Viruses, among the leading agents of infectious diseases, are one of the most important causes of respiratory tract infections that can lead to severe morbidity and mortality in childhood.\(^1\) Recognised as respiratory tract viruses, adenovirus (AoV), human bocavirus (HBoV), human coronaviruses (HCoV), human metapneumovirus (HMPV), human parainfluenza 1–4 viruses (HPIV 1–4), human rhinovirus/enterovirus (HRV/EV), influenza virus (IV) A and B, respiratory syncytial virus (RSV), and parechovirus play a predisposing role in the development of acute respiratory tract infections and are widely circulated.\(^2\)

The epidemiology of respiratory viruses in acute respiratory tract infections varies seasonally from region to region around the world.\(^2,3\) The annual virus distribution in the northern hemisphere is as follows: IV and RSV in December-January-February (winter); rhinoviruses in spring and fall; HPIV type 1 in...
winter, type 3 in spring-summer; some non-rhinovirus enteroviruses in summer; HMPV year-round, especially in spring; AoV and HBOV year-round. HCoV infections are primarily detected in the winter and spring months in temperate climates and can be detected at low levels throughout the year. While HCoV OC43 and 229E subtypes are more common in winter and spring, there are reports that NL63 and HKU1 subtypes are more predominantly detected in winter.

COVID-19, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the seventh known coronavirus to infect humans, has caused humanity to face a coronavirus pandemic for the first time. Uncertainties about the disease in the early stages of the pandemic, the lack of available treatment or protective vaccines against the virus, and the rapid spread of the infection around the world have created a serious burden on health services.

Serious public health measures have been taken to slow the spread of infection and gain time to increase knowledge about SARS-CoV-2 and the disease caused by this virus. These measures led to changes in people’s lifestyles and caused serious disruptions in interpersonal social activities within the community.

In the first year of the COVID-19 pandemic, significant changes were noticed in the circulation of other respiratory viruses. The aim of this study was to evaluate the change in the seasonal dynamics of respiratory viruses and its reflection on the clinic, especially between the first year when the bans were completely lifted and face-to-face education started and the following year.

Material and Methods

Data from this single-center, cross-sectional study was collected between February 1, 2021 and December 1, 2022 at Prof. Dr. Cemil Taşçıoğlu City Hospital, a tertiary education and research hospital with a capacity of approximately 100 pediatric beds in Istanbul. This hospital is a reference training and research hospital serving approximately 350,000 pediatric outpatients and 3500 pediatric inpatients annually. The study did not include almost the first year of the pandemic because we started evaluating respiratory tract viruses by multiplex PCR in our hospital approximately one year after the pandemic.

All children included in the study were pediatric patients between the ages of 1 month and 18 years who were hospitalized with infectious causes and whose etiology could not be determined. Swabs from the nasopharyngeal region were tested for SARS-CoV-2 and other respiratory viruses by multiplex polymerase chain reaction (PCR).

The ethical committee of Prof. Dr Cemil Taşçıoğlu City Hospital approved the study (No: 2022/315).

Demographic characteristics, hospitalization diagnoses, intensive care requirements of the patients, and months and seasons in which viruses were detected were obtained from hospital medical records.

Non-pharmacological pandemic control in the world and in Türkiye

Personal non-pharmacological measures included the use of face masks, respiratory etiquette, and the importance of hand hygiene, maintaining a social distance of at least 2 meters between individuals, screening and isolating sick individuals, identifying and quarantining people in contact.

Non-pharmacological measures taken by the society were making the use of face masks mandatory in public indoor environments and public transportation, closing schools and childcare facilities and switching to online education, postponing indoor meetings and major events, closing non-essential businesses within the scope of stay-at-home and quarantine measures, prohibiting eating indoors in restaurants, tracking contacts, and
informing the public about the routes and dynamics of virus transmission through various communication methods.

The environmental non-pharmacological measure was the disinfection of contacted surfaces.

Non-pharmacological measures taken by countries were travel restrictions between cities and countries, border closures, health screenings at entry and exit points, quarantine measures at entrances, and compulsory screenings.

In our country, almost all of the above-mentioned measures were implemented in the early stages of the pandemic, and curfews were imposed on certain age groups (such as those over 65 and under 20) at certain hours.

**Statistical analysis**

The relationship between the measures taken in terms of public health in our country and the detected data was analyzed. SPSS 15.0 for Windows program was used for statistical analysis. Descriptive statistics were given as numbers and percentages for categorical variables, and numerical variables were presented as mean, standard deviation, minimum and maximum. The rates in the groups were compared with the chi-square test. The statistical alpha significance level was accepted as p<0.05.

**Results**

A total of 1173 patients who were hospitalized, followed and treated with prediagnoses of fever etiology, acute gastroenteritis, febrile seizures, encephalitis, sepsis, rash, myocarditis, soft tissue infections, lymphadenopathy or eye infections, and especially acute respiratory tract infections, were tested for SARS-CoV-2 and other respiratory tract viruses by multiplex PCR. 659 patients were male and 514 were female, with a median age of 29.5 months (1-216 months). Five hundred fifty-seven patients were between 1 month and 24 months, 255 patients between 25 and 60 months, 183 patients between 61 and 120 months, and 178 patients were 121 months and older.

Five hundred thirteen viruses were detected in 631 patients tested in 2021 and 526 viruses were detected in 542 patients tested in 2022. The five most frequently detected viruses in 2021 were SARS-CoV-2, HRV/EV, RSV, HPIV (47 patients, 43 of which had type 3 HPIV), and HBoV, while those detected in 2022 were SARS-CoV-2, HRV/EV, AoV, RSV, HCoVs (42 patients, most frequently OC43 in 17 patients). The distribution of detected viruses by months and years is shown in Fig. 1.

In the five-month period between February 1, 2021 and July 1, 2021, when school attendance was online and the restrictions were not entirely lifted, 88 patients were detected to be positive. Of these, 75% (66 patients) were positive for SARS-CoV-2. Among the rest, HRV/EV (12 patients) was the most commonly detected, followed by AoV (7 patients), HCoV OC43 (3 patients), HPIV type 3 and HBoV (2 patients each) and RSV and Parechovirus (1 patient each). Seven hundred fifty-seven viruses were detected in the first one-year period (July 1, 2021- July 2022), when the restrictions were lifted completely and face-to-face education started at all levels of schools. SARS-CoV-2 was detected in 270 patients, followed by HRV/EV (164 patients), RSV (69 patients), AoV (49 patients), HPIV type 3 (47 patients), HBoV (39 patients), influenza A (32 patients), HMPV (30 patients), and HCoV OC43 (21 patients).

In 834 patients, at least one SARS-CoV-2 or other viruses, alone or in combination, were detected. The viruses detected and their numbers are shown in Table I. Among the patients with confirmed HPIV, 51 had type 3, 20 had type 1, 6 had type 4 and 5 had type 2. The subtypes detected in patients with HCoV were OC43 in 33, NL63 in 11, HKU1 in 10, and 229E in 7. The mean age of SARS-CoV-2 positive patients was 64.3 months (1-214 months), and 50.4% were between 1 month-24 months (188 patients) and 22.5% were 121 months and older (84 patients).
Fig. 1. Distribution of SARS-CoV-2 and other viruses by year and month.

Fig. 2. Distribution of SARS-CoV-2 and other viruses by age.
In a total of 178 patients aged 121 months and older, 47.2% were positive for SARS-CoV-2, which was statistically significant. The distribution of SARS-CoV-2 and other viruses by age groups is shown in Fig. 2. Co-infection with other respiratory viruses was detected in 46 of a total of 373 SARS-CoV-2 positive patients detected during the study period. In 38 patients, SARS-CoV-2 was co-infected with a single other respiratory virus, while in the remaining patients, two viruses were co-infected. The most common respiratory virus accompanying SARS-CoV-2 was HRV/EV in 18 patients, followed by RSV in 10 patients and influenza and human coronaviruses (OC43 in 5 and 229E in 1) in 6 patients each. Co-infection with other respiratory viruses was detected in 55 of 109 RSV-positive patients. In 43 patients, RSV was accompanied by a single virus, while at least two respiratory viruses were found in the remaining 12 patients. The most common concomitant virus in RSV-positive patients was HRV/EV, which was recorded in 22 patients. This was followed by HCoVs, which were positive in 14 patients (OC43 in 12 patients and 229E and NL63 in two patients). Interactions between respiratory viruses are shown in Table I.

During the study period, 386 patients were hospitalized for lower respiratory tract infections (acute bronchiolitis, pneumonia), 238 patients for upper respiratory tract infections (cryptic tonsillitis, sinusitis, acute otitis media, sinusitis, epiglottitis, croup), 202 patients for fever etiology, 111 patients for acute gastroenteritis and 236 patients for other diagnoses (such as convulsions, encephalitis, sepsis, rash, myocarditis, soft tissue infections, lymphadenopathy, eye infections).

During the study period, 113 patients (9.6%) required intensive care. The need for intensive care was statistically significantly higher for infections with Bocavirus and Respiratory Syncytial Virus between 1 month and 24 months, in the period July 1, 2021-July 1, 2022 (the first period when schools were held full-time face-to-face at all levels).

Discussion

The development and worldwide dissemination of effective vaccines and the development of a certain level of immunity to the disease among people have led to a reduction in the frequency of infections caused by SARS-CoV-2 and the gradual withdrawal of bans. In our country, interactions between respiratory viruses are shown in Table I.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Co-Infections</th>
<th>Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>Only one</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>327  (87.6%)</td>
<td>38</td>
</tr>
<tr>
<td>HRV/EV</td>
<td>133  (58.6%)</td>
<td>74</td>
</tr>
<tr>
<td>RSV</td>
<td>54   (49.5%)</td>
<td>43</td>
</tr>
<tr>
<td>HPIV (1,2,3,4)</td>
<td>41   (50.6%)</td>
<td>35</td>
</tr>
<tr>
<td>AoV</td>
<td>30   (42.8%)</td>
<td>29</td>
</tr>
<tr>
<td>HCoV</td>
<td>18   (30.5%)</td>
<td>27</td>
</tr>
<tr>
<td>HBoV</td>
<td>18   (40%)</td>
<td>20</td>
</tr>
<tr>
<td>Influenza</td>
<td>22   (62.8%)</td>
<td>11</td>
</tr>
<tr>
<td>HMPV</td>
<td>20   (62.5%)</td>
<td>7</td>
</tr>
<tr>
<td>Parechovirus</td>
<td>-</td>
<td>5</td>
</tr>
</tbody>
</table>

the full removal of the bans took place on July 1, 2021. Starting with the 2021-2022 academic year, face-to-face education was provided to students at all levels. In our study, the detection of viruses other than SARS-CoV-2 (66) was relatively low (28 respiratory viruses) before schools started face-to-face education. There was a significant increase in other respiratory viruses when schools started face-to-face education. During this period, while 270 SARS-CoV-2 viruses were detected, 487 were detected from other respiratory viruses. This is important because it shows that schools are a significant environmental factor for children in the spread of respiratory viruses.

Strict non-pharmaceutical measures taken worldwide have not only had an impact on SARS-CoV-2. They were also expected to have effects on other respiratory viruses transmitted directly or indirectly by contact or through airborne or droplet transmission. The debate on whether prolonged non-pharmaceutical measures affect community immunity to common pathogens and the circulation of viruses in the community has become more evident with recently published studies. In studies reported from Europe, the United States and Canada, a decrease of 45-70% was observed in emergency room admissions for children in the few months following the first COVID-19 case detection or the declaration of the pandemic. In the two-month period after the pandemic was declared and the first case was seen in our country, on March 11, 2020, a significant decrease was detected in our hospital emergency room admissions compared to the same periods of previous years. For the two months between March 11 and May 11, hospital, the visits to the green zone among pediatric patients were very high in the pre-pandemic period. While there were 613 green zone visits per day in 2018 and 919 green zone visits in 2019, it decreased to 224 visits in 2020. With the pandemic, the intense coverage of uncertainties about the disease in the visual and written media and the reports of the serious course of the disease, especially in adults, may have caused fear in parents. Due to this fear, we think that patients with non-severe illnesses were treated at home instead of coming to the hospital.

With the pandemic, it has been reported that there has been a dramatic decline in diseases caused by respiratory viruses in many countries around the world. A study by Vittucci et al. showed a significant reduction (around 80%) in illnesses caused by respiratory viruses. It was observed that influenza (0 patients) and RSV (5 patients) infections were almost eliminated. In a study reported by Ippolito et al. between November 1, 2020 and February 28, 2021, there was an 80% decrease in pediatric patients hospitalized for lower respiratory tract infections compared to previous years, with no hospitalizations due to influenza and RSV. In another study reported from Italy, it was shown that hospitalizations due to acute respiratory tract infection decreased by 82.2% between March 9, 2020, and February 28, 2021, with the lowest detection of other respiratory viruses including RSV (4 cases) and HRV/EV (1 case). According to the weekly influenza surveillance reports of our country, a significant decrease in respiratory tract viruses evaluated within the scope of Sentinel SARI Surveillance among inpatients due to severe acute respiratory infection (SARI) between April 2020 and December 2021 (which coincides with the first autumn and winter seasons after the pandemic) was detected. In the first season after this pandemic, no influenza positivity was reported in our country as in most countries, a decrease of over 99% in RSV-related infections was detected and 1 case was
reported. In our hospital, a significant decrease in hospitalizations due to lower respiratory tract infectious diseases caused by respiratory viruses other than COVID-19 was observed during this period.

During the initial period of the pandemic, there were significant changes in the seasonal dynamics of respiratory viruses due to several factors, most notably the strictly implemented non-pharmaceutical measures. In many countries, a decline in the circulation of most respiratory viruses other than HRV/EV was observed. The magnitude, timing, and duration of this reduction varied by respiratory virus. Historically, the lowest levels were detected in influenza virus and RSV infections. These low levels did not persist in the second year of the pandemic as non-pharmaceutical measures were relaxed.

In a study conducted in France, it was observed that respiratory tract viruses were detected intensively. HRV/EV was detected most frequently, followed by RSV, AoV, HPIV types and HCoV types, respectively. Compared to previous periods, RSV had its first peak unexpectedly between February-April 2021 and its second significant wave between November 2021-January 2022. The influenza virus started to circulate in the 38th-40th weeks and peaked in the 51st week. In a study reported from Slovenia, almost all viruses were observed to return between April 2021 and March 2022. This is explained by the complete removal of bans, and the opening of schools. The RSV peak detected in September and October 2021 was earlier than in previous years. Influenza A infections were detected later than expected. HBoV1 was found to be high in April, May, June and July, HPIV types in May and June, HCoV types in February and March, AoVs were detected almost all year round, and HRV/EV was detected at the highest levels in September, March and July, but above certain levels throughout the year. Another study reported from Germany examined the seasonal dynamics of respiratory viruses between January 2016 and January 2020. It was found that the detection rates of HBoV, HCoV, HPIV, and RSV viruses decreased during periods when non-pharmaceutical measures were intensively implemented. Unlike other seasonal viruses, INV, HMPV and HRV/EV remained at low levels even after the measures were removed. After the restrictions were lifted, an unseasonal increase in HBoV and RSV, a peak in HPIV detection rate delayed by two months compared to the pre-pandemic period, and a peak in HCoV detection with a delay of several months were observed. In our study, no influenza virus was detected until December 2021. In 2021, RSV was observed for the first time in 1 patient in June and 1 patient in July, and increased from August onwards, the highest number of patients (27 patients) was reached in October and was statistically significant, then decreased and disappeared throughout the winter. In 2022, RSV was most common in November. HBoV was detected in a small number of patients in June, September, and October 2021, but the statistically greatest rates were found in November. The number of cases continued to decline in December and was detected sporadically throughout the study period in 2022. HMPV was detected in 3 patients only in December 2021, and in 2022, it continued to be seen until August, with the highest rates in January, March, and April. Among the HPIV types, especially type 3 was observed significantly in the summer and fall of 2021. In 2021, the highest rate was detected in November, with the second highest rate in August. While HPIV Type 1 was not seen at all in 2021, it was seen in the summer and fall of 2022. HPIV type 4 was detected in 3 patients each in the fall in both 2021 and 2022, while type 2 was detected in 1 patient only in October 2021 and in a total of 4 patients in all seasons except summer in 2022. Adenovirus was found throughout the year, more frequently in 2022. Among HCoV types, OC43 was observed in the summer and fall of 2021 and in winter 2022, while NL63 and HUK1 were not observed in 2021, but were observed in spring 2022. HRV/EV, on the other hand, was detected in all four seasons in 2021 and 2022, peaking in the fall and spring in both years.
In studies reported from many countries, HRV/EV were shown to be less affected by non-pharmaceutical measures taken against COVID-19.\textsuperscript{17,18,20} In a study conducted in Türkiye within the first year of the pandemic, HRV/EV was the most frequently detected virus, even more frequently than SARS-CoV-2.\textsuperscript{26} In our study, HRV/EV and AoV were less common in circulation with SARS-CoV-2 before the complete cessation of the restrictions. Shortly after, a significant increase in HRV/EV, HBoV, RSV, HCoV OC43, and HPIV was observed. A study reported from Germany found that HBoV, HCoV, HPIV, and RSV viruses showed an unseasonal increase in the period when measures were removed, similar to ours.\textsuperscript{25}

The fact that viruses are non-enveloped makes them more resistant to external environments and allows them to live longer on surfaces. This allows them to be transmitted through direct or indirect contact. It has also been reported that non-enveloped viruses can pass through surgical masks.\textsuperscript{27} For these reasons, especially HRV/EV has found a place in circulation in most countries even in the first period of the pandemic. However, in most reported studies, including our study, HBoV has not been shown to circulate like HRV/EV in the first year of the pandemic. We think that this may be related to viral interference. When the host is infected with more than one virus, complex interactions between these viruses are likely to occur. Among different virus-virus interactions, so-called viral interference is the direct or indirect antagonistic effect of one virus against another virus. It can do this by affecting the ability of the other virus to cause infection or disease in the host. Competition between viruses for resources can occur, leading to the down-regulation of receptors required for intracellular entry in the host, or by stimulating the innate immune response and interferon release.\textsuperscript{4} In the 2009 H1N1 pandemic, such an association between influenza and HRV was observed.\textsuperscript{28} A similar association was also shown in a study that found that previous HRV infection prevented SARS-CoV-2 replication by accelerating the responses of interferon-stimulated genes in the upper respiratory tract.\textsuperscript{29} The influenza virus has remained low throughout the pandemic, even at its historically lowest level in most countries. Even when the measures were relaxed or even removed, and the expected peak did not occur. This was also observed in our study. The failure of influenza to reach its expected peak during the first year of the pandemic and even after the relaxation of bans may be due to viral interference with SARS-CoV2 or other respiratory viruses.

Following the lifting of restrictions around the world, it was observed that seasonal viruses did not follow the seasonal pattern they exhibited in the pre-pandemic period. At the end of 2020, an increase in inter-seasonal RSV infection rates was reported in Australia. In the same report, an increase was found in RSV infections between 2-4 years of age.\textsuperscript{30} A study reported from Slovenia revealed that an early RSV epidemic peak was observed between September and October 2021. In addition, more hospitalizations occurred between the ages of 0-4 years compared to previous years.\textsuperscript{24} RSV activity has increased since April 2021 in the publication reported from the United States. In some areas, there was an increase at an unexpected time.\textsuperscript{21} While RSV was not seen in Tokyo in 2020, the largest increase in RSV cases occurred in 2021.\textsuperscript{31} In our study, the average age of patients found to be RSV-positive was 14.4 months (1-146 months). The patient with the oldest age was SARS-CoV-2-positive and had a co-infection. Twenty-seven patients (24.7%) who were found to be RSV-positive received intensive care support. The mean age of patients receiving intensive care support was 8.7 months (1-86 months) and 70% of the patients were 4 months or younger. Co-infection with HBoV was detected in 4 of the RSV-positive patients requiring intensive care. We think that the increase in the rate of RSV infection at unexpected times and in an older age group and again the higher need for intensive care may be due to a lack of contact with RSV infection during pregnancy and early childhood, and immune-debt occurring in
children as a result of decreased immunity in older children and in the general population.

This study has some limitations. First of all, this was a single-center retrospective study. The hospital where the study was conducted is one of the many hospitals in the city where pediatric patients are hospitalized and followed up. The lack of data from other hospitals shows that the results will not cover the whole city. However, it can give a rough idea. The study does not include almost the first year of the pandemic, as respiratory tract multiplex PCR was not performed in our hospital. Therefore, data on other respiratory viruses are missing in almost the first year of the pandemic when public health measures were intensively implemented. In addition, evaluation of respiratory tract viruses by multiplex PCR was performed in our hospital approximately one year after the pandemic. Therefore, we could not make a comparison with the pre-pandemic period due to the lack of data from the years before the pandemic in our hospital. However, it is important that it includes the 5 months before the full removal of bans and the period immediately after. Since only inpatient data were used in our study, the results cannot be attributed to the entire population. However, due to the concerns of families early in the pandemic, more young children were hospitalized for follow-up due to COVID-19, leading to mild illnesses in hospitalization diagnoses.

Although it was concluded that with the removal of the bans, infections with other respiratory viruses, especially PIV, RSV and BoV, increased significantly earlier than the expected months when they were frequently detected before the pandemic, RSV and BoV-associated infections led to higher intensive care needs, even HRV/EV-associated infections led to intensive care needs, and influenza virus-associated infections did not increase as expected, it will be important to confirm this with more comprehensive prospective studies and to compare it with the pre-pandemic period with multicenter data.

**Ethical approval**

All procedures, including the informed consent process, were conducted per the National Health and Medical Research Council of Turkey’s ethical standards and the Helsinki Declaration. The ethical committee of Prof. Dr Cemil Tascioglu City Hospital approved the study (No:2022/315). Informed consent was obtained from participants.

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: AK, DKİ, ÖK; data collection: AK, ÇK, DKİ, ÖK, IE, LB, AB, GAT, EA; analysis and interpretation of results: AK, ZA; draft manuscript preparation: AK, DKİ, ÖK, AB. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare the study received no funding.

**Conflict of interest**

The authors declare that there is no conflict of interest.

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