

## ORIGINAL RESEARCH

# Upper respiratory viral load in asymptomatic individuals and mildly symptomatic patients with SARS-CoV-2 infection

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Received 14 April 2020

Revised 10 August 2020

Accepted 17 August 2020



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**To cite:** Ra SH, Lim JS, Kim G, et al. *Thorax* Epub ahead of print: [please include Day Month Year]. doi:10.1136/thoraxjnl-2020-215042

## ABSTRACT

**Background** Asymptomatic individuals with SARS-CoV-2 infection have viable viral loads and have been linked to several transmission cases. However, data on the viral loads in such individuals are lacking. We assessed the viral loads in asymptomatic individuals with SARS-CoV-2 infection in comparison with those in symptomatic patients with COVID-19.

**Methods** Study participants were recruited from a community facility designated for the isolation of patients with mild COVID-19 in South Korea. The presence of symptoms was evaluated with a questionnaire-based survey. Viral loads in the upper respiratory tract were measured with real-time reverse transcription-PCR (RT-PCR) targeting the *E*, *RdRp* and *N* genes of SARS-CoV-2, with a cycle threshold (Ct) value of 40 for determining positivity.

**Results** In 213 patients with SARS-CoV-2 infection, 41 (19%) had remained asymptomatic from potential exposure to laboratory confirmation and admission; of them, 39 (95%) underwent follow-up RT-PCR testing after a median 13 days. In 172 symptomatic patients, 144 (84%) underwent follow-up RT-PCR testing. Twenty-one (54%) asymptomatic individuals and 92 (64%) symptomatic patients tested positive for SARS-CoV-2 at follow-up. Asymptomatic individuals and symptomatic patients did not show any significant differences in the mean Ct values of the *E* (31.15 vs 31.43;  $p>0.99$ ), *RdRp* (32.26 vs 32.93;  $p=0.92$ ) and *N* (33.05 vs 33.28;  $p>0.99$ ) genes.

**Conclusion** Approximately one-fifth of the individuals without severe symptoms were asymptomatic, and their viral loads were comparable to those in symptomatic patients. A large proportion of mildly symptomatic patients with COVID-19 or asymptomatic individuals with SARS-CoV-2 showed persistent positive upper respiratory RT-PCR results at follow-up.

## INTRODUCTION

The presence of asymptomatic individuals with positive reverse transcription-PCR (RT-PCR) results for SARS-CoV-2 has been noted even in the early reports of the COVID-19 outbreak.<sup>1,2</sup> Moreover, several case reports have suggested that the transmission of SARS-CoV-2 from asymptomatic individuals is possible.<sup>1-3</sup> However, owing to the rapid spread of COVID-19 and the resulting shortage in PCR testing capabilities, asymptomatic individuals with SARS-CoV-2 infection are likely

## Key questions

### What is the key question?

- ▶ Do asymptomatic individuals with SARS-CoV-2 infection have the potential to significantly contribute to the community spread of COVID-19?

### What is the bottom line?

- ▶ In a series of 213 patients with COVID-19 without severe symptoms, 19% were asymptomatic from potential exposure to laboratory confirmation and admission. Asymptomatic individuals had comparable loads of SARS-CoV-2 genes to symptomatic patients.

### Why read on?

- ▶ Asymptomatic individuals were frequent among those infected with SARS-CoV-2, but harboured a comparable viral load compared with that of symptomatic patients and may thus act as a meaningful driving force for the community spread of COVID-19.

to go unnoticed by healthcare workers. Therefore, asymptomatic individuals have not been systematically evaluated in terms of their viral load and infectivity, and thus, the extent of their possible contribution to the community spread of COVID-19 is unknown.

In South Korea, a large cluster of COVID-19 cases was identified in Daegu City; this cluster was soon revealed to be related to a single religious group.<sup>4</sup> Thereafter, Korean epidemiologic teams thoroughly traced the close contacts of this religious group and discovered more than 3000 cases of COVID-19 with a wide range of symptom severity, from none to severe. Those without severe symptoms were admitted to dedicated care facilities for isolation and monitoring. As a result of this unique situation, we were able to assess many asymptomatic individuals who were otherwise unlikely to be identified as cases of COVID-19.

Here, we assessed the viral load in the upper respiratory tract of asymptomatic individuals infected with SARS-CoV-2 using the cycle threshold (Ct) values of RT-PCR targeting SARS-CoV-2 genes

and compared the results with those from patients with mild symptoms of COVID-19.

## METHODS

### Study participants

We recruited study participants from a community care facility designated for patients with COVID-19 without severe symptoms. The admission criteria were as follows: COVID-19 national early warning score <4; without high-risk profiles such as age  $\geq 65$  years, chronic illness, current smoker and those requiring supplemental oxygen due to low oxygen saturation <90%. This facility is located approximately 50 miles from Daegu City and housed a total of 260 patients at the time of this study.

In March 2020, we conducted a survey on the patients to gather information on any COVID-19 symptom as well as age, sex and underlying disease. Asymptomatic individuals were defined as those who did not report any of the following symptoms: fever ( $>37.5^{\circ}\text{C}$ ), chills, myalgia, fatigue, rhinorrhoea, nasal congestion, hyposmia, hypogeusia, sore throat, dyspnoea, cough, sputum production, haemoptysis, headache, dizziness, anorexia, nausea, vomiting, abdominal pain and diarrhoea. Patients participated in this study by filling out the survey, and written informed consent was obtained from each patient. Missing data were obtained with telephone interviews by trained physicians. This study was approved by the institutional review board of Asan Medical Center.

### Sample acquisition

All sample acquisitions for follow-up RT-PCR at the care centre were carried out by experienced physicians donning the complete personal protective equipment. According to the Center for Disease Control guidelines<sup>5</sup> for obtaining clinical specimens for confirming COVID-19, we obtained swab samples from both the nasopharynx and oropharynx and combined them in a single tube to maximise the sensitivity of the RT-PCR test and minimise the use of resources. The nasopharyngeal samples were obtained according to a previously reported method,<sup>6</sup> in which swabs were inserted through the nostril to a distance equivalent to the outer opening of the ear canal and gently rubbed for several seconds to absorb the secretions. For oropharyngeal samples, the tonsillar pillars were swabbed. The nasopharynx and oropharynx were swabbed once each with separate swabs, placed in a single tube containing 300  $\mu\text{L}$  of viral transport medium and transported to a reference laboratory under cold conditions for subsequent RNA extraction and RT-PCR testing.

### RT-PCR for SARS-CoV-2 genes

Viral RNA from the upper respiratory tract swab samples was extracted using the MagNA Pure 96 System (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. The resulting samples were tested for the *E*, *RdRp* and *N* genes of SARS-CoV-2 with real-time RT-PCR using the Allplex 2019-nCoV Assay kit (Seegene)<sup>7</sup> with CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, California, USA) under the following condition: 20 min at  $50^{\circ}\text{C}$  for reverse transcription and 15 min at  $95^{\circ}\text{C}$  for denaturation as the initial step, followed by 45 cycles of 15 s each at  $94^{\circ}\text{C}$  and 30 s at  $58^{\circ}\text{C}$ . A Ct value of <40 was used as the cut-off for determining positivity, according to the manufacturer's instructions.

### Statistical analysis

Categorical variables were compared using the Fisher's exact test or  $\chi^2$  test as appropriate, and continuous variables were

**Table 1** Characteristics of asymptomatic individuals with SARS-CoV-2 infection and symptomatic patients with COVID-19\*

	Asymptomatic individuals (n=39)	Symptomatic patients (n=144)	P value
Age, median (IQR), y	25.0 (21.5–34.0)	26.5 (22.0–46.0)	0.22
Male sex, n (%)	22 (56)	62 (43)	0.14
Underlying diseases, n (%)	3 (8)	26 (18)	0.12
Hypertension	0	5	
Asthma	0	4	
Liver disease	0	2	
COPD	0	1	
Solid cancer	0	1	
Rheumatologic disease	1	0	
Other	2†	13‡	
Interval from the first swab for diagnosis to the start of isolation, median (IQR), days	6.0 (4.5–7.0)	6.0 (4.0–9.0)	0.84
Interval from initial confirmation to follow-up RT-PCR testing, median (IQR), days	12 (9–14)	13 (11–15)	0.06
Interval from symptom onset to follow-up RT-PCR testing, median (IQR), days	NA	26 $\pm$ 6	NA
Positive result on follow-up RT-PCR testing, n (%)	21 (54)	92 (64)	0.25
Ct values, mean $\pm$ SD			
<i>E</i> gene	31.15 $\pm$ 2.72	31.43 $\pm$ 2.80	>0.99
<i>RdRp</i> gene	32.26 $\pm$ 2.86	32.93 $\pm$ 2.87	0.92
<i>N</i> gene	33.05 $\pm$ 2.52	33.28 $\pm$ 2.48	>0.99

\*Categorical variables were compared using the Fisher's exact test or  $\chi^2$  test, as appropriate, and continuous variables were compared using the Mann-Whitney U test or Student's t-test. All tests of significance were two-tailed.

†Includes thyroid disease (n=1) and allergic rhinitis (n=1).

‡Includes allergic rhinitis (n=8), hyperlipidaemia (n=2), thyroid disease (n=2) and nephrotic syndrome (n=1).

NA, cycle threshold; NA, not available; RT-PCR, reverse transcription-PCR.

compared using the Mann-Whitney U test or Student's t-test as appropriate. All tests of significance were two-tailed, and p values <0.05 were considered statistically significant. Continuous variables are represented as median values with IQR for non-normally distributed variables and as mean $\pm$ SD values for normally distributed variables. All statistical analyses were carried out in IBM SPSS Statistics for Windows, V.23.0 (IBM Corp., Armonk, New York, USA).

## RESULTS

Of the 260 patients with COVID-19, 213 (82%) participated in this study. During the observation period, 41 (19%; 95% CI 15% to 25%) individuals remained asymptomatic, of whom 39 (95%) underwent follow-up RT-PCR testing at the care facility. Of the 172 (81%; 95% CI 75% to 86%) patients with mild symptoms of COVID-19, 144 (94%) underwent follow-up RT-PCR testing. Thus, a total of 183 patients (asymptomatic, n=39; symptomatic, n=144) with follow-up RT-PCR results were included in the analysis.

The baseline clinical characteristics of asymptomatic individuals and symptomatic patients are shown in table 1. On follow-up RT-PCR testing, 21 (54%) asymptomatic individuals and 92 (64%) symptomatic patients tested positive for SARS-CoV-2 genes (p=0.25). The mean Ct values of the SARS-CoV-2 genes in asymptomatic individuals and symptomatic patients were as

follows: *E* gene, 31.15 versus 31.43 ( $p > 0.99$ ); *RdRp* gene, 32.26 versus 32.93 ( $p = 0.92$ ) and *N* gene, 33.05 versus 33.28 ( $p > 0.99$ ) (table 1).

## DISCUSSION

A recent study on 72 314 Chinese patients reported that the proportion of asymptomatic individuals with SARS-CoV-2 was approximately 1%.<sup>8</sup> However, this study was based on patients from the Hubei Province, where the sudden shortage of health-care resources is presumed to have led to a selection bias towards the exclusion of asymptomatic individuals and patients with mild symptoms. Therefore, the actual prevalence of asymptomatic cases of COVID-19 is most likely higher than 1%. Accordingly, we found that 19% of patients with COVID-19 had remained asymptomatic from potential exposure to laboratory confirmation and facility admission.

In our study, 54% of asymptomatic individuals and 64% of patients with mild symptoms showed positive results in the follow-up PCR testing conducted at a median of 13 days from diagnosis. Similarly, Xiao *et al* reported that in 301 patients with mild-to-moderate symptoms of COVID-19, the median duration from symptom onset to an effective negative SARS-CoV-2 result was 20 days.<sup>9</sup> A recent Chinese study also reported that the median duration of viral shedding assessed by SARS-CoV-2 RT-PCR was 21 days in patients with mild COVID-19.<sup>10</sup> These results suggest that patients with COVID-19 may experience a more protracted course than that initially hypothesised. However, our results should be interpreted with caution because positive RT-PCR results do not necessarily indicate the presence of viable virus, for which cell culture is needed for confirmation. Indeed, recent studies have shown that viable virus was isolated within 10 days from the symptom onset.<sup>11 12</sup> Therefore, further studies are needed to determine whether the prolonged duration of positive RT-PCR results in asymptomatic individuals with SARS-CoV-2 infection or mildly symptomatic patients with COVID-19 can be translated into a continuous need for precaution and quarantine.

Previous studies have shown that viable SARS-CoV-2 was isolated from asymptomatic individuals and that a certain number of cases are likely to have been transmitted from asymptomatic individuals.<sup>12-15</sup> Indeed, we found that asymptomatic individuals had RT-PCR Ct values for SARS-CoV-2 genes comparable with those of patients with mild symptoms of COVID-19. Considering that most asymptomatic individuals with COVID-19 are likely to go unnoticed by healthcare workers and continue to reside within communities, such individuals may act as an essential driving force for the community spread of COVID-19 and the ongoing pandemic state. Our data thus support the general public use of face masks as well as broadening the scope of SARS-CoV-2 testing to include asymptomatic persons in certain high-risk settings until such time as further data become available regarding the duration and transmissibility of viable virus shedding from asymptomatic individuals with SARS-CoV-2 infection to support these recommendations.

This study is limited in that the study population largely comprised those in their 20s and 30s, a characteristic that is likely linked to the demographic of the Shincheonji religious group. Therefore, the prevalence of asymptomatic individuals and their viral load may be different in other age groups. Moreover, there is a possibility of recall bias concerning the self-reports of symptoms; nevertheless, as the study participants were highly cooperative and well educated on the gravity

of the current pandemic, we believe that such bias is not likely to have significantly affected our main results. Additionally, we could not obtain the results of the initial RT-PCR tests of the patients because each of them underwent RT-PCR tests at separate triages. Finally, the duration from the initial confirmation to follow-up RT-PCR testing for symptomatic patients was slightly longer than that for asymptomatic individuals; such a discrepancy occurred because the guideline of the community care facility recommended conducting follow-up RT-PCRs for patients after symptom resolution and at least 7 days after diagnosis. However, this bias is not likely to have substantially affected our main findings on the Ct values in the follow-up RT-PCR testing.

In conclusion, we report that as many as one-fifth of individuals with SARS-CoV-2 infection without severe symptoms were asymptomatic and the viral load in their nasopharynx was comparable to that in patients with mild symptoms. In addition, a large proportion of mildly symptomatic patients or asymptomatic individuals showed persistent positive upper respiratory RT-PCR results at follow-up. Asymptomatic individuals with SARS-CoV-2 infection may contribute to the ongoing community spread of COVID-19, but further studies with cell culture are needed to evaluate the transmissibility of COVID-19 from asymptomatic individuals to inform a recommendation on universal personal protective equipment.

**Acknowledgements** We thank the participants in this study.

**Contributors** Administrative, technical or logistic support: SHR, JSL, MJK, JJ; analysis and interpretation of the data: SHR, JSL, S-HK; collection and assembly of data: SHR, JSL, MJK, JJ; concept and design: S-HK; clinical revision for important intellectual content: SHR, JSL, MJK, JJ, S-HK.

**Funding** This study was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number H120C0073).

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Ethics approval** IRB of Asan Medical Center (2020-0336).

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available upon reasonable request. Deidentified participant data.

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