Original Investigation / *Özgün Araştırma*

DOI: 10.5578/ced.20219606 • *J Pediatr Inf 2021;15(4):e235-e238*

Tracking SARS-CoV-2, İzmir, Turkey

SARS-CoV-2 İzinin Sürülmesi, İzmir, Türkiye

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Cite this article as: Appak Ö, Güzel I, Gülmez A, Arslan N, Duman M, Belet N, et al. Tracking SARS-CoV-2, İzmir, Turkey. J Pediatr Inf 2021;15(4):e235-e238.

Abstract

Objective: With the rapid spread of SARS-CoV-2, a new coronavirus, around the world, a pandemic was declared by World Health Organization on March 2020. The first cases were reported in March 2020 from Turkey. In our hospital, the first pediatric case was detected on April 2, 2020. However, there is no data on whether this virus had been present in our region or not before this date. The aim of our study was to determine the first entry of SARS-CoV-2 virus to our region for pediatric patients.

Material and Methods: SARS-CoV-2 positivity was investigated retrospectively with the RT-qPCR method in the pediatric respiratory tract specimens taken between the October 1, 2019 and March 31, 2020. In the specimens, SARS-CoV-2 RNA was studied using real-time PCR based "COVID-19 RT-qPCR Detection Kit".

Results: 886 samples were included in the study. Of the respiratory tract specimens, 97.1% were nasopharyngeal swabs, 2.8% were bronchoalveolar lavage. Most frequently, rhinovirus (28.6%), influenza A subtype H1N1 (pandemic H1N1) (18.5%) and influenza B (16%) were detected. Rhinovirus and enterovirus were the most frequent double agents seen together. No SARS-CoV-2 positivity was detected in the respiratory tract specimens studied.

Conclusion: SARS-CoV-2 PCR test was conducted in a limited number of centers at the beginning of the pandemics may have affected the detection of the first case in Turkey. Multicenter studies of archived samples would enable more realistic results in tracking SARS-CoV-2 in our country.

Keywords: SARS-CoV-2, respiratory viruses, pediatric

Öz

Giriş: Yeni bir koronavirüs olan SARS-CoV-2'nin dünya çapında hızla yayılmasıyla birlikte, Mart 2020'de Dünya Sağlık Örgütü tarafından bir pandemi ilan edildi. İlk vakalar Mart 2020'de Türkiye'den bildirildi. Hastanemizde ilk pediatrik vaka 2 Nisan 2020 tarihinde tespit edildi. Ancak bu virüsün bölgemizde bulunup bulunmadığına dair bu tarihten önce herhangi bir veri bulunmamaktadır. Çalışmamızın amacı, pediatrik hastalar için SARS-CoV-2 virüsünün bölgemize ilk girişini belirlemektir.

Gereç ve Yöntemler: SARS-CoV-2 pozitifliği, 1 Ekim 2019 ile 31 Mart 2020 tarihleri arasında alınan pediatrik solunum yolu örneklerinde RT-qPCR yöntemi ile retrospektif olarak araştırıldı. Örneklerde, SARS-CoV-2 RNA, gerçek-zamanlı PCR tabanlı "COVID-19 RT-qPCR Tespit Kiti" kullanılarak çalışıldı.

Bulgular: Çalışmaya 886 örnek dahil edildi. Solunum yolu örneklerinin %97.1'i nazofaringeal sürüntü, %2.8'i bronkoalveolar lavajdı. En sık rinovirüs (%28.6), influenza A alt tipi H1N1 (pandemik H1N1) (%18.5) ve influenza B (%16) tespit edildi. Rinovirüs ve enterovirüs birlikte görülen en sık çift etkenlerdi. İncelenen solunum yolu örneklerinde SARS-CoV-2 pozitifliği tespit edilmemiştir.

Sonuç: SARS-CoV-2 PCR testinin, Türkiye'de pandeminin başlangıcında sınırlı sayıda merkezde yapılmış olması ilk vakanın tespitini etkilemiş olabilir. Arşivlenmiş örnekler ile yapılacak çok merkezli çalışmalar, ülkemizde SARS-CoV-2'nin izlenmesinde daha gerçekçi sonuçlar sağlayacaktır.

Anahtar Kelimeler: SARS-CoV-2, solunum virüsleri, pediatrik

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Received: 11.05.2021 **Accepted:** 05.08.2021 **Available Online Date:** 29.12.2021

Introduction

World Health Organization (WHO) China Country Office reported pneumonia cases with unknown aetiology in Wuhan city of Hubei State of China on December 31, 2019. Its cause was revealed on January 7, 2020 as a new coronavirus (2019-nCoV) that had not been detected in humans before. The virus was named SARS-CoV-2 because of its close resemblance to SARS-CoV and the disease was named as COVID-19 (1).

Coronaviruses are single-stranded, positive-sense, enveloped RNA (+ssRNA) viruses with diameters of 80-220 nm. They are the members of *Coronavirinae* subfamily of the *Coronaviridae* family of the *Nidovirales* order. This subfamily is divided into four genera as alphacoronavirus (Alpha CoV), betacoronavirus (beta-CoV), gammacoronavirus and deltacoronavirus. Seven coronaviruses are known today as causing disease in humans [Alpha CoV; (HCoV)-NL63, 229E, beta-CoV; (HCoV)-OC43, HKU1, SARS-CoV, MERS-CoV ve SARS-CoV-2)]. While Alpha CoV and beta-CoVs in general cause respiratory diseases in humans and gastroenteritis in animals, gamma and deltacoronaviruses cause infection in avian species (2,3).

The disease is transmitted via air and droplets. Also, it is transmitted when sick individuals disperse droplets by coughing and sneezing, and others come into contact with these droplets through their hands and then touch their mouth, nose or eye mucosae. The most frequent symptoms of the infection are fever, cough and dyspnea. In more severe cases, pneumonia, severe acute respiratory tract infection, kidney failure may develop and the disease can result in death (4). In our country, the first COVID-19 case was detected on March 11, 2020. In our hospital, the first adult case was detected on March 18, 2020 and the first pediatric case was detected on April 2, 2020. However, there is no data on whether this virus had been present in our region or not before this date.

In our study, it was planned to evaluate pediatric patients who presented to our hospital with findings of respiratory tract infection retrospectively, to scan SARS-CoV-2 RNA in the respiratory tract specimens in our archive and to conduct an investigation by sequence analysis in case positivity was detected. Our study will provide data on when the virus entered our region and make a contribution to understanding the dynamics of the pandemics.

Materials and Methods

886 respiratory tract specimens that had been sent to the Central Laboratory from the pediatric inpatients and outpatients of Dokuz Eylul University Faculty of Medicine (DEU) Hospital between October 1, 2019 and March 31, 2020 for the investigation of the causes for viral respiratory tract infection and that had been kept at -70°C were included in the study. In these samples pertaining to upper and lower respiratory tract (nasopharyngeal swab, nasopharyngeal aspirate and bronchoalveolar lavage), the viruses causing respiratory infection were studied using real-time PCR based Fast Track Diagnostics/Respiratory Pathogens 21 (FTD-21) [Junglinster, Luxemburg] kit.

In the specimens, SARS-CoV-2 RNA was studied using real-time PCR based "COVID-19 RT-qPCR Detection Kit" (Biospeedy, Ankara, Turkey). Viral target region in the kit is the SARS-CoV-2 RNA-dependent RNA polymerase (RbRp) gene fragment, and human ribonuclease P (RNase P) gene fragment was used as internal control. Samples with "cycle thresholds" (Ct) <40 were evaluated as positive. The extraction procedure was carried out with "Viral Nucleic Acid Isolation Kit" (Biospeedy, Ankara, Turkey) in accordance with the instructions of the producer. The results of the study were evaluated qualitatively. PCR test was repeated for samples in which reaction was detected in the first test using nucleic acid extraction EZ1 Virus Mini Kit V 2.0 (QIAGEN, Germany). The extraction procedure was carried out in EZ1 Advanced XL (QIAGEN) device in accordance with the instructions of the production company.

Our study was conducted upon the approval of Dokuz Eylül University Non-Invasive Research Ethical Committee (Decision no: 2020/13-27, Date: 15.06. 2020). Necessary study permissions were taken from the https://bilimselarastirma.saglik.gov.tr/ address of Health Services Directorate, T.R. Ministry of Health, and from the Medical Directorate of DEU Research and Application Hospital (date: May 14, 2020 and no: 72292585-00.99-E.41313).

Results

The mean age of the patients whose 886 samples were included in the study was 61.4 months (minimum 0.2 - maximum 246.2 months), and 45.7% (405/886) were females and 54.3% were males. 53.6% (475/886) of the patients were outpatients and 46.4% of them were inpatients.

Of the respiratory tract specimens, 97.1% (861/886) were nasopharyngeal swabs, 2.8% were bronchoalveolar lavage. Causative agents were detected with FTD-21 kit in 70.4% (624/886) of these samples. 83.3% (520/624) of these were with a single agent, while two or more agents were together in 16.7% (104/624). Most frequently, rhinovirus (28.6%), influenza A subtype H1N1 (pandemic H1N1) (18.5%) and influenza B (16%) were detected (Table 1). Among the co-infections, two agents were positive in 88.5% (92/104), three agents in 8.6% (9/104), and four agents in 2.9% (3/104). Rhinovirus and enterovirus were the most frequent double agents seen together.

In eight specimens, weak reaction was detected by Biospeedy, COVID-19 RT-qPCR test with a Ct value between 35 and 40. The samples of these patients were extracted with

Table 1. Respiratoy panel results

Positive agents	Total (n)	% of positive
IFN A	2	0.4
IFN A H1N1 (pandemic H1N1)	96	18.5
IFN B	83	16
rhinovirus	149	28.6
Cor	17	3.2
Cor 229E	2	-
Cor NL63	5	-
Cor OC43	-	-
Cor HKU-1	10	-
PIV	35	6.7
PIV-1	13	-
PIV-2	3	-
PIV-3	8	-
PIV-4	11	-
Mycoplasma pneumoniae	2	0.4
bocavirus	13	2.5
MPV	43	8.3
RSV A/B	57	11
adenovirus	19	3.7
enterovirus	3	0.5
parechovirus	1	0.2
Total	**520**	**100**

IFN A: Influenza A, IFN B: Influenza B, Cor: Coronavirus, PIV: Parainfluenza virus, MPV: Metapneumovirus, RSV: Respiratory syncytial virus.

Table 2. SARS-CoV-2 suspected positive patients

Respiratory panel date	Sex	Samples type	Symptomps	Respiratory panel results
31.12.2019	F	NS	Fever	metapneumovirus
3.01.2020	F	NS	Fever, cough, otalgia	influenza B virus
7.01.2020	M	NS	Fever, otalgia, nasal congestion	influenza A (H1N1)
9.01.2020	M	NS	Nasal congestion, sore throat	coronavirus NL63
16.01.2020	M	NS	Fever, nasal congestion	negative
23.01.2020	M	NS	Fever, erythema, itching	negative
21.02.2020	M	NS	Fever, runny nose	rhinovirus
25.02.2020	M	NS	Fever, cough, weakness	rhinovirus

F: Female, M: Male, NS: Nasopharyngeal swab.

EZ-1, and SARS-CoV-2 RT-qPCR tests were repeated and the results were detected as negative. In six of these specimens, other respiratory viruses were positive (Table 2).

Discussion

In our study, airway samples taken from pediatric patients in the period between October 2019 and March 2020 were scanned to provide epidemiological data and to detect the entry date of SARS-CoV-2 to our region. No SARS-CoV-2 RNA presence was detected in the specimens. In our study encompassing the flu season of 2019-2020, the most frequent causative agent was detected to be rhinovirus with a percentage of 28.6%. In another study conducted in our center, which examined the 7-years distribution of respiratory tract viruses in pediatric patients, rhinovirus was detected to be the agent that was most frequently detected each year (5).

As far as we know, no other study in which respiratory tract specimens of pediatric cases were scanned for SARS-CoV-2 retrospectively has been reported in our country. In the literature, there are similar studies in which respiratory tract specimens pertaining to adult and/or pediatric patients were evaluated in various other countries. In France, although the first official COVID-19 case was reported in January 24, 2020, SARS-CoV-2 RNA test of a patient with pneumonia findings on December 27, 2019 was detected to be positive in a retrospective study (6,7). The absence of a travel to China or another country in this patient's health history shows that SARS-CoV-2 had been in circulation in France at the end of December 2019 (7). In a study conducted in Spain where the first case was detected on February 25, 2020, specimens of 170 patients between January 1, 2020 and February 25, 2020 were studied for SARS-CoV-2. Similar to our study, SARS-CoV-2 was detected in none of the samples. Other respiratory viruses were detected in 87% of the samples in the same study and these were found as influenza A, rhinovirus, coronavirus and respiratory syncytial virus in the order of frequency (8). In a study that investigated 1700 respiratory tract specimens pertaining to the last two months of 2019 in San Francisco, where the first case was detected in February 6 2020, for SARS-CoV-2, all specimens were detected to be negative (9).

In our study, weak reaction was detected in the first SARS-CoV-2 RT-qPCR test results of eight specimens. While six of these specimens were positive for other respiratory viruses, no positivity was detected in two of them with the test used. The extraction system routinely used for SARS-CoV-2 caused solely cell lysis in only five minutes and revealed the nucleic acid. Because the rapid method used was not a classical extraction method, tests for suspicious reactive specimens were repeated with a different method that provided extraction and nucleic acid concentration. SARS-CoV-2 was detected to be negative in all specimens with RT-qPCR that was studied like this. In a study in Scotland that investigated SARS-CoV-2 in 174 respiratory tract specimens that were archived before the first case was seen on March 1st, 2020, 166 samples were detected to be negative with RT-PCR that targeted RbRp and E gene regions. Eight samples were negative for RbRp but because suspicious reaction was detected in the E gene region, the test was repeated with another RT-PCR commercial kit, and similar to our study, they were detected to be negative (10).

In conclusion, our study is important with regard to tracking SARS-CoV-2 in our region. The restrictive aspect of our study is that it includes the data of a single center. The fact that SARS-CoV-2 PCR test was conducted in a limited number of centers at the beginning of the pandemics may have affected the detection of the first case in Turkey. Therefore, the study of the archived samples as we did in our study and disclosure of the local data would enable a greater number of samples to be studied and more realistic conclusions to be reached.

Ethics Committe Approval: The study was approved by Dokuz Eylül University Non-Interventional Research Ethics Committee (Decision no: 2020/13-27, Date: 15.06.2020).

Informed Consent: Patient consent was obtained.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - ÖA, AAS, MD, NB; Design - ÖA, AAS; Supervision - ÖA, AAS, MD, NB; Resource - AAS; Data Collection and/or Processing - AG, IG, NA, ÖA; Analysis and/or Interpretation - AG, IG, NA, ÖA; Literature Search - AG, IG, NA, ÖA; Writing - ÖA, AAS; Critical Review - AAS, MD, NB.

Conflict of Interest: All authors declare that they have no conflicts of interest or funding to disclose.

Financial Disclosure: The authors declared that this study has received no financial support.

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