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Evaluation of the prevalence and seasonality of human parainfluenza virus over five year period in pediatric patients

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ABSTRACT

Objective: Human Parainfluenza viruses (HPIVs) cause respiratory tract infections, and the second most common cause of acute respiratory illness-related hospitalizations after the respiratory syncytial virus in children <5 years of age. The aim of the study; determination of HPIVs positivity and common types in pediatric patients with respiratory tract infection; investigation the distribution of HPIV positivity by age groups, months and seasons, respectively.

Material and Method: HPIV results of 1613 pediatric patients who were sent to the molecular virology laboratory from various pediatric clinics of Gazi Hospital between March 2016 and February 2021 (five years period) were investigated. Nucleic acid isolation was performed on the EZ1 Advanced (Qiagen, Germany) device using the EZ1 Virus Mini Kit by the manufacturer's protocol.

Results: HPIV positivity was detected as 4.1% in clinical samples and, the most common HPIV type was found to be HPIV-3 (55%). The distribution of other HPIV types were; HPIV-2, HPIV-4 and HPIV-1 with 26%, 23% and 14%, respectively. HPIV-3 is the most common type in 2016, 2017, 2018 and 2019; however, HPIV-1 is the most common type in 2020. HPIVs co-infection was detected with other respiratory tract viruses in 51% of samples. The highest HPIV co-infection was detected in Rhinovirus. The highest HPIV positivity rate (45%) were determined in the 0-2 age group compared to other age groups ($p<0.05$). The highest positivity rate was in October in the autumn season ($p<0.05$), the lowest was in January and February in winter. The highest rate (8.1%) of HPIV positivity was found in 2016 and the lowest rate (0.7%) was in 2020.

Conclusions: Since it is not possible to diagnose viral etiology of respiratory tract infections based on clinical findings, viral respiratory tract panel and Multiplex real-time PCR test are a fast and useful method in early diagnosis, treatment decision and prevention of unnecessary antibiotic use. HPIVs positivity is seen at higher rates in children aged 0-2 and in autumn months with seasonal differences.

Keywords: Respiratory tract infections, Human Parainfluenza virus (HPIV), Multiplex Real-Time polymerase chain reaction

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INTRODUCTION

Human parainfluenza viruses (HPIVs) are single-stranded, enveloped RNA viruses in the Paramyxoviridae family. HPIVs can cause upper/lower respiratory tract infections and after respiratory syncytial virus (RSV), the second most common cause of acute respiratory illnesses related hospitalizations in children <5 years of age (**1, 2**). Human parainfluenza viruses with four distinct types known to infect humans: HPIV-1, HPIV-2, HPIV3, and HPIV-4; and included into two genera, Respirovirus (HPIV-1 and HPIV-3) and Rubulavirus (HPIV-2 and HPIV-4). HPIV-1 and HPIV-2 both cause croup, upper and lower respiratory illnesess. HPIV-3 is more often associated with bronchitis, bronchiolitis and pneumonia; HPIV-4 is recognized less often but may cause mild to severe respiratory illnesses (**3**).

Human parainfluenza viruses spread via the air by sneezing or coughing, personal contact, such as touching hands, objects or surfaces that have HPIVs on them then touching nose, mouth or eyes from an infected person to others. Currently, there is no vaccine to protect and specific antiviral treatment for HPIV infection (**4, 5**). Parainfluenza viruses replicate in the ciliated epithelial cells of the respiratory tract. Infection begins in the nose, oropharynx and then spreads to the lower airways 2-5 days after the initial infection. Once epithelial cells of the respiratory tract become infected, inflammatory infiltrates develop and the immune response is thought to contribute to disease pathogenesis. Although these infections are rather mild in healthy individuals, they may lead to serious respiratory diseases in children and immunocompromised individuals (**4, 5**).

In the respiratory system infections caused by viruses are clinically similar and the agent could be determined with only laboratory diagnosis. The Multiplex polymerase chain reaction can be used easily and efficiently to detect and diagnose the HPIV infection. Based on the sensitivity, specificity, and rapidity of the test, it is the most accurate test for HPIV diagnosis. Compared to viral culture and IFA testing, the PCR has been reported to have superior sensitivity and specificity (**6**).

Human parainfluenza virus is one of the main pathogens of respiratory tract infection in children. In the present study, we aimed the determination of HPIVs positivity and common types in pediatric patients who were sent to molecular virology laboratory due to the symptoms of respiratory tract infection; investigation the distribution of HPIV positivity by age groups, months and seasons, respectively.

MATERIAL and METHODS

In the study, the Human Parainfluenza virus results of 1613 pediatric patients, between 0-18 age, who were sent to the molecular virology laboratory due to the symptoms of acute respiratory tract infection from pediatric clinics of Gazi University Medical Faculty Hospital between March 2016 and February 2021 (five years period) were investigated retrospectively.

Collection of Samples

Totally 1648 clinical samples (nasopharyngeal swab, throat swab, nasal swab, and bronchoalveolar lavage (BAL)) of 1613 patients were included in the study. Throat swab, nasal swab and nasopharyngeal swab samples in transport medium (UTM-RT Transport, Copan Diagnostics, Italy); bronchoalveolar lavage samples were sent to the laboratory in a sterile transport container.

Nucleic Acid Isolation and Viral DNA Amplification

Nucleic acid isolation was performed on the EZ1 Advanced (Qiagen, Germany) device using the EZ1 Virus Mini Kit by the manufacturer's protocol. Viral DNAs were stored at -80 °C until amplification.

The amplification of the obtained DNA was performed by using Fast Track Diagnostics Respiratory Pathogen 21 (FTD, Luxembourg) and multiplex Real-Time PCR method. Fast Track Diagnostics® Respiratory Pathogen 21 test detects a total of 21 respiratory tract pathogens, including Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Influenza A, Influenza B, Influenza A (H1N1), Coronavirus NL63, Coronavirus OC43, Coronavirus HKU1, Coronavirus 229E, Human metapneumovirus A/B, Rhinovirus, Human bocavirus, Respiratory syncytial virus A/B, Adenovirus, Enterovirus, Human parechovirus and Mycoplasma pneumoniae.

FTD® Respiratory Pathogen 21 is ready to use test kit containing TaqMan probes and primers and results in the measurement of the fluorescence signal. Sample results with a FAM (fluorophore) fluorescence signal as a result of the test are considered positive.

Statistical Analysis: Statistical analysis was performed through SPSS 20.0. The data were evaluated using the Chi-Square and Mann-Whitney U test, and the $p<0.05$ value was considered significant in the analyses.

Ethical permission: This study was approved by Gazi University Medical Faculty Non-Interventional Clinical Research Ethics Committee.

RESULTS

A total of 1648 samples (throat swabs, nasal swabs, nasopharyngeal swabs and bronchoalveolar lavage (BAL) were obtained from 1613 patients; 872 male (%54) and 741 female (%46) were sent to the Molecular Virology Laboratory from pediatric clinics were investigated retrospectively.

Human parainfluenza virus positivity was detected as 4.1% (69/1648) in clinical samples. Among the HPIV positive cases, 50.7% (35/69) were female, 49.3% (34/69) were male. Gender distribution of HPIV positivity did not show any statistically significant differences ($p>0.05$).

The HPIV positive samples sent from Department of Pediatric Infectious Diseases 28/69 (40%), Pediatric Hematology 11/69 (16%), General Pediatrics 7/69 (10%), Pediatric Pulmonology 5/69 (7%), Pediatric Neurology 4/69 (6%), Pediatric Oncology 4/69 (6%), Neonatology 4/69 (6%), Pediatric Metabolism 1/69 (1.5%), Pediatric Endocrinology 1/69 (1.5%), Pediatric Gastroenterology 1/69 (1.5%), Pediatric Cardiology 1/69 (1.5%), Pediatric Nephrology 1/69 (1.5%), Pediatric Rheumatology 1/69 (1.5%) clinics. It was determined that the most frequent reasons for the patients were cough (48.7%) and fever (21.4%).

The distribution of human parainfluenza virus-positive patients were examined by age groups; it was found as 45% (31/69) in the age group 0-2; 27.5% (19/69) in the age group 3-5; 17.4% (12/69) in the age group 6-8; 5.8% (4/69) in the age group 9-12; 4.3% (3/69) in 13-16 years old. HPIV positivity was not detected in patients aged 16-18 years.

It was found that there is a higher rate (45%) of positivity in the 0-2 age group compared to other age groups. When the distribution of human parainfluenza virus positivity among age groups was examined, human parainfluenza virus positivity was found to be statistically significant in 0-2 years of age group patients ($p <0.05$).

The most common HPIV type was found to be HPIV-3 with 55% (38/69). The distribution of other HPIV types was detected HPIV-2, HPIV-4 and HPIV-1 with 26% (18/69),

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23% (16/69) and 14% (10/69), respectively. Co-positivity of the two HPIV types was detected as 18% (13/69) in samples. HPIV-3 was found the most common HPIV type as a percentage, but it was not statistically significant (p>0.05).

Among the HPIV positive cases, HPIV-3 was the most common type in 0-2, 3-5 and 9-12 years of age with 27.5%, 21.7% and 2.9%, respectively. However, HPIV-4 was the most common type in 6-8 and 13-16 years of age with 7.2% and 1.5%. Human parainfluenza virus types positivity rates according to age groups are given in **Figure1**.

The highest positivity rate was in October (13.2%; 15/113) and November (8.7%; 11/126), the lowest was in February (0%; 0/94) and January (0.6%; 1/166). When the distribution of HPIV positivity according to the seasons it was observed that the highest positivity rate was in the autumn season (9.3%) and the lowest rate was in the winter season (2%). In our study, a statistically significant difference was found between the autumn and winter seasons (p <0.05). The distribution of HPIV types positivity according to the months is shown in **Figure2**.

HPIV-1 and HPIV-2 were commonly detected in autumn; HPIV-3 was detected in early summer to autumn and HPIV-4 autumn and winter months. The distribution of HPIV types positivity according to the months and season shows in **Table1**.

Human parainfluenza virus positivity was found as 8.1% (13/160) in 2016, 5.9% (13/220) in 2017, 7.8% (22/281) in 2018, 3.6% in 2019 (17/465) and 0.7% (4/522) in 2020. The highest rate of HPIV positivity was found in 2016, and the lowest rate was in 2020. The distribution of HPIV positivity according to years is shown in **Figure3**. When the distribution of HPIV types according to the years were investigated; it was seen that HPIV-3 is the most common type in 2016, 2017, 2018 and 2019 with 3.1%, 3.1%, 4.9% and 2.3% respectively. However, HPIV-1 is the most common type with 0.3% in 2020. The distribution of HPIV types according to the years are shown in **Figure4**. Among the HPIV positive samples, only HPIV positivity was found as 49% (34/69) and HPIV co-infection with other viruses in 51% (35/69) of samples. The highest HPIV co-infection was detected in Rhinovirus, 37.1% (13/35); the lowest HPIV co-infection was found in Human parechovirus, 2.8% (1/35) (Table 1). Co-positivity of the two HPIV types was detected as 18% in samples. Among human parainfluenza virus-positive samples, co-infection with the triple agent was detected in six samples. HPIV-2/ HPIV3/ Adenovirus association was detected in two samples, HPIV-3/ Bocavirus/ Adenovirus; HPIV-1/ Adenovirus/ Metapneumovirus A/B; HPIV-3/ CoronavirusOC43/ Rhinovirus; HPIV-3/ Adenovirus/ Human parechovirus were detected in each of four sample. The distribution of co-infection with triple agents is given in **Table2**.

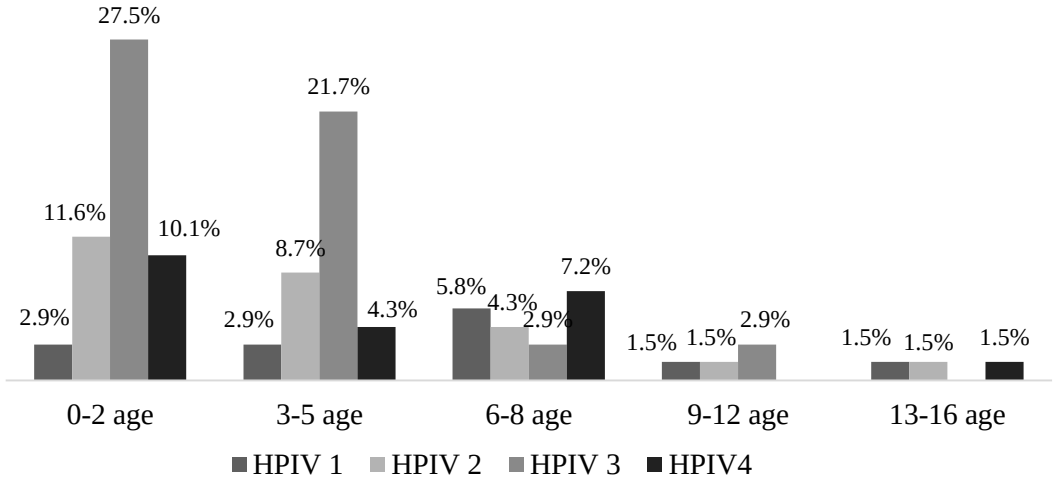

Figure 1: Human parainfluenza virus type's positivity rates according to age groups

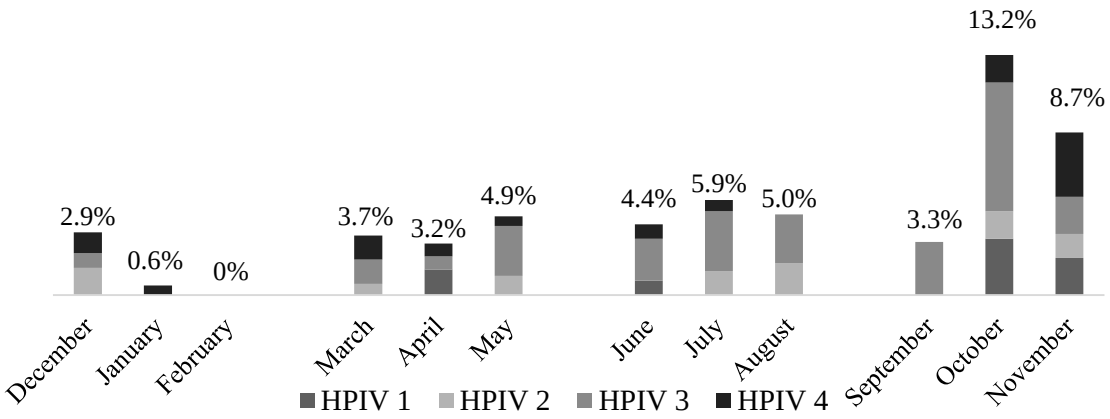

Figure 2: The distribution of HPIV positivity according to the months

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Table 1: The distribution of HPIV types positivity according to the months

%	January	February	March	April	May	June	July	August	September	October	November	December
HPIV-1	0.0	0.0	0.0	1.6	0.0	0.9	0.0	0.0	0.0	3.0	1.7	0.0
HPIV-2	0.0	0.0	0.7	0.0	1.2	0.0	1.5	2.0	0.0	1.6	1.3	1.2
HPIV-3	0.0	0.0	1.5	0.8	3.1	2.6	3.7	3.0	3.3	7.0	1.7	0.8
HPIV-4	0.6	0.0	1.5	0.8	0.6	0.9	0.7	0.0	0.0	1.6	4.0	0.9
TOTAL	0.6	0.0	3.7	3.2	4.9	4.4	5.9	5.0	3.3	13.2	8.7	2.9

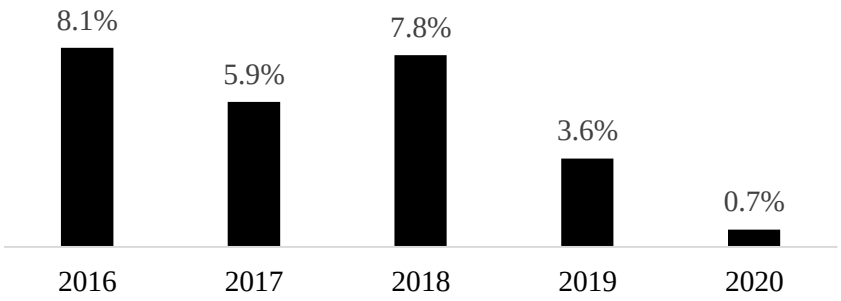

Figure 3: The distribution of HPIV positivity according to years

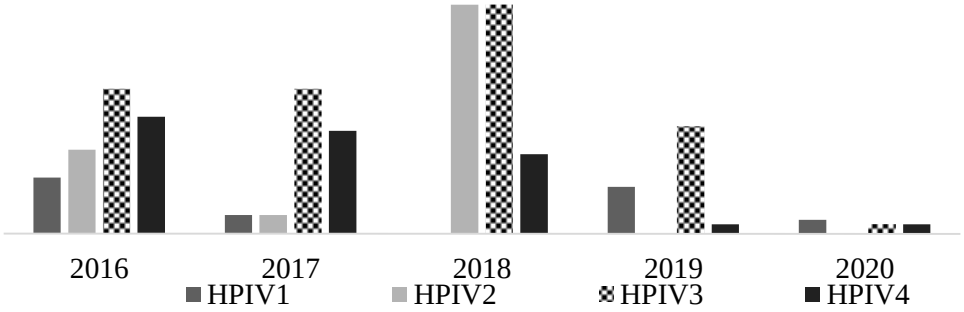

Figure 4: The distribution of HPIV types according to the years

Table 2: The distribution of HPIVs and co-infection with dual and triple agents

Co-infection with HPIVs ***Dual (n:35)***	**Patients n (%)**
Rhinovirus	13 (37.1)
HPIV-2/HPIV3	11 (31.4)
Adenovirus	3 (8.6)
Coronavirus (OC43, 229E, HKU1)	3 (8.6)
Human bocavirus	1 (2.8)
Enterovirus	1 (2.8)
İnfluenza A	1 (2.8)
Respiratory synctial virus A/B	1 (2.8)
Human paraechovirus	1 (2.8)
TOTAL	35 (100)
Triple co-infection agents (n:6)	
HPIV-2/HPIV3/Adenovirus	2 (33)
HPIV-3/Bocavirus/Adenovirus	1 (16)
HPIV-1/Adenovirus/MetapneumovirusA/B	1 (16)
HPIV-3/CoronavirusOC43/Rhinovirus	1 (16)
HPIV-3/Adenovirus/Human parechovirus	1 (16)
TOTAL	6 (100)

DISCUSSION

Acute respiratory infections are one of the most common diseases in children worldwide, and it is known that viruses are responsible for approximately 80% of these infections. After RSV, HPIVs are the second most common cause of acute respiratory illness-related hospitalizations in children <5 years of age (**6, 7**).

In the present study, we investigated the HPIVs positivity, common types and the distribution of HPIVs types positivity by age groups, months and seasons, in pediatric patients. Using multiplex real-time PCR technique we demonstrated that 4.1% of samples were positive for any HPIV types and, the most common HPIV type was HPIV-3 (55%) followed HPIV-2, HPIV-4 and HPIV-1. Aykaç et al. (**8**) investigated the prevalence of respiratory viruses in 1240 pediatric patients by multiplex real-time PCR and HPIV positivity was found as 3.7% (46/1240) in pediatric patients in Turkey. DeGroote et al. (**9**) retrospectively investigated HPIV test results belong to about 3 million pediatric patients during 2011-2019 in the USA. They reported that HPIV positivity was 5% in children and HPIV-3 was the most common type. Similarly, in lots of studies on HPIV epidemiology, HPIV-3 was reported to be the most common HPIV type and our results are in concordance with the literature (**8-12**).

The studies showing that viral co-infections of HPIV and other respiratory viruses are common in respiratory tract infections (12, **13**). In the present study, HPIV co-infection with other viruses detected in 51% of samples, and triple co-infection HPIV and other respiratory viruses were detected in six samples. Rhinovirus and Adenovirus were the most frequently coinfecting virus with 37% and 31%, respectively in our study. GU et al. (**12**) reported that rhinovirus was the most commonly detected virus with HPIV coinfection in children in Korea. In Turkey, it was reported that RSV, Rhinovirus and Adenovirus were the most common viral pathogens in respiratory tract infections in children (**11, 14, 15**). Rhinovirus and Adenovirus are one of the leading causes of respiratory diseases and are often isolated from respiratory tract infections. Also, even though the infection has been recovered clinically, the virus can be detectable for a long time. It is thought to be associated with the sensitivity of the multiplex real-time PCR method and detecting more than one virus simultaneously.

The human parainfluenza virus is one of the pathogens associated with respiratory tract infection and is responsible for substantial morbidity and mortality in children between 0 and 2 years of age. Aykaç et al. (8) found HPIV positivity in <18 years of age and they reported that 41 of 46 HPIV positive children were 0-2 years old. In China, 11,398 respiratory samples were collected from pediatric patients with acute respiratory illness and the median age of HPIV positive patients was detected as 1.7 years (0.6-2.5). In the present study, HPIV positivity was found as 45% in the 0-2 years of age ($p<0.05$). Due to incomplete immunity, children may be more susceptible to infection. The analysis of age distribution according to viral infection shows that the largest number of positive cases of HPIV, occurs in children less than 2 years of age, which is consistent with the international literature (8, **16, 17**).

Human parainfluenza virus infections are seen throughout the year, however, their highest rates were found in autumn. In our study, the highest positivity rate was in October and November (Autumn) the lowest was in February and January (Winter) and statistically, significant difference was found between the autumn and winter seasons ($p <0.05$). HPIV-1 and HPIV-2 were commonly detected in autumn; HPIV-3 was detected early summer to autumn and HPIV-4 autumn and winter. The seasonality of HPIV types differs by region. In literature HPIV-1 is usually detected in the autumn; HPIV-2 does not show seasonal variation but detects more commonly in autumn. There are generally more cases of HPIV-3 in spring and early summer; HPIV-4 usually occurs during late autumn and winter in temperate countries (18-20). Our study supports that although HPIVs are seen all year round, they reach their highest rates in autumn months by showing seasonal variation.

CONCLUSIONS

The human parainfluenza virus positivity was found between 3.6% and 8.1% in 2016-2019; however, in 2020 HPIV positivity was detected as 0.7%. The COVID-19 pandemic and related mitigation strategies and pandemic restrictions such as face mask use, disinfection and social distancing have possibly exerted a strong impact on the circulation of respiratory viruses. Not only HPIV but also all respiratory virus positivity rate was decreased all over the World (21, 22). Nevertheless, interventions used to limit person-to-person transmission of SARS-CoV-2 are the same as those that would be recommended in a parainfluenza pandemic and should therefore be expected to limit the spread of seasonal parainfluenza as well.

Author contributions: MC, SY, AT, HM, KY, HT, GB; Literature search and study design, Patient examination and therapies, experiments, statistical analyzes, **MC;** Article write up and revisions.

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Ethical issues: All authors declare originality of research.

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