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Original Article

Can Some Viral Respiratory Infections Observed Before the Pandemic Announcement Be Related to SARS-CoV-2?

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Abstract

OBJECTIVE: There have been doubts that SARS-CoV-2 has been circulating before the first case was announced. The aim of this study was to evaluate the possibility of COVID-19 in some cases diagnosed to be viral respiratory tract infection in the pre-pandemic period in our center.

MATERIAL AND METHODS: Patients who were admitted to our hospital's pulmonary diseases, infectious diseases, and intensive care clinics with the diagnosis of viral respiratory system infection within a 6-month period between October 2019 and March 12, 2020, were screened. Around 248 archived respiratory samples from these patients were analyzed for SARS-CoV-2 ribonucleic acid by real-time-quantitative polymerase chain reaction. The clinical, laboratory, and radiological data of the patients were evaluated.

RESULTS: The mean age of the study group was 47.5 (18-89 years); 103 (41.5%) were female and 145 (58.4%) were male. The most common presenting symptoms were cough in 51.6% (n = 128), fever in 42.7% (n = 106), and sputum in 27.0% (n = 67). Sixty-nine percent (n = 172) of the patients were pre-diagnosed to have upper respiratory tract infection and 22.0% (n = 55) had pneumonia, one-third of the patients (n = 84, 33.8%) were followed in the service. Respiratory viruses other than SARS-CoV-2 were detected in 123 (49.6%) patients. Influenza virus (31.9%), rhinovirus (10.5%), and human metapneumovirus (6.5%) were the most common pathogens, while none of the samples were positive for SARS-CoV-2 RNA. Findings that could be significant for COVID-19 pneumonia were detected in the thorax computed tomography of 7 cases.

CONCLUSION: The negative SARS-CoV-2 real-time-quantitative polymerase chain reaction results in the respiratory samples of the cases followed up in our hospital for viral pneumonia during the pre-pandemic period support that there was no COVID-19 among our cases during the period in question. However, if clinical suspicion arises, both SARS and non-SARS respiratory viral pathogens should be considered for differential diagnosis.

KEYWORDS: 2019 novel coronavirus disease, polymerase chain reaction, non-severe acute respiratory syndrome, pandemic, severe acute respiratory syndrome coronavirus 2

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INTRODUCTION

Coronaviruses (CoVs) have been well-known as a causative agent of animal diseases. They were reported to cause upper respiratory tract infections in humans in 1960s and mortal outbreaks of human diseases have emerged such as severe acute respiratory syndrome coronavirus (SARS-CoV-1) and The Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) in the last 20 years.[1] SARS-CoV-2 is the third highly pathogenic coronavirus in the 21st century, which appeared in December 2019 in China[2] following a cluster of viral pneumonia cases soon after resulting a pandemic globally.[3] The World Health Organization (WHO) named the disease caused by the SARS-CoV-2 as coronavirus disease-2019 (COVID-19).

Turkey has announced the first case on March 11, 2020, and subsequently, new cases have been reported one after another. On March 18, 2020, the first COVID-19 patient was determined by a positive real-time-quantitative polymerase chain reaction (RT-qPCR) for SARS-CoV-2 in Dokuz Eylül University Hospital in İzmir, Turkey. The clinical and radiological signs of COVID-19 in the early stages of the disease are like other viral pneumonia; therefore, the differential diagnosis is not easy. Therefore, there may be possible COVID-19 cases among the viral pneumonia treated before the date of March 11, 2020, when the first case was detected in Turkey. Thus, there are reports that SARS-CoV-2 emerged and circulated earlier than previous announcement of the first case, scientists explained. There are studies that have reported SARS-CoV-2 in the USA, Europe, and Brazil prior to the identification of the first case.[4-7]The aim of this study was to evaluate the

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possibility of some cases considered as viral respiratory tract infections in the close pre-pandemic period to be COVID-19.

MATERIAL AND METHODS

Study Population

Patients who were admitted to our hospital's pulmonary diseases, infectious diseases, and clinical microbiology and intensive care clinics within a 6-month period from October 2019 to March 12, 2020, and treated for the diagnosis of viral pneumonia were screened. There were 394 patients sampled for respiratory pathogens, and 248 samples analyzed for SARS-CoV-2 were included in the study. The clinical, laboratory, and radiological data of these patients related to COVID-19 were retrospectively scanned and evaluated through the records of the hospital database system after obtaining an non-interventional research ethics committee approval of Dokuz Eylül University Faculty of Medicine.

Clinical features (symptoms and vital signs; fever, respiratory rate, blood pressure, pulse), laboratory findings (leukocyte and lymphocyte count, C-reactive protein, procalcitonin, ferritin, lactate dehydrogenase, D-dimer), radiological features, and prognosis (recovery, intensive care transport, exitus) were the parameters that were analyzed.

Respiratory Viruses and Severe Acute Respiratory Syndrome Coronavirus 2 Testing

Real-time-quantitative polymerase chain reaction for SARS-CoV-2 RNA was performed blindly on samples collected from the patients with suspected viral pneumonia and stored at –80˚C. Nucleic acid extraction was done by EZ-1 virus mini kit using EZ-1Advanced XL platform (Qiagen, Hilden, Germany). Severe acute respiratory syndrome coronavirus 2 RNA was tested by a 1-step RT-PCR assay targeting viral RdRp (Biospeedy SARS CoV-2 qPCR detection kit, Bioeksen, Turkey). The test was performed on the RotorGene Q 5plex HRM. Human RNase P gene amplification was used as an internal control.

Each sample was tested by a syndromic panel-based multiplex RT-PCR assay for the detection of the following respiratory pathogens as a part of the routine diagnostics: Influenza A/B virus, rhinovirus, coronaviruses (NL63, 229E, OC43, HKU1), parainfluenza viruses,[1-4] human metapneumoviruses (hMPV) (A/B), bocavirus, RSV (A/B), adenovirus, enterovirus, parechovirus, and *Mycoplasma pneumonia* (Fast Track Diagnostics [FTD]respiratory pathogens-21 Assay, Siemens Healthcare GmbH, Erlangen, Germany).

MAIN POINTS

- Viruses other than SARS-CoV-2 were circulating in the pre era of COVID-19.
- There are studies on the existence of COVID-19 before its detection in other countries.
- When we evaluated our own local data, we could not show the presence of SARS-COV-2 during the pre-COVID-19 period.

Statistical Analysis

For descriptive findings, categorical variables were indicated by numbers and percentages, and variables determined by measurement will be indicated by mean ± standard deviation, (SD) median, minimum value, and maximum value. The distribution property of the variables specified in the measurement will be evaluated with the Kolmogorov–Smirnov test. The chi-square test will be used for the categorical variables specified by counting to evaluate the relationship between the independent variables and the dependent variable. In cases where the values observed in the cells do not satisfy the chi-square test assumptions, the differences in frequencies between the groups will be compared using Fisher's exact test. The *t*-test or Mann–Whitney *U* test will be used to evaluate the relationship between the variables specified in the measurement and the dependent variable, considering the normal distribution of the data. Data were evaluated with Statistical Package for Social Sciences (SPSS) version 22.0 package program (IBM Corp.; Armonk, NY, USA). Significance level $P < .05$ will be accepted.

RESULTS

The mean age of 248 patients whose SARS-CoV-2 RT-qPCR test could be analyzed was 47.5 (18-89 years); 103 (41.5%) were female and 145 (58.4%) were female. Respiratory viruses were isolated in 123 (49.6%) patients.

The presenting symptoms were cough in 51.6% (n = 128), fever in 42.7% (n = 106), sputum in 27.0% (n = 67), fatigue in 25.4% (n = 63), and sore throat in 25% (n = 62). Dyspnea (19.4%, n = 48), myalgia (13.7%, n = 34), headache (10.1%, n = 25), and nausea—vomiting (6.5%, n = 16) were the least common symptoms.

About 63% percentage (n = 158) of the patients were treated in the outpatient clinic, 33.8% (n = 84) in the service, and 2.4% (n = 6) in the intensive care unit. Sixteen cases (6.5%) were transferred from the ward to the intensive care unit. Sixty-nine percent (n = 172) of the patients were pre-diagnosed to have upper respiratory tract infection and 22.0% (n = 55) had pneumonia. Six percent (n = 15) of the patients died during the treatment period.

Non-SARS-CoV-2 respiratory viruses detected in nasal swab samples of the patients are presented in Table 1 And none of them were found to be SARS-CoV-2-positive in PCR test. Findings that could be significant for COVID-19 pneumonia were detected in the thorax computed tomography (CT) of 7 cases. The data of these cases are presented in Figure 1 and Table 2.

DISCUSSION

When the pandemic emerged soon after COVID-19 was identified in China, doubt about the existence of cases that could be COVID-19 before the detection of the first case in each country has been a matter of curiosity. Therefore, we reviewed the viral pneumonia cases in our clinic that may be associated with COVID-19 just before March 11, 2020, when the first COVID-19 case was announced in Turkey. Samples collected for the respiratory virus panel with a preliminary

Table 1. The Distribution of the Respiratory Pathogens Obtained from Nasal Swabs of the Study Population

Respiratory Pathogens	n (%)
Respiratory viruses	123 (49.6)
Non-severe acute respiratory syndrome coronavirus	11 (4.4)
Influenza	79 (31.9)
Rhinovirus	26 (10.5)
Metapneumovirus	16 (6.5)
Parainfluenza	5 (2)
Respiratory syncytial virus	5 (2)
Adenovirus	4 (1.6)
Mycoplasma pneumonia	3 (1.2)
Influenza + *Mycoplasma pneumonia*	1 (0.4)
Influenza + metapneumovirus	1 (0.4)
Parainfluenza + rhinovirus	1 (0.4)

diagnosis of viral pneumonia were then re-examined for SARS-CoV-2 PCR and none were found positive. Another viral agent was detected in 4 of 6 cases with radiological findings, suggestive of COVID-19.

The mean age of our study population was relatively young (47.5), and gender distribution was in favor of women. There were respiratory viruses in 49.6% of the patients. In a prospective study of European primary care including adults with symptoms of lower respiratory tract infection, 45.8% of the patients had microbiologically confirmed common respiratory virus infection (influenza virus, hMPV, respiratory syncytial virus (RSV), coronavirus (CoV) or rhinovirus) with a mean age of 50, mostly consisting of females similarly with our findings.[8] Another study has also reported the mean age of 45.2 with a slight male predominance.[9] On the other hand, the demographic data on COVID-19 reveal that all age groups are at risk of getting COVID-19; however, patients aged > 60 years and males are prone to severe infection in COVID-19.[10]

The presentation of lower respiratory tract infection of viral etiology has been defined as mostly self-limiting, and influenza virus, hMPV, RSV, CoV, or rhinovirus have been reported to have a significantly higher symptom score. Cough, sputum, and rhinorrhea were the most commonly reported symptoms.[8] In another study including 5859 individuals suspected of influenza-like illness, cough, rhinorrhea, and headache were the most common symptoms and dyspnea was especially associated with RSV (odds ratio: 2.33, 95% CI: 1.73-3.12).[11] In our study, cough, fever, and sputum were the most common symptoms. Therefore, although cough was

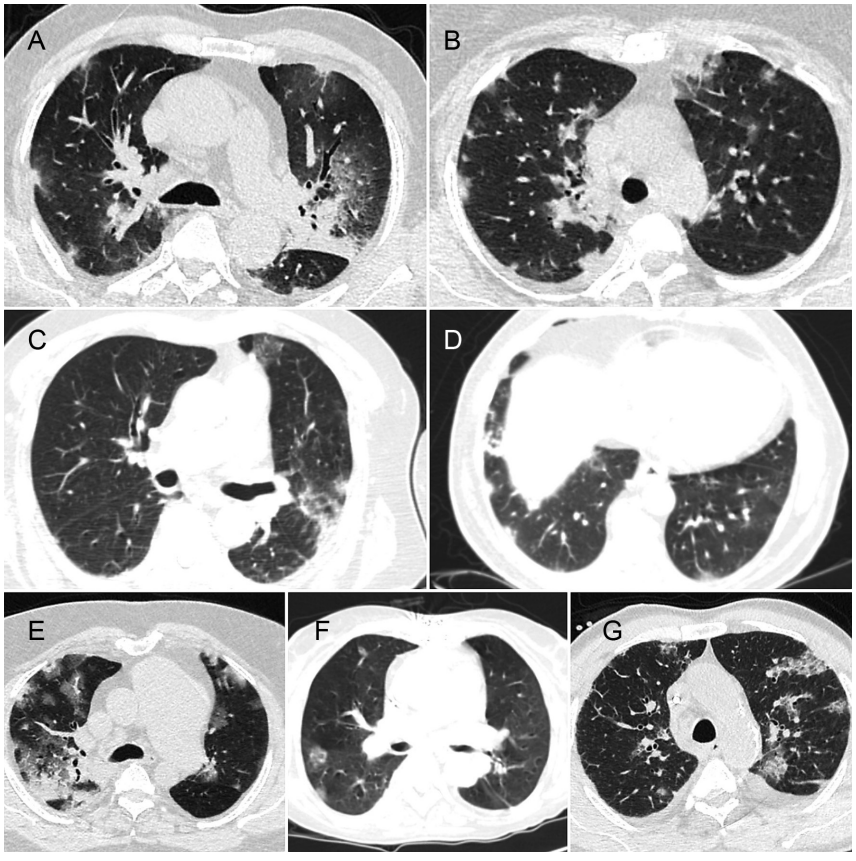

Figure 1. (A) Consolidated areas of ground-glass density in the upper lobes of both lungs and peripheral ground-glass density infiltrates in the lower lobes. (B) Peripherally located nodular infiltration areas in the upper and lower lobes of both lungs. (C) Peripheral weighted patchy ground-glass densities in both lungs. (D) Peripheral predominantly faintly circumscribed peripheral ground-glass density nodular infiltrates in the lower lobe of both lungs. (E) Peripheral-peribronchovascular consolidations and areas of ground-glass density in the upper and lower lobes of both lungs. (F) Ground-glass areas in both lungs, with focal patchy centrilobular distribution more prominent in the right lung. (G) Infiltration areas of more prominent ground-glass density in the left upper lobe in both lungs.

Table 2. Characteristics of 7 Cases Whose Radiological Findings Were Suggestive of COVID-19 Pneumonia

Case No	Age/Sex	Co-morbidity	Detected Microorganism in Nasal Swab	Lymphocyte Count (10 × 3/μL)	CRP (mg/L)	Procalcitonin (mg/L)	Prognosis
1	70M	+	Influenza A	0.5	434	42	Exitus
2	52/F	–		0.9	22	-	Discharged from service
3	70/M	+		1	97	0.13	Discharged from service
4	59/M	+		1.1	328	-	Discharged from service
5	60/M	+	Influenza A	0.9	360	0.32	Discharged from service
6	65/F	+	Metapneumovirus	0.3	45	0.21	Exitus
7*	68/M	–		1.1	84	-	Discharged from service

All were negative for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) except *Whose specimen was not adequate for reinvestigation of SARS-CoV-2 polymerase chain reaction.
CRP; C reactive protein.

the most frequent presentation for both SARS-CoV-2 and non-SARS-CoV-2 agents, symptoms like rhinorrhea and headache should be considered in viral pneumonia. Fever and fatigue have been reported more in COVID-19.[10]

Sixty-nine percent (n = 172) of the patients were pre-diagnosed to have upper respiratory tract infection and 22.0% (n = 55) had pneumonia. Six percent (n = 15) of the patients died during the treatment period.

Most of the patients evaluated for viral respiratory tract infection in our study had moderate clinical severity and one-third required hospitalization with a 6% mortality. This may be due to the fact that the study population consisted of patients whose viral respiratory tract specimens were studied without distinction of upper-lower respiratory tract infection. In our study, 22.0% of the patients had pneumonia. In a study including 954 patients suspected to have respiratory viruses, there was 27.5% positivity, and the patients had a mean pneumonia severity index (PSI) score of 42, H1N1 was found to have highest PSI.[9] Viral pneumonia has been reported to account for approximately one-third of community-acquired pneumonia cases, in a wide range of clinical course of mild to severe.[12] Generally, adenovirus, hMPV, para-influenza virus, RSV, and influenza A and B viruses suffer mild symptoms and have a low mortality.[13] Poor outcomes are both associated with viral and host factors including older age, comorbidities, solid organ transplant, and hematopoietic stem cell transplant.[14] The clinical spectrum of SARS-CoV-2 ranges from asymptomatic to moderate or severe disease with multiorgan failure. Asymptotic infections have been reported between 27% and 40% and hospitalization rates were between 4% and 7% depending on the population characteristics.[15]

In our study, the most common viral agents were influenza virus (31.9%), rhinovirus (10.5%), and hMPV (6.5%) of the tested population. In other studies, similarly, influenza was also the most common pathogen (9.9%-48%) depending on the influenza season, followed by rhinovirus (4.3%-7.9%) and hMPV (1.8%-4.1%).[9-11] In a large systemic review of community-acquired pneumonia in European adults, influenza virus A and B were most frequently (9%) identified viruses, followed by rhinovirus, coronavirus, and parainfluenza (2%-7%).[16]

Research is being conducted in many countries to investigate the question marks about the global circulation of SARS-CoV-2 before December 2019. Several studies by different groups retrospectively demonstrated the presence of antibodies and viral RNA in clinical samples, and SARS-CoV-2 community circulation was demonstrated by detecting viral RNA in wastewater at a time inconsistent with November 2019.[17] In USA, antibodies for SARS-CoV-2 were determined in more than 100 blood samples in early December 2019.[4] In France, SARS-CoV-2 antibodies were detected in serum samples and a respiratory sample before December 2019.[5,18] In Brazil, SARS-CoV-2 community spread was demonstrated at the end of November 2019 in wastewater.[7] The presence of SARS-CoV-2 in different parts of the world at an earlier stage than currently accepted will provide basic clues for the assessment of this pandemic and make a very important contribution to future pandemic preparedness.

Findings that could be significant for COVID-19 pneumonia were detected in the thorax CT of 7 cases. The data of these cases are presented in Figure 1 and Table 2. Although some clinical (sputum was less frequent in COVID-19) and laboratory parameters (leukocyte, lymphocyte, and C-reactive protein levels were lower in COVID-19) may guide to distinguish COVID-19 from other viral pathogens, radiological appearance might be more helpful.[19] Peripheral involvement, pure ground-glass opacity, and apicobasal gradient were more likely to be present in COVID-19. On the contrary, thickening of the bronchial walls and micronodules were more frequently associated with other respiratory viruses.[20,21] Of the 7 patients having radiological signs of COVID-19, influenza was positive in 2, and hMPV in 1, C-RP was highest in influenza cases.

CONCLUSION

The absence of positive PCR results for SARS-CoV-2 in the nasal swab samples of the patients who were followed up with a viral pneumonia clinic in the pre-pandemic period in our hospital supports the fact that there was no COVID-19 among our cases at that time. Cases with significant findings in terms of COVID-19 on thorax CT suggest that other viral pneumonia may be confused with COVID-19 clinically and radiologically; when clinical suspicion arises, PCR testing should be performed for both SARS and non-SARS respiratory viral pathogens for differential diagnosis.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Dokuz Eylül University Faculty of Medicine (approval No: 2020/13-46).

Informed Consent: Informed consent was not obtained due to the retrospective design of the study. Data analysis permission was obtained from the hospital administration, provided that it is used by anonymizing.

Peer-review: Externally peer-reviewed.

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