The disappearance of respiratory syncytial virus and influenza viruses in children during the second year of the COVID-19 pandemic – are non-pharmaceutical interventions as effective as vaccines?

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Abstract. – OBJECTIVE: Respiratory viral diseases are common in children. A viral diagnostic test is necessary, because COVID-19 shows signs and symptoms similar to those of common respiratory viruses. The article aims at analyzing the presence of respiratory viruses that were common before the pandemic in children who were tested for suspected COVID-19, and is also concerned with how common respiratory viruses were impacted by COVID-19 measures during the second year of pandemic.

PATIENTS AND METHODS: Nasopharyngeal swabs were examined to detect the presence of respiratory viruses. The respiratory panel kit included SARS-CoV-2, influenza A and B, rhinovirus/enterovirus, parainfluenza 1, 2, 3 and 4, coronaviruses NL 63, 229E, OC43, and HKU1, human metapneumovirus A/B, human bocavirus, respiratory syncytial virus (RSV) A/B, human parechovirus, and adenovirus. Virus scans were compared during and after the restricted period.

RESULTS: No virus was isolated from the 86 patients. SARS-CoV-2 was the most frequently observed virus, as expected, and rhinovirus was the second, and coronavirus OC43 was the third. Influenza viruses and RSV were not detected in the scans.

CONCLUSIONS: Influenza and RSV viruses disappeared during the pandemic period and rhinovirus was the second most common virus after the CoVs during and after the restriction period. Non-pharmaceutical interventions should be established as a precaution to prevent infectious diseases even after the pandemic.

Key Words:
COVID-19, SARS-CoV-2, Respiratory viruses, Respiratory syncytial virus, Influenza, Children.

Introduction

Respiratory viral diseases are common in children. Although their incidence varies in different reports[1-3], respiratory syncytial virus (RSV), influenza viruses, human rhinovirus (HRV), parainfluenza viruses (PIV), coronaviruses (CoVs) and adenovirus are frequently observed. Viral, bacterial and non-infectious respiratory pathologies should also be considered in the differential diagnosis. The clinical presentation usually consists of respiratory symptoms like fever, headache, cough, weakness, and nasal discharge, which presents a similar clinical picture[1-3]. In 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been declared a pandemic by the World Health Organization (WHO)[4-6] and it is also known to cause respiratory tract disease in children. Polymerase chain reaction (PCR) tests are recommended for suspicious cases as the symptoms are not specific[7,8].

In this study, we investigated the presence of respiratory viruses that were commonly seen before the pandemic in children who were tested for suspicion of the COVID-19. We tried to understand the accuracy of the COVID-19 screening criteria. During the pandemic, measures like social distance, face masks, hand hygiene, and ventilation of indoor spaces [non-pharmaceutical interventions (NPI) were implemented in Turkey, as in many other countries]. We aimed to reveal how the common respiratory viruses were affected by the measures during the second year of the pandemic. We attempted to determine the prevalence

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of influenza and RSV viruses in particular, which were important pathogens before the pandemic. We also had the opportunity to make comparisons between the period of restrictions and the period after several restrictions were lifted.

Patients and Methods

Our study included patients who applied to the Pediatric Emergency service of a Health Center in Kecioren (approximately 1 million population), one of the largest districts of Ankara, the capital of Turkey. We prospectively analyzed 207 patients (108 girls and 99 boys) aged 1 month to 18 years who visited the Pediatric Emergency Outpatient Clinic between April 2021 and November 2021. The patients were grouped according to their age as 1 month, 5 years, 5-12 years, and ≥ 12 years. According to the SARS-CoV-2 Management and Treatment Guidelines[9] for Pediatric Patients by the Turkish Ministry of Health, patients who were recommended to undergo a viral PCR diagnostic test were selected as the case group. We recorded the patients' contact history, age, sex, complaints, symptoms, and physical examination findings. We then performed laboratory tests, including complete blood count, creatine phosphokinase (CPK), C-reactive protein (CRP), ferritin, troponin, and D-dimer tests, as well as chest radiography. Chest radiographs were classified as normal, mild, moderate, or severe involvement, all with reticular infiltration. Patients with chronic diseases or a more severe clinical picture were excluded from the study.

The nasopharyngeal swab sample was transferred by the transport medium to the center where the respiratory virus panel was studied. The samples (combined throat and nose swab) were transported to the laboratory in a transport medium (VNAT-Bioeksen, Istanbul, Turkey) and stored at -80°C. The samples were analyzed by multiplex RT-PCR (BiospeeedyR, Bioksen, Istanbul, Turkey), which analyzes 21 respiratory tract viruses simultaneously, at the National Virology Reference Laboratory of the General Directorate of Public Health. The reactions were performed on a Bio-Rad CFX96 qPCR system. The respiratory panel kit included SARS-CoV-2, influenza A and B, rhinovirus/enterovirus, parainfluenza (PIV) 1,2,3 and 4, coronaviruses (CoVs) NL 63, 229E, OC43, and HKU1, human metapneumovirus (HMPV) A/B, human bocavirus (HBoV), respiratory syncytial virus (RSV) A/B, human parechovirus (HPeV), and adenovirus. RT-PCR was used to detect the viruses.

Complete blood counts were studied with an auto-hematology analyzer (Mindray Model: BC 6800- Chicago, IL, USA). Biochemistry tests were performed with an AU5800 analyzer (Beckman Coulter, Tokyo, Japan). D-dimer tests were performed with Sysmex CS-2100i (Siemens, Tokyo, Japan). Ferritin and troponin tests were performed using Advia Centaur XPT (Siemens, Tokyo, Japan).

While the study was ongoing, the public lockdown was gradually lifted after May 17, 2021 (curfew restrictions for children under 18 were also removed). Therefore, we separated the data as before and after May 17, 2021. Also, while our study continued, primary and secondary schools were reopened for face-to-face education in September 2021, and the vaccination program was started for those over the age of 12. Thus, we were able to compare the data before and after the schools were reopened.

Statistical Analysis

SPSS for Windows, version 22.0 (IBM Corp., Armonk, NY, USA) was used to analyze the data. $p < 0.05$ was considered significant. For skewed distributions, continuous data were expressed as mean, and standard deviation (SD). Number of cases (%) was used to describe categorical data. The Mann-Whitney U test was used to compare statistical differences in non-normally distributed variables between two independent groups. Pearson's Chi-square test was used to compare categorical variables. On all statistical analyses, a p-value < 0.05 was accepted as the significant level.

Results

The demographic data of the patients are given in Table I. The predominant patients group in the study was those aged ≥ 12 years. No virus was isolated from the 86 patients; those who were positive for SARS-CoV-2 constituted approximately one-third of the total patient group (n = 60). According to the virus scan results, SARS-CoV-2 ranked first, as expected, followed by rhinovirus and CoV-OC43. Influenza viruses, RSV, and HPeV were not detected in the scans (Table II). More than one virus was detected in six cases: HRV+ enterovirus in 4, PIV-3+HRV in 1, and CoV-OC43+HMPV in 1.

SARS-CoV-2 was mostly isolated from patients' ≥ 12 years of age, and it was isolated the least from the 1 month-5 years age group, with a statistically significant difference ($p \leq 0.003$). Table III shows the virus results according to age groups.

Table I. Demographic characteristic of the patients.

Age in years, mean (range)	11 (3-18 months)
Age groups	**n (%)**
1 month-5 y	39 (18.8)
5-12 y	55 (26.6)
≥12 y	113 (54.6)
Gender	
Female	108 (52.2)
Male	99 (47.8)

The patients were questioned and compared according to their complaints of fever, sore throat, headache, weakness, diarrhea, abdominal pain, muscle-joint pain, cough, respiratory distress, and loss of taste and smell. Among the complaints, sore throat and headache were found to be significantly higher among SARS-CoV-2-positive cases; diarrhea and respiratory distress were significantly higher among SARS-CoV-2-negative cases. No significant difference was found between the other complaints. Laboratory results showed that white blood cell, lymphocyte, and platelet counts were significantly lower in the SARS-CoV-2-positive group. Besides, CRP levels were higher in the SARS-CoV-2-negative group. There was no significant difference in the results of chest radiography.

With similar practices all over the world, Turkey began to gradually lift the lockdown measures taken against SARS-CoV-2 as of May 17, 2021. The curfew for individuals under the age of 18 was gradually lifted and public areas, including restaurants and shops, were reopened. Because this change took place during our study, we had the opportunity to obtain and analyze the virus profiles during and after the restriction period (Table IV). The remarkable change after May 17 was a significant increase in the incidence of rhinovirus and SARS-CoV-2 and the changes in SARS-CoV-2 variants ($p \leq 0.05$).

Table II. Viral PCR results of patients.

	n (%)
Virus negative	86 (41.5)
HRV	29 (13.9)
HEV	1 (0.5)
SARS-CoV-2/B1.1.7	10 (4.8)
CoV-OC43	14 (6.8)
CoV-229E	1 (0.5)
SARS-CoV-2/E484K	1 (0.5)
Adenovirus	1 (0.5)
SARS-CoV-2	49 (23.7)
PIV-3	3 (1.4)
PIV-4	2 (1.0)
HBoV	4 (1.9)
HRV+HEV	4 (1.9)
HRV+PIV-3	1 (0.5
HMPV+CoV-OC43	1 (0.5)
Influenza virus A, B	0 (0.0)
HPeV	0 (0.0)
RSV A/B	0 (0.0)

PCR: Polymerase chain reaction, HRV: Human Rhinovirus, HEV: Human Enterovirus, PIV: Parainfluenza virus, HBoV: Human Bocavirus, HAdV: Adenovirus, HMPV: Human Metapneumovirus, HPeV: Human Parechovirus, RSV: respiratory syncytial virus.

Table III. Test results for different age groups.

Age	1 month-5 years n (%)	5 year-12 years n (%)	≥ 12 years n (%)
Virus negative	17 (40.4)	23 (41.8)	46 (40.0)
SARS-CoV-2	3 (7.1)	14 (25.4)	32 (27.8)
HRV	10 (23.8)	11 (20.0)	13 (11.3)
SARS-CoV-2/B1.1.7	0 (0.0)	1 (1.8)	9 (7.8)
CoV-OC43	4 (9.5)	3 (5.5)	8 (6.9)
SARS-CoV-2/E484K	0 (0.0)	0 (0.0)	1 (0.8)
HEV	2 (4.7)	0 (0.0)	3 (2.6)
CoV-229E	1 (2.3)	0 (0.0)	0 (0.0)
HAdV	1 (2.3)	0 (0.0)	0 (0.0)
PIV-3	2 (4.7)	2 (3.6)	0 (0.0)
PIV-4	0 (0.0)	0 (0.0)	2 (1.7)
HBoV	2 (4.7)	1 (1.8)	1 (0.8)

HRV: Human Rhinovirus, HEV: Human Enterovirus, PIV: Parainfluenza virus, HBoV: Human Bocavirus, HAdV: Adenovirus, HMPV: Human Metapneumovirus, HPeV: Human Parechovirus, RSV: respiratory syncytial virus.

Table IV. Comparison of the incidence of the most commonly occurring viruses during and after the restriction period.

	During the restrictions n (%)	After the restrictions n (%)	*p*
Virus negative	18 (38.3)	68 (42.5)	
SARS-CoV-2	5 (10.6)	44 (27.5)	**0.020**
HRV	6 (12.8)	28 (17.5)	0.510
SARS-CoV-2/B1.1.7	10 (21.3)	0 (0.0)	**<0.010**
CoV-OC43	5 (10.6)	10 (6.3)	0.330
SARS-CoV-2/E484K	1 (2.1)	0 (0.0)	0.220
SARS-CoV-2 total	16 (34.0)	44 (27.5)	0.460
Non-SARS CoV	6 (12.8)	10 (6.3)	0.200
HEV	3 (6.4)	2 (1.3)	0.070
CoV-229E	1 (2.1)	0 (0.0)	0.220
HAdV	0 (0.0)	1 (0.6)	1.000
PIV-3	0 (0.0)	4 (2.5)	0.570
PIV-4	0 (0.0)	2 (1.3)	1.000
PIV total	0 (0.0)	6 (3.8)	0.340
HBoV	0 (0.0)	4 (2.5)	0.570
HMPV	0 (0.0)	1 (0.6)	1.000

HRV: Human Rhinovirus, HEV: Human Enterovirus, PIV: Parainfluenza virus, HBoV: Human Bocavirus, HAdV: Adenovirus, HMPV: Human Metapneumovirus, HPeV: Human Parechovirus.

Again, while our study was continuing, schools were reopened in mid-September and face-to-face education was re-initiated. Also, children over the age of 12 were included in the scope of vaccination. So, we had the opportunity to separately evaluate and analyze the impact of opening the schools (Table V). Although there was no significant change in the incidence of the other viruses, there were changes in the SARS-CoV-2 variants.

Table V. Comparing the incidence of the most common viruses before and after schools are opened.

	Before, *n* (%)	After, *n* (%)	*p*
Virus negative	52 (42.3)	34 (40.5)	
SARS-CoV-2	23 (18.7)	26 (31.0)	0.040
HRV	22 (17.9)	12 (14.3)	0.560
SARS-CoV-2/B1.1.7	10 (8.1)	0 (0.0)	< 0.010
CoV-OC43	8 (6.5)	7 (8.3)	0.780
SARS-CoV-2/E484K	1 (0.8)	0 (0.0)	1.000
SARS-CoV-2 total	34 (27.6)	26 (31.0)	0.640
Non-SARS CoV	9 (7.3)	7 (8.3)	0.790
HEV	5 (4.1)	0 (0.0)	0.080
CoV-229E	1 (0.8)	0 (0.0)	1.000
HAdV	1 (0.8)	0 (0.0)	1.000
PIV-3	3 (2.4)	1 (1.2)	0.640
PIV-4	0 (0.0)	2 (2.4)	0.160
PIV total	3 (2.4)	3 (3.6)	0.680
HBoV	2 (1.6)	2 (2.4)	1.000
HMPV	0 (0.0)	1 (1.2)	0.400

HRV: Human Rhinovirus, HEV: Human Enterovirus, PIV: Parainfluenza virus, HBoV: Human Bocavirus, HAdV: Adenovirus, HMPV: Human Metapneumovirus, HPeV: Human Parechovirus.

Discussion

COVID-19 is less common and has a milder disease course among younger children. Despite the various complaints and symptoms of children, it has been reported[10,11] that the most common complaints are cough and fever. We observed no difference between girls and boys, as well as most of the cases were over the age of 12 years. Sore throat and headache were found to be significantly higher among SARS-CoV-2-positive cases. The laboratory findings mostly involved elevated CRP levels, leukopenia, lymphopenia, and elevated fibrinogen[12,13]. Consistent with the literature, significant levels of leukopenia, lymphopenia, and thrombocytopenia were observed in the present study. In our study, CRP levels were found to be higher among SARS-CoV-2-negative patients. According to our results, there was no specific complaint or symptom of COVID-19, but some laboratory tests may prove useful in the differential diagnosis.

A viral diagnostic test is necessary for COVID-19 because it shows sign and symptoms similar to those of common respiratory viruses, but its follow-up and treatment must be different[11,14]. The viruses that cause acute respiratory disease and that were common before the pandemic are influenza, RSV, parainfluenza, and rhinovirus. The reports[15,16] in our country are similar; in previous periods, RSV and influenza were the most common viruses in the childhood age group. Studies[17-19] from many countries have reported that this picture has changed after the pandemic and that respiratory viruses, which are frequently observed in children, have decreased, disappeared, or their frequency has changed. We obtained similar data from our study. We found that influenza, RSV, PIV, adeno, metapneumo and bocaviruses were rare after the onset of restrictions and precautions against the COVID-19 pandemic. SARS-CoV-2 was the most isolated virus, while rhinovirus increased during the pandemic and became the most frequently isolated virus after CoVs.

Rhinovirus activity was not affected by SARS-CoV-2. Similar studies[20,21] reported that rhinovirus, RSV, and adenovirus were prominent in children and adults. The fact that the rhinovirus continues to exist during the pandemic and that it has raised the most as the restrictive measures are lifted makes us think it is highly contagious. Rhinoviruses are in the *Picornaviridae* family and are non-enveloped viruses so might be less susceptible to inactivation by soap and-water hand washing.

The most striking result of our study was that the RSV and influenza viruses, which were the most common respiratory viruses before the pandemic, were not isolated from any of the patients. We realized that this result did not change after the social restrictions were lifted or after the schools re-initiated face-to-face education. Similarly, several studies[22-24] have reported that the influenza virus has almost completely disappeared. In our health center where the study was conducted, a small number of influenza and RSV cases started to be seen as of December 2021, after our case collection process was completed. Since CoVs and other viruses spread by droplet transmission, non-pharmaceutical interventions have reduced the presence of non-coronal respiratory viral agents. Besides, public lockdowns and the closure of public areas, including schools, universities, restaurants, and shops were very effective[25,26]. Fortunately we had the opportunity to examine the cases during and after the public lockdown and before and after the schools were reopened; thus, we were able to observe the effects of different stages of restrictions on children. Accordingly, the rhinovirus was the most seen after SARS-CoV-2 during the lockdown period and after the restrictions were lifted. Thus, isolation and NPIs were highly effective during the second year of the pandemic. We also observed that the reopening of schools had no significant effect on the virus profiles. This could be associated with the lesser impact of the pandemic on children, the simultaneous initiation of vaccination, and a higher awareness for children.

Conclusions

Once the containment measures are completely lifted, other respiratory viruses may appear again with more intensity. This could be caused by a weakened immune response due to the precautions taken during the pandemic; prolonged exposure to viral antigens is thought to weaken the immune response. Even though the SARS-CoV-2 pandemic is over, it is clear that NPIs are effective at preventing the transmission of other common respiratory viruses. Besides vaccination, NPIs against respiratory viruses may stop infections. Given that we live in crowded environments, it seems logical that these measures should still be maintained in a certain way.

Conflict of Interest

The authors declare that they have no conflicts of interests.

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Authors' Contributions

Conception and design: Kışlal FM; acquisition of data: Hanilçe Y, Büyükbaşaran ZE; analysis and interpretation of data: Kışlal FM, Altaş B, Güven D; drafting the article: Hanilçe Y; Güven D; supervision: Altaş B; validation and final approval: all authors.

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Availability of Data and Materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Informed Consent

Consent was obtained from the families of the patients participating in the study.

Ethical Approval

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethics Committee approval was obtained from Ankara Atatürk Sanatorium Training and Research Hospital's Ethics Committee (Approval number and date: No. 2222, dated March 09).

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