significant.

In 2020, when the new timing requirements were applied, the compliance rates were 95.6%, 97.5%, and 96.1% in the areas of outbreak A, outbreak B, and outbreak C, respectively (Table 7). The rates of other areas in the same period as each outbreak were 97.8%, 96.0% and 95.1%, respectively. The only statistically significant difference was found for outbreak A, where the compliance rate in the outbreak area was 2.2%p lower than in other areas ($p = 0.0148$). For PPM hospitals, there was no substantial difference in compliance rates between the outbreak areas and the other areas. For public health centers, the rate was 13%p lower in outbreak A than in other areas ($p = 0.0003$), while the rates in the areas of outbreak B and outbreak C were similar to those in other areas.

Table 6. Compliance rate with the required timing for individual case investigation nationwide and in each outbreak compared to the same area in the same period in the previous 3 years (2017–2019)

Variable	2017–2019		2020		*p*
	n	Compliance rate (%)	*n*	Compliance rate (%)	
Nationwide	76,286	98.4	19,624	98.9	< 0.0001
PPM	55,682	99.4	15,652	99.6	0.0004
Non-PPM	17,314	95.5	3,711	96.1	0.108
PHC	3,290		261		
Outbreak A	1,379	98.4	341	98.2	0.8302
PPM	1,064	99.2	275	99.6	0.6972
Non-PPM	264	95.9	65	92.4	0.2143
PHC	51		1		
Outbreak B	3,193	98.4	707	99.0	0.2082
PPM	2,515	99.2	614	99.4	1
Non-PPM	538	95.3	89	96.8	0.7893
PHC	140		4		
Outbreak C	3,337	98.4	822	99.0	0.2077
PPM	2,654	99.4	705	99.9	0.2264
Non-PPM	546	94.7	108	94.0	0.7523
PHC	137		9		

Outbreak A occurred in Daegu and Gyeongbuk province from February to March 2020. Outbreaks B and C occurred in Seoul, Incheon, and Gyeonggi province in May and August 2020, respectively. Compliance rate with the time to individual case investigation based on the requirement to perform the investigation within 3 days for sputum smear-positive patients and within 7 days for sputum smear-negative patients.
PPM, public-private mix (tuberculosis control project hospitals); PHC, public health centers.

Table 7. Compliance rate with the required timing for individual case investigations in each COVID-19 outbreak area and other areas in 2020

Variable	Outbreak areas, 2020		Other areas, 2020		*p*
	n	Compliance rate (%)	*n*	Compliance rate (%)	
Outbreak A	341	95.6	2,738	97.8	0.0148
PPM	275	99.3	2,215	98.8	0.7621
Non-PPM	65	80.3	471	93.3	0.0003
PHC	1		52		
Outbreak B	707	97.5	828	96.0	0.1168
PPM	614	98.5	591	98.5	0.935
Non-PPM	89	90.3	228	89.9	0.9026
PHC	4		9		
Outbreak C	822	96.1	857	95.1	0.3145
PPM	705	97.3	647	98.2	0.3024
Non-PPM	108	88.9	201	85.7	0.4155
PHC	9		9		

Outbreak A occurred in Daegu and Gyeongbuk province from February to March 2020. Outbreaks B and C occurred in Seoul, Incheon, and Gyeonggi province in May and August 2020, respectively. Compliance rate with the time to individual case investigation based on the requirement to perform the investigation within 3 days after the notification.
PPM, public-private mix (tuberculosis control project hospitals); PHC, public health centers.

Discussion

Our study showed that the diagnosis of TB was delayed during the COVID-19 pandemic in 2020. A delay of 3 days was found during the COVID-19 pandemic in the time from the onset of TB symptoms to TB treatment initiation. This TB diagnostic delay can be explained by the delay in patients' first visits to the medical institutions, as well as health system delays (i.e., those related to the medical system) [12,15]. It is possible that patients' first visits to

https://doi.org/10.24171/j.phrp.2021.0063

medical institutions to see a doctor were delayed despite the symptoms of TB patients due to concerns regarding exposure to COVID-19 patients. The pandemic has adversely affected public sentiment by creating fear and anxiety [16,17]. According to a recent survey among medical staff (doctors or dedicated TB nurses) in PPM hospitals in the ROK, a substantial proportion of respondents (17.4%) stated that TB patients were afraid to visit medical institutions due to fear of becoming infected with COVID-19 [18], while some TB patients who needed hospitalization refused hospitalization [10]. Nonetheless, patients having respiratory symptoms could find it difficult to see doctors since they would be redirected to COVID-19 screening clinics [19], patients had to undergo procedures such as checking for fever and filling out questionnaires before seeing a doctor for TB diagnosis and treatment. In other words, the diagnosis of TB might have been delayed because COVID-19 was prioritized as a potential diagnosis in patients with respiratory symptoms rather than TB [19]. Furthermore, according to news articles in the ROK, a few medical institutions did not even accept patients from Daegu, where the first large outbreak of COVID-19 occurred [20]. When COVID-19 patients were diagnosed at medical institutions and public health centers, these facilities were immediately closed and their medical services were discontinued since they had many high-risk patients. Therefore, the delay in TB diagnosis may have been due to decreased access to medical care, as well as patient-related factors [5,6,10,11].

In almost all age groups, the time to TB treatment initiation was longer during the COVID-19 pandemic in 2020. However, it was much shorter (11.5 days) than before the pandemic in the 0- to 9-year-old age group, unlike other age groups. Due to the young age of these patients, the COVID-19 outbreak may have spurred their parents or guardians to visit medical institutions more urgently. Since the symptoms of COVID-19 are almost identical to those of TB, their parents and guardians might have suspected COVID-19, prompting them to visit medical institutions early, which in turn led to an earlier TB diagnosis. At the first clinical presentation a wide range of differential diagnoses must be considered, providing an opportunity to diagnose other diseases early [21]. We evaluated the time taken for TB diagnosis by the type of medical institution. PPM hospitals accounted for around 80% of all TB patients in the ROK. Among the 67 designated infectious disease control institutions in 2020, 22 (32.8%) were PPM hospitals, including Masan National Tuberculosis Hospital [22]. For TB cases notified by PPM hospitals, the diagnosis of TB was delayed by 4 days compared to the previous 3 years, whereas it was not delayed for non-PPM hospitals and public health centers, which had fewer TB patients than before. Fortunately, the coverage of acid-fast bacilli smear and culture tests and drug susceptibility testing was above 90% at PPM hospitals, which implies that TB management was carried out at PPM hospitals under the national PPM TB control project in the ROK regardless of the pandemic [23].

In the areas affected by each outbreak, we found that there was a delay in TB diagnosis (Figure 2). This was only significant for the outbreak in Seoul, Incheon, and Gyeonggi province in August (outbreak C), which had a delay of 4 days in TB diagnosis compared to before the pandemic. Meanwhile, a few province-level areas, including the city of Daegu where the first larger outbreak occurred and some areas without large-scale outbreaks had delays in TB diagnosis throughout 2020, but some areas had shorter times to TB treatment initiation (Figure 1). Given these circumstances, COVID-19 outbreaks in the context of the pandemic were considered to have had impacts on TB nationally in 2020, with geographic variation due to differences in how local health authorities carried out prevention strategies and resource distribution depending on local conditions and based on the central health authority's policy.

We confirmed that there was no delay in TB notification during the COVID-19 pandemic, including the outbreak areas, compared to the previous 3 years. Individual case investigations were not delayed overall in 2020 compared to before the pandemic. However, the compliance rate with the required timing for individual case investigations in Daegu and Gyeongbuk province from February to March, 2020 was lower than in other areas in the same period. Furthermore, it was clearly shown that individual case investigations carried out at public health centers were delayed during the outbreaks in Daegu and Gyeongbuk province, with a 13%p lower compliance. The dedicated TB staff could not deal with the corresponding tasks in time, while PPM hospitals were not affected by the COVID-19 pandemic in terms of compliance with the required timing for TB individual case investigations. Public health centers in the ROK prioritized responding to COVID-19 in terms of staff and resources, which may have affected TB management, particularly at the beginning of the pandemic. However, our analysis showed that there were no delays in TB notifications and individual case investigations in PPM hospitals during the COVID-19 pandemic. A major reason for this may be the maintenance of dedicated TB nurses in PPM hospitals, even when medical resources were concentrated on the COVID-19 response [14]. In the analysis of TB management indicators at PPM medical institutions, it was reported that the initial TB test rate and the initial compliance rate

with treatment guidelines during the COVID-19 pandemic were well maintained, without any substantial differences compared to the rates before COVID-19 [23]. In addition, it is necessary to consider the decrease in the number of people diagnosed with TB during the COVID-19 pandemic compared to the same period in 2017–2019 [11]. Therefore, continuing support should be provided for medical resources dedicated to TB management to flexibly respond to any possible changes, including a potential temporary increase in TB notifications after the COVID-19 pandemic [11,19]. Through our study, we learned that adequate preparation to address other diseases during the prolonged pandemic is required [5–7,24].

This study has several limitations. First, it was a retrospective study and only notified cases were included in the analysis. It is likely that some patients with TB died before diagnosis and that some TB cases were not notified, and those cases were impossible to include. Another limitation is that we could not evaluate the respective impact of patient delay and health system delay because we did not have information on the date of patients' first visits to medical institutions, which would have been necessary to distinguish these delays. The information was also insufficient to determine specific factors related to delays in diagnosis, such as underlying diseases or knowledge, patients' attitudes towards TB, and the local environment related to health care. We should scrutinize where exactly delays in diagnosis occur and investigate various factors causing delays in the diagnosis of TB, such as health strategies, infrastructure related to health care, personnel in each local region, and so on. To achieve this goal, we should gather the corresponding information for future analysis. Despite these limitations, this study is meaningful in that the data analyzed were nationally representative, and the findings will be helpful for developing an evidence-based policy for TB management and prevention of delayed diagnoses.

Conclusion

The diagnosis of TB during the COVID-19 pandemic in the ROK was delayed nationwide, especially for patients notified by PPM hospitals, which cover about 80% of all TB patients. Delays in TB notification did not occur. Some delays in TB individual case investigations in Daegu and Gyeongbuk province, where an outbreak occurred, were confirmed. Especially in public health centers, there was a relatively large delay in individual case investigations in the outbreak areas. To establish a policy and strategies for minimizing delays in TB diagnosis, follow-up studies are needed to identify patient-related and health care system factors that cause delays. Understanding factors in detail through future studies and developing strategies accordingly will be helpful for sustainable TB management during outbreaks of other infectious diseases in the future.

Notes

Ethics Approval

The study was approved by the KDCA Institutional Review Board (IRB No: 2021-06-14-PE-A). The requirement for written informed consent from patients was waived.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

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

Authors' Contributions

Conceptualization: YK, JY, HP, ES; Data curation: JK; Formal analysis: JK; Investigation: YJ, JH, DK, HJ; Methodology: YK; Writing-original draft: JY, YK; Writing-review & editing: YK.

Additional Contributions

We would like to express our deep appreciation to the health care providers at the public-private mix hospitals and public health centers for making efforts to ensure that tuberculosis patients were welltreated despite the COVID-19 situation in the Republic of Korea.

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2021.0177
Osong Public Health Res Perspect 2021;12(5):304-313

Original Article

Effects of activities of daily living-based dual-task training on upper extremity function, cognitive function, and quality of life in stroke patients

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ABSTRACT

Objectives: The aim of this study was to investigate the effect of daily living dual-task training focused on improving attention and executive function of the upper extremities, cognitive function, and quality of life in stroke patients.
Methods: We included 30 stroke patients who were hospitalized between July 2020 and October 2020. They were divided into experimental and control groups through randomization. The experimental group performed 20 minutes of dual-task training and received 10 minutes of conventional occupational therapy, while the control group performed 20 minutes of single-task training and received 10 minutes of conventional occupational therapy. Both groups underwent their respective rehabilitation for 30 minutes per session, 5 times per week for 5 weeks.
Results: Both groups showed significant improvements in upper extremity function, cognitive function, and quality of life; the experimental group showed higher results for all items. A significant between-group difference was observed in the magnitude of the changes.
Conclusion: In stroke patients, dual-task training that combined attention and executive function with daily living activities was found to be meaningful, as it encouraged active participation and motivation. This study is expected to be used as a foundation for future interventions for stroke patients.

Keywords: Cognitive function; Dual-task; Quality of life; Stroke; Upper extremity function

Received: July 5, 2021
Revised: August 11, 2021
Accepted: August 18, 2021

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This paper was written by revising and supplementing the first author's master's thesis.

Introduction

Stroke is a cerebrovascular disease in which a blood vessel supplying the brain is ruptured or blocked, resulting in complex dysfunction and neurological function impairment following brain damage [1,2]. The type and level of the disorder are determined based on the area and

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extent of brain damage [2]. Common major dysfunctions after stroke include motor disorders, upper extremity dysfunction, cognitive disorders, emotional disorders, and speech disorders [3,4]. In particular, upper extremity dysfunction and cognitive impairment cause many difficulties in achieving successful rehabilitation [5].

In the field of occupational therapy, improving the function of the injured upper extremities has been regarded as one of the most important goals, as impaired upper extremity function limits independent performance in daily life [6–8]. Among stroke patients, 16% to 30% show deficits in cognitive functions such as orientation, attention, memory, language ability, executive function, and spatiotemporal cognition within 1 year [9]. Stroke patients with cognitive impairment experience difficulties in processing meaningful stimuli from the environment, making it difficult for them to self-structure and organize their environment [10]. Specifically, impaired attention and executive function have been reported as factors that negatively affect the patient's return to society, in addition to resulting in serious deterioration of the ability to perform daily activities, such as academic tasks and leisure activities [11].

Attention is the ability to elicit an efficient behavioral response by accepting and selecting external stimuli from the surrounding environment [12,13]. Attention constitutes the first step of the brain's information processing process; thus, problems in maintaining proper attention result in difficulty in the execution of other cognitive functions [14,15]. Executive function is a comprehensive concept that integrates processes related to purposeful and goal-directed behavior, and it involves initiation, planning, sequencing, control and suppression of impulses, thinking flexibility, problem-solving, abstract thinking, organization, and social judgment [16–18]. The frontal lobe of the brain is responsible for executive function. In particular, neural networks in the prefrontal cortex are involved in learning, which plays an essential role in recovery and rehabilitation after brain injury. These networks are, therefore, the main target for effective rehabilitation [19,20]. If executive function is impaired, the ability to perform complex daily life activities decreases, negatively affecting social communication and thereby reducing social participation [21,22].

The objective of rehabilitation treatment is to allow stroke patients to live independent daily lives by recovering maximum residual function after injury [23]. Thus, if rehabilitation programs for stroke patients include training focused on attention and executive function, more efficient improvement in performing daily life activities can be expected [24]. A variety of effective training methods for stroke patients have been suggested. In particular, dual-task training involving the simultaneous execution of cognitive tasks and motor tasks in relation to the recovery of motor control in patients with neurological impairment (e.g., stroke) has been implemented in clinical settings [25]. In dual-task training, the patient performs 2 or more complex tasks of different characteristics that frequently occur in everyday life [26,27]. The objective of dual-task training is to improve the subject's ability to process information by performing 2 different tasks simultaneously [28]. Most daily life movements involve performing 2 motor tasks simultaneously, and performing various daily life activities involves the concurrent execution of high-level cognitive function and motor tasks [29,30]. However, despite the high frequency of dual tasks in daily life, rehabilitation of stroke patients is mainly performed under single-task conditions [31]. Because a training method that focuses on a single task does not reflect the complex activities of daily life, it has been reported that recovering fragmentary functions such as upper extremity function and balance alone does not significantly change the level at which individuals perform daily life activities [32]. Therefore, it is necessary to actively utilize dual-task training in which 2 or more different tasks are simultaneously handled in order to improve stroke patients' performance of daily activities [28].

Most existing studies conducted with stroke patients have involved the simultaneous performance of balance or gait training with upper extremity training or cognitive tasks, and many cases demonstrated positive effects of training [33–35]. Only a handful of studies on dual-task training have applied daily life activities and upper extremity movements together with cognitive tasks for stroke patients. Therefore, this study aimed to examine the effects of repeated dual-task training coupling daily life activities with attention and executive function on upper extremity function, cognitive function, and quality of life in stroke patients.

Materials and Methods

Participants

This study was conducted among 30 subjects who met the selection criteria among patients who were hospitalized after being diagnosed with stroke based on magnetic resonance imaging or computed tomography scans taken at the H hospital in Cheongju between July 2020 and October 2020. The specific inclusion criteria were as follows: (1) those with disease onset within the past 6 months, (2) those who scored at least 20 points on the Korean version of the Mini-Mental State Examination (K-MMSE) and were

able to follow the researcher's instructions, (3) those in Brunnstrom Recovery Stage (BRS) 3 or higher, and (4) those who had no issues in communication or problems with vision or hearing and could follow instructions.

Protocol

The study period was from July 2020 to October 2020. The 30 subjects who met the inclusion criteria were randomized into experimental and control groups. The experimental group performed dual tasks for 20 minutes in the treatment room and received 10 minutes of conventional occupational therapy for a total of 30 minutes per session, 5 times a week for 5 weeks. The control group performed a single task for 20 minutes and received 10 minutes of conventional occupational therapy, for a total of 30 minutes per session, 5 times a week for 5 weeks. The dual tasks combined attention or executive function with daily life activities, whereas the single tasks consisted of sensory stimulation training on the paralyzed side, upper extremity muscle strength training, cognitive and perceptual training, and fine hand movement using tools. Occupational therapists with more than 4 years of experience conducted the evaluation (pre-assessment, intervention, and post-assessment, in sequential order). The design of this study is presented in Figure 1.

Outcome Measurements

Manual Function Test

The Manual Function Test (MFT) is widely used for the measurement of upper extremity function and motor ability in the early-stage rehabilitation of stroke patients [36]. Measurements are made on both the paralyzed and the non-paralyzed sides, with a total of 8 items in the areas of upper extremity movement (4 items), grip strength (2 items), and finger manipulation (2 items). For each sub-test that can be performed, a score of 1 is assigned up to a total of 32 points, and 0 is assigned for sub-tests that could not be performed. The MFT score is calculated by converting the initial score out of 32 points to a score out of 100 by multiplying it by 3.125. The test-retest concordance was 0.99 for the paralyzed side and 0.83 for the non-paralyzed side [37,38].

The Digit Span Test

The Digit Span Test (DST) consists of the DST-Forward and DST-Backward. The DST-Forward starts with 3 digits to repeat with 1 additional digit to memorize at each stage, for a total of 9 digits to memorize and repeat at the last stage. A higher number of digits indicates a higher level of attention. In the DST-Backward, the test is conducted in the same manner for scoring, and a higher number of digits indicates better working memory. The reliability was found to be 0.72 for the DST-Forward and 0.57 for the DST-Backward [39].

Executive Function Performance Test Korean version

Executive Function Performance Test Korean version (EFPT-K) evaluates executive function and instrumental daily life performance by measuring the degree of impairment of executive function, individual competence in independent function, and the level of assistance to complete tasks. For each task, points are given for elements of executive function such as initiation, preparation, ordering, judgment and safety, and termination, and each element is given a minimum of 0 points and a maximum of 5 points. The total score is scored from 0 to 100 points, and a lower score indicates a higher level of independence. The inter-tester reliability was 0.91 and the internal consistency was found to be high (0.94) [40].

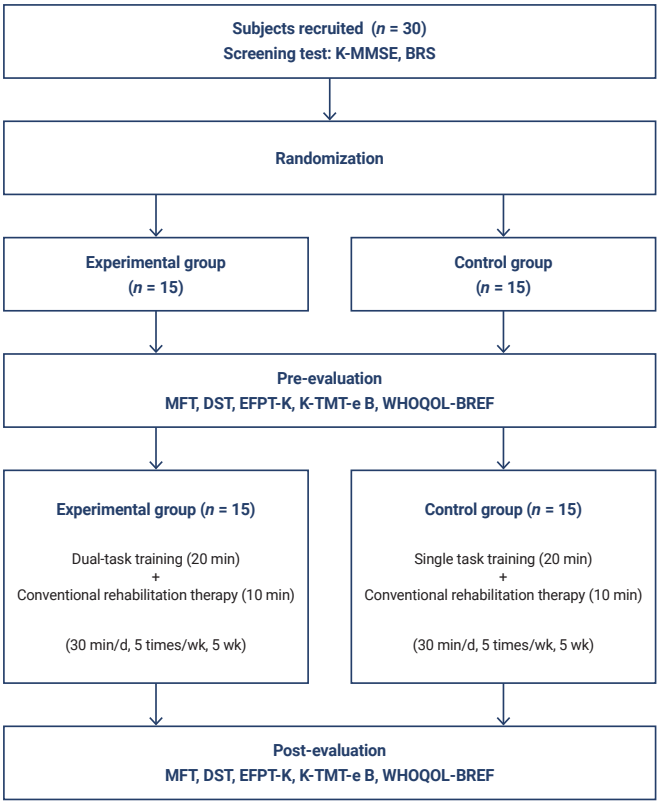

Figure 1. Flow chart of the study.
K-MMSE, Korean version of the Mini-Mental State Examination; BRS, Brunnstrom Recovery Stage; MFT, Manual Function Test; DST, Digit Span Test; EFPT-K, Executive Function Performance Test Korean version; K-TMT-e B, Korean version of the Trail Making Test for the Elderly B; WHOQOL-BREF, World Health Organization Quality of Life-BREF.

https://doi.org/10.24171/j.phrp.2021.0177

Korean version of the Trail Making Test for the Elderly B

To measure the improvement in the speed of cognitive processing, the Korean version of the Trail Making Test for the Elderly B (K-TMT-e B) was conducted. Cognitive processing and executive function are measured through visual concept tracking and visual movement tracking. There are 2 types of the test (type A and B); however, only type B, which reflects attention movement and retrograde suppression, was used to determine cognitive processing speed. The instructions for the test are to connect items composed of numbers and alphabetical letters in alternating order. The frequency and type of errors are not separately recorded, but the instructions state that the test should be stopped if it takes more than 5 minutes [26,27]. The test-retest reliability of the K-TMT-e B was found to be relatively high (0.89) [41].

World Health Organization Quality of Life-BREF

Quality of life was measured using the World Health Organization Quality of Life-BREF (WHOQOL-BREF). The WHOQOL-BREF is divided into 4 subscales (physical health, psychology, social relations, and environment) and consists of a total of 26 items. It is measured on a 5-point scale (1 to 5 points), and higher scores indicate higher quality of life. The test-retest reliability ranged from 0.43 to 0.73, and the value of Cronbach's alpha, which indicates the degree of internal consistency, was found to be 0.89 [42].

Intervention

The dual-task training used in this study was designed based on previous studies [43,44] that examined training for attention and daily life activities for stroke patients, as well as the study by Park et al. [45], which conducted interventions for executive function in stroke patients. Attention training consisted of continuous subtraction, simple addition or subtraction, counting the numbers 1 to 20 forward or backward in order, reading words backwards, and a simple word game of coming up with a word that starts with the last letter of the previous word. Daily living-based activities included climbing the stairs, making tea or coffee, folding tops or bottoms, buttoning and unbuttoning, and moving beans. The activities for executive function consisted of presenting a virtual situation, explaining the order of wearing clothes, describing a daily routine, making a shopping list, and talking about categorization types. The activities of executive function were composed of items related to daily life activities. Because executive function is a high-level cognitive domain, it can cause confusion in patients with brain damage when performing the dual task of applying items that require higher-order thinking and daily life activities together. Therefore, for example, when "buttoning and unbuttoning" is performed as a daily activity, the corresponding item of the executive function would be "explaining the order of wearing clothes." Dual-task training was conducted by combining the aforementioned executive function and daily life activities (Table 1, Table S1).

Statistical Analysis

The statistical analysis was conducted using IBM SPSS ver. 24.0 (IBM Corp., Armonk, NY, USA). The normality of the distribution of data across the study subjects was determined using the Shapiro-Wilk test, and if normality could not be established, a nonparametric analysis was conducted. Among general characteristics, sex, stroke type, and the paralyzed side were analyzed using the chi-square and Mann-Whitney U-tests to test the homogeneity of the subjects before the intervention. Changes after the intervention within the experimental and the control groups were analyzed using the Wilcoxon signed rank test, whereas between-group differences before and after the intervention were analyzed using the Mann-Whitney U-test. The statistical significance level for all data was set to 0.05.

Results

General Characteristics of Research Subjects

The general characteristics of the subjects who participated in the study are presented in Table 2. There were 8 male (53.3%) and 7 female patients (46.7%) in the experimental group, and 7 male (46.7%) and 8 female patients (53.3%) in the control group. The mean age was 65.20 ± 12.17 years in the experimental group and 65.27 ± 12.73 years in the control group. The right and left sides were paralyzed in 6 (40.0%) and 9 patients (60.0%), respectively, in the experimental group, and the right side was paralyzed in 7 patients (46.7%) and the left side was paralyzed in 8 (53.3%) in the control group. The mean duration of disease was 4.07 ± 0.88 months in the experimental group and 4.20 ± 0.77 months in the control group. There were no significant differences in sex, age, the paralyzed side, disease duration, K-MMSE scores, and BRS between the 2 groups ($p > 0.05$).

Comparison of the MFT Results Before and After the Intervention

Table 3 shows the post-intervention changes in upper extremity function in the experimental and the control groups. MFT measures both the affected and unaffected sides, but in this study, only the affected side was measured and reflected in the results. In the experimental group,

Table 1. Dual-task training that combined attention and executive function tasks with daily activities

Daily living activity-based task	Attention task	Executive function task
Climbing stairs	Continuous subtraction e.g., 20–3–3–3	Presenting a virtual situation e.g., You're in a taxi but do not have your wallet.
Making tea/coffee	Simple addition/subtraction e.g., 5+4, 9–6	Explaining the order of wearing clothes e.g., Dress, skirt, pants, shirts, tie
Folding tops/bottoms	Counting numbers forward/backward in order e.g., Counting from 1 to 20 forwards/backwards in order	Talking about daily routine e.g., Describe your daily routine for today.
Buttoning/unbuttoning	Reading words backwards e.g., Hat, pencil, soap, ballpoint pen	Making a shopping list e.g., Make a shopping list for grocery shopping, pharmacy, bookstore.
Moving beans	Simple word game of coming up with a word that starts with the last letter of the previous word e.g., Standing-train-garage	Categorization e.g., Types of land animals, types of drinks, colors

Perform attention tasks while performing daily living activity, or perform executive function task while performing daily living activity.

Table 2. General characteristics of the research subjects

Characteristic	Experimental group (n = 15)	Control group (n = 15)	p
Mean age (y)	65.20 ± 12.17	65.27 ± 12.73	0.868
Sex			0.715
Male	8 (53.3)	7 (46.7)	
Female	7 (46.7)	8 (53.3)	
Stroke type			0.464
Infarction	8 (53.3)	6 (40.0)	
Hemorrhage	7 (46.7)	9 (60.0)	
Paralyzed side			0.713
Right	6 (40.0)	7 (46.7)	
Left	9 (60.0)	8 (53.3)	
Disease duration (mo)	4.07 ± 0.88	4.20 ± 0.77	0.691
K-MMSE (score)	24.00 ± 2.00	24.00 ± 1.96	0.916
BRS (phase)	5.33 ± 0.61	5.00 ± 0.65	0.161

Data are presented as mean ± standard deviation or n (%).
K-MMSE, Korean version of the Mini-Mental State Examination; BRS, Brunnstrom Recovery Stage.

all of the MFT items showed statistically significant improvements after the intervention ($p < 0.05$, $p < 0.01$). Similarly, the control group showed statistically significant improvements in all items after the intervention ($p < 0.05$, $p < 0.01$). Significant differences between the 2 groups were found in the changes in upper extremity function for all items, excluding the item for upper extremity movement ($p < 0.05$).

Comparison of the DST, EFPT-K, and K-TMT-e B Results Before and After the Intervention

The results for changes in cognitive function before and after the intervention in the experimental and control groups are presented in Table 4. The experimental group showed statistically significant improvements in both the DST-Forward and DST-Backward after the intervention ($p < 0.01$). The control group also showed significant improvements in all items after the intervention ($p < 0.05$, $p < 0.01$). Statistically significant improvements were seen for all items in the EFPT-K in both the experimental and the control groups ($p < 0.01$). For the K-TMT-e B, there was a statistically significant reduction in the time taken for completion after the intervention in both the experimental and the control groups ($p < 0.01$). On the cognitive function assessment, the experimental group showed slightly higher scores than the control group for all of the items, and there was a significant difference when the changes between the groups were compared ($p < 0.01$).

Comparison of the WHOQOL-BREF Results Before and After the Intervention

Table 5 shows the results for changes in the WHOQOL-

https://doi.org/10.24171/j.phrp.2021.0177

Table 3. Changes in Manual Function Test scores after the intervention within groups and differences between groups ($n = 30$)

Variable	Intervention with groups				Difference between groups		
	Before (mean ± SD)	After (mean ± SD)	*Z*	*p*	Mean ± SD	*Z*	*p*
Upper extremity training							
EG	14.40 ± 1.35	15.53 ± 0.74	−2.724	0.006**	1.13 ± 1.18	−0.932	0.351
CG	14.40 ± 1.45	15.20 ± 1.01	−2.414	0.016*	0.80 ± 1.14		
Grip strength							
EG	5.33 ± 0.48	5.87 ± 0.35	−2.530	0.011*	0.67 ± 0.48	−2.159	0.031*
CG	5.47 ± 0.51	5.73 ± 0.45	−2.000	0.046*	0.27 ± 0.45		
Finger manipulation							
EG	4.60 ± 0.91	6.20 ± 1.08	−3.520	0.000**	1.60 ± 0.50	−2.361	0.018*
CG	4.60 ± 0.82	5.53 ± 0.99	−2.889	0.004**	0.93 ± 0.79		
Total score							
EG	24.53 ± 1.99	27.73 ± 1.58	−3.438	0.001**	3.20 ± 1.52	−2.430	0.015*
CG	24.47 ± 2.06	26.40 ± 1.95	−3.088	0.002**	1.93 ± 1.79		

SD, standard deviation; EG, experimental group; CG, control group.
*$p < 0.05$, **$p < 0.01$.

Table 4. Changes in the DST, EFPT-K, K-TMT-e B after the intervention within groups and differences between groups ($n = 30$)

Variable	Intervention with groups				Difference between groups		
	Before (mean ± SD)	After (mean ± SD)	*Z*	*p*	Mean ± SD	*Z*	*p*
DST-Forward							
EG	5.27 ± 0.88	6.93 ± 0.88	−3.542	0.000**	1.67 ± 0.48	−3.181	0.001**
CG	5.20 ± 1.08	6.07 ± 1.10	−3.127	0.002**	0.87 ± 0.64		
DST-Backward							
EG	2.60 ± 0.73	3.93 ± 0.45	−3.397	0.001**	1.33 ± 0.61	−3.500	0.000**
CG	3.00 ± 0.75	3.40 ± 0.73	−2.449	0.014*	0.40 ± 0.50		
EFPT-K							
EG	12.70 ± 2.51	9.96 ± 1.30	−3.413	0.001**	−2.74 ± 1.29	−4.720	0.000**
CG	12.70 ± 2.63	11.94 ± 2.58	−3.474	0.001**	−0.77 ± 0.19		
K-TMT-e B							
EG	89.13 ± 10.85	67.00 ± 12.16	−3.411	0.001**	−22.13 ± 4.86	−3.199	0.001**
CG	89.60 ± 12.33	77.00 ± 13.26	−3.412	0.001**	−12.60 ± 7.83		

DST, Digit Span Test; EFPT-K, Executive Function Performance Test Korean version; K-TMT-e B, Korean version of the Trail Making Test for the Elderly B; SD, standard deviation; EG, experimental group; CG, control group.
*$p < 0.05$, **$p < 0.01$.

BREF quality of life scale after the intervention for the experimental and the control groups. Statistically significant improvements were found for all items in both the experimental and the control groups after the intervention ($p < 0.01$), and there was a significant difference in the change in the quality of life between the 2 groups ($p < 0.01$).

Discussion

Dual-task training involves performing 2 or more complex tasks with different characteristics that frequently occur in daily life. The training requires performing 1 additional task while performing 1 basic task, or performing 2 or more tasks simultaneously [26,27]. To carry out functional movements in daily life, it is necessary to be able to perform multiple tasks at once [46–48]. According to previous studies on stroke, dual-task training was more effective in improving the subject's information processing ability than performing only 1 task at a time for the same amount of time [28]. However, most studies have used upper extremity functions together for balance and gait training, or have dealt with cognitive tasks for balance and gait

Table 5. Changes in WHOQOL-BREF after the intervention within groups and differences between groups ($n = 30$)

Variable	Intervention with groups				Difference between groups		
	Before (mean ± SD)	After (mean ± SD)	*Z*	*p*	Mean ± SD	*Z*	*p*
Physical health							
EG	19.47 ± 3.50	26.00 ± 2.59	−3.391	0.001**	6.53 ± 2.61	−3.158	0.002**
CG	19.93 ± 4.14	23.60 ± 4.18	−3.309	0.001**	3.67 ± 2.09		
Psychology							
EG	15.60 ± 2.44	22.33 ± 3.71	−3.423	0.001**	6.73 ± 2.86	−3.617	0.000**
CG	15.60 ± 2.92	18.47 ± 4.08	−3.196	0.001**	2.87 ± 1.88		
Social relations							
EG	8.73 ± 2.21	10.87 ± 1.99	−3.455	0.001**	2.13 ± 0.99	−3.094	0.002**
CG	8.67 ± 1.58	9.60 ± 1.76	−2.913	0.004**	0.93 ± 0.88		
Environment							
EG	23.53 ± 2.64	27.93 ± 3.15	−3.428	0.001**	4.40 ± 1.95	−3.524	0.000**
CG	23.73 ± 2.28	25.20 ± 2.65	−2.842	0.004**	1.47 ± 1.72		
Total score							
EG	68.13 ± 8.55	86.73 ± 9.52	−3.411	0.001**	18.60 ± 4.05	−3.596	0.000**
CG	66.93 ± 9.27	76.87 ± 11.74	−3.414	0.001**	9.93 ± 5.68		

WHOQOL-BREF, WHO Quality of Life-BREF; SD, standard deviation; EG, experimental group; CG, control group.
**$p < 0.01$.

training [30,31,33,34]. Therefore, in this study, we devised a dual task in which a cognitive task including attention and executive function was combined with daily living-based activities, and the effect of this activity on stroke patients was investigated. Dual-task activities were generated based on previous studies [43–45]. In each session, dual-task training appropriately integrated attention and executive function tasks with daily living activities.

The results revealed that both the experimental group, which performed daily life-based dual-task training, and the control group, which performed single-task training, showed improvements in upper extremity function after the intervention. A study by Kim et al. [49], investigated the effects of a dual task in which the experimental group moved the upper extremity of the paralyzed side while standing, and the results showed improvement in upper extremity function on the paralyzed side. In a study by Jang et al. [50], significant improvements were observed in functional movement of the upper extremity after performing tasks in dual-task and single-task groups, suggesting that upper extremity function can be improved with single-task training and conventional occupational therapy. Similarly, the improvement in upper extremity function in both groups of this study may have been due to the activities that heavily involved the upper extremity, such as sensory stimulation of the affected side and upper extremity movement using the affected side, in the daily life-based single and dual tasks.

When changes in cognitive function after the intervention in the experimental and the control groups were examined, significant improvements were observed for both groups in the DST, which assessed attention, the EFPT-K, which tested executive function, and the K-TMT-e B. However, an analysis of between-group differences showed that the change was significantly greater in the experimental group than in the control group. Kim and Kim [51] conducted concurrent motor tasks, such as ball throwing, clapping, and elastic bands, with cognitive tasks, including matching picture cards, finding vocabulary, and calculations in the experimental group, and showed a significant improvement in executive function compared to the control group, which performed single motor or cognitive tasks; this was consistent with the results of the present study. The results of the K-TMT-e B test used in this study showed a statistically significant reduction in the time taken for completion in the experimental group compared to the control group. The study by Mirelman et al. [52] reported that cognitive processing speed improved when motor function improved, while Han [44] reported an improvement in high-dimensional cognitive function as a result of activation of the cerebral cortex during dual-task training involving cognitive tasks. In this study, daily life activity training was consistently performed in addition to cognitive tasks that focused on attention and executive function, and the improved motor function is thought to have exerted a positive effect on cognitive processing speed. This combination of daily living

https://doi.org/10.24171/j.phrp.2021.0177

and cognitive activities could have had a greater effect on the improvement of cognitive function in the experimental group.

When changes in the quality of life in the experimental and the control groups were examined, both the experimental group, which performed dual tasks, and the control group, which performed single tasks and received conventional occupational therapy, showed significant improvements in quality of life. In a study by Meester et al. [53], the experimental group was asked to perform dual tasks in which cognitive tasks were carried out or daily life activities were explained while on the treadmill, and the control group was asked to perform a single task on the treadmill. When quality of life was measured, both groups showed an improvement in confidence in their quality of life, but the quality of life in the experimental group was significantly higher. Similarly, in this study, the magnitude of the improvement after the intervention was significantly higher in the experimental group than in the control group. This seems to suggest that dual tasks have a greater effect on quality of life in stroke patients than single tasks, which is in line with the results of previous studies.

Although we were able to demonstrate that dual-task training involving daily living activities with a focus on attention and executive function can have positive effects on upper extremity function, cognitive function, and the quality of life in stroke patients, there are several limitations of this study. First, the results are difficult to generalize as there was only a small number of subjects. Second, the dual tasks could not be presented in accordance with individual competency. Third, follow-up tests were not performed, which could have identified continuing effects of the intervention. To supplement these limitations, additional research should be conducted with more subjects and with a comparison of the effects between the groups based on the individual competency of the subjects, as the impact of dual-task training may vary depending on individual competency [54]. Despite these limitations, this study has clinical significance in that executive function training was implemented in addition to attention in the cognitive function tasks, and that the study elicited active participation and motivation from the subjects since the daily living activities consisted of familiar activities.

Conclusion

This study demonstrated that dual-task training that focused on attention and executive function combined with daily living activities had a positive effect on upper extremity function, cognitive function, and the quality of life in stroke patients. This dual-task training represents a new method to help stroke patients return to their daily routines, and it is expected to be used as a foundation for the treatment of stroke patients in the future.

Supplementary Material

Table S1. Dual task programs per session. Supplementary data are available at https://doi.org/10.24171/j.phrp.2021.0177.

Notes

Ethics Approval

This study was approved by the Institutional Review Board (IRB) from the Cheongju University (IRB No: 1041107-202006-HR-031-01). Written informed consent was obtained from the subject before the intervention.

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 authors would like to express their appreciation to all the participants and their families for their cooperation and participation in this study.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2021.0208
Osong Public Health Res Perspect 2021;12(5):314-323

Original Article

Analysis of risk factors affecting suicidal ideation in South Korea by life cycle stage

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ABSTRACT

Objectives: This study analyzed risk factors for suicidal ideation in South Koreans from a life cycle perspective.
Methods: A secondary analysis was conducted of data collected in 2015 as part of the 6th Korea National Health and Nutrition Examination Survey (KNHANES). The participants comprised 5,935 individuals aged 12 years or older. The statistical analysis reflected the complex sampling design of the KNHANES, and the Rao-Scott chi-square test and multiple logistic regression analysis were performed.
Results: The prevalence of suicidal ideation was 5.7% in adolescents, 3.7% in young adults, 5.4% in middle-aged adults, and 7.0% in older adults. Depression and stress were risk factors in every stage of the life cycle. In those aged 12 to 19 years, activity restrictions were significantly associated with suicidal ideation. Education and subjective health status were risk factors in adults aged 20 to 39 years, and education, activity restrictions, and quality of life were the major risk factors in those aged 40 to 64 years. For adults 65 years of age or older, the risk of suicidal ideation was higher among those with inappropriate sleep time.
Conclusion: The risk factors for suicidal ideation were found to be different across stages of the life cycle. This suggests a need for individualized suicide prevention plans and specific government policies that reflect the characteristics of each life cycle stage.

Keywords: Life cycle stages; Risk factors; Suicidal ideation

Received: July 29, 2021
Revised: September 15, 2021
Accepted: October 12, 2021

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This paper is a revised and supplemented version of the first author's master's thesis from Uiduk University.

Introduction

The death rate due to suicide in South Korea is 24.6 per 100,000 persons, ranking first among Organization for Economic Cooperation and Development (OECD) countries [1]. Suicide is among the top 5 causes of death in South Korea according to national statistics [2]. In 2017, it ranked first among causes of death in 10s, 20s and 30s, second in aged 40s and 50s, and fourth in aged 60 or older [2]. Therefore, recognizing suicide as a serious social problem (i.e., not just a personal problem), identifying the main factors associated with suicide, and preparing

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measures to prevent suicide are important policy issues in South Korea. Research on suicide is actively underway. Because it is impossible to enroll people who have actually committed suicide as participants in studies, researchers have analyzed suicide notes or conduct research among people who survived suicide attempts [3–5], but it is difficult to obtain representative results using these methods [6].

Suicidal ideation, which is used as the primary indicator for predicting suicide, is a decisive factor that leads to actual suicide by functioning as a prerequisite for suicide planning and suicide attempts [7,8]. A study of risk factors for suicidal thoughts, suicide plans, and suicide attempts in 17 countries reported that participants with suicidal ideation were at high risk of planning and attempting suicide within a year [9]. Therefore, if a study aims to identify risk factors for suicide with the goal of applying the results for suicide prevention, it may be more effective to study those who have considered suicide rather than focusing only on those who have attempted suicide [10].

In 2010, Japan had the second-highest suicide rate among OECD countries, but its suicide rate thereafter declined for 6 years, and it ranked fifth in 2017 [1]. It has been reported that Japan's government-wide suicide program had a significant impact on the continuing decline in the suicide rate. In particular, this anti-suicide program implemented generation-specific measures to prevent suicide beyond efforts targeting national and regional levels [11]. Japan's systematic suicide prevention strategy has substantial implications for suicide prevention measures and related policies in South Korea. In order to establish an anti-suicide policy, it is necessary to select a clear and specific target and develop and implement a program accordingly. Most previous studies on suicide-related factors in South Korea were conducted in specific age groups, and some previous studies were limited to certain stages of the life cycle, such as adolescence, adulthood, and old age [12–16]. Thus, the range of selection of subjects for research has been quite limited.

Suicide is an extreme act stemming from various motives, negative life events, or stress. It is difficult to explain suicide in terms of any single specific reason, biological, psychological, or socioeconomic cause, or cultural values [17].

Developmental characteristics, developmental tasks to be achieved, and experiences of the surrounding environment vary throughout the life cycle [18]. Therefore, individuals also face different psychological, social, economic, and cultural issues at various stages in life. For this reason, studies aiming to identify risk factors for suicidal ideation should analyze this phenomenon with more subdivided from a life cycle perspective, instead of limiting the analysis to specific regions and age groups.

This study analyzed risk factors for suicidal ideation among adolescents (12–19 years), young adults (20–39 years), middle-aged adults (40–64 years), and older adults (65 years or older) from a life cycle perspective. Based on the results of this study, we discuss the necessity of establishing policies for life cycle–specific suicide prevention measures and present directions for future initiatives.

Materials and Methods

Study Design

This study is a secondary analysis of data collected in the 6th Korea National Health and Nutrition Examination Survey (KNHANES), which was conducted in 2015. The KNHANES is a nationally representative cross-sectional and population-based survey, conducted by the Korea Centers for Disease Control and Prevention (now known as the Korea Disease Control and Prevention Agency).

Participants

The potential participants in the present study comprised 7,380 individuals aged 12 years or older who took part in the survey in 2015. After the exclusion of individuals who did not respond to the question on suicidal ideation, 5,935 participants were included in our analysis.

Research Variables

The dependent variable, suicidal ideation, was measured by responses (yes or no) to the following question: "Have you ever seriously thought about committing suicide in the past year?" The independent variables were demographic, socioeconomic, and health-related factors. Demographic factors included sex (male or female), family structure (single person or cohabitation of 2 or more persons), residential area (urban area or rural area). Socioeconomic factors included household income (low, middle, or high), education level (less than primary school graduate, middle school graduate, or high school graduate or higher). Health-related factors included the presence or absence of depressed mood, stress level (low or high), subjective health status (poor or good), and the presence or absence of activity restrictions. Quality of life was divided into percentiles based on answers to the EQ-5D Questionnaire (≤0.70, 0.71–0.90, or >0.90). Sleep time was evaluated using answers to a question about the average number of sleep hours per day; based on those responses, the participants were categorized based on the recommended time by age group [19]. Physical activity was categorized based on whether participants indicated that they had engaged in medium-intensity activity for more

than 10 minutes in the last week.

Statistical Analysis

Data were analyzed using SAS ver. 9.4 (SAS Institute, Cary, NC, USA). The statistical analysis reflected the complex sampling design, and all analyzes used survey sample weights to derive unbiased estimates for data analysis. The Rao-Scott chi-square test was performed to analyze differences in suicidal ideation according to demographic, socioeconomic, and health-related factors by life cycle stage. Multiple logistic regression analysis was used to identify the factors influencing suicidal ideation according to life cycle stage. The model's predictive accuracy was identified using C-statistics. The overall framework of the study is shown in Figure 1.

Results

Participant Characteristics by Life Cycle

The number of participants was 5,935 among those aged 12 and older who took part in the 6th KNHANES, which was conducted in 2015, after the exclusion of those who did not answer the suicidal ideation question. By life cycle stage, 10.3% of participants were adolescents (12–19 years), 22.7% were young adults (20–39 years), 43.7% were middle-aged (40–64 years), and 23.3% were older adults (over 65 years).

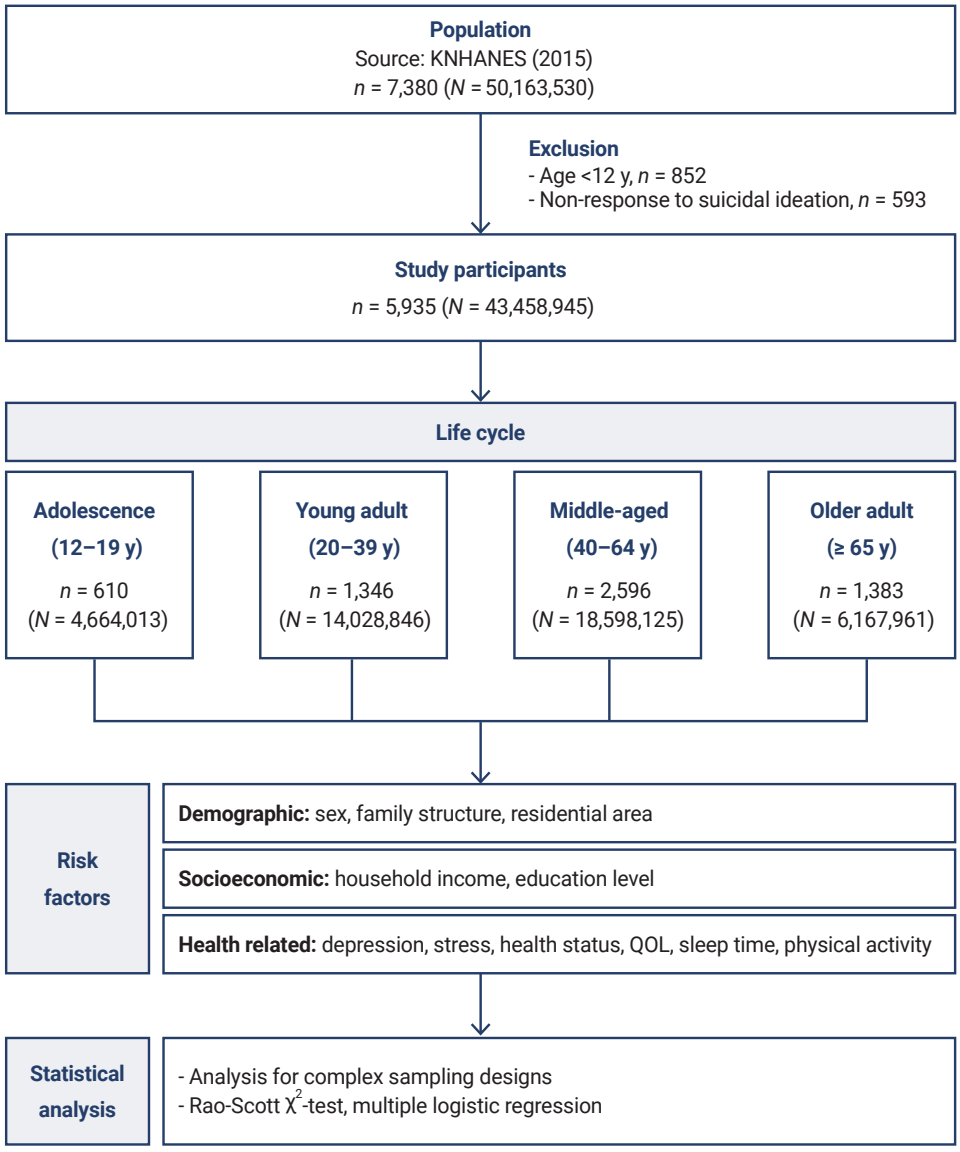

Figure 1. Framework of the study.
KNHANES, Korea National Health and Nutrition Examination Survey; *N*, weighted *n*; QOL, quality of life.

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In all age groups except for those aged 65 and older, the number of male participants exceeded the number of female participants. There were more households where 2 or more people lived together than single households, and most participants lived in cities. These demographic characteristics differed significantly by life cycle ($p < 0.001$). The most common household income level was middle, followed in descending order by low and high, with the exception of those aged 65 and older. The most common education level in the young and middle-aged group was high school graduation or higher, whereas elementary school graduation or lower was the most common in older adults ($p < 0.001$). Those aged 65 or older were most likely to experience depression, have poor subjective health status, have restrictions on activity, and not engage in exercise. Stress was highest in the 20 to 39 age group, and in the 12 to 19 age group, sleep was the most inadequate and participants were most likely to perceive themselves as having a low quality of life ($p < 0.001$) (Table 1).

Prevalence of Suicidal Ideation by Life Cycle Stage

The prevalence of suicidal ideation was the highest among older adults. The rate of suicidal ideation was 5.7% in participants who were 12 to 19 years old, 3.7% in those aged 20 to 39 years, 5.4% in those aged 40 to 64 years, and 7.0% in those who were 65 years and older ($p = 0.012$) (Figure 2).

Table 2 presents a comparison of the prevalence of suicidal ideation by participants' characteristics. In the 12

Table 1. General characteristics of the study population ($n = 5,935$)

Characteristic	Category	12–19 y ($n = 610$)	20–39 y ($n = 1,346$)	40–64 y ($n = 2,596$)	≥65 y ($n = 1,383$)	χ^2 (p)
Demographic factor						
Sex	Male	329 (51.7)	594 (52.0)	1,109 (49.7)	608 (42.4)	22.23 (< 0.001)
	Female	281 (48.3)	752 (48.0)	1,487 (50.3)	775 (57.6)	
Family structure	Single person	1 (0.4)	68 (6.1)	196 (6.3)	303 (19.2)	102.67 (< 0.001)
	≥ Two persons	609 (99.6)	1,278 (93.9)	2,400 (93.7)	1,080 (80.8)	
Residential area	Urban area	533 (86.7)	1,173 (86.9)	2,114 (83.1)	1,010(73.7)	27.19 (< 0.001)
	Rural area	77 (13.3)	173 (13.1)	482 (16.9)	373 (26.3)	
Socioeconomic factor						
Household income	Low	72 (14.4)	97 (7.6)	313 (11.5)	608 (45.1)	486.78 (< 0.001)
	Middle	360 (58.7)	784 (58.2)	1,331 (52.2)	630 (45.6)	
	High	173 (26.9)	460 (34.2)	936 (36.2)	135 (9.3)	
Education level	≤ Primary school	294 (42.1)	6 (0.5)	379 (13.2)	766 (62.5)	1,593.26 (< 0.001)
	Middle school	210 (37.7)	14 (1.2)	356 (13.6)	173 (12.2)	
	≥ High school	100 (20.2)	1,241 (98.4)	1,664 (73.2)	333 (25.3)	
Health-related factor						
Depressed mood	Yes	46 (7.2)	177 (12.6)	343 (12.8)	231 (16.2)	23.04 (< 0.001)
	No	564 (92.8)	1,169 (87.4)	2,253 (87.2)	1,152 (83.8)	
Stress level	High	160 (27.9)	506 (38.4)	685 (26.8)	258 (18.5)	106.26 (< 0.001)
	Low	450 (72.1)	840 (61.6)	1,911 (73.2)	1,124 (81.5)	
Subjective health status	Poor	23 (4.7)	176 (14.1)	446 (17.8)	390 (30.1)	120.85 (< 0.001)
	Good	581 (95.3)	1,088 (85.9)	1,971 (82.2)	900 (69.9)	
Activity restriction	Yes	14 (3.1)	45 (3.3)	180 (7.5)	215 (19.1)	134.42 (< 0.001)
	No	590 (96.9)	1,218 (96.7)	2,226 (92.5)	1,065 (80.9)	
Quality of life	≤ 0.70	535 (87.7)	92 (7.4)	237 (9.4)	224 (17.0)	1,699.96 (< 0.001)
	0.71–0.90	7 (1.1)	82 (6.0)	306 (10.4)	431 (31.7)	
	> 0.90	68 (11.1)	1,172 (86.6)	2,053 (80.2)	728 (51.3)	
Sleep time	Inappropriate	154 (26.9)	135 (10.6)	412 (15.9)	197 (13.9)	80.77 (< 0.001)
	Appropriate	456 (73.1)	1,211 (89.4)	2,182 (84.1)	1,181 (86.1)	
Medium-intensity physical activity	No	396 (68.2)	863 (67.3)	1,731 (70.7)	1,107 (87.9)	94.40 (< 0.001)
	Yes	205 (31.8)	397 (32.7)	668 (29.3)	169 (12.1)	

Data are presented as *n* (%). The discrepancies between *n* and total *n* are due to missing values.
n, unweighted sample size; %, weighted percent

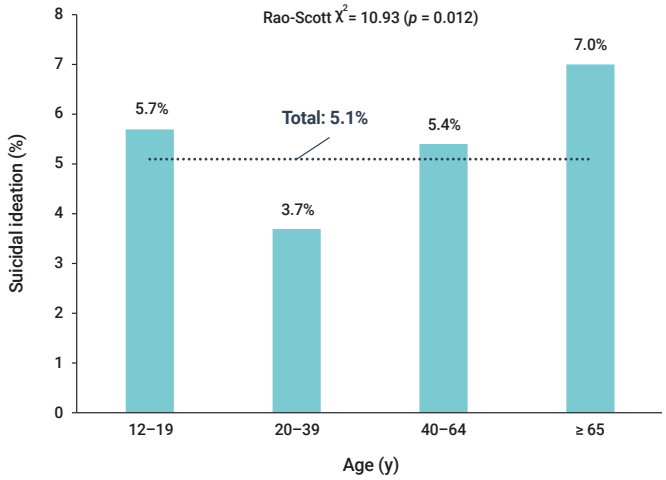

Figure 2. Suicidal ideation by life cycle stage.

to 19 age group, the prevalence of suicidal ideation was higher in female participants (χ^2=4.68, p=0.031), people with depression (χ^2=49.64, p<0.001), high levels of stress (χ^2=35.67, p<0.001), poor perceived health status (χ^2=17.56, p<0.001), and activity restrictions (χ^2=26.89, p<0.001). The percentage of participants with suicidal ideation was also significantly higher among people with a quality-of-life score of 0.71 to 0.90 (χ^2=73.83, p<0.001). In the 20 to 39 age group, the prevalence of suicidal ideation differed significantly depending on participants' level of education (χ^2=22.32, p<0.001). Suicidal ideation was also more prevalent among those with depression (χ^2=178.88, p<0.001), high levels of stress (χ^2=45.58, p<0.001), and poor perceived health status (χ^2=56.68, p<0.001). The percentage of participants with suicidal ideation was also significantly higher among people with a quality-of-life score of 0.71 to 0.90 (χ^2=20.92, p<0.001) and inappropriate sleep time (χ^2=4.89, p=0.027). In the middle-aged group (40–64 years), suicidal ideation was more prevalent among those living alone (χ^2=9.28, p=0.002) and participants with a low household income (χ^2=50.82, p<0.001) and low education level (χ^2=49.07, p<0.001). The prevalence of suicidal ideation was significantly higher in participants with depression (χ^2=310.84, p<0.001), high levels of stress (χ^2=85.15, p<0.001), poor perceived health status (χ^2=43.18, p<0.001), and activity restrictions (χ^2=60.01, p<0.001). Significantly higher rates of suicidal ideation were found among people with a quality-of-life score of 0.71 to 0.90 (χ^2=42.60, p<0.001), inappropriate sleep time (χ^2=18.89, p<0.001), and no medium-intensity physical activity (χ^2=4.30, p=0.038). In older adults (over 65 years), the prevalence of suicidal ideation was significantly higher in women (χ^2=4.28, p=0.039), participants who lived alone (χ^2=11.65, p=0.001), and those who lived in rural areas (χ^2=6.23, p=0.013). Suicidal ideation was more prevalent among those with depression (χ^2=130.43, p<0.001), high levels of stress (χ^2=86.97, p<0.001), poor perceived health status (χ^2=25.82, p<0.001), and activity restrictions (χ^2=12.63, p<0.001). The rate of suicidal ideation was significantly higher among people with a quality-of-life score lower than 0.7 (χ^2=13.74, p=0.001), inappropriate sleep time (χ^2=14.41, p<0.001), and no medium-intensity physical activity (χ^2=5.82, p=0.016).

Factors Contributing to Suicidal Ideation by Life Cycle Stage

Multiple logistic regression analysis showed that the factors contributing to suicidal ideation were different for each life cycle stage (Table 3). The first model was developed to identify factors affecting suicidal ideation in adolescents (12–19 years). The model's predictive accuracy was 88.2%, and suicidal ideation was 10.98 times more common among those who had depression than among those who did not (95% confidence interval [CI], 2.98–40.40). The risk of suicidal ideation was 5.33 times higher among participants with high levels of stress than among those with low levels of stress (95% CI, 1.51–18.85) and 5.91 times greater among those with activity limitations than among those who did not (95% CI, 1.60–21.84). In other words, the factors affecting suicidal ideation at the age of 12 to 19 were depression, stress, and activity restrictions.

The second model was developed to identify factors contributing to suicidal ideation in young adults (20–39 years), and the model's predictive accuracy was 90.4%. Suicidal ideation risk was 6.56 times higher in those with a middle school education than in the high school graduation or higher group (95% CI, 1.33–32.32). Suicidal ideation was more common among those who reported depression (odds ratio [OR], 17.99; 95% CI, 6.51–49.68), high levels of stress (OR, 4.41; 95% CI, 1.65–11.79), and poor health status (OR, 2.75; 95% CI, 1.24–6.09).

The predictive accuracy of the third model, which was constructed to predict the risk of suicidal ideation in middle-aged adults, was 87.5%. In this group (40–64 years), suicidal ideation risk was 2.76 times higher in those with an elementary school or lower education than in the high school graduation or higher group (95% CI, 1.46–5.20). The risk for suicidal ideation was 10.98 times higher among those who had depression than those who did not (95% CI, 6.30–19.16) and was 2.11 times greater among those with high levels of stress than among those with low levels of stress (95% CI, 1.14–3.87). Activity restrictions (OR, 1.86; 95% CI, 1.00–3.45) and low perceived quality of life (OR, 2.77; 95% CI, 1.01–7.59) also significantly affected the likelihood of

https://doi.org/10.24171/j.phrp.2021.0208

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Table 2. Prevalence of suicidal ideation based on general characteristics by life cycle stage (n = 5,935)

Characteristic	Category	12–19 y			20–39 y			40–64 y			≥65 y		
		Yes	No	χ^2(p)	Yes	No	χ^2(p)	Yes	No	χ^2(p)	Yes	No	χ^2(p)
Demographic factor													
Sex	Male	9 (3.4)	320 (96.6)	4.68	14 (2.5)	580 (97.5)	3.80	49 (4.5)	1,060 (95.5)	3.70	37 (5.2)	571 (94.8)	4.28
	Female	19 (8.1)	262 (91.9)	(0.031)	44 (4.9)	708 (95.1)	(0.051)	92 (6.3)	1,395 (93.7)	(0.055)	60 (8.2)	715 (91.8)	(0.039)
Family structure	Single person	0	1 (100.0)	-	2 (4.2)	66 (95.8)	0.05	25 (10.7)	171 (89.3)	9.28	33 (11.7)	270 (88.3)	11.65
	≥ Two persons	28 (5.7)	581 (94.3)		56 (3.6)	1,222 (96.4)	(0.821)	116 (5.1)	2,284 (94.9)	(0.002)	64 (5.8)	1,016 (94.2)	(0.001)
Residential area	Urban area	27 (6.3)	506 (93.7)	1.86	46 (3.4)	1,127 (96.6)	1.27	113 (5.5)	2,001 (94.5)	0.25	62 (5.8)	948 (94.2)	6.23
	Rural areas	1 (1.8)	76 (98.2)	(0.173)	12 (5.2)	161 (94.8)	(0.26)	28 (5.0)	454 (95.0)	(0.621)	35 (10.3)	338 (89.7)	(0.013)
Socioeconomic factor													
Household income	Low	8 (11.9)	64 (88.1)	4.59	7 (5.2)	90 (94.8)	1.70	46 (16.7)	267 (83.3)	50.82	55 (8.9)	553 (91.1)	5.40
	Middle	12 (4.2)	348 (95.8)	(0.101)	36 (4.0)	748 (96.0)	(0.427)	70 (5.0)	1,261 (95.0)	(< 0.001)	34 (4.9)	596 (95.1)	(0.067)
	High	8 (5.6)	165 (94.4)		15 (2.7)	445 (97.3)		25 (2.7)	911 (97.3)		6 (5.8)	129 (94.2)	
Education level	≤ Primary school	14 (6.0)	280 (94.0)	1.05	1 (12.0)	5 (88.0)	22.32	43 (14.2)	336 (85.8)	49.07	58 (7.8)	708 (92.2)	4.12
	Middle school	8 (4.5)	202 (95.5)	(0.593)	4 (28.6)	10 (71.4)	(< 0.001)	30 (10.3)	326 (89.7)	(< 0.001)	9 (3.9)	164 (96.1)	(0.127)
	≥ High school	6 (7.6)	94 (92.4)		49 (3.4)	1,192 (96.6)		60 (3.2)	1,604 (96.8)		19 (5.7)	314 (94.3)	
Health-related factor													
Depressed mood	Yes	13 (34.2)	33 (65.8)	49.64	46 (23.3)	131 (76.7)	178.88	103 (29.8)	240 (70.2)	310.84	61 (26.1)	170 (73.9)	130.43
	No	15 (3.4)	549 (96.6)	(< 0.001)	12 (0.8)	1,157 (99.2)	(< 0.001)	38 (1.9)	2,215 (98.1)	(< 0.001)	36 (3.3)	1,116 (96.7)	(< 0.001)
Stress level	High	22 (16.3)	138 (83.7)	35.67	51 (8.3)	455 (91.7)	45.58	91 (13.4)	594 (86.6)	85.15	56 (21.5)	202 (78.5)	86.97
	Low	6 (1.5)	444 (98.5)	(< 0.001)	7 (0.7)	833 (99.3)	(< 0.001)	50 (2.5)	1,861 (97.5)	(< 0.001)	41 (3.7)	1,083 (96.3)	(< 0.001)
Subjective health status	Poor	6 (30.3)	17 (69.7)	17.56	24 (13.0)	152 (87.0)	56.68	61 (13.8)	385 (86.2)	43.18	48 (12.5)	342 (87.5)	25.82
	Good	22 (4.5)	559 (95.5)	(< 0.001)	30 (2.2)	1,058 (97.8)	(< 0.001)	73 (3.8)	1,898 (96.2)	(< 0.001)	39 (4.3)	861 (95.7)	(< 0.001)
Activity restriction	Yes	4 (38.2)	10 (61.8)	26.89	5 (8.1)	40 (91.9)	1.97	36 (22.1)	144 (77.9)	60.01	33 (13.3)	182 (86.7)	12.63
	No	24 (4.7)	566 (95.3)	(< 0.001)	49 (3.6)	1,169 (96.4)	(0.161)	97 (4.3)	2,129 (95.7)	(< 0.001)	53 (5.2)	1,012 (94.8)	(< 0.001)
Quality of life	≤ 0.70	23 (5.1)	512 (94.9)	73.83	6 (3.6)	86 (96.4)	20.92	26 (10.7)	211 (89.3)	42.60	32 (13.3)	192 (86.7)	13.74
	0.71–0.90	4 (68.7)	3 (31.3)	(< 0.001)	11 (12.6)	71 (87.4)	(< 0.001)	38 (13.3)	268 (86.7)	(< 0.001)	32 (7.2)	399 (92.8)	(0.001)
	> 0.90	1 (1.0)	67 (99.0)		41 (3.1)	1,131 (96.9)		77 (3.8)	1,976 (96.2)		33 (4.7)	695 (95.3)	
Sleep time	Inappropriate	7 (5.6)	147 (94.4)	0.00	12 (7.1)	123 (92.9)	4.89	45 (10.2)	367 (89.8)	18.89	28 (13.8)	169 (86.2)	14.41
	Appropriate	21 (5.7)	435 (94.3)	(0.993)	46 (3.3)	1,165 (96.7)	(0.027)	95 (4.5)	2,087 (95.5)	(< 0.001)	69 (5.9)	1,112 (94.1)	(< 0.001)
Medium-intensity physical activity	No	22 (6.9)	374 (93.1)	1.96	44 (4.0)	819 (96.0)	0.46	107 (6.5)	1,624 (93.5)	4.30	80 (7.3)	1,027 (92.7)	5.82
	Yes	6 (3.4)	199 (96.6)	(0.162)	10 (3.1)	387 (96.9)	(0.497)	26 (3.5)	642 (96.5)	(0.038)	6 (2.7)	163 (97.3)	(0.016)

Data are presented as n (%). The discrepancies between n and total n are due to missing values.
n, unweighted sample size; %, weighted percent; χ^2, Rao-Scott χ^2-test.

Table 3. Factors contributing to suicidal ideation by life cycle (n = 5,935)

Characteristic	Category	12–19 y	20–39 y	40–64 y	≥ 65 y
Demographic factor					
Sex	Male	0.39 (0.13–1.15)	0.99 (0.43–2.30)	0.83 (0.49–1.42)	1.27 (0.69–2.34)
	Female	1.00	1.00	1.00	1.00
Family structure	Single person			0.74 (0.35–1.56)	1.54 (0.83–2.84)
	≥ Two persons			1.00	1.00
Residential area	Rural area				1.56 (0.83–2.94)
	Urban area				1.00
Socioeconomic factor					
Household income	Low			1.86 (0.81–4.25)	
	Middle			1.26 (0.66–2.40)	
	High			1.00	
Education level	≤ Primary school		2.51 (0.45–14.00)	2.76 (1.46–5.20)*	
	Middle school		6.56 (1.33–32.32)*	1.85 (0.95–3.63)	
	≥ High school		1.00	1.00	
Health-related factor					
Depressive mood	Yes	10.98 (2.98–40.40)*	17.99 (6.51–49.68)*	10.98 (6.30–19.16)*	5.98 (3.39–10.54)*
	No	1.00	1.00	1.00	1.00
Stress level	High	5.33 (1.51–18.85)*	4.41 (1.65–11.79)*	2.11 (1.14–3.87)*	3.25 (1.69–6.24)*
	Low	1.00	1.00	1.00	1.00
Subjective health status	Poor	2.87 (0.71–11.62)	2.75 (1.24–6.09)*	1.34 (0.81–2.22)	1.45 (0.78–2.67)
	Good	1.00	1.00	1.00	1.00
Activity restriction	Yes	5.91 (1.60–21.84)*		1.86 (1.00–3.45)*	1.35 (0.67–2.70)
	No	1.00		1.00	1.00
Quality of life	≤ 0.70		1.57 (0.42–5.92)	2.77 (1.01–7.59)*	0.96 (0.32–2.85)
	0.71–0.90		1.08 (0.42–2.77)	0.98 (0.55–1.75)	0.80 (0.40–1.60)
	> 0.90		1.00	1.00	1.00
Sleep time	Inappropriate		1.32 (0.53–3.29)	1.27 (0.68–2.39)	2.01 (1.08–3.73)*
	Appropriate		1.00	1.00	1.00
Medium-intensity physical activity	No			0.94 (0.44–2.01)	0.48 (0.20–1.19)
	Yes			1.00	1.00
C-statistics		0.882	0.904	0.875	0.829

Data are presented as odds ratio (95% confidence interval).
*p < 0.05.

suicidal ideation.

The final model was developed to show factors affecting suicidal ideation in older adults (65 years and older). Suicidal ideation among older people was found to be affected by depression (OR, 5.98; 95% CI, 3.39–10.54), stress (OR, 3.25; 95% CI, 1.69–6.24), and sleep time (OR, 2.01; 95% CI, 1.08–3.73), with 82.9% predictive accuracy.

Discussion

This study aimed to contribute to the development of suicide prevention and suicide management programs and the generation of basic data for policy establishment by identifying factors that affect suicidal ideation from a life cycle perspective. Statistically significant differences in suicidal ideation were found across life cycle stages. Suicidal ideation was most common (7.0%) in older adults, followed by adolescents (5.7%), middle-aged adults (5.4%), and young adults (3.7%). These results are similar to the findings of previous studies that above the age of 20 years, suicidal ideation increased with age. A previous study using Korean Health Panel data reported that the prevalence of suicidal ideation was 4.4% in the 19 to 39 years group and 5.6% in the 40 to 64 years group [20]. Lee's study [8] using Korean Psycho-Social Anxiety Survey data reported that the prevalence of suicidal ideation was 4.5% in the 19 to 39 years group, 6.7% in the 40 to 64 years group and 10.5% in the 65 years and older group. This shows that policies to prevent suicide in older adults should be prioritized. The results of this study are different from those of a previous study that

https://doi.org/10.24171/j.phrp.2021.0208

reported suicidal ideation in 12.8% of adolescents [21]. This discrepancy is thought to have been caused by differences in the method and time of sampling.

The analysis in the present study of factors affecting suicidal ideation by life cycle stage showed that suicidal ideation was higher among those who were depressed or stressed in all age groups. Suicide reflects an individual's internal psychological state, and depression and stress are representative variables that indicate this psychological state. The effects of depression and stress on suicidal ideation have already been demonstrated in several previous studies [8,12,13,21]. Depression refers to the results of negative perceptions of oneself and emotional problems such as anxiety, depression, failure, loss, lethargy, and worthlessness [22], which are very closely related to suicide, as studies have shown that about 50% to 87% of people who commit suicide have depression at the time of suicide. In addition, stress refers to the psychological and physical tension a person feels when he or she is in an environment to which adaptation is difficult. If stress accumulates, people experience anxiety and tension, and long-term stress can lead to serious thoughts of suicide [23].

The impact of activity restrictions on suicidal ideation was significant in adolescents. These results are consistent with the findings of a prior study reporting that activity-restricted adolescents were 3.86 times more likely to consider suicide than their counterparts [21]. In a study of protective factors against youth suicide, the result that physical and mental health were the personal health characteristics with the most important impact on suicide supports the finding of this study that activity-restricted adolescents had a high risk of suicidal ideation [24]. This finding suggests the need for suicide prevention programs aiming to improve the physical health of adolescents and policies that can support adolescents with activity restrictions.

In the 20 to 39 age group, the factors influencing suicidal ideation were education level and subjective health status, and in the 40 to 64 age group, significant relationships were found for education level, activity restrictions, and quality of life. These results are consistent with the findings of a previous study, which found that educational level was a risk factor for suicidal ideation in young and middle-aged adults [8]. In general, education helps people achieve their life goals [25], and it can be inferred that low education levels among young and middle-aged adults may have affected suicidal ideation because of the potential link between low education levels and low social status and a vulnerable working environment. The previous finding that 84.2% of those who attempted suicide were high school graduates or below suggests the need to pay close attention to the possibility of suicidal ideation in people with low education levels [26]. In previous studies, subjective health status has been reported as a risk factor for suicidal ideation [27,28]. Subjective health status refers to an individual's judgment of one's health, which may differ from the individual's actual health status. Not only does this individual judgment affect physical and psychological health, daily life, and quality of life [29,30], but dissatisfaction with health conditions can also cause problems in social interaction, potentially contributing to suicidal ideation. Activity restrictions in middle-aged women were found to have a significant impact on health-related quality of life [31], and low quality of life functioned as a mediating factor in suicidal behavior in people with depression [32]. Therefore, health policies for middle-aged adults should aim not only to prevent and manage diseases, but also to enhance physical functionality, thereby preventing activity restrictions and promoting the maintenance of high quality of life.

Sleep time significantly affected suicidal ideation in those aged 65 years or older. In a study of adults in Taiwan, a U-shaped curve was proposed for the relationship between sleep time and suicide, with both short sleep time and sleep times of more than 8 hours associated with an increased risk of suicide, supporting this study [33]. This finding also partially aligns with previous studies that shorter sleep times in older adults can significantly increase the likelihood of suicidal ideation [34]. It remains unclear whether sleep problems are a symptom of depression or whether sleep problems themselves can lead to suicide, but several studies have established that sleep has a significant association with suicide [35]. Sleep patterns can be changed; therefore, identifying and implementing ways to improve sleep problems could make an important contribution to reducing the risk of suicide. Insomnia is triggered by various causes such as biological factors (e.g., a genetic predisposition), environmental factors (e.g., economic difficulties), and psychological causes (e.g., death of children and family members). When a clinician encounters an older adult who complains of insomnia, the focus should not be limited to insomnia symptoms; instead, the clinician should try to listen to and empathize with the psychological phenomena that underlie insomnia symptoms [36].

Suicidal ideation is caused by a combination of factors. Thus, it is necessary to identify risk factors specific to each generation and adopt a multifaceted approach to prevent suicide. The conclusions based on the results of this study are as follows. First, establishing a suicide prevention plan requires the development and implementation of individualized programs for each stage of the life cycle. The

results of Japan's generational suicide prevention measures support the need to implement such a policy [11]. Second, it is necessary for the state to prepare depression and stress management measures to prevent suicide and to actively put them into practice. Third, sleep time should be monitored and managed in older adults, and efforts should be made to identify the causes and effects of both excessive and insufficient sleep. In this study, KNHANES data were used to comprehensively identify the prevalence of suicidal ideation and related factors by life cycle. However, the data used in this study should not be compared on a yearly basis, as the wording of questions about suicide has varied across waves of the KNHANES. As a result, a limitation of this study is that it only used data from the 2015 KNHANES. Another limitation of this study is that it only analyzed suicidal ideation as a predictor of suicide because few participants reported suicide plans and suicide attempts. Therefore, follow-up research should identify variations in suicidal ideation over time and analyze suicide plans, suicide attempts, and factors that lead to suicide. Furthermore, to support the development of more specific and efficient policies, in-depth research should be conducted from a life cycle perspective that considers regional characteristics.

Notes

Ethics Approval
Not applicable.

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

Funding
None.

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

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2021.0207
Osong Public Health Res Perspect 2021;12(5):324-332

Original Article

Yes-associated protein 1 as a prognostic biomarker and its correlation with telomerase in various cancers

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ABSTRACT

Objectives: The aims of this study were to investigate the expression of Yes-associated protein 1 (*YAP1*), its prognostic significance, and the correlation between *YAP1* and telomerase in various cancers.

Methods: The Gene Expression Profiling Interactive Analysis database was used to analyze RNA sequencing data and the survival rate of patients with various cancers in The Cancer Genome Atlas (TCGA) database. PrognoScan was used to analyze the prognostic value of *YAP1* expression in various cancers. Tumor Immune Estimation Resource was used to determine the correlation between *YAP1* expression and telomerase in various cancer types based on TCGA data.

Results: The analysis suggested that *YAP1* was differentially expressed between tissues of various cancers and non-tumor tissues. High *YAP1* expression was also related to a poor prognosis in adrenocortical carcinoma, bladder urothelial carcinoma, and pancreatic adenocarcinoma. Moreover, *YAP1* expression was correlated with the expression of telomerase reverse transcriptase and telomerase RNA component in various cancer types.

Conclusion: These results suggest that *YAP1* is a potential biomarker with prognostic significance and relevance for oncogene research in various cancer types. The correlation between the expression of *YAP1* and telomere-associated genes will help to understand their cancer-promoting mechanisms and interactions.

Keywords: Correlation study; Neoplasms; Prognosis; Telomerase; Telomerase RNA; Yes-associated protein 1

Received: July 26, 2021
Revised: August 31, 2021
Accepted: September 2, 2021

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Introduction

Yes-associated protein 1 (*YAP1*) expression and nuclear localization have been found to be increased in various cancers [1–4]. *YAP1* is one of the most important effectors of the Hippo signaling pathway and is involved in crosstalk with other cancer-promoting pathways. The

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Hippo pathway plays a crucial role in organ size control and tissue regeneration [5]. The roles of Hippo pathway dysregulation in tumorigenesis and cancer progression have been widely reported [6]. As a potent oncogene activated in many cancers, *YAP1* has a negatively regulated downstream target in the Hippo signaling pathway and functions as a transcriptional coactivator involved in the regulation of cell growth, proliferation, and apoptosis [2, 7–9]. *YAP1* plays a key role as a tumor suppressor in the Hippo signaling pathway and enhances gene transcription by binding to transcription factors [10]. Specifically, *YAP1* contributes to cancer development by promoting malignant phenotypes, the expansion of cancer stem cells, and drug resistance of cancer cells. *YAP1* is considered a potent oncogene closely linked to the progression of several cancer types [11,12], and *YAP1* overexpression in cancer cell lines can also promote tumor growth [13,14]. Therefore, *YAP1* promotes tumorigenesis, but the underlying mechanisms by which *YAP1* exerts this effect require further exploration.

Telomeres are cellular nucleoprotein complexes, and their main function is to maintain chromosomal integrity and genomic stability [15]. A telomere is a ribonucleoprotein complex composed of 2 main core subunits: telomerase reverse transcriptase (*TERT*), which constitutes the catalytic subunit, and a functional telomerase RNA component (*TR* or *TERC*) that provides a template for telomerase elongation [16]. A positive correlation between *TERT* mRNA levels and telomerase activity has been reported, suggesting that telomerase is primarily regulated by *TERT* expression [17]. Telomerase is active in adult germ-line tissues, immortal cells [18], and most malignant tumors [19]. *TERT* induces stemness of cancer cells to promote metastasis and recurrence [20]. In cancer cells, the upregulation of *TERT* transcriptional activity has been reported [21]. *TERT* overexpression has been detected in more than 80% to 90% of human cancers [15]. Thus, *TERT* overexpression may represent the mechanism by which cancer cells prevent telomere shortening and become immortal [22]. Zhang et al. [23] recently reported that *YAP1* regulates *TERT* expression, and that hyperactivation of *YAP1* promotes telomerase activity and increases telomeric length, causing an increase in *TERT* expression. They also showed that *TERT* expression was positively correlated with *YAP1* activation in liver cancer tissues. Several studies have reported that *TERT* overexpression contributes to cancer progression [24]. Therefore, *YAP1* promotes *TERT* expression, which may contribute to tumor progression [25]. However, several studies have highlighted the importance of *TERC* in cancer because of findings indicating that *TERC* expression may be highly upregulated in a variety of cancers [26–30]. Although *TERC* is associated with the development of several diseases, its underlying mechanisms in cancer are poorly understood. In addition, the correlation between the expression of *YAP1* and telomerase-associated genes in cancer has not been completely explored.

In the present study, we analyzed *YAP1* expression in normal and different types of tumor tissues based on The Cancer Genome Atlas (TCGA) data using online databases and tools. We also evaluated the prognostic value of *YAP1* expression its correlation with the expression of 2 major telomerase components (*TERT* and *TERC*) in various cancer types on the basis of TCGA data.

Materials and Methods

Gene Expression Profiling Interactive Analysis Database Analysis

The Gene Expression Profiling Interactive Analysis (GEPIA) database (https://gepia.cancer-pku.cn/index.html), which is a web server tool consisting of 8,587 normal and 9,736 tumor tissue samples from the TCGA and GTEx projects [31–33], was used to analyze differences in *YAP1* expression between normal and tumor tissue based on RNA sequencing. We represented expression of the *YAP1* profile across various cancers and paired normal tissues. We also analyzed the survival curves, including overall survival (OS), which refers to the duration of patient survival from the date of disease treatment, and disease-free survival (DFS), which denotes relapse-free survival, according to *YAP1* gene expression by using the log-rank and Mantel-Cox tests for different cancer types via the GEPIA database.

PrognoScan Database Analysis

The PrognoScan database (http://www.abren.net/PrognoScan/), a platform for evaluating potential tumor markers, is widely used to evaluate biological relationships between gene expression and patient prognosis such as OS and DFS [34]. It includes a large-scale collection of publicly available cancer microarray datasets with clinical information. We used this PrognoScan database to analyze the prognostic value of *YAP1* in various cancers based on the hazard ratio (HR) and log-rank *p*-values.

Tumor Immune Estimation Resource Database Analysis

The Tumor Immune Estimation Resource (TIMER) database (https://cistrome.shinyapps.io/timer/) for systematic analysis was used to explore gene correlations in various cancers. The TIMER database consists of 10,897 samples across 32 cancer types from TCGA to estimate the relationship

of cancer signaling pathway genes. Spearman correlation analysis of these samples was performed to determine the relationship between *YAP1* expression and telomerase (*TERT* and *TERC*) [35].

Statistical Analysis

Gene expression data from the GEPIA were explored with online tools. Survival curves were generated with GEPIA and PrognoScan online tools. The correlations of gene expression were evaluated in the TIMER database using Spearman correlation analysis. All results are presented with *p*-values from the log-rank test. Statistical significance of the data (*p*-values) was provided by the program.

Results

mRNA Expression Levels of *YAP1* in Various Types of Cancer

To determine differences in *YAP1* expression between tumor and normal tissue, *YAP1* expression in normal samples and multiple cancer types was analyzed using the GEPIA database. The mRNA expression levels of *YAP1* were higher in cholangiocarcinoma (CHOL), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), glioblastoma multiforme (GBM), pancreatic adenocarcinoma (PAAD), stomach adenocarcinoma, and thymoma (THYM) than in non-tumor tissues (Figure 1A). However, the mRNA expression levels of *YAP1* were lower in adrenocortical

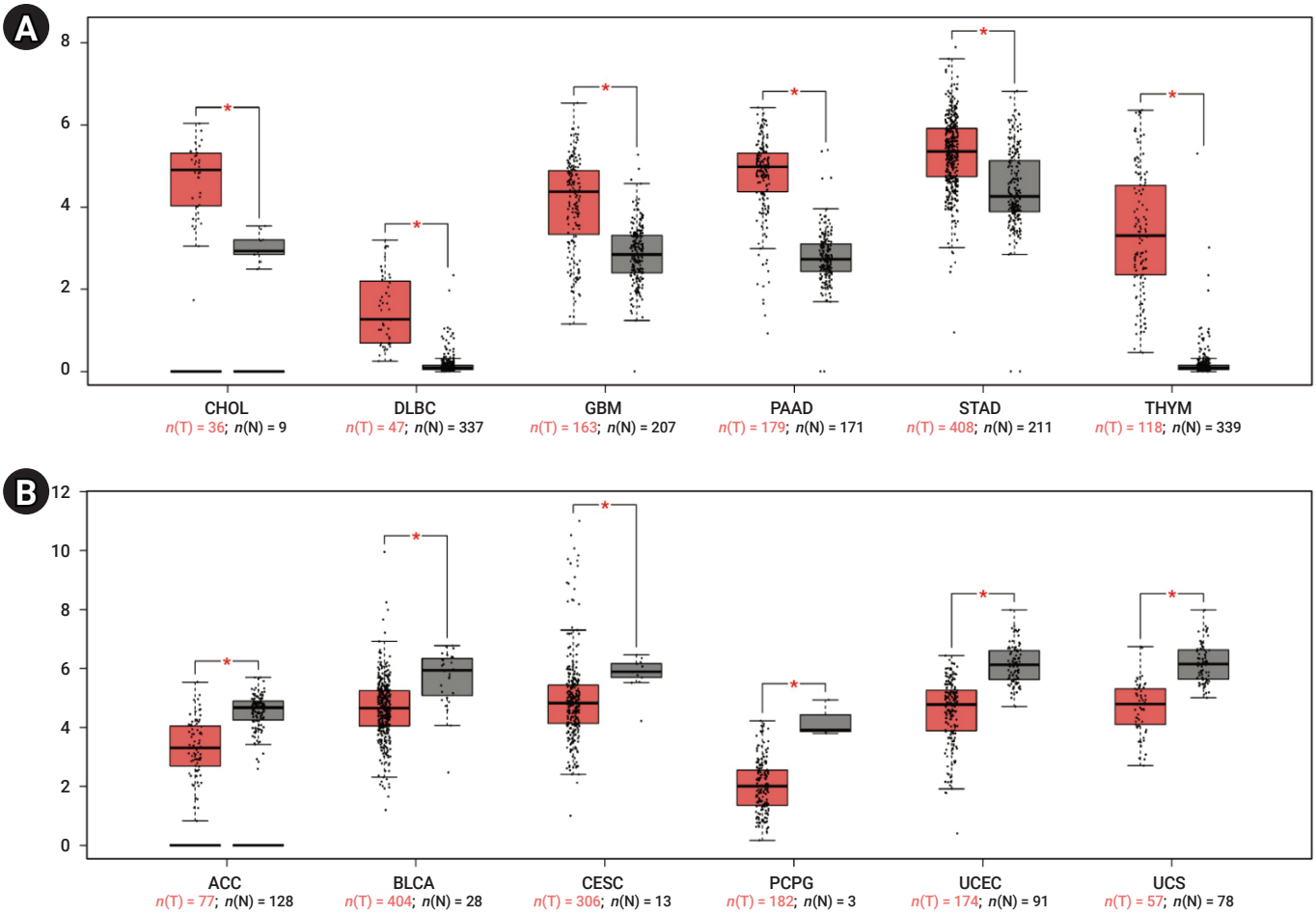

Figure 1. The mRNA expression levels of Yes-associated protein 1 (*YAP1*) in various cancer types. The expression levels of *YAP1* were analyzed using the Gene Expression Profiling Interactive Analysis database.
(A) High expression of *YAP1* in various cancer tissues compared with normal tissues. (B) Low expression of *YAP1* in various cancer tissues compared with normal tissues. **p*<0.05. T, tumor; N, normal; CHOL, cholangiocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; GBM, glioblastoma multiforme; PAAD, pancreatic adenocarcinoma; STAD, stomach adenocarcinoma; THYM, thymoma; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; CESC, cervical squamous cell carcinoma; PCPG, pheochromocytoma and paraganglioma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma.

https://doi.org/10.24171/j.phrp.2021.0207

carcinoma (ACC), bladder urothelial carcinoma (BLCA), cervical squamous cell carcinoma, pheochromocytoma and paraganglioma, uterine corpus endometrial carcinoma, and uterine carcinosarcoma than in non-tumor tissues (Figure 1B). These results suggested that *YAP1* was differentially expressed between tissue samples of various cancers and non-tumor tissues.

Prognostic Significance of *YAP1* Expression in Various Types of Cancer

We investigated whether *YAP1* expression was correlated with the prognosis in various cancer types. Therefore, the effect of *YAP1* expression on survival rates was evaluated using the GEPIA and PrognoScan databases. The OS rates of patients with different types of cancers that overexpressed or underexpressed *YAP1* were compared. The results revealed shorter OS with a worse prognosis in patients with high *YAP1* expression than in those with ACC (HR, 0.009; $p = 0.006$) and PAAD (HR, 0.006; $p = 0.005$) who had low *YAP1* expression (Figure 2A, B). Moreover, the DFS rates between patients with low and high *YAP1* expression were compared. High *YAP1* expression was associated with poorer DFS in

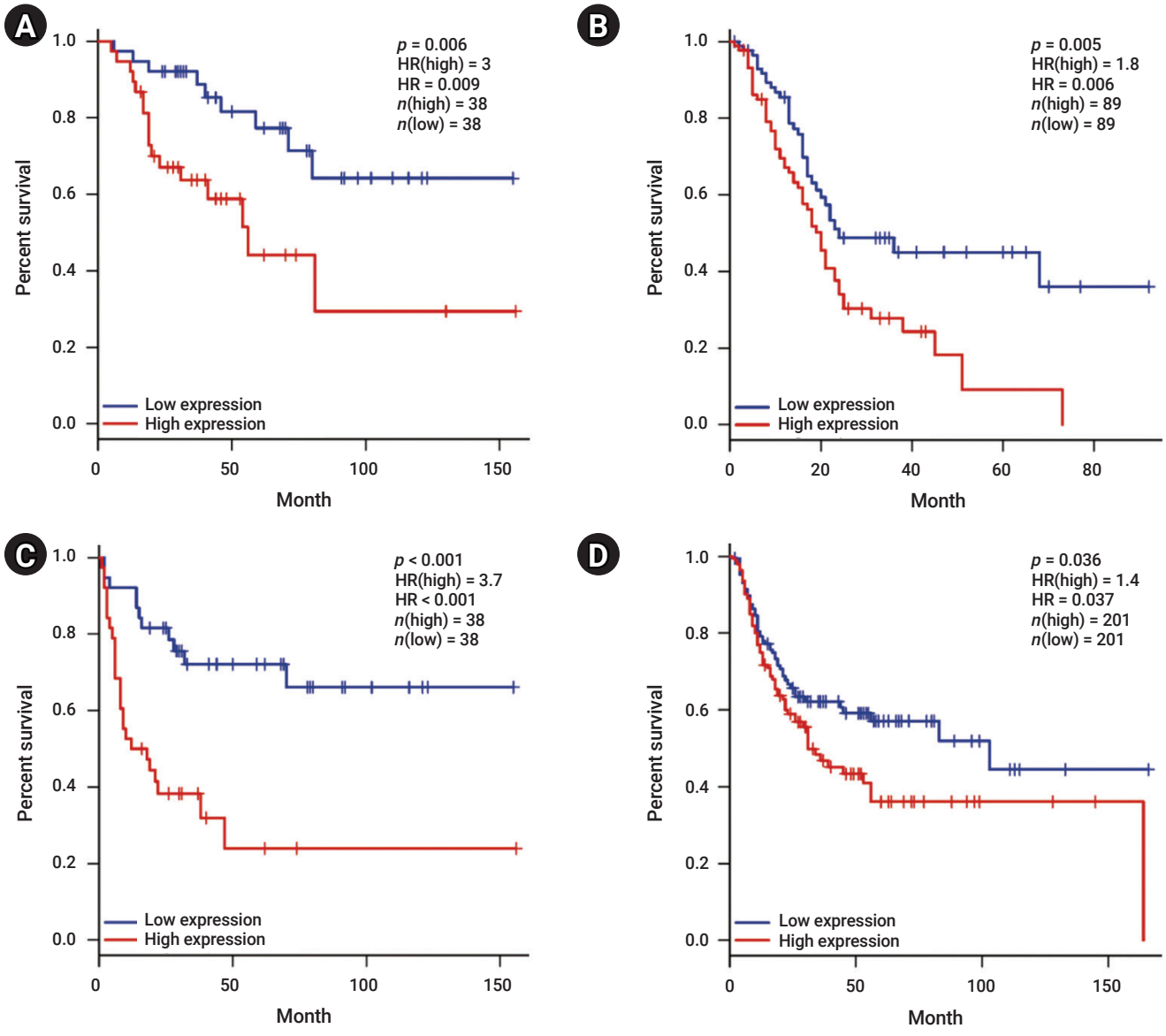

Figure 2. The prognostic significance of high expression of Yes-associated protein 1 (*YAP1*) in different cancer types. *YAP1* expression and survival rates were evaluated using the Gene Expression Profiling Interactive Analysis and PrognoScan databases.
(A, B) Overall survival curves according to *YAP1* expression in adrenocortical carcinoma (ACC) and pancreatic adenocarcinoma.
(C, D) Disease-free survival curves according to *YAP1* expression in ACC and bladder urothelial carcinoma. HR, hazard ratio.

patients with ACC (HR < 0.001; $p < 0.001$) and BLCA (HR, 0.037; $p = 0.036$) (Figure 2C, D). In other cancer types, *YAP1* did not have any prognostic value (Table S1). To further examine the prognostic potential of *YAP1* in different cancer types, we analyzed the PrognoScan database. The analysis indicated a worse prognosis in cancers of the bladder, brain, breast, colorectal, esophagus, and lung (Table S2). These results suggest that *YAP1* expression affects cancer prognosis.

Correlation between *YAP1* Expression and Telomerase in Various Types of Cancer

To determine the correlation between *YAP1* expression and *TERT* and *TERC*, we analyzed the data included in the TIMER database. As shown in Table 1, the analysis indicated *YAP1* expression was negatively correlated with *TERT* in BLCA, breast invasive carcinoma (BRCA), CHOL, colon adenocarcinoma (COAD), brain lower grade glioma (LGG), lung adenocarcinoma (LUAD,), mesothelioma (MESO), prostate adenocarcinoma (PRAD), rectal adenocarcinoma (READ), sarcoma (SARC), testicular germ cell tumors (TGCT), thyroid carcinoma (THCA), and THYM. Moreover, *YAP1* expression was negatively correlated with *TERC* in BLCA, BRCA, DLBC, GBM, head and neck squamous cell carcinoma (HNSC), LUAD, lung squamous cell carcinoma (LUSC), MESO, ovarian serous cystadenocarcinoma (OV), PRAD, READ, TGCT and THYM (Table 2). However, *YAP1* expression was positively correlated with *TERT* activity in OV and uveal melanoma (UVM). These results suggested that *YAP1*

Table 1. Correlations between *YAP1* and *TERT* expression in different types of cancer

Cancer type	R	*p*
Adrenocortical carcinoma	0.10	0.384
Bladder urothelial carcinoma	−0.14	0.005*
Breast invasive carcinoma	−0.14	0.000*
Cervical squamous cell carcinoma and endocervical adenocarcinoma	0.03	0.547
Cholangiocarcinoma	−0.35	0.038*
Colon adenocarcinoma	−0.12	0.012*
Lymphoid neoplasm diffuse large B-cell lymphoma	0.05	0.750
Esophageal carcinoma	0.03	0.729
Glioblastoma multiforme	0.01	0.908
Head and neck squamous cell carcinoma	−0.04	0.326
Kidney chromophobe	0.07	0.604
Kidney renal clear cell carcinoma	−0.08	0.062
Kidney renal papillary cell carcinoma	0.04	0.509
Brain lower grade glioma	−0.28	0.000*
Liver hepatocellular carcinoma	−0.08	0.142
Lung adenocarcinoma	−0.09	0.045*
Lung squamous cell carcinoma	0.01	0.839
Mesothelioma	−0.25	0.022*
Ovarian serous cystadenocarcinoma	0.20	0.000*
Pancreatic adenocarcinoma	−0.08	0.298
Pheochromocytoma and paraganglioma	−0.04	0.636
Prostate adenocarcinoma	−0.24	0.000*
Rectum adenocarcinoma	−0.29	0.000*
Sarcoma	−0.22	0.000*
Skin cutaneous melanoma	0.04	0.397
Stomach adenocarcinoma	−0.08	0.100
Testicular germ cell tumors	−0.18	0.031*
Thyroid carcinoma	−0.13	0.004*
Thymoma	−0.50	0.000*
Uterine corpus endometrial carcinoma	0.01	0.760
Uterine carcinosarcoma	0.08	0.553
Uveal melanoma	0.31	0.005*

The correlation between Yes-associated protein 1 (*YAP1*) and telomerase reverse transcriptase (*TERT*) expression was analyzed by using Tumor Immune Estimation Resource database.
*$p < 0.05$.

https://doi.org/10.24171/j.phrp.2021.0207

Table 2. Correlations between *YAP1* and *TERC* expression in different types of cancer

Cancer type	R	*p*
Adrenocortical carcinoma	−0.07	0.568
Bladder urothelial carcinoma	−0.14	0.005*
Breast invasive carcinoma	−0.18	0.000*
Cervical squamous cell carcinoma and endocervical adenocarcinoma	−0.03	0.587
Cholangiocarcinoma	0.06	0.742
Colon adenocarcinoma	−0.03	0.468
Lymphoid neoplasm diffuse large B-cell lymphoma	−0.31	0.035*
Esophageal carcinoma	−0.07	0.336
Glioblastoma multiforme	−0.18	0.023*
Head and neck squamous cell carcinoma	−0.11	0.016*
Kidney chromophobe	0.18	0.156
Kidney renal clear cell carcinoma	−0.07	0.101
Kidney renal papillary cell carcinoma	−0.07	0.243
Brain lower grade glioma	−0.01	0.748
Liver hepatocellular carcinoma	0.05	0.345
Lung adenocarcinoma	−0.16	0.000*
Lung squamous cell carcinoma	−0.12	0.007*
Mesothelioma	−0.28	0.007*
Ovarian serous cystadenocarcinoma	−0.14	0.012*
Pancreatic adenocarcinoma	−0.12	0.122
Pheochromocytoma and paraganglioma	−0.05	0.484
Prostate adenocarcinoma	−0.18	0.000*
Rectum adenocarcinoma	−0.39	0.000*
Sarcoma	−0.09	0.144
Skin cutaneous melanoma	−0.03	0.588
Stomach adenocarcinoma	−0.07	0.173
Testicular germ cell tumors	−0.23	0.005*
Thyroid carcinoma	−0.04	0.381
Thymoma	−0.56	0.000*
Uterine corpus endometrial carcinoma	0.08	0.071
Uterine carcinosarcoma	0.16	0.236
Uveal melanoma	−0.05	0.642

The correlation between Yes-associated protein 1 (*YAP1*) and telomerase reverse transcriptase (*TERC*) expression was analyzed by using Tumor Immune Estimation Resource database.
*$p < 0.05$.

expression was correlated with *TERT* and *TERC* in different cancer types.

Discussion

In the past decade, previous studies have focused on determining *YAP1* expression to improve the understanding of its prognostic significance and potential effect on various cancer types. *YAP1* is a potent oncogene [24], and its levels are frequently increased in many cancer types [1,14,36–38]. The expression and role of *YAP1* in cancer are cell type-dependent, and its expression may contribute to cancer development [2,6,9,39]. Upregulation of *YAP1* expression has been observed in multiple cancer types. *YAP1* overexpression has been reported in patients with hepatocellular carcinoma (HCC), colorectal cancers, LUAD, ovarian cancer, and prostate cancer [2,3,14,40]. These findings suggest the potential oncogenic role of *YAP1* in multiple cancer types.

Zhang et al. [41] performed the immunohistochemical analyzes of primary esophageal squamous cell carcinoma tumor resection samples from patients, and reported that overexpression of *YAP1* was associated with tumor relative to adjacent tissue samples. In addition, Collak et al. [42] identified overexpression of *YAP1* in nuclear and cytosolic of benign prostates using immunohistochemistry, whereas moderate expression of *YAP1* was found in cellular locations of prostate intraepithelial neoplasia and prostate cancer. These findings show differences in expression levels of *YAP1* across cancer tissue samples, and are consistent with those presented in this study.

In this study, we showed that the prognostic value of *YAP1* expression was significant in various cancer types. Importantly, our data provide evidence that *YAP1* expression is correlated with telomerase (*TERT* and *TERC*) expression in various cancer types. Previous studies have reported that *YAP1* is a prognostic marker for OS and DFS in HCC [2]. *YAP1* expression is a remarkable predictor of poor prognosis in HCC patients with negative keratin 19 cells [9]. *YAP1* expression has also been significantly correlated with a poor prognosis in OV [6,36,43,44]. However, our results showed that *YAP1* expression did not have a prognostic role in various cancers, including HCC and OSC, based on TCGA data. Interestingly, higher expression of *YAP1* predicted poorer OS in patients with ACC and PAAD, and poorer DFS in patients with ACC and BLCA. According to these results, we suggest that higher expression of *YAP1* may be significantly correlated to a poorer prognosis in various cancers.

Telomerase is active in adult germ-line tissues, immortal cells [18], and most types of malignant tumors [19]. It is upregulated during tumorigenesis through the transcriptional regulation of *TERT* in up to 90% of cancers [45–48]. Upregulation of *TERC* is an early event in tumorigenesis, and *TERC* could be more closely correlated with tumor grade than telomerase activity or *TERT* expression [49–55]. *TERC* activity is associated with cancer, but its underlying mechanisms are poorly understood. This is the first study to explore the correlations between *YAP1* expression and telomerase expression (*TERT* and *TERC*) in various cancer types. Our data indicated that *YAP1* expression was

negatively correlated with *TERT* in BLCA, BRCA, CHOL, COAD, LGG, MESO, PRAD, READ, SARC, TGCT, THCA, and THYM. *YAP1* expression was positively correlated with *TERT* in OV and UVM, but negatively correlated with *TERC* in BLCA, BRCA, DLBC, GBM, HNSC, LIHC, LUSC, MESO, OV, PRAD, READ, TGCT, and THYM. Our findings may suggest that *YAP1* expression affects *TERT* and *TERC* expression in different cancer types. However, further investigation should be performed to elucidate the potential role of *YAP1* and telomere-related gene expression, which may contribute to novel research and therapies for treating various cancer types. Our results indicate that *YAP1* expression is correlated with 2 major components of telomerase, and is associated with a poor prognosis in various cancer types.

In this study, we attempted to confirm the clinical value of *YAP1* expression in various cancer types and its effect on telomerase. Although *YAP1* expression was different in many cancer types, the detailed mechanism underlying *YAP1* regulation should be established in future studies.

Conclusion

We suggest that *YAP1* could be a potential prognostic biomarker, which may stimulate novel cancer research. Understanding the correlation between *YAP1* expression and telomerase may provide insights into telomere-related diseases, including different types of cancers. Therefore, future oncology research could seek to understand the biological functions of *YAP1* and the correlation between *YAP1* and telomere-associated gene expression.

Supplementary Material

Table S1. Survival analysis of *YAP1* in different types of cancer using Gene Expression Profiling Interactive Analysis database; **Table S2.** Survival analysis of *YAP1* in different types of cancer using PrognoScan database. Supplementary data are available at https://doi.org/10.24171/j.phrp.2021.0207.

Notes

Ethics Approval
Not applicable.

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

Funding
This research was supported by the National Research Foundation Grant funded by the Korean Government (NRF-2021R1C1C1003333 to HSJ).

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

Authors' Contributions
Conceptualization: SJH, JK; Data curation: SJH, JK; Formal analysis: SJH, JK; Funding acquisition: SJH; Investigation: HRK, KY, CWS; Methodology: HRK, KY, CWS; Project administration: HRK, KY, CWS; Resources: HRK, KY, CWS; Software: HRK, KY, CWS; Supervision: HRK, KY, CWS; Validation: HRK, KY; Visualization: HRK, KY; Writing–original draft: all authors; Writing–review & editing: all authors.

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https://doi.org/10.24171/j.phrp.2021.0207

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2021.0181
Osong Public Health Res Perspect 2021;12(5):333-341

Original Article

Comparative study of the intestinal parasitism profiles between communities across the 5 municipalities of the Barranquilla metropolitan area, Colombia

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ABSTRACT

Objectives: The aim of this study was to compare the rate of parasitism and intestinal parasitism profiles of children who live in relatively proximal communities across the 5 municipalities of the Barranquilla metropolitan area, Colombia.
Methods: In total, 986 fecal samples from children aged 1 to 10 were analyzed using a direct method (physiological saline and lugol) and the zinc sulfate flotation technique. A comparative analysis of the parasitism profiles between sampling locations (7 schools and 3 health centers providing growth and development services) was conducted using principal component analysis (PCA). The presence of *Taenia solium* antibodies was evaluated by the enzyme-linked immunosorbent assay method in 269 serum samples from the same group of children.
Results: The overall prevalence of intestinal parasitism was 57.6% (range, 42.1%–77.6%) across the sampling areas. The prevalence of helminthiasis was between 4.1% and 23.7%, and that of intestinal protozoa was between 38.2% and 73.5%. PCA showed that the parasite profiles of each sampling location shared no common characteristics. A total of 3.0% of the serum samples were positive for *T. solium* antibodies.
Conclusion: The intestinal parasitism profiles between relatively proximal sampling locations with similar geographic conditions were vastly different, indicating the need to study each small ecological niche on a localized scale to develop more cost-effective interventions for controlling intestinal parasitism.

Keywords: Helminthiasis; Neglected diseases; Parasites; Taenia solium

Received: July 9, 2021
Revised: August 24, 2021
Accepted: September 23, 2021

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Introduction

Intestinal parasites are widely distributed in the world and mainly affect developing countries

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in tropical and subtropical regions where environmental, social, and cultural conditions, including inadequate basic sanitation, a lack of sewage, and inadequate waste management, increase the likelihood of their transmission. School-age children are one of the most vulnerable populations to gastrointestinal parasite infections. Parasitism in children is associated with growth retardation, severe anemia, and impaired learning ability [1]. Approximately 1.5 billion people in the world (24.0% of the global population) are affected by soil-transmitted helminths, 270 million of whom are preschool-age children. In Latin America, 46 million school-age children live in areas with a high risk of transmission of these parasites [2]. In Colombia, the results of 3 national parasitism surveys carried out in 1965, 1980, and 2014 all found a prevalence rate of more than 80% for intestinal parasitism among the population despite their vastly different methodological processes [3]. The Colombian Massive Anthelmintic Deworming Guidelines were created in 2013, based on the helminth control guide published by the World Health Organization (WHO). Guidelines include deworming using annual or biannual single doses of albendazole (400 mg) or mebendazole (500 mg) for eligible groups in the population according to their risk of soil-helminth transmission, with school-age children (5 to 14 years old) being a prioritized group [4,5]. In the areas examined in this study, this program was only implemented until the end of 2015.

Several studies have found significant differences in the parasitic profiles of population groups in the same geographic area across different points in time, which gives insight into the dynamics of the evolution of parasitic infections depending on various factors related to the parasite, the host, and the environment [6–8].

The WHO has affirmed that intestinal parasites should be controlled based on chemotherapeutic strategies suited to the target population, but also through routine monitoring and the control of infection dynamics [9,10]. An understanding of the ecology and epidemiology of parasitic diseases and the environmental changes that affect them enables more cost-effective parasite control measures. In this study, the parasite profiles of children under 10 years of age from 10 different locations across the 5 municipalities of the Barranquilla metropolitan area (BMA) were examined, and the results between locations were compared.

Materials and Methods

In this descriptive, correlational cross-sectional study, 986 fecal samples were collected from children under 10 years of age (482 females and 504 males) at 10 locations (7 schools and 3 health centers providing growth and development services). All sites were located in urban areas in the 5 municipalities of the BMA. Samples were collected between June 2014 and September 2015.

Since species such as *Taenia solium* are difficult to diagnose using conventional coproanalysis techniques and Colombia is considered an endemic area for this parasite, the presence of anti-*T. solium* immunoglobulin G (IgG) antibodies was evaluated in 269 serum samples from the group of children examined in this study.

Area Description

The BMA is located in the Department of Atlántico, Colombia, and includes the municipalities of Soledad, Galapa, Malambo, Puerto Colombia, and Barranquilla district. The BMA borders the Caribbean Sea to the north, the Magdalena River to the east, and other municipalities to the southwest. The BMA has an area of 520 km^2 and a dry tropical climate, with an average temperature of 28°C. Figure 1 shows a map of the BMA including its municipalities and the sample locations in this study. The population of the Department of Atlántico

Figure 1. Municipalities of the Barranquilla metropolitan area and sampling locations within the Department of Atlántico, Colombia.

https://doi.org/10.24171/j.phrp.2021.0181

according to the National Department of Statistics for 2015 was 2,461,001 inhabitants, of which approximately 82.3% were located in the BMA and 648,884 were under 14 years of age [11]. With 986 fecal samples, a confidence level of greater than 95% was achieved. The differences between municipalities related to factors such as poverty, education, health services, access to home public services, basic sanitation, and housing are reflected in the multidimensional poverty index (MPI) of each municipality. In 2015, the MPI was 37.2% in the District of Barranquilla, 78.3% in Galapa, 62.5% in Malambo, 42.3% in Puerto Colombia, and 45.4% in Soledad [12].

Collection and Analysis of Samples

After obtaining prior authorization and the directives from each participating institution, the project was explained to as many children's parents as possible. The appropriate way to collect the stool sample was explained and the containers for sample collection were provided to them. The exclusion criterion was children with severe gastrointestinal symptoms that required urgent medical attention. At a previously determined date and time, the parents who voluntarily agreed to take part in the study delivered the fecal samples of the children which were immediately transported under refrigerated conditions to laboratories for processing. The sample analysis was conducted via a direct examination with a physiological saline solution and a 3% iodine solution. Additionally, the fecal concentration technique was performed with a zinc sulfate concentration of 33.0% for the concentration of eggs (2 g of feces were homogenized in 10 mL of water, filtered through gauze, and centrifuged in a 15 mL tube for 2 minutes at 400×*g*, and the sediment was resuspended in a zinc sulfate solution) [13,14].

In addition, 269 serum samples were collected from a portion of the children who were in a good state of health after obtaining consent from their parents. The venous blood samples were taken from the children after fasting. Using a closed vacuum tube without anticoagulant, the samples were transported under refrigeration to the clinical laboratory and immediately centrifuged, and the serum was stored at −80°C until use.

The presence of anti-*T. solium* IgG antibodies in serum was determined using the commercial anti-*T. solium* IgG human enzyme-linked immunosorbent assay (ELISA) kit (ab108770; Abcam, Cambridge, UK). This kit is specified for the detection of antibodies against *T. solium* by indirect ELISA, using a crude cysticercus lysate of *T. solium* as an antigen. The test has a sensitivity of 93.8% and a specificity greater than 95%. The analysis protocol was performed according to the manufacturer's instructions using the Stat Fax 303 Plus Microstrip Reader (Minneapolis, MN, USA).

Statistical Analysis

An exploratory descriptive analysis of the results was conducted to determine the absolute and relative frequency of the parasites present in the samples, and principal component analysis (PCA) was conducted to compare the results between sampling locations using the XLSTAT program for Excel (Addinsoft Inc., Paris, France). This test allowed us to evaluate the similarities between the parasite profiles of each location and municipality. The results concerning the frequency of parasitism, helminthiasis, and intestinal parasitic protozoa from each sampling location and municipality were compared using the chi-square test for categorical variables with the same statistical program. Descriptive analysis was used to determine the presence of anti-*T. solium* IgG antibodies in the serum.

Ethical Considerations

This study was approved by the Ethics Committee of the Metropolitan University (Ref. 2014–11), and it was based on the Declaration of Helsinki and Resolution 8430 of 1993 of the Ministry of Health and Social Protection in Colombia, which establishes the scientific, technical, and administrative standards for research in health. It was classified as minimal risk. Blood samples were taken after obtaining informed consent from the children's parents or legal representative. Participation in the project was voluntary, and the results of the fecal analyzes were shared with the participants so that they could seek in the Public Health Service, corresponding treatment if necessary. Positive findings regarding the presence of *T. solium* IgG antibodies in serum samples did not suggest the presence of an active infection and only had epidemiological value. Since the children showed apparent good health, the ELISA test results were not provided. The analysis and publication of the results were carried out without any data that could have revealed the identities of individual participants or information that could compromise their privacy.

Results

We analyzed 986 stool samples from children under 10 years of age at 10 sampling locations across the 5 municipalities of the BMA. In total, 568 of the children had some intestinal parasites (57.6%), with the District of Barranquilla showing the lowest frequency of parasitism (47.6%) and Soledad municipality showing the highest (73.5%). Soil-transmitted helminth infection was present in 12.0% of the total study population, with the highest percentages being found

in Puerto Colombia and Malambo, at 16.5% and 15.9%, respectively, followed by Soledad (8.2%), Barranquilla district (7.5%), and Galapa (5.7%), where lower rates were observed. Only 1 child had 4 different helminth species, and 1 had 3 different types of helminth species. However, 18 children had 2 different helminth species (1.8%), while 98 (9.9%) had 1 species only. A total of 53.5% of the children analyzed had intestinal parasitic protozoa, with a maximum of 4 different species ($n=3$) being found. A combination of helminths and protozoa was found in 7.9% of the children. Of the 568 parasitized children, 41.4% ($n=235$) had more than 1 intestinal parasite. Table 1 shows the frequency of parasitism by municipality. Certain similarities were observed in the rates of parasitism between Barranquilla district and Galapa and between Malambo and Puerto Colombia, but such similarities were not observed when comparing the sampling locations in each municipality (Table 2). No statistically significant differences were found in parasitism according to sex.

The most frequent helminth in the BMA was *Trichiuris trichiura* (7.6%) followed by *Ascaris lumbricoides* (4.5%). The sampling location with the highest rate of helminthiasis was a school in Puerto Colombia (23.7%), where a single case of hookworm was also found. Across all of the municipalities in the BMA, *Strongyloides* sp. infection was observed in only 1 child in the Barranquilla district. This study did not test for the presence of eggs or proglottids of *Taenia* sp. in the evaluated samples.

The highest proportion of infection with intestinal parasite protozoa was observed at a school in Malambo (77.6%), and the lowest proportion was observed at a health center in Galapa (38.2%). The most common protozoa were *Blastocystis* sp. (26.1%), the proportion of which varied between 5.9% and 51.0% across different sampling locations, and *Giardia* sp. (15.2%), the proportion of which varied between 8.6% and 32.1% (Table 2). The highest frequency of *Blastocystis* sp. was found in Soledad (51.0%), and the highest frequency of *Giardia* sp. was found in Malambo (18.5%).

PCA was conducted to identify relationships between the variables (the frequency of parasite species and sampling locations) according to their similarities using Pearson's correlation coefficient. Figure 2 is a graphic representation of factors 1 and 2, which had a cumulative variance of 51.3%. The main components of factor 1 (cumulative variance, 31.3%) were *Enterobius* sp. (20.9%), *T. trichiura* (15.7%), and *Uncinaria* (15.0%). The main components of factor 2 (cumulative variance, 20.0%) accumulated variance were *A. lumbricoides* (22.9%), *Blastocystis* sp. (18.3%), and *Entamoeba coli* (14.1%). A cumulative variance of 69.7% was reached by the inclusion of factor 3 and

Table 1. The pravelence of parasitism by municipality

Parasitism type	Municipalities of the BMA					
	District of Barranquilla ($n=359$)	Galapa ($n=70$)	Malambo ($n=157$)	Puerto Colombia ($n=351$)	Soledad ($n=49$)	Total BMA ($n=986$)
Parasitism	47.6[a)]	50.0[a)]	64.3[b)]	64.1[b)]	73.5[c)]	57.6
Helminths	7.5[a)]	5.7[b)]	15.9[a)]	16.5[a)]	8.2[a)]	12.0
Protozoa	44.6[a)]	47.1[a)]	60.5[b)]	58.1[b)]	73.5[c)]	53.5
Poly-infection	34.5[a)]	40.0[a)]	49.5[a)]	41.8[a)]	50.0[a)]	41.4
Parasite species						
Entamoeba hystolitica	5.6	7.1	6.4	6.6	6.1	6.2
Entamoeba coli	7.5	21.4	19.1	15.7	16.3	13.7
Iodamoeba sp.	0.3	1.4	0.6	0.3	2.0	0.5
Blastocystis sp.	26.7	27.1	29.3	20.2	51.0	26.1
Giardia sp.	13.7	11.4	18.5	15.7	18.4	15.2
Endolimax nana	7.8	8.6	14.7	19.1	24.5	13.8
Ascaris lumbricoides	2.5	1.4	3.2	8.3	0	4.5
Trichiuris trichiura	3.6	1.4	12.1	10.8	8.2	7.6
Enterobius sp.	0.8	0	0.6	1.1	0	0.8
Hymenolepis nana	1.1	2.9	1.9	0.9	0	1.2
Uncinaria sp.	0	0	0	0.3	0	0.1
Strongyloides sp.	0.3	0	0	0	0	0.1

BMA, Baranquilla metropolitan area.
[a–c)]Frequencies (%) in the same row that do not share the superscript letter are statistically significantly different (chi-square test, $p<0.05$). The frequency of poly-infections was calculated in relation to the number of children with a positive result for more than 1 species of intestinal parasites.

https://doi.org/10.24171/j.phrp.2021.0181

Table 2. The rate of parasitism for each sampling location in each municipality of the BMA

Parasitism type	Barranquilla district		Galapa		Malambo		Puerto Colombia			Soledad
	HCS (*n* = 221)	School (*n* = 138)	HCS (*n* = 34)	School (*n* = 36)	HCS (*n* = 81)	School (*n* = 76)	School 1 (*n* = 226)	School 2 (*n* = 97)	School 3 (*n* = 28)	School (*n* = 49)
Parasitism	42.1[a)]	56.5[b)]	44.1[a)]	55.6[a)]	51.8[b)]	77.6[c)]	61.5[b)]	70.1[c)]	64.3[b)]	73.5[c)]
Helminths	4.1[a)]	13.0[b)]	5.9[a)]	5.6[a)]	17.3[b)]	14.5[b)]	15.5[b)]	23.7[c)]	0[a)]	8.2[b)]
Protozoa	39.4[a)]	52.9[b)]	38.2[a)]	55.6[b)]	44.4[a)]	77.6[c)]	54.4[b)]	64.9[b)]	64.3[b)]	73.5[c)]
Poly-infection	26.9[a)]	43.6[b)]	20.0[a)]	55.0[b)]	33.3[a)]	61.0[b)]	38.1[a)]	52.9[b)]	27.8[a)]	50.0[b)]
Parasite species										
Entamoeba hystolitica	2.3	10.9	2.9	11.1	1.2	11.8	5.8	10.3	0	6.1
Entamoeba coli	7.2	8.0	11.8	30.6	12.3	26.3	17.3	13.4	10.7	16.3
Iodamoeba sp.	0.5	0	2.9	0	1.2	0	0.4	0	0	2.0
Blastocystis sp.	24.4	30.4	5.9	47.2	22.2	36.8	17.3	27.8	17.9	51.0
Giardia sp.	11.3	17.4	11.8	11.1	8.6	28.9	10.6	22.7	32.1	18.4
Endolimax nana	7.7	8.0	14.7	2.8	9.9	19.7	19.9	16.5	21.4	24.5
Ascaris lumbricoides	1.4	4.3	2.9	0	4.9	1.3	7.5	12.4	0	0
Trichiuris trichiura	1.4	7.2	0	2.8	12.3	11.8	9.7	16.5	0	8.2
Enterobius sp.	0.9	0.7	0	0	0	1.3	0.9	2.1	0	0
Hymenolepis nana	0.9	1.4	2.9	2.8	3.7	0	0.9	1.0	0	0
Uncinaria sp.	0	0	0	0	0	0	0	1	0	0
Strongyloides sp.	0	0.7	0	0	0	0	0	0	0	0

BMA, Baranquilla metropolitan area; HCS, health centers providing services.
[a–c)]Frequencies (%) in the same row that do not share the superscript letter are statistically significantly different.

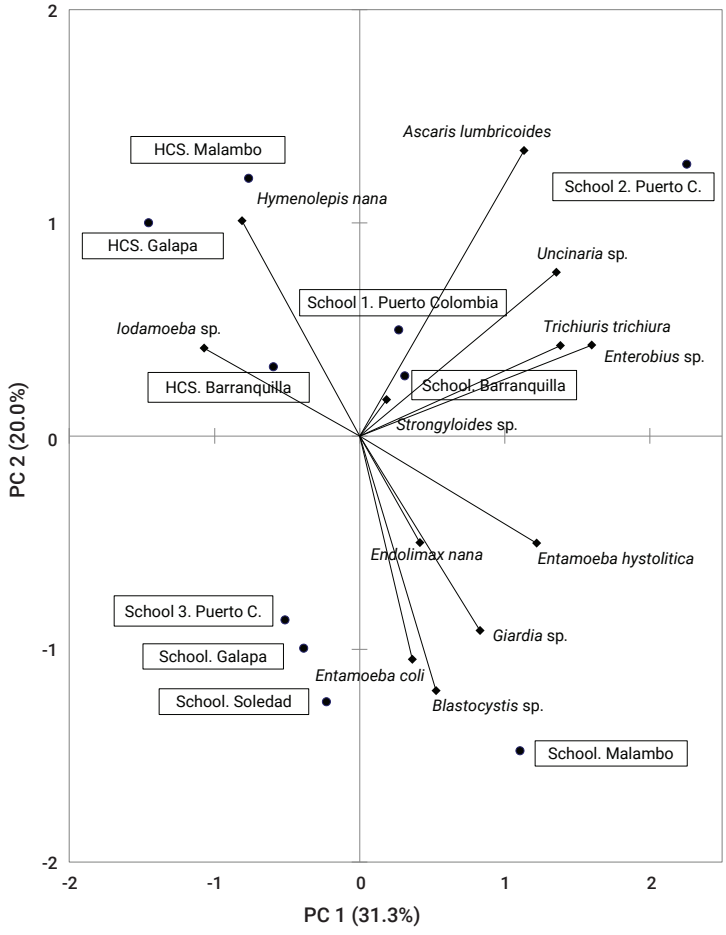

Figure 2. Principal component analysis revealing the relationships between the rates of parasites and sampling locations.
The graphic shows the cumulative variability for Principal Componet (PC) 1 and 2 (51.3%). The parasite profiles for each sampling location shared no common characteristics that enabled them to be grouped by municipality. HCS, health centers providing services.

the variables *Endolimax nana* (30.0%), *Hymenolepis nana* (14.9%), and *Entamoeba hystolitica* (14.7%). PCA showed that the parasite profiles for each sampling location shared no common characteristics that enabled them to be grouped by municipality. When this analysis was conducted with intestinal protozoa parasites or helminths set as the independent variables, no relationship was observed.

The sites with the lowest rates of parasitism were located in the upper left quadrant of the chart, which corresponded to health centers providing growth and development services. In the lower left quadrant were schools, where the main type of intestinal parasitism found was intestinal parasitic protozoa. Most of the sites with high rates of helminth infection were located in the right quadrants, particularly at a school in Malambo where high frequencies of both protozoa and helminths were found.

The results of the indirect ELISA immunoassay for IgG *T. solium* antibodies detection were positive in 8 (3.0%) of the 269 serum samples analyzed (90 samples from Barranquilla district, 45 from Galapa, 45 from Malambo, 44 from Puerto Colombia, and 45 from Soledad). The positive results were from 6 children from the Soledad municipality (13.3%), 1 child from Barranquilla district (1.1%), and 1 child from Galapa (2.2%). The results for 5 children were in the borderline zone of the test (values between 9 and 11 standard units) according to the manufacturer's guidelines related to the technique. Two of the children were from Soledad, 1 was from the Barranquilla district, and 2 were from Galapa.

Discussion

Soil-transmitted helminth infections in general and the taeniasis/cysticercosis complex in particular are pathologies currently prioritized by the WHO, since they are considered reemerging diseases with a significant negative impact on the social and economic development of populations [15]. In this study, the prevalence of intestinal parasitism in children under 10 years of age in the BMA was 57.6%, with a prevalence of helminthiasis of 12.0% and a prevalence of protozoan parasites of 53.5%.

The PCA of the parasite profiles for each sampling location did not show any similarities that would have enabled the classification of parasite profiles by municipality. The individuality of the parasitic profiles of relatively proximal locations with similar climatic and cultural conditions correlates to multiple factors implicit in parasite infection. The specific conditions of small ecological niches, where genetic, physiological, nutritional, and cultural factors could have subtle variations and the complexity of the life cycle of each parasite could be favored or obstructed by specific actions, determine the status of parasitic infection at each location [16]. The variability found in this study is consistent with the findings from the most recent national survey on parasitism, which found a diversity of profiles across municipalities in the Caribbean Arid Belt region, where the BMA is located [3].

No relationship between the MPI value and parasitism was found in any of the municipalities. Lower rates of parasitism, helminthiasis, and protozoa were observed in the Barranquilla district and Galapa, which had the lowest and highest MPI values, respectively. The municipalities of Malambo and Puerto Colombia, which also had significantly different MPI values (62.5% for Malambo and 42.3% for Puerto Colombia), showed similar rates of parasitism. In this study, the MPI value was not specified for each individual household; however, it is important to highlight that MPI values indicate the coverage of the sewage network and unsanitary environmental conditions in homes, among other factors, and that the condition of poverty is generally associated with intestinal parasitism [17–19].

Blastocystis sp., which is the most common intestinal protozoan in the world [20], was also the most common intestinal protozoan found in this study, followed by *Giardia* sp. These 2 parasites are primary causes of diarrhea, especially among children and immunosuppressed people. Various studies have observed zoonotic transmission of these parasite species, although the environmental conditions that favor transmission must be investigated in greater detail [21,22]. *Blastocystis* sp. and *Giardia* sp. have been found in domestic animals in different tropical regions of the world [23,24]. In 2018 a study of the Colombian Caribbean, Sarmiento-Rubiano et al. [25] found a high rate of zoonotic transmission of intestinal parasites among dogs and cats with owners from Barranquilla city, which is an epidemiological situation that could be related to the rate of parasites found in this study. Different studies reported the presence of *Blastocystis* sp. in environmental water sources such as rivers and lakes, mainly in tropical areas where the water temperature favors its survival. As such, this could be a source of regular contamination with *Blastocystis* sp. for people who live along the river. These findings have led to the suggestion to include the detection of *Blastocystis* sp. in the WHO Guidelines for Drinking Water Quality [26,27]

Although few studies have sought to directly link infection with intestinal parasites and exposure to contaminated water sources, a multivariate analysis conducted in Vietnam found a statistically significant increase in the risk of helminthiasis among populations that were in direct contact with sources of contaminated water [28]. In 2018, Amoah et al. [29] found epidemiological evidence for a higher rate

https://doi.org/10.24171/j.phrp.2021.0181

of parasitic infections associated with direct exposure to sewage or sludge and the consumption of produce grown in contaminated soil. It is possible that the Magdalena River and the swamps surrounding the BMA, which have higher rates of microbiological contamination than are permitted for agricultural and fishing use [30], can be a determining factor associated with the high frequencies of parasitism found in the municipalities of Malambo and Puerto Colombia. These municipalities are significantly affected by the Mesolandia and Mallorquín swamps, respectively—water sources that show high levels of microbiological contamination and organic matter [31,32]. In contrast, the municipality of Galapa, which has the highest MPI value in the BMA at 79.8% and the worst indicators of excreta elimination of the evaluated municipalities [12] but is not close to any water source, had the lowest rate of helminthiasis and one of the lowest rates of parasitism.

In this study, almost 3% of children were found to have antibodies against *T. solium*. The presence of these antibodies does not necessarily indicate active infection with the parasite; rather, it can indicate contact with the parasite at any point in an individual's life. Contact can be with the intestinal parasite form (taeniasis) or with the larval form (cysticercosis), and the presence of the antibody can be associated with non-infectious contact with the parasite [33]. The results related to the rate of IgG anti-*T. solium* antibodies found in this study are consistent with the 7% rate of antibodies found in a national study from 2008 of the general population of the Department of Atlántico [34]. The national rate ranges between as low as 0.5% in the Department of Caldas to as high as 40.2% in the Department of Vaupés [34]. Epidemiological studies of the rate of antibodies related to *T. solium* have limited diagnostic value since the presence of such antibodies only indicates contact with the parasite and not necessarily active infection. In this study, 6 of the positive cases found were children who lived in the municipality of Soledad who attended the same educational institution, which may indicate the location of an epidemic focus that should be studied further.

The results in the borderline zone of the ELISA immunoassay could be associated with cross-reactions in the test, since individuals could have been infected with *H. nana* and *Echinococcus granulosus*, which are also parasites that, like *Taenia* sp., are members of the Cestoda class [29]. In this study, the presence of *H. nana* was found in a stool sample from 1 of the children whose serological results were in the borderline zone. The difficulty of diagnosing *T. solium* infections reported by different authors in different contexts indicates the need to create new and more specific diagnostic tests due to the significant impact of parasites on public health.

Conclusion

The results of this study show that intestinal parasitism affects more than 50% of the population under 10 years of age in the BMA, and although the sampling locations in this analysis were both physically and culturally close and shared vastly interdependent relationships, significant differences were found in the rates of parasitism and the parasitism profiles of each location. Deficiencies in basic sanitation, poverty conditions, and the proximity to contaminated water sources were factors associated with the prevalence of intestinal parasites among children, but specific factors related to each local ecological niche also led to differences in the parasitic profiles observed, and such factors must be studied further. It is necessary to devise and implement programs for health promotion and the prevention of parasitism at a local level.

The prevalence of anti-*T. solium* antibodies in children was 3%, which indicates early contact with the parasite and has implications related to health outcomes. New and more efficient diagnostic strategies for the detection of *T. solium* infections must be sought.

Notes

Ethics Approval

This study was approved by the Ethics Committee of the Metropolitan University (Reference 2014-11 of June 10, 2014) and was based on the Declaration of Helsinki and Resolution 8430 of 1993 of the Ministry of Health and Social Protection of Colombia, which establishes the scientific, technical and administrative standards for research in health. The study was classified as minimal risk.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

All data generated or analyzed in this study were included in this published article. Further data may be requested from the corresponding author.

Additional Contributions

Conceptualization: all authors; Data curation: MF, LG, MSM; Formal analysis: MCS, JEB; Investigation: all authors; Methodology: all authors; Project administration: LASR; Resources: all authors; Software: LASR; Supervision: LASR, JEB; Validation: all authors; Visualization: all author; Writing–original draft: LASR, JEB; Writing–review & editing: all authors.

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

Instruction for authors

Enacted January 1, 2010
Last revised April 1, 2021

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