Differences in seroprevalence between epicenter and non-epicenter areas of the COVID-19 outbreak in South Korea

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To compare the standardized severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) seroprevalence of high epicenter region with non-epicenter region, serological studies were performed with a total of 3,268 sera from Daegu City and 3,981 sera from Chungbuk Province. Indirect immunofluorescence assay (IFA) for SARS-CoV-2 IgG results showed a high seroprevalence rate in the Daegu City (epicenter) compared with a non-epicenter area (Chungbuk Province) (1.27% vs. 0.91%, P = 0.0358). It is noteworthy that the highest seroprevalence in Daegu City was found in elderly patients (70's) whereas young adult patients (20's) in Chungbuk Province showed the highest seroprevalence. Neutralizing antibody (NAb) titers were found in three samples from Daegu City (3/3, 268, 0.09%) while none of the samples from Chungbuk Province were NAb positive. These results demonstrated that even following the large outbreak, the seropositive rate of SARS-CoV-2 in the general population remained low in South Korea.

Keywords: SARS-CoV-2, COVID-19, epicenter, seroprevalence, South Korea

Introduction

The 2019 coronavirus disease (COVID-19) which had emerged in December of 2019 is an ongoing pandemic. The first case of COVID-19 in South Korea was confirmed on January 20, 2020 (COVID-19 National Emergency Response Center, Epidemiology and Case Management Team, Korea Centers for Disease Control and Prevention, 2020). From February to March 2020, South Korea had experienced a huge COVID-19 outbreak in Daegu City which constituted the first wave in the epi curve of South Korea (Fig. 1). With rigorous contact tracing and strict patient isolation, South Korea was able to control the outbreak in Daegu City (Korean Society of Infectious Diseases *et al*., 2020; Peck, 2020), and maintained the low rate of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection afterward until the second wave in August (KDCA, 2020) (Fig. 1A).

Since a high proportion of COVID-19 cases are asymptomatic or have only mild clinical manifestations (Sakurai *et al*., 2020; Wu and McGoogan, 2020), serologic tests should be performed to detect subclinical or undiagnosed COVID-19 cases in the population, which are not included in the original confirmed case counts. Some cross-sectional studies of the seroprevalence of SARS-CoV-2 in South Korea (using leftover sera or non-random sampling of participants with a limited number of specimens) have been reported (Noh *et al*., 2020; Song *et al*., 2020).

In this study, we aimed to investigate a large-scale seroprevalence in high- and low-cumulative case rate-confirmed COVID-19 regions (Daegu City [the epicenter of 1st wave] and Chungbuk Province) using serum samples from two tertiary referral hospitals in each region (Fig. 1B). Daegu City is the area with the highest cumulative COVID-19 patient number in South Korea (6,929 cumulative cases on July 17, 2020; 234.38 cases per 100,000 people), whereas Chungbuk Province has the second lowest cumulative COVID-19 patient numbers in South Korea (71 cumulative cases on July 17, 2020; 4.44 cases per 100,000 people) (KDCA, 2020).
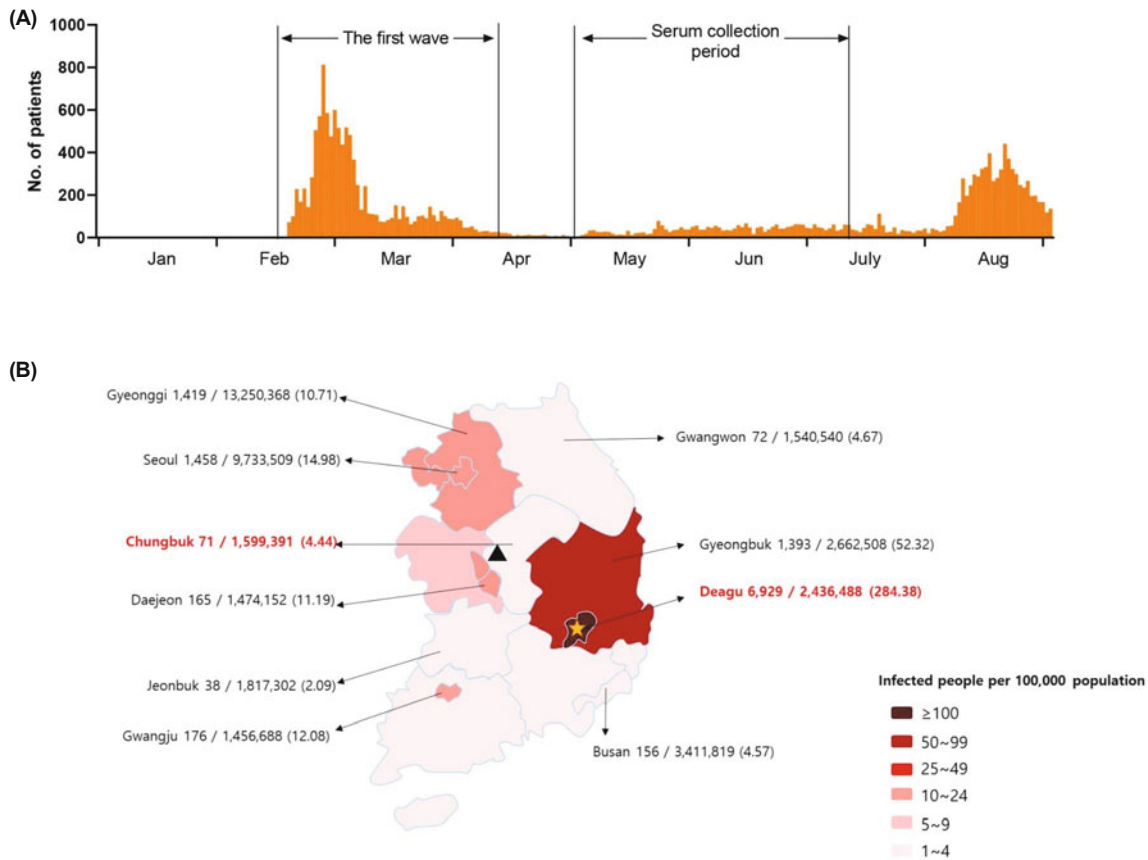
Fig. 1. (A) Number of confirmed COVID-19 cases as of September 4, 2020, in South Korea. Study samples were collected from May 1 to July 17, 2020 (indicated as sample collection period). (B) Map of South Korea colored according to the cumulative confirmed COVID-19 cases per 100,000 population (▲ CBNUH, ★ KNUH). Number of cumulative cases / population of each region (number of cumulative cases per 100,000 people in each area) as of July 17, 2020.

Materials and Methods

Collection of outpatient sera

Excess outpatient sera were randomly selected daily (around 130 serum samples per day) from two hospitals, Kyungpook National University Hospital (KNUH) in Daegu City and Chungbuk National University Hospital (CBNUH) in Chungbuk Province, between May 1, 2020 and July 17, 2020 (Fig. 1B).

Establishment of IFA against SARS-CoV-2 IgG

To established an indirect immunofluorescence assay (IFA) to detect SARS-CoV-2 IgG by checking possible cross-reactivity of the assay using negative control sera from patients who had recovered from respiratory virus infections other than SARS-CoV-2 and confirmed there was no cross-reactive response as previously described (Ko *et al.*, 2020). We confirmed that IFA SARS-CoV-2 IgG detection can provide 99.5% sensitivity, 100% specificity, and 100% positive predictive value using sera from 200 hospitalized COVID-19 patients (Table 1).

Immunofluorescence assay (IFA)

To investigate the IgG immune response against SARS-CoV-2, an IFA assay for IgG antibody was conducted in Vero cells with 100 TCID$_{50}$ of SARS-CoV-2 for 2 h at 37°C and incubated for 2 days. The infected cells were fixed for 10 min with 4% formaldehyde before permeabilization with 1% Triton X-100 (Sigma) in PBS and blocking with 3.5% bovine serum albumin (BSA, Sigma) in PBS. After washing five times, cells were then treated with diluted serum (1 : 20) samples and incubated for 1 h at room temperature, followed by incubation with the fluorescein-labeled antibody against human IgG (H+L) (Abcam; Cat# ab97224; 1:2,000 dilution) for 1 h at room temperature. Fluorescence was observed using an Olympus IX 71 (Olympus) microscope and DP controller software to capture images (Jeong *et al.*, 2020).

Table 1. Evaluation of the indirect immunofluorescence assay (IFA) for detect specific severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgG antibody with the sera of laboratory-confirmed 2019 coronavirus disease (COVID-19) patients and control sera from patients who had recovered from respiratory virus infections other than SARS-CoV-2.

	Sera of laboratory confirmed COVID-19 patients (n = 200)	Sera of patients who had recovered from respiratory virus infections other than SARS-CoV-2 (n = 21)
Positive	199	0
Negative	1	21
Total	200	21

Serum neutralizing antibody (NAb) assay

For serum neutralizing antibody (NAb) titer measurement, the heat-inactivated serum samples were 2-fold serially diluted and an equal volume of SARS-CoV-2 (CBNU-nCoV-1) at 100 tissue culture infective dose ($TCID_{50}$) was added to all diluted samples, and then the mixture was incubated at 37°C for 1 h. Following the Vero cell infection in a 96-well tissue culture plate, the infected cells were incubated at 37°C in 5% CO_2 for 4 days, supernatant were removed and fixed of 10% formalin, and then NAb titer was measured with crystal violet staining as previously described (Park *et al.*, 2020).

Statistical analysis

Seroprevalence was determined for all subjects and the exact binomial 95% confidence interval (CI) was calculated from the test for one proportion using z-statistics. In addition, standardized seroprevalence was calculated based on the 2019 population of Daegu City and Chungbuk Province. All statistical analyses were performed using SPSS 25 (IBM Corp.) and $P < 0.05$ was considered as statistically significant.

Results and Discussion

A total of 7,248 outpatients' serum samples, which were not diagnosed as COVID-19 positive at the time of this study, were collected from the two hospitals (3,268 from KNUH and 3,981 from CBNUH) between May 1, 2020 and July 17, 2020. Collection sites and age distribution of the patients sampled, and standardized seroprevalence of each area are summarized in Table 2. The IFA results revealed that the samples from KNUH (Daegu City) showed 1.25% seropositivity while those from CBNUH showed 0.83% seropositivity against SARS-CoV-2. Interestingly, the 70–79-year-old age group showed the highest seroprevalence in Daegu City, while patients 20–29 years of age showed the highest seroprevalence in Chungbuk Province. Twenty-four of 41 (58.5%) seropositive samples from KNUH were ≥ 60 years old, while 10 of 33 (30.3%) seropositive samples were from ≥ 60-year-old age group of CBNUH. NAb titers were found in three samples from KNUH (3/3, 268, 0.09%) while none of the CBNUH samples were NAb positive.

SARS-CoV-2 infection results in a wide range of clinical manifestations ranging from asymptomatic to fatal cases (Chen *et al.*, 2020; Guan *et al.*, 2020). Recently, Trinité *et al.* (2021) reported that although SARS-CoV-2 infection elicited a rapid neutralizing antibody response in hospitalized patients, mild- symptomatic and asymptomatic patients developed lower and sometimes undetectable neutralizing antibodies. Further, Liu *et al.* (2020) reported that the clinical features and disease prognosis vary among patients of different ages, and aged COVID-19 patients showed heavier clinical manifestations compared with those under 60 years of age. In this study, we tested outpatients' sera which were collected randomly from outpatients who do not show any suspected COVID-19 clinical symptoms. We found that even young people occupied a larger portion of SARS-CoV-2 seropositive population in Chungbuk province compared to those of Daegu City, the age factor and asymptomatic clinical manifestation might explain the no detectable neutralizing antibody in CBNUH sera from 20–29 years old outpatients.

In this study, we compared seroprevalence within the general population at the epicenter of the COVID-19 outbreak in South Korea with that of a non-epicenter area. Interestingly, the seroprevalence of SARS-CoV-2 in Daegu City was only 1.5 times higher than that of Chungbuk Province while the incidence of COVID-19 (number of cumulative cases per 100,000 people in each area) in Daegu City was 64 times higher than that of Chungbuk Province. Further, the highest seroprevalence in Daegu City was found in elderly patients compared to the young adult patients in Chungbuk Province. These findings suggest that undiagnosed, asymptomatic COVID-19 cases have been occurring in young population in Chungbuk Province. The spread of COVID-19 includes asymptomatic and subclinical transmission as well as extensive transmission during the symptomatic phase (Arons *et al.*, 2020). Therefore, this can result in the silent spread of SARS CoV-2 in the society without detection by established surveillance systems.

The standardized SARS-CoV-2 seroprevalence in Daegu City was 1.27% while that of Chungbuk Province was 0.91% ($P = 0.036$). The standardized SARS-CoV-2 seroprevalence of Daegu City in < 60 years age group was 1.73 and that of Chungbuk province was 1.29, respectively ($P = 0.331$). In ≥ 60 years age group, the standardized SARS-CoV-2 seroprevalence of Daegu City was 0.64 and that of Chungbuk Pro-

Table 2. Seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies in Daegu City and Chungbuk Province by age (crude seroprevalence/standardized seroprevalence according to the population composition of each area) measured in the sera from outpatients at major referring hospitals in each region.

Age groups	Seropositive samples/tested samples (crude prevalence, 95% CI)		Standardized seroprevalence (95% CI)		
	KNUH samples Total 3268	CBNUH samples Total 3981	Seroprevalence of Daegu City	Seroprevalence of Chungbuk Province	*P*-value
0–9	0/56 (0.00, 0.00–0.00)	1/230 (0.43, 0.00–1.29)	0.00 (0.00–0.00)	0.59 (0.00–1.48)	0.1927
10–19	1/58 (1.72, 0.00–5.07)	2/145 (1.38, 0.00–3.28)	9.32 (5.32–13.32)	3.60 (1.04–6.16)	0.0151
20–29	2/158 (1.27, 0.00–3.01)	5/263 (1.90, 0.25–3.55)	3.51 (1.75–5.36)	3.87 (2.02–5.71)	0.7774
30–39	3/187 (1.60, 0.00–3.41)	3/306 (0.98, 0.00–2.08)	3.55 (1.92–5.18)	1.64 (0.52–2.76)	0.0558
40–49	1/374 (0.27, 0.00–0.79)	6/491 (1.22, 0.25–2.19)	0.37 (0.00–0.77)	1.46 (0.66–2.26)	0.0168
50–59	10/732 (1.37, 0.53–2.21)	6/874 (0.69, 0.14–1.23)	1.06 (0.56–1.55)	0.52 (0.17–0.87)	0.0818
60–69	11/855 (1.29, 0.53–2.04)	5/908 (0.55, 0.07–1.03)	0.60 (0.24–0.97)	0.29 (0.04–0.55)	0.1678
70–79	11/622 (1.77, 0.73–2.80)	4/526 (0.76, 0.02–1.50)	0.64 (0.18–1.10)	0.41 (0.04–0.77)	0.4430
> 80	2/226 (0.88, 0.00–2.11)	1/238 (0.42, 0.00–1.24)	0.58 (0.00–1.27)	0.41 (0.00–1.00)	0.7113
Total	41/3268 (1.25, 0.87–1.64)	33/3981 (0.83, 0.55–1.11)	1.27 (1.01–1.53)	0.91 (0.69–1.13)	0.0358

Abbreviations: CI, confidence interval; KNUH, Kyungpook National University Hospital; CBNUH, Chungbuk National University Hospital.

vince was 0.36 (P = 0.905). This level of SARS-CoV-2 sero-prevalence in South Korea is much lower compared to other COVID-19 hotspots in the world (Eckerle and Meyer, 2020). Thus, this suggests that rigorous contact tracing with strict patient isolation strategies employed in South Korea effectively suppressed the silent spread of COVID-19 in Daegu City during the first wave (Peck, 2020). In addition, the attainment of herd immunity through natural infection would be very difficult in South Korea due to this low seroprevalence. Further, although Sweden attempted to reach natural herd immunity, the seroprevalence within the community as of July 2020, only ranged from 7.5% to 10% (Ramachandran, 2020), which is far less than the 60 to 70% believed to be required for herd immunity. Therefore, this suggests that even in highly affected areas, majority of the population remains seronegative to SARS-CoV-2. Considering that asymptomatic infections make up an estimated 15% of SARS-CoV-2 infections (Mizumoto *et al.*, 2020; He *et al.*, 2021), the formation of herd immunity through natural infection is expected to be a difficult task which will take quite a long time.

In summary, we compared the SARS-CoV-2 seroprevalence rates between the geographic area with the highest and lowest COVID-19 case rate in South Korea. The results revealed that Daegu City (epicenter) showed higher seroprevalence rate than that of the region with the lowest cumulative number of infections, Chungbuk Province. However, following the large outbreak during the first wave of SARS-CoV-2 infection, the seropositive rate of SARS-CoV-2 in the general population remained low in South Korea.

Acknowledgments

Conflict of Interest

We have no conflict of interest to report.

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