original article

Impact of non-pharmaceutical interventions on circulating respiratory viruses during the COVID-19 pandemic in Turkey

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Citation: Kirca F, Aydoğan S, Gozalan A, Güler E, Uyan Erten AZ, Özşen Uygur AS, et al. Impact of non-pharmaceutical interventions on circulating respiratory viruses during the COVID-19 pandemic in Turkey. Ann Saudi Med 2023; 43(3): 143-153. DOI: 10.5144/0256-4947.2023.143
Received: March 3, 2023

Accepted: May 12, 2023

Published: June 1, 2023

Funding: None.

BACKGROUND: Non-pharmaceutical interventions (NPIs) applied to limit the SARS-CoV-2 pandemic also affect the circulation and seasonal characteristics of other respiratory viruses.
OBJECTIVES: Assess the impact of NPIs on the spread and seasonal characteristics of non-SARS-CoV-2 respiratory viruses and examine viral respiratory co-infections.
DESIGN: Retrospective cohort
SETTING: Single center in Turkey.
PATIENTS AND METHODS: Syndromic multiplex viral polymerase chain reaction (mPCR) panel results of patients admitted to the Ankara Bilkent City Hospital with symptoms of acute respiratory tract infection between April 1, 2020 and October 30, 2022 were evaluated. Two study periods before and after 1 July 2021, when the restrictions were discontinued, were statistically analyzed and compared to determine the effect of NPIs on circulating respiratory viruses.
MAIN OUTCOME MEASURES: Prevalence of respiratory viruses as determined by syndromic mPCR panel.
SAMPLE SIZE: 11 300 patient samples were evaluated.
RESULTS: At least one respiratory tract virus was detected in 6250 (55.3%) patients. Of these, at least one respiratory virus was detected in 5% in the first period (between April 1, 2020 and June 30, 2021, when NPIs were applied), and in 95% in the second period (between July 1, 2021 and October 30, 2022, when NPIs were relaxed). After the removal of NPIs, there was a statistically significant increase in hRV/EV, RSV-A/B, Flu A/H3, hBoV, hMPV, PIV-1, PIV-4, hCoV-OC43, PIV-2 and hCoV-NL63 ($P<.05$). In the 2020-2021 season, when strict NPIs were applied, all respiratory viruses evaluated did not have the usual seasonal peak and there were no seasonal influenza epidemics during this period.
CONCLUSIONS: NPIs resulted in a dramatic decrease in the prevalence of respiratory viruses and notable disruption of seasonal characteristics.
LIMITATIONS: Single-center study and retrospective.
CONFLICT OF INTEREST: None.

The Coronavirus Disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), was first detected in Wuhan, China in December 2019. The World Health Organization (WHO) declared the outbreak as pandemic on March 11, 2020, and following this date, the first case was reported in Turkey.[1] To limit the transmissibility of COVID-19, WHO recommended personal measures (e.g., face masks, hand hygiene), social distancing measures (e.g., quarantining, staying at home, school closures), movement measures and special populations measures (e.g., elderly and healthcare workers).[2] Accordingly, a number of strict non-pharmaceutical interventions (NPIs) and lockdowns of varying severity were also adopted in Turkey[1] and most of the mitigation strategies were strictly applied for 15 months following the declaration of the pandemic. However, NPIs were officially discontinued after July 1, 2021, except for mask mandates and social distancing.[3,4]

Although the purpose of implementing NPIs is to limit the transmissibility of COVID-19, studies in other countries have reported that such restrictions are also effective in limiting the spread of non-SARS-CoV-2 respiratory viruses.[5,6] The aim of this study was to assess the impact of NPIs on the spread and seasonal characteristics of non-SARS-CoV-2 respiratory viruses and also to examine viral respiratory co-infections in Turkey. Thus, the circulation patterns of respiratory viruses were examined in a total of 11 300 patient samples for a 2.5-year period by comparing the data obtained before and after July 1, 2021.

PATIENTS AND METHODS

This single center, retrospective study was conducted between April 1, 2020 and October 30, 2022 in the Ankara Bilkent City Hospital, which is a tertiary care hospital. Syndromic multiplex viral polymerase chain reaction (mPCR) panel results of patients who presented to the emergency department and infectious diseases clinic with acute respiratory tract symptoms and in whom the indication for the test was decided after clinical examination were evaluated retrospectively. In addition, the study period was divided into two periods, before and after July 1, 2021, when the restrictions were discontinued, and these two periods were compared in order to determine the impact of NPIs on circulating respiratory viruses during the COVID-19 pandemic. Demographic and microbiological data of the patients were obtained from the hospital medical records. This study was carried out with the approval of the Ankara Bilkent City Hospital Ethics Committee (no.E2-22-2745 dated November 9, 2022) and in accordance with the principles of the Declaration of Helsinki of the World Medical Association.

Oropharyngeal and nasopharyngeal (OP/NP) swabs were collected from patients and were placed in a viral transport media (VTM; various manufacturers). Pathogen detection was performed by the QIAstat-Dx Respiratory SARS-CoV-2 Panel, a point-of-care (POC) multiplex test (Qiagen, Hilden, Germany). Combining nucleic acid extraction and real-time polymerase chain reaction (RT-PCR), the QIAstat-Dx analyzer is a fully automatic diagnostic device which uses a multiplexed RT-PCR test for the detection of 19 viral and 3 bacterial pathogens. The assay was performed according to the manufacturer's instructions. The OP/NP samples in the transport media were loaded into a QIAstat-Dx analyzer test cartridge (Qiagen, Hilden, Germany) and then into the QIAstat-Dx analyzer module (Qiagen, Hilden, Germany); results were available in approximately 70 minutes.

The respiratory panel kit included *Mycoplasma pneumoniae, Clamydophila pneumoniae, Bordetella pertussis*, influenza A virus (Flu A), influenza A subtype H1N1/2009 virus (Flu A/ H1N1(pdm09), influenza A subtype H1 virus (Flu A/H1), influenza A subtype H3 virus (Flu A/H3), influenza B virus (Flu B), coronavirus HKU1 (hCoV-HKU1), coronavirus 229 E (hCoV-229E), coronavirus OC43 (hCoV-OC43), coronavirus NL63 (hCoV-NL63), adenovirus (hAdV), parainfluenza virus 1 (PIV-1), parainfluenza virus 2 (PIV-2), parainfluenza virus 3 (PIV-3), parainfluenza virus 4 (PIV-4), human metapneumovirus A/B (hMPV), bocavirus (hBoV), rhinovirus/enterovirus (hRV/EV), respiratory syncytial virus A/B (RSV-A/B), and SARS-CoV-2.

Statistical analysis was performed by the SPSS software version 23.0 (IBM Corporation, Armonk, NY, US). The normality of continuous data was assessed by histogram and Kolmogorov-Smirnov methods. Median and interquartile range values were used for non-normally distributed variables. Differences between the groups were evaluated by the chi-squared test or Fisher's exact test, as appropriate. Values of $P<.05$ were considered statistically significant.

RESULTS

A total of 11 300 OP/NP samples were evaluated. At least one respiratory tract virus was detected in 6250 (55.3%) patients. The number of detected viruses was 7469 in total. The most prevalent virus was hRV/EV in 2572 isolates (34%), followed by RSV-A/B in 1126 (15%) and Flu A/H3 in 506 (6.7%) (**Table 1**). The virus isolation rate was 55% in men and 45% in women. When the

gender distribution of the isolated viruses was evaluated, hRV/EV, RSV-A/B, SARS-CoV-2, Flu A/H3, hAdV, hCoV-NL63 and Flu A/H1N1(pdm09) were statistically significantly higher in men ($P<.05$), and hCoV-OC43 was statistically significantly higher ($P=.001$) in women. The median age of virus-positive patients was 4 years (interquartile range [IQR], 9; range, 0 to 97), with 75% of positive patient samples in children 0-10 years old (**Table 2**). When the distribution of viral etiology according to age groups was examined, hRV/EV, RSV-A/B, hMPV, PIV-3 and hCoVs were the most common viruses at the age of 1 year, and influenza, hAdV, PIV-2 and PIV-4 in children aged between 6 and 10 years (**Table 3**).

In 1109 of 6250 positive samples, two or more respiratory pathogens were co-detected with a rate of 17.6%. hRV/EV was the most frequently detected virus in mixed infections. The most common pathogen combination was hRV/EV + RSV-A/B in dual co-infections, and hRV/EV + RSV-A/B + hBoV in triple co-infections. The most frequently detected pathogen combinations in co-infections are shown in **Table 4**. Co-infection with SARS-CoV-2 was detected in 2% (126/6250) of the positive samples. The most frequent combination with SARS-CoV-2 was with hRV/EV in 48 patients (0.8%), hAdV in 13 patients (0.2%), RSV-A/B in 11 patients (0.2%), hBoV in 10 patients (0.2%).

To determine the effect of NPIs on circulating respiratory viruses, respiratory syndromic panel results were compared during the period of restriction and relaxation. At least one respiratory virus was detected in 5%

Table 1. Viral pathogen and gender distributions of patients during the COVID-19 pandemic.

Viral pathogen distribution	Isolated viruses	Gender Distribution		*P* value
		Female (%)	Male (%)	
Virus				
hRV/EV	2572 (34.4)	40.9	59.1	**.001**
RSV- A/B	1126 (15)	47.4	52.6	**.034**
SARS-CoV-2	834 (11.1)	47.7	52.3	**.050**
Flu A/H3	506 (6.7)	49.4	50.6	**.023**
hBoV	473 (6.3)	44.6	55.4	.988
hAdV	441 (5.9)	39.9	60.1	**.041**
PIV-3	413 (5.5)	40.7	59.3	.099
hMPV	225 (3)	41.3	58.7	.319
PIV-1	220 (2.9)	48.2	51.8	.273
PIV-4	171 (2.2)	37.4	62.6	.057
hCoV-OC43	138 (1.8)	60.9	39.1	**.001**
hCoV-229E	105 (1.4)	51.4	48.6	.154
PIV-2	77 (1)	53.2	46.8	.123
hCoV-NL63	76 (1)	32.9	67.1	**.039**
hCoV-HKU1	44 (0.5)	31.8	68.2	.088
Flu A	38 (0.5)	47.4	52.6	.728
Flu A/ H1N1 (pdm09)	9 (0.1)	11.1	88.9	**.049**[a]
Flu B	1 (0.1)	0	100	-
Total	7469	44.6% (2786/6250)	55.4% (3464/6250)	

Data are n (%) based on the total number of detected viruses for isolated viruses or % based on the number of positive samples for gender. [a]Calculated with the Fisher exact test.

hRV/EV: rhinovirus/enterovirus; RSV-A/B: respiratory syncytial virus A/B, SARS-CoV-2: severe acute respiratory syndrome coronavirus-2; hBoV: bocavirus, hAdV: adenovirus PIV-3: parainfluenza virus 3, hMPV: human metapneumovirus A/B, PIV-1: parainfluenza virus 1, PIV-2: parainfluenza virus 2, PIV-4: parainfluenza virus 4, hCoV-OC43: coronavirus OC43, hCoV-229E: coronavirus 229 E, hCoV-NL63: coronavirus NL63, hCoV-HKU1: coronavirus HKU1, Flu A: influenza A virus, Flu A/H1N1(pdm09): influenza A subtype H1N1/2009 virus, Flu A/H1: influenza A subtype H1 virus, Flu A/H3: influenza A subtype H3 virus, Flu B: influenza B virus.

Table 2. Distribution of respiratory viral infections by age group.

Age group	Isolated specimens
<1	401 (6.4)
1	1307 (20.9)
2	787 (12.6)
3	585 (9.4)
4	433 (6.9)
5	370 (5.9)
6-10	832 (13.3)
11-15	338 (5.4)
16-20	173 (2.8)
21-30	343 (5.5)
31-40	279 (4.5)
41-50	166 (2.7)
51-60	122 (2.0)
≥61	114 (1.8)
Total	6250 (100)

Data are number (%) based on the number of positive samples.

(313) of the samples in the first period between April 1, 2020 and June 30, 2021, when strict NPIs were applied, and in 95% (5937) in the second period between July 1, 2021 and October 30, 2022, when NPIs were relaxed. Of the total 7469 viruses detected in this study, 342 (5%) were detected in the first period, and 7127 (95%) in the second period (**Table 5**). After the removal of NPIs, there was a statistically significant increase in hRV/EV, RSV-A/B, Flu A/H3, hBoV, hMPV, PIV-1, PIV-4, hCoV-OC43, PIV-2 and hCoV-NL63 ($P<.05$). Between the two periods, there was no statistically significant difference despite the numerical increase in hAdV, PIV-3, hCoV-229E, hCoV-HKU1, Flu A, Flu A/H1N1(pdm09) and Flu B viruses.

When pathogens were evaluated according to the study periods, the most common respiratory viruses were hRV/EV (60%), hAdV (10%) and PIV-3 (9%), respectively in the first period, and hRV/EV (40%), RSV-A/B (19%) and Flu A/H3 (9%), respectively in the second period. The prevalence of respiratory virus over time; during the periods of adoption and relaxation of NPIs was determined in this study, and according to this, in the 2020-2021 season, when strict NPIs were applied, all respiratory viruses evaluated did not have the usual seasonal peak and there were no seasonal influenza epidemics during this period (**Figures 1 to 6**).

Table 3. Percentage distribution of viral infections according to age group.

Age (years)	hRV/EV	RSV- A/B	SARS-CoV-2	Flu	hBoV	hAdV	hMPV	PIV-1	PIV-2	PIV-3	PIV- 4	hCoVs
<1	7	2.9	10.2	0.2	2.1	4.1	2.7	22.3	11.7	9.9	0.6	5.2
1	18.5	52	11.4	7.6	19.9	13.6	19.6	14.1	13	21.8	0.6	13.2
2	15.6	11.6	5	4.7	25.2	16.8	14.2	7.3	13	18.2	7.6	9.9
3	10.1	7.7	4	5.8	19.2	15.6	11.1	15.9	9.1	16.5	8.8	9.6
4	7.7	5.1	2.4	6.3	12.9	11.6	10.2	11.4	9.1	7.3	7.6	8.3
5	6.4	5	3.5	7.9	7.8	9.3	7.6	6.8	3.9	6.1	11.1	4.7
6-10	14.4	7.5	7	30.8	10.4	18.1	16.9	10	22.1	9.2	19.9	8.3
11-15	5.8	1.5	9.5	8.3	0.6	3.4	3.6	2.3	3.9	4.6	3.5	6.3
16-20	2.8	0.5	4.4	3.8	0.4	1.6	0	2.7	1.3	2.7	0	4.1
21-30	4.4	2.4	13.8	9	0.4	0.7	3.1	1.4	3.9	1	2.9	9.6
31-40	3.3	1.9	9.7	7.6	0.6	2	5.8	3.6	2.6	1.9	2.3	6.6
41-50	1.7	0.6	7.3	3.4	0.4	1.4	1.3	0.9	3.9	0	0	8.3
51-60	1.2	0.4	6.4	1.6	0	1.1	1.8	0.5	0	0.5	1.2	3.9
≥61	1	0.7	5.5	2.9	0	0.7	2.2	0.9	2.6	0.5	1.2	1.9

Data are percentages of total number of detected viruses. For abbreviations, see footnote Table 1.

DISCUSSION

This retrospective study, carried out in the Ankara Bilkent City Hospital, which is the largest hospital in Europe with a total bed capacity of 4119, indicates that the COVID-19 pandemic had a significant impact on the circulation of common respiratory viruses in Turkey. The positivity rate of respiratory samples examined during this study period was 55.3%. The fact that the respiratory syndromic panel kit also detected SARS-CoV-2 contributed to the high rate of positivity. During the study period of the pandemic, the QIAstat-Dx Respiratory panel was applied to a only a limited number of patients after clinical examination; therefore, the SARS-CoV-2 results from the respiratory panel used in this study cannot reflect the actual data for all patients in the hospital. For that reason, the prevalence of SARS-CoV-2 was excluded in this study and we were unable to report on the age group distribution and prevalence. The QIAstat-Dx Respiratory panel results for SARS-CoV-2 were used to detect co-infections, which was the aim of this study.

Respiratory syndromic panel results were compared by dividing the pandemic period before and after July 1, 2021, when strict NPIs were relaxed. Of the 6250 patient samples with at least one respiratory virus, 5% were detected in the first period and 95% in the second period. Members of the same viral genus, RVs and respiratory enteroviruses are non-enveloped viruses, and therefore cannot be distinguished by most of molecular tests.[7] hRV/EV was the most frequently detected virus (34%) in our 2.5-year study period, which is compatible with the findings of other surveillance studies (21.4% and 35.8%) conducted in our country.[8] In this study, a statistically significant difference was found in the increase in hRV/EV, RSV-A/B, Flu A/H3, hBoV, hMPV, PIV-1, PIV-4, hCoV OC43, PIV-2 and hCoV-NL63 viruses after NPIs were discontinued ($P<.05$). Therefore, our findings showed that strict NPIs caused a drastic decrease in viral respiratory infections and changes in seasonal characteristics.

Consistent with many studies investigating respiratory viral pathogens during the pandemic period, hRV/EV was 60% in the first period and 40% in the second.[3,5,9,10] Despite strict measures, hRV/EV continued to circulate at low frequency but started to increase rapidly when the restrictions were removed, and continued rising sharply when schools were opened, peaking in the following autumns in 2021 and 2022 (**Figure 1**). It was observed that applying NPIs reduced the number of RV infections but was not effective in preventing their spread. RV are non-enveloped viruses and might be inherently less susceptible to inactivation by handwashing only (soap-and-water). The quality of handwashing by children can be poor as well. Such factors may have contributed to RV infection being less affected by NPIs.[11] Also, Leung et al showed that surgical face masks can prevent transmission of seasonal coronaviruses in humans (hCoVs) and influenza viruses but cannot prevent RV transmission from symptomatic individuals with acute respiratory disease via respiratory droplets and aerosols.[12]

In our study, the second most frequently detected virus was RSV-A/B with a rate of 15%, which usually oc-

Table 4. Pathogens detected in co-infections (n=6250 combination infections).

Single	5141 (82.3)
Double Infections	1006 (16.0)
hRV/EV + RSV-A/B	181 (2.9)
hRV/EV + hBoV	119 (1.9)
hRV/EV + hAdV	99 (1.6)
hRV/EV + PIV3	69 (1.1)
hRV/EV + PIV1	49 (0.8)
Others	489 (8.0)
Triple Infections	96 (1.5)
hRV/EV + RSV-A/B + hBoV	12 (0.2)
hRV/EV + hAdV + hBoV	9 (0.1)
hRV/EV + hAdV + PIV3	6 (0.1)
hRV/EV + RSV-A/B + PIV4	6 (0.1)
hRV/EV + hBoV + PIV3	5 (0.1)
Others	58 (0.9)
Quadruple Infections	7 (0.07)
hRV/EV + hBoV + hAdV + PIV4	1 (0.1)
hRV/EV + hBoV + PIV3 + hCoV-OC43	1 (0.1)
hRV/EV + hBoV + PIV3 + PIV4	1 (0.1)
hRV/EV + RSV-A/B + hAdV + hCoV-NL63	1 (0.1)
hRV/EV + RSV-A/B + hAdV + hMPV	1 (0.1)
hRV/EV + RSV-A/B + hAdV + hBoV	1 (0.1)
RSV-A/B + hAdV + hBoV + hMPV	1 (0.1)
Total	6250 (100)

Data are n (%) based on number of positive samples. For abbreviations, see footnote Table 1.

Table 5. Prevalence of respiratory tract viruses detected before and after discontinuation of non-pharmaceutical interventions.

Virus	1 April 2020-30 June 2021	1 July 2021-30 October 2022	Total	*P* value
hRV/EV	188 (60)	2384 (40)	2572	**.001**
RSV-A/B	4 (1)	1122 (19)	1126	**.001**[a]
SARS-CoV-2	52 (17)	782 (13)	834	.081
Flu A/H3	0 (0)	506 (9)	506	**.001**[a]
hBoV	8 (3)	465 (8)	473	**.001**
hAdV	30 (10)	411 (7)	441	.073
PIV-3	27 (9)	386 (7)	413	.140
hMPV	2 (0.6)	223 (4)	225	**.001**[a]
PIV-1	0 (0)	220 (4)	220	**.001**[a]
PIV-4	0 (0)	171 (3)	171	**.001**[a]
hCoV-OC43	12 (4)	126 (2)	138	**.045**
hCoV-229E	3 (1)	102 (2)	105	.493[a]
PIV-2	0 (0)	77 (1)	77	**.033**[a]
hCoV-NL63	9 (3)	67 (1)	76	**.006**
hCoV-HKU1	5 (2)	39 (0.7)	44	.052
Flu A	2 (0.6)	36 (0.6)	38	.716[a]
Flu A/H1N1(pdm09)	0 (0)	9 (0.2)	9	1.0[a]
Flu B	0 (0)	1 (0)	1	1.0[a]
Total	342 (5)	7127 (95)	7469	

Data are n (%) based on the total number of detected viruses. [a]Calculated with Fisher's exact test. For abbreviations, see footnote Table 1.

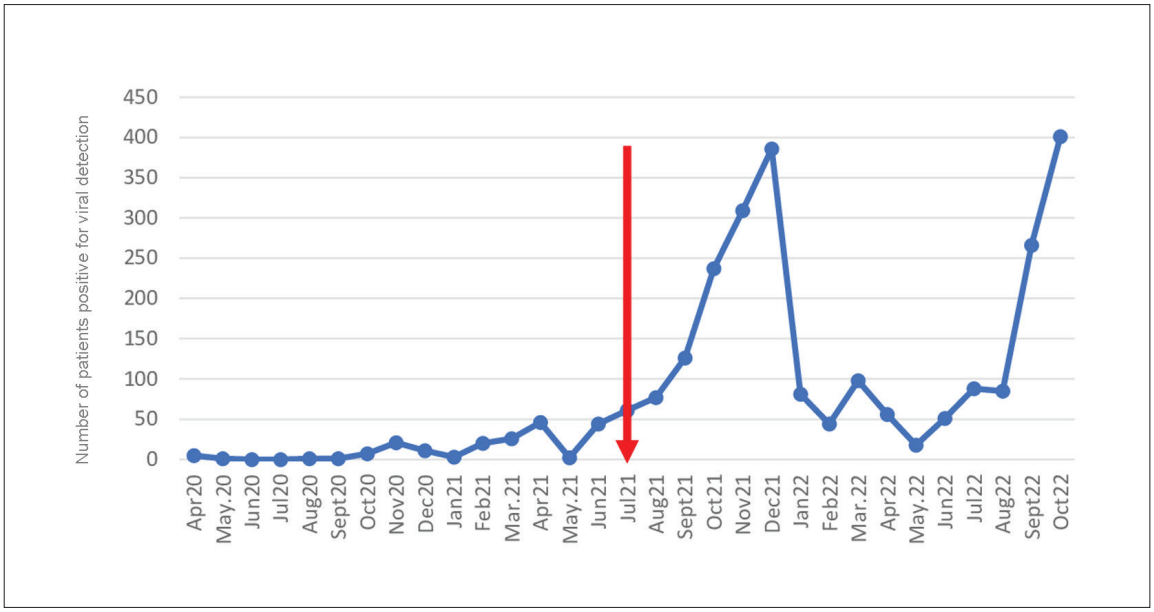

Figure 1. Temporal pattern of rhinovirus/enterovirus infection after discontinuation of non-pharmaceutical interventions (red line is date of discontinuation).

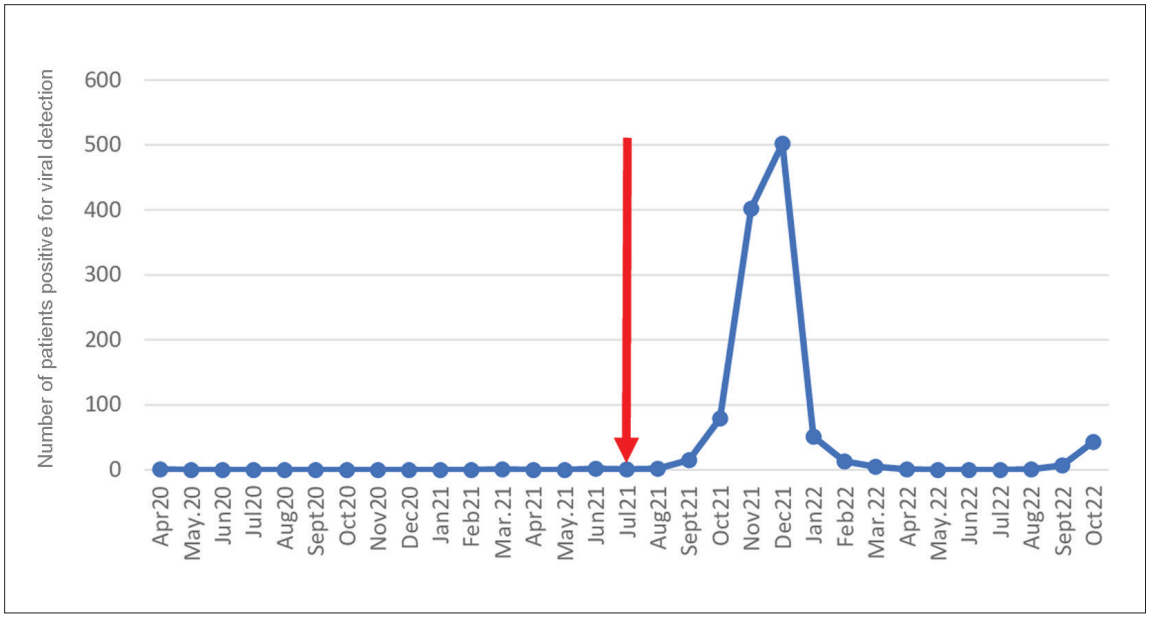

Figure 2. Temporal pattern of respiratory syncytial virus infection A/B after discontinuation of non-pharmaceutical interventions (red line is date of discontinuation).

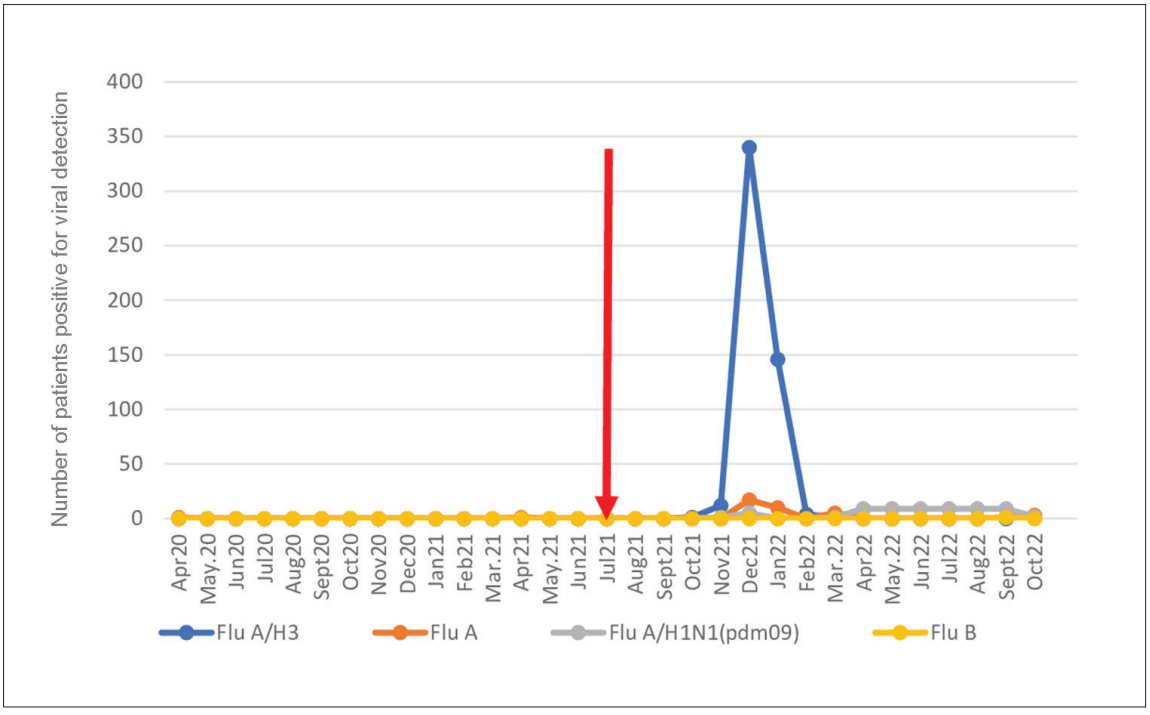

Figure 3. Temporal pattern of influenza virus infection after discontinuation of non-pharmaceutical interventions (red line is date of discontinuation). Flu A/H3: Influenza A subtype H3 virus, Flu A: Influenza A virus, Flu A/H1N1(pdm09): Influenza A subtype H1N1/2009 virus, Flu B: Influenza B virus.

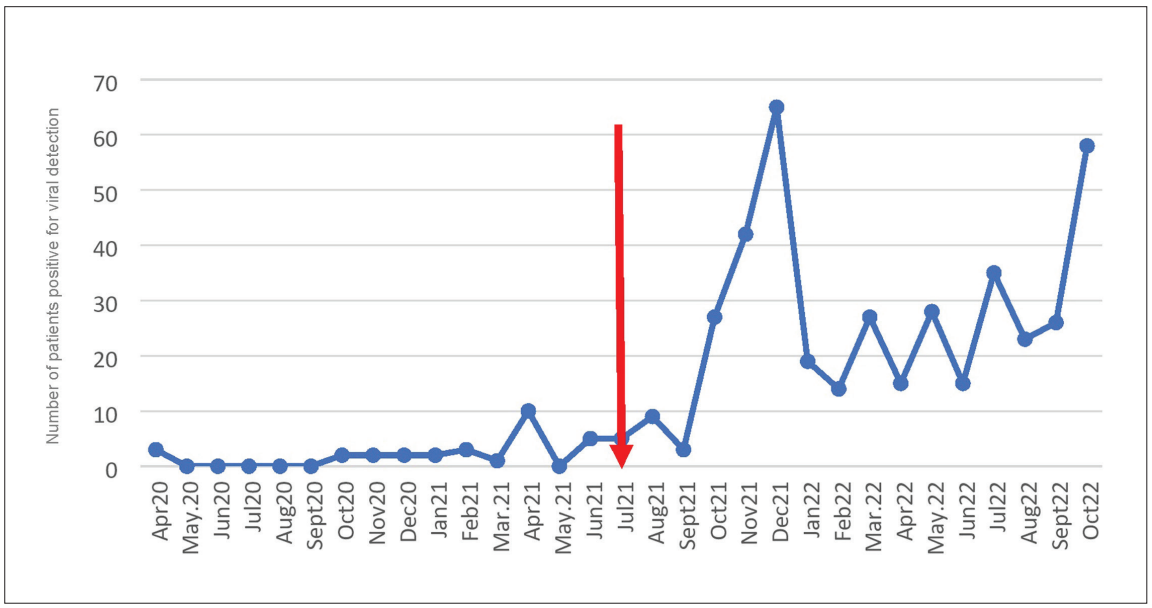

Figure 4. Temporal pattern of adenovirus infection after discontinuation of non-pharmaceutical interventions (red line is date of discontinuation).

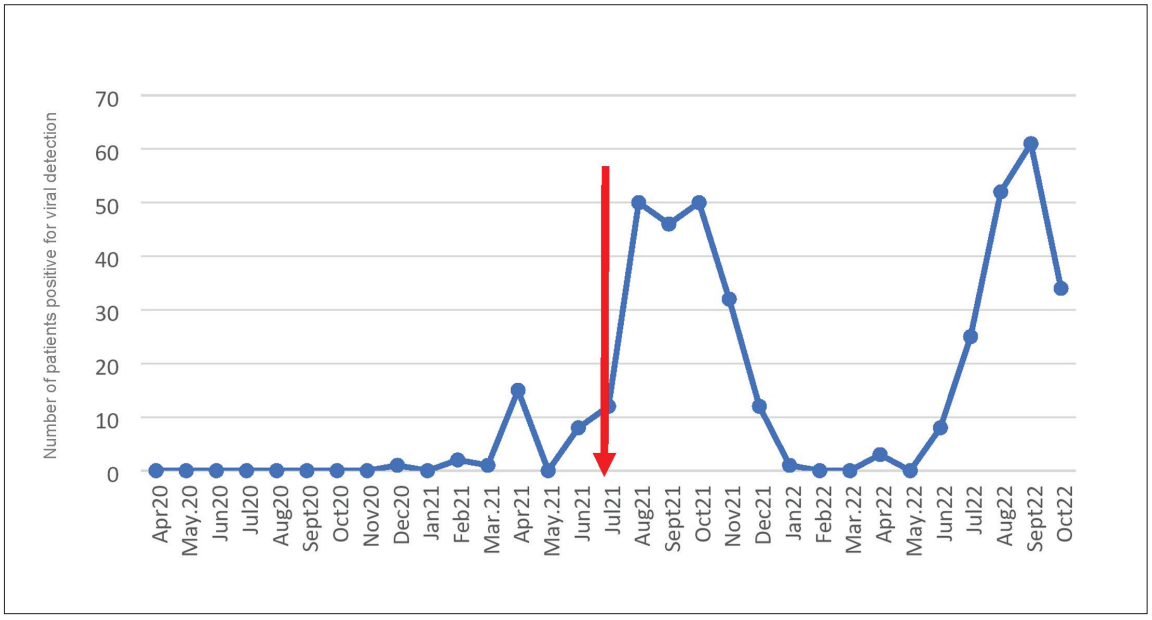

Figure 5. Temporal pattern of parainfluenza virus 3 infection after discontinuation of non-pharmaceutical interventions (red line is date of discontinuation).

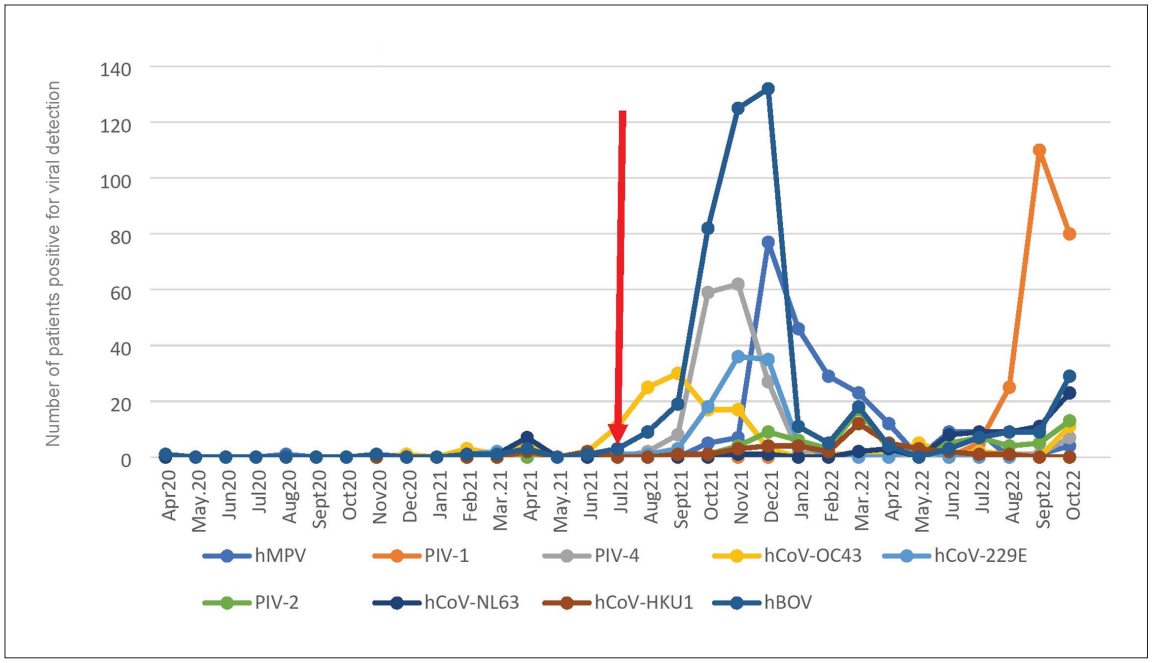

Figure 6. Temporal pattern of respiratory virus infection after discontinuation of non-pharmaceutical interventions (red line is date of discontinuation). hMPV: human metapneumovirus A/B, hBoV: bocavirus, PIV-1: parainfluenza virus 1, PIV-2: parainfluenza virus 2, PIV-4: parainfluenza virus 4, hCoV-OC43: coronavirus OC43, hCoV-229E: coronavirus 229E, hCoV-NL63: coronavirus NL63, hCoV-HKU1: coronavirus HKU1.

curs in the form of epidemics in late autumn, winter or early spring.[13] However, due to the effect of NPIs, RSV-A/B peak was not seen, and RSV-A/B was detected at a very low rate of 1% in the first study period. With the relaxation of NPIs, RSV-A/B peaked sharply in the following autumn in 2021 and winter in 2022, and then showed seasonal characteristics as before (**Figure 2**). In the relaxation period, RSV-A/B was the second most frequently detected virus with a rate of 19%.

According to the results of this study, influenza (Flu A) was detected in only two patients during the period of strict NPIs, and there was no seasonal influenza epidemic in the period of 2020-2021 (**Figure 3**). Weekly Influenza Surveillance results of the Ministry of Health of the Republic of Turkey, reported only one positivity for influenza (Flu B) in 1076 samples, which also supports our findings.[14] Considering the total positive virus rate in the first and second study periods, Flu A/H3 increased from 0% to 9%, peaking in the following winter season in 2022 (**Figure 3**). One possible reason for the absence of a seasonal flu epidemic is that NPIs also suppress influenza, since influenza and SARS-CoV-2 are transmitted in much the same way primarily through respiratory droplets.[15] Another reason can be viral interference. During a Flu A virus epidemic that occurred in 2009, it was reported from several European countries that a seasonal RV epidemic delayed transmission of influenza virus.[16-18] Anchi et al reported that previous RV infection suppressed Flu A virus replication in cell culture research, explaining that infection with RV and other RNA viruses is detected by innate immune sensors in human airway cells and leads to the induction of antiviral interferon-stimulated genes and the production of type I and type III interferons, an effective defense mechanism against many viruses.[19]

It is not yet known what impact the SARS-CoV-2 pandemic will have on other respiratory viruses in the coming years. The decrease in circulating influenza viruses may result in poor vaccination coverage. There may also be a decrease in immunity due to low antibody titers or a decrease both in prior infections and in vaccine content. This may cause increased influenza infection and viral replication, creating additional opportunities for mutations, which might in turn lead to the emergence of new variants.[7]

In this study, hAdV was detected at a rate of 10% in the first period when NPIs were applied, and 7% in the second period when restrictions were relaxed. As reported in other studies where hAdV was less affected by NPIs,[6,7,20] there was no statistically significant difference between the first and second periods in our study (P=.073). With the relaxation of NPIs, hAdV peaked in

autumn 2021 and winter 2022 and was in continuous circulation in other seasons (**Figure 4**). The effectiveness of NPIs may have been reduced due to the fact that hAdVs are non-enveloped viruses with long-term survival on environmental surfaces and can be transmitted directly or indirectly through respiratory secretions and the fecal-oral route; they are relatively resistant to disinfectants.[7]

During the period when NPIs were applied, face-to-face education was started for a short period of time, and then distance education was implemented again. Between March 1 and April 26, 2021, in line with the controlled normalization decisions, face-to-face education was started in all preschool educational institutions and schools across the country. During the strict NPIs period, detection of RV/EV, hBoV, hAdV, PIV-3 and non-SARS-CoV-2 coronavirus strains was highest in spring 2021, the period when schools were opened.

In this study, PIV-3 was detected at the rate of 9% in the first period, and 7% in the second. Unlike other parainfluenza viruses, there was no statistically significant difference despite the numerical increase in the second period of the study (*P*=.14). PIV-3, which increased in prevalence with the opening of preschool institutions and schools in the first period, showed seasonal characteristics in summer and autumn both in 2021 and 2022 in the second period (**Figure 5**). The findings of earlier studies carried out in Turkey showed that PIV-3 peaked during summer and winter months.[21,22] While the PIV-3 rate was close to zero with strict NPIs, it showed a considerable rise with the opening of schools, most likely because young children adhere inadequately to mask mandates and hygiene rules. Consistent with our results, an outbreak caused by PIV-3 was reported in a nursery in Japan during the COVID-19 pandemic.[23]

Although it was reported in earlier studies that non-enveloped respiratory viruses were mostly isolated during the pandemic period and that these features contributed to their stability, in our study, the enveloped virus PIV-3 was the third most common infection agent during the strict NPIs.[24] Therefore, when evaluating the effect of NPIs on respiratory viruses, along with the structural features of the pathogen, the lack of full compliance with NPIs should also be considered.

Respiratory pathogens were co-detected at a rate of 18%, with hRV/EV being the most frequently detected virus in co-infections. The most common pathogen combination was hRV/EV + RSV-A/B. Detection of more than one pathogen in a clinical sample by a molecular method does not always mean co-infection, but may be the result of asymptomatic infection or prolonged shedding. Especially after respiratory tract infection, the prolongation of the virus excretion period can lead to this result. In such cases, nucleic acids of both the current infection and the past infection agent can be detected in the same sample with the mPCR test.[25-27] Quantitative PCR tests may be useful for the diagnosis of co-infections, but defining thresholds are still unclear, although a high viral load is associated with infection.[13] Therefore, it is important to perform clinical evaluation and interpretation.

When the viral etiology was evaluated according to age groups after the removal of NPIs, respiratory pathogens were detected most often in the 0-10 year age group, which comprised 75% of patients. Since children are more susceptible and vulnerable to viral respiratory infections, viral resurgence would be expected to be more common at this age.[28] It should also be noted that due to the crowded and closed environment in schools, the spread of respiratory viruses may be increased.

Respiratory tract infections usually have similar symptoms, and therefore it is necessary to diagnose the pathogen to order agent specific antiviral and antibiotic therapy.[29] Rapid and accurate diagnosis of COVID-19 has become more critical as the prevention of virus transmission through isolation of positive patients and quarantine of contacts is the foremost requirement of pandemic management. rRT-PCR is the standard test for the diagnosis of SARS-CoV-2.[30] These assays are time and labor intensive and require advanced laboratory infrastructure. POC multiplex tests, also used to diagnose SARS-CoV-2, test multiple pathogens at the same time and help in the timely differential diagnosis of infectious diseases.[31] Despite a higher cost compared to routine screening tests, the POC multiplex test results in a faster workflow in our hospital, especially in the emergency service, during the pandemic period.

In conclusion, the goal of the implementations of NPIs during the COVID-19 pandemic was intended to limit community transmission of SARS-CoV-2, reduce the need for healthcare resources, lessen the workload, and buy time to develop vaccines and treatments. NPIs introduced in Turkey have resulted in a dramatic decrease in the prevalence of respiratory viruses and a notable disruption of seasonal circulation patterns specific to respiratory virus infections. This situation may have emerged with the widespread use of masks and social isolation measures as well as the contribution of viral interference mechanisms. The strength of our study is the large patient population, which can provide insight into the distribution of other viral respira-

tory pathogens during the pandemic. Understanding the mechanisms underlying the dramatic reductions in seasonal respiratory viruses is of paramount importance as such data will guide the planning public health measures in the event of future seasonal respiratory virus epidemics or pandemics.

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