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Original Scientific Paper

SCREENING OF ASYMPTOMATIC COVID-19 CASES FROM THE ASPECT OF VIRUS SPREAD

Mesut Ortatatli[1], Zahir Kilic[1], Ahu Pakdemirli[1,2] and Levent Kenar[1]

[1]Department of Medical CBRN Defense, University of Health Sciences, Etlik, Ankara, Turkey;
[2]Department of Physiology, Gulhane Medical Faculty, University of Health Sciences, Ankara, Turkey

SUMMARY – The COVID-19 pandemic has hit the entire world. While symptomatic patients can be taken out of the infection chain, asymptomatic cases are very important for the spread of SARS-CoV-2. We investigated the relationship between isolating asymptomatic COVID-19 cases with close contacts by identifying and breaking the infection chain. This study included 3 asymptomatic COVID-19 cases identified during screening and 25 close contacts as a result of filiation. Twenty-five people with close contact were classified into groups A (n=8), B (n=6) and C (n=11). On SARS-CoV-2 PCR tests performed in close contacts, all of them were negative on day 0, whereas two group C subjects were positive on day 7. On antibody test applied on day 28, six subjects were positive in group A, two subjects were positive in group B, and one subject was positive in group C. A statistically significant decrease was observed in the number of infected people in the group where asymptomatic cases were identified and removed (p=0.04). It was determined that asymptomatic COVID-19 cases who were not isolated from the community had a 5-fold increased risk of infection. Screening and identification of asymptomatic cases is also essential to augment the effectiveness of quarantine and isolation measures.

Keywords: *COVID-19; Asymptomatic infection; Filiation; Close contact; Transmission*

Introduction

Viral pneumonia exhibiting a severe acute respiratory syndrome (SARS) clinical picture that nobody could have predicted how much it would affect the entire world was identified in China in the last days of 2019. The virus, which is 79% similar to SARS-CoV from the coronavirus (CoV) family and 50% similar to the Middle East respiratory syndrome (MERS-CoV), was first named as 2019 new coronavirus (2019-nCoV)[1,2]. Then, the 2019-nCoV exhibiting similar SARS clinical picture but being a virus different from SARS-CoV was named SARS-CoV-2 by the coronavirus study group[3]. The name of the new disease was designated as coronavirus disease 2019 (COVID-19) by the World Health Organization.

Until identification of SARS-CoV in the last days of 2002, coronaviruses were known as an agent isolated from many mammal animals (bat, camels, dogs and masked palm civets) causing upper respiratory disease with mild common cold symptoms in humans[4]. SARS-CoV caused 9.6% mortality with 8096 cases and 774 deaths during the epidemic in 2003[5]. MERS-CoV which emerged in 2012 caused 34%

Correspondence to: *Mesut Ortatatli, MD, PhD*, Gülhane Eğitim ve Araştırma Hastanesi KBRN AD. Bşk.lığı, Ankara, Turkey
E-mail: mortatatli@gmail.com

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mortality with 2494 cases and 858 deaths[6,7]. Although the mortality of SARS-CoV and MERS-CoV was comparatively high, no pandemics occurred because the basic reproductive numbers (R_0) were lower than SARS-CoV-2 (R_0 of SARS-CoV=3, MERS-CoV≤1, and SARS-CoV-2= 4.7-6.6)[4,6].

The receptor used by MERS-CoV on entering the cell is dipeptidyl peptidase 4 (DPP4), while SARS-CoV and SARS-CoV-2 bind to the angiotensin-converting enzyme 2 (ACE2) receptor[4,5]. The binding affinity of SARS-CoV-2 Spike (S) protein to the ACE2 receptor is 10 to 20 times higher than SARS CoV[4]. Although the clinical course of COVID-19 varies (81% mild, 14% severe, and 5% critical course), asymptomatic course of the disease is thought to be higher than expected[7,8]. The most common reported symptoms in COVID-19 patients are fever and cough, and ground-glass opacity findings on computed tomography in the lungs due to viral pneumonia aid the diagnosis[4,9]. The gold diagnostic standard of COVID-19 is to identify the SARS-CoV-2 viral nucleic acid by polymerase chain reaction (PCR) in respiratory tract swab samples. Display of antibodies (IgM and/or IgG) against the virus is also used in serologic confirmation of COVID-19[10,11].

While the contact story was at the forefront of SARS-CoV-2 infection in initial cases seen in Wuhan, it was understood that the virus could have transmitted from person to person *via* droplets[4]. Our knowledge and experience gained from previous outbreaks have shown that personal protective equipment, distance, and hand cleaning have great importance, as well as isolation, quarantine, and filiation measures in the prevention of COVID-19 pandemics.

The aim of our study was to show that the disease transmission chain could be broken with isolation measures by determining the persons contacted with COVID-19 positive cases found as the result of filiation although they were asymptomatic.

Patients and Methods

In our study, 3 asymptomatic cases with positive SARS-CoV-2 PCR test during screening and 25 suspicious cases supposed to be in close contact with confirmed cases *via* filiation were examined retrospectively. Three cases with positive PCR results were hospitalized for follow up and further treatment. According to contact history to three confirmed patients, 25 suspected cases were divided into case-matched groups A, B and C, and isolated from the public with Cohort Method. SARS-CoV-2 PCR tests were administered to 25 suspects in close contact on day 0 and 7, and antibody test was performed on day 28. PCR tests of nasopharyngeal and/or oropharyngeal swab samples were carried out in the Ministry of Health Reference Laboratory. Antibody tests were conducted using fingertip blood samples by the COVID-19 IgM + IgG Rapid Test Kit (Colloidal Gold) (Beijing Hotgen Biotech Co. Ltd., China) according to the manufacturer's instructions. All confirmed and suspected cases with COVID-19 were face-to-face interviewed to identify and follow-up the symptom history.

This study was approved by decisions of the University of Health Sciences Ethics Committee (June 9, 2020, numbered 2020-252) and Turkish Ministry of Health (2020-05-14T17_44_16).

Statistical analysis

Data were statistically analyzed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Categorical data were presented as frequencies and percentages, while continuous data that had normal distribution were presented as means and standard deviations (SD). The distribution of variables was controlled using the Kolmogorov-Smirnov test. Continuous values, in cases of normal distribution, were analyzed with Student's t-test for two independent groups. Fisher exact test was used to compare categorical data. A p value of $p<0.05$ between groups was considered statistically significantly.

Results

A total of 28 cases, 3 of which were found positive in the COVID-19 PCR screening test and 25 were found to be in close contact *via* the filiation, were included in the study. All of our cases divided into A, B and C groups were male and their mean age was 22.32±1.57 years (age range, 20-25 years). The age factor was not statistically significant between the groups (p=0.16).

The SARS-CoV-2 PCR test applied to 25 patients with close contact was negative in all on day 0 runs. However, two positive results were found in group C, while other cases were negative on day 7. Two patients with positive PCR test results were removed from the group and referred to the pandemic hospital for further examination and treatment. In addition to that, 6 cases in group A, 2 cases in group B and one case in group C were found positive on SARS-CoV-2 antibody tests performed on day 28 (Table 1).

During daily follow up screenings and isolation, two patients in group A complained of smell and taste deterioration on day 10 for two days. In all other cases, no symptom or complaint including fever, cough, respiratory distress, sore throat, nausea, vomiting or diarrhea was recorded during isolation.

In order to determine the effect of early removal of PCR positive cases from the group on transmission (contamination, infection) rate, groups A and B were combined and regrouped. There was only one in 9 cases of COVID-19 transmission in asymptomatic positive cases detected in the early period, and 8 in 14 cases in the undetectable group. There was a statistically significant difference in SARS-CoV-2 infection rate between those two groups (p=0.04). It was also found that detection of asymptomatic cases in the early period and their isolation from the public decreased the risk of COVID-19 infection 2 times (95% confidence interval (CI), 1.086-3.963) and increased it 5 times (95% CI, 0.767-34.479) if these cases were not detected (odds ratio, 10.667 both).

Discussion

The COVID-19 first emerged as atypical pneumonia cases characterized by fever, dry cough, and progressive dyspnea[12-14]. Initially, asymptomatic cases were somewhat neglected in the spread of outbreak and infection while only symptomatic COVID-19 patients were taken into consideration. There was even an expectation that when fever was below 38 °C, it would exclude the diagnosis of COVID-19. The possible case definitions such as "finding at least one of the signs and symptoms of fever or acute respiratory disease" were included in diagnostic criteria[15].

However, the contribution of asymptomatic cases to the infection spread rate was discussed amongst physicians. The estimated rate of asymptomatic COVID-19 cases then ranged from 17.9% to 30.8%, as reported in various studies[16,17]. Bai *et al.* showed that 6 people were infected with COVID-19 *via* an asymptomatic carrier[18]. In our study, we determined that 6 subjects in group A, 2 subjects in group B, and 3 subjects in group C were infected *via* 3 asymptomatic COVID-19 cases.

The presence and severity of symptoms in cases with COVID-19 were generally correlated with age and prior health conditions such as chronic respiratory disease, cardiovascular disease, diabetes, and immunosuppression[7,18,19]. Therefore, more asymptomatic cases are also expected to be amongst young age group. All asymptomatic cases in our study were in young age group and their mean age was 22.32±1.57 years.

Long *et al.* followed-up 164 cases with COVID-19 close contact for PCR and virus-specific IgG and/or IgM 30 days later. They determined antibody positivity in 16 (9.8%) cases with SARS-CoV-2 PCR positivity and 7 (4.3%) cases without PCR positivity. Ten out

Table 1. Laboratory follow-up tests applied to 25 cases with close contact and their results

Test	Day	Group A (n=8)		Group B (n=6)		Group C (n=11)	
		Positive	Negative	Positive	Negative	Positive	Negative
PCR	0	0	8	0	6	0	11
	7	0	8	0	6	2*	9
Antibody test	28	6	2	2	4	1	8

PCR = polymerase chain reaction; *two positive cases were referred to hospital for follow-up and treatment

of 23 positive cases were evaluated as asymptomatic[20]. In our study, all of our PCR and/or antibody positive cases were determined as asymptomatic except for 2 cases with nonspecific symptoms (deterioration in smell and taste). Antibody positivity was observed in 9 of 25 (36%) patients while PCR positivity was found in two (8%) cases with close contact. No abnormality was found in 5 cases with PCR positivity and they were referred to the hospital for advanced diagnostic procedures such as lung imaging (chest radiography and computed tomography) and blood tests. It was evaluated that performing the antibody test with PCR in the follow up of close contact cases had great importance in terms of determining undetected cases.

Personal protective measures have great importance in epidemic disease control and prevention in addition to isolation and quarantine. While isolation of symptomatic and confirmed cases from the public is effective in preventing the epidemic, neglecting the asymptomatic cases has negative influence on the effect of isolation measures. Thus, detection and isolation of asymptomatic cases with filiation screening is vital for public health. In our study, it was determined that non-isolated asymptomatic cases increased the risk of infection 5 times. As asymptomatic and presymptomatic COVID-19 cases are in the population, personal hygiene, wearing face mask, and proper social distancing between individuals will reduce transmission.

Since this study had some limitations including the small number of cases and lack of some laboratory data, further investigations are needed to identify the role of asymptomatic cases in the COVID-19 epidemic.

Conclusion

Our study suggests a conclusion that infection control measures such as early diagnosis, isolation, filiation, and use of personal protective equipment can slow down the rate of COVID-19 outbreak. It is estimated that the infection chain can also blocked and reduce the rate of virus transmission by regularly testing the people providing public services under risk of exposure (hairdressers, waiters, public transport drivers, salesperson, cabin crew, etc.) once a week for asymptomatic COVID-19.

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Sažetak

PROBIR ASIMPTOMATSKIH SLUČAJEVA COVID-19 U ODNOSU NA ŠIRENJE VIRUSA

M. Ortatatli, Z. Kilic, A. Pakdemirli i *L. Kenar*

Pandemija COVID-19 pogodila je cijeli svijet. Dok je simptomatske bolesnike moguće izdvojiti iz infektivnog lanca, asimptomatski slučajevi vrlo su važni za širenje virusa SARS-CoV-2. Ispitivali smo odnos izolacije asimptomatskih slučajeva COVID-19 s bliskim kontaktima kroz identifikaciju i prekidanje infektivnog lanca. Istraživanje je uključilo tri asimptomatska slučaja COVID-19 identificirana tijekom probira i 25 njihovih srodnika u bliskom kontaktu. Tih 25 osoba klasificirano je u skupine A (n=8), B (n=6) i C (n=11). U PCR testovima na SARS-CoV-2 sve su te osobe bile negativne 0. dana, dok su dvije osobe iz skupine C bile pozitivne 7. dana. U testu na protutijela provedenom 28. dana šest je osoba bilo pozitivno u skupini A, dvije osobe u skupini B i jedna osoba u skupini C. Zabilježen je statistički značajan pad broja zaraženih ljudi u skupini u kojoj su asimptomatski slučajevi identificirani i izdvojeni (p=0,04). Utvrđeno je da su asimptomatski slučajevi COVID-19 koji nisu bili izolirani iz zajednice imali 5 puta veći rizik infekcije. Probir i identifikacija asimptomatskih slučajeva bitna je i zbog povećanja učinkovitosti mjera karantene i izolacije.

Ključne riječi: *COVID-19; Asimptomatska infekcija; Srodstvo; Bliski kontakt; Prijenos*