

Original Article

Investigation of human bocavirus in pediatric patients with respiratory tract infection

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Abstract

Introduction: Human bocavirus (HBoV) is a linear single-stranded DNA virus belonging to the *Parvoviridae* family. This study aimed to investigate the incidence of HBoV and co-infections in pediatric patients with symptoms of viral respiratory tract infection.

Methodology: This study included 2,310 patients between the ages of 0-18 in whom HBoV and other respiratory tract viral pathogens were analyzed in nasopharyngeal swab specimens.

Results: In the pediatric age group, HBoV was found in 4.5% (105/2310) of the patients and higher in children between the ages of 1 and 5. Mixed infection was detected in 43.8% (46/105) of HBoV positive patients ($p = 0.10$). Mono and mixed infection rates were higher in outpatients than in inpatients ($p < 0.05$). Respiratory syncytial virus was significantly higher than the other respiratory viral pathogens ($p < 0.001$).

Conclusions: This study is important as it is one of the rare studies performed on the incidence of HBoV in the Marmara region. In pediatric age group, the incidence of HBoV was found 4.5%. The incidence rate of HBoV in this study was similar to those in studies around the world, but close to low rates. The incidence of HBoV was found higher especially among children between the ages of 1-5 in this study. In addition to the incidence of HBoV, accompanying co-infections in the pediatric age group were also investigated in this study. Since concurrence of RSV, HRV and hMPV with HBoV was the most common it must be considered that there may be more than one agents in patients with symptoms of respiratory tract infection.

Key words: Human bocavirus; respiratory tract infections; respiratory syncytial virus.

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Introduction

Lower respiratory tract infection (LRTI) is a major cause of morbidity and mortality worldwide especially in children. Many pathogens including bacteria, viruses and fungi can cause LRTI [1]. However, about 80% of LRTIs are caused by viruses [2]. Human bocavirus (HBoV) causing infection in the vertebrate is a linear single-stranded DNA virus belonging to *Parvoviridae* family, *Parvovirinae* sub-family and *Bocavirus* genus [3]. Infection with HBoV is common during winter and spring although it is seen throughout the whole year [4]. Worldwide prevalence of HBoV was estimated to be 6.3% for respiratory tract infections in the studies performed between 2005 and 2016 [3]. The incidence of HBoV was 5-10% in the respiratory tract samples of young children with respiratory tract infection. HBoV accompanied by another pathogen may be detected in respiratory tract specimens at a rate of 30-50% [3,4]. It was reported in studies performed on HBoV prevalence

between 2005 and 2016 that the prevalence rates ranged between 4.1-25.1% in South America, 4.1-9.8% in North America, 1.5-29.8% in Europe, 1.0-56.8% in Africa, 1.6-24.5% in the Asia, and 1.9-18.3% in the Middle East [3].

HBoV1, the first identified species, was detected in nasopharyngeal aspirate specimens of children under the age of 2 with acute respiratory tract infection in 2005. Following HBoV1, three types of HBoV, HBoV2, HBoV3 and HBoV4, were isolated in children with acute gastroenteritis [5]. Although HBoV1 was detected in both respiratory and stool specimens it was predominantly detected in respiratory specimens. HBoV2, HBoV3 and HBoV4 were mostly detected in stool specimens. In a large number of studies, HBoV1 has been identified in respiratory tract samples of patients with colds, pharyngitis, acute otitis media, asthma, bronchitis, and pneumonia [6]. As a result of molecular studies, it has been reported that high viral

load in respiratory specimens is probably associated with respiratory symptoms and that low viral load is associated with asymptomatic course [7]. HBoV1 infection may be more severe in patients with B cell response deficiency. It has been reported that immunosuppressed conditions and underlying diseases such as congenital heart diseases, heart failure, asthma, and prematurity are important risk factors for severe HBoV infections [8,9]. Important risk factors for young infants include mother's smoking, having birth in winter and tendency to have asthma [10,11].

In seroepidemiological studies, the rate of specific antibodies against HBoV is over 90% in children with the ages up to four years and between 64% and 95% in adults [12,13].

There are limited number of studies on HBoV prevalence in Turkey. In this study, the incidence of HBoV was investigated in nasopharyngeal swab specimens of pediatric age-group patients pre-diagnosed with viral respiratory tract infection. In addition to the incidence of HBoV infection, age groups, sex, seasonal differences, comorbid diseases, and abnormal findings on auscultation in patients with LRTI were investigated.

Clinical effects of viruses such as respiratory syncytial virus (RSV), human metapneumovirus (hMPV), influenza virus A (INF A), human parainfluenza virus (PIV), adenovirus (ADV), human coronavirus (HCoV)-OC43, HCoV-229E, and human rhinovirus (HRV) in respiratory tract infection are known. In addition, there is evidence that HBoV is pathogenic [14]. This study aimed to investigate HBoV in respiratory system samples and correlate them with respiratory findings.

Methodology

Study population

This cross-sectional study included 2,310 patients between the ages of 0-18 in whom HBoV and other respiratory tract viral pathogens were analyzed in nasopharyngeal swab specimens in Virology Laboratory between April 2015 and December 2017.

In this retrospective cohort study, patients' age, gender, complaints during admission, physical examination findings, laboratory results (hemogram and CRP), viral PCR results of respiratory tract swab, radiological data, status of hospitalization and length of hospital stay were recorded from their files.

The patients were diagnosed with LRTI and URTI (acute bronchitis, bronchiolitis, and pneumonia) after the assessment of symptoms, physical examination findings and radiological data together.

In addition to the incidence of HBoV infection, age groups, gender, and comorbid diseases of the patients and seasonal differences were investigated. Clinical data of the patients were obtained from the hospital database and patient files. This present study was approved by Ethics Committee of Istanbul University, Faculty of Medicine (Reference number: 2018/994/12).

Molecular analysis

Samples were sent to the laboratory in a viral transport medium (VTM, Vircell, Spain) following the cold chain rules.

Extraction of viral nucleic acids was performed on EZ1 Advanced XL using EZ1 Virus Mini Kit V 2.0 (QIAGEN, Germany) in accordance with the recommendations of manufacturer. HBoV and other viruses of the respiratory panel such as influenza virus A, influenza virus H1N1, influenza B, parainfluenza virus 1-4, adenovirus (ADV), human rhinovirus (HRV), respiratory syncytial virus (RSV) A/B, enterovirus, human metapneumovirus (hMPV), parechovirus and human coronaviruses (HCoV-229E, HCoV-HKU1, HCoV-NL63, HCoV-OC43) were investigated with multiplex real-time PCR using FTD Respiratory pathogens 21 (Fast-Track Diagnostics, Luxembourg) kit on Rotor-Gene Q (QIAGEN, Hilden, Germany). Negative and positive controls were used in all tests.

Statistical Analysis

Data analysis was performed with MedCalc Statistical Software version 12.7.7 (MedCalc Software Bvba, Ostend, Belgium; <http://www.medcalc.org>; 2013). Descriptive statistics were used in defining continuous variables (mean, standard deviation, minimum, median, maximum). Independent and non-normally distributed continuous variables were compared with Mann-Whitney U test. Comparison between frequency distribution of a categorical variable and the expected frequency distribution was performed with single sample Pearson Chi-square test. Pearson chi square test (Fisher's Exact test was used in relevant parts) was used to evaluate the relationship between the categorical variables. Statistical significance was accepted as $p < 0.05$.

Results

In this study, PCR results of 2,310 nasopharyngeal swab samples of the patients diagnosed with respiratory tract infection within about two years were analyzed.

Median age of 2,310 pediatric patients was 1 year (IQR = 0-5). Of the patients, 1,323 (57.3%) were male

and 987 (42.7%) were female. The incidence of HBoV was 4.5% (105/2310) in pediatric patients.

The incidence of HBoV was 4.8% (64/1,323) in boys and 4.2% (41/987) in girls, and no statistically significant difference was found between the genders ($p = 0.48$). Median age of patients with HBoV infection was 1.6 (IQR = 0-2) years. The highest incidence rate of HBoV among the age groups was 7.7% (64/826) in the age group between 1-5 ($p < 0.001$) (Figure 1).

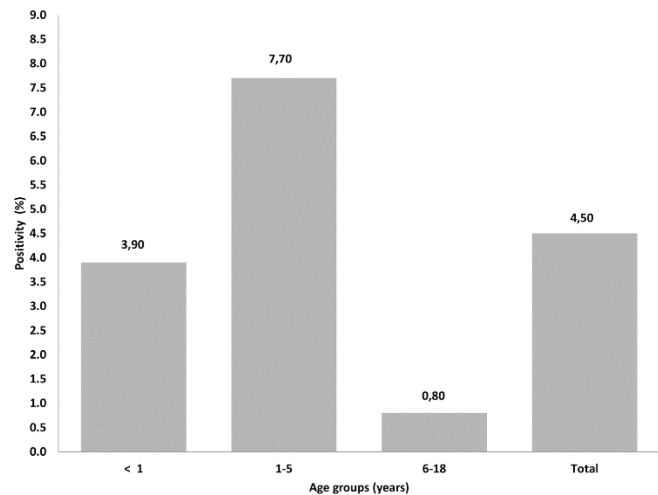
Among all the patients, mono infection with HBoV was in 2.6% (59/2310) and mixed infection was in 2% (46/2310). Together with HBoV, double infection was detected in 42 samples and triple infection in 6 samples.

The most commonly observed pathogens accompanying HBoV were RSV, hMPV, HRV, and ADV respectively. Among these mixed infection pathogens, RSV had the highest rate and its incidence was statistically higher than that of the other three viruses ($p < 0.001$) (Table 1). When the distribution of HBoV positivity according to the months was evaluated it was found that HBoV was mostly seen in December (26.7%), followed by January (16.2%) and November (14.2%) ($p < 0.001$). The lowest positivity rates were detected in April and August. Distribution of HBoV according to months is given in Figure 2.

Incidence rates of mono and mixed infection were higher in outpatients than in inpatients ($p < 0.05$). Pathological lung auscultation findings suggesting LRTI were present in 35.6% (21/59) of patients with mono-infection accompanying HBoV and 26.1% (12/46) of patients with mixed infections (Table 2).

In this study, comorbid diseases were also investigated. The most common comorbid diseases in

Figure 1. Distribution of human Bocavirus incidence rates in children according to age groups.



The highest incidence rate of HBoV among the age groups was 7.7% (64/826) in the age group between 1-5 and it was statistically significant ($p < 0.001$).

patients with HBoV infection were chronic pulmonary diseases (asthma, cystic fibrosis), cardiac diseases, neurological disorders, and hereditary metabolic diseases (Table 2).

Discussion

Respiratory tract viruses, especially HBoV, are commonly detected in asymptomatic patients and as HBoV is accompanied by other respiratory tract viruses its role in pathogenesis is still under discussion [15]. Throughout the world, HBoV infections are associated with upper and lower respiratory tract infections.

Table 1. Demographic and laboratory features of the patients.

		% (n)	p* values
HBoV positive patients	Total	4.5 (105/2310)	
	Male	4.8 (64/1323)	0.48
Age (median)		1 (IQR= 0-5)	
Mono infection		2.6 (59/2310)	
Mixed infection		2 (46/2310)	
Double pathogens	HBoV + RSV	34.8 (16/46)	< 0.001
	HBoV + HRV	17.4 (8/46)	
	HBoV + hMPV	13.04 (6/46)	
	HBoV + ADV	10.9 (5/46)	
	HBoV + PIV-4	4.3 (2/46)	
	HBoV + HCoV- HKU	4.3 (2/46)	
	HBoV + INF-A H1N1	4.3 (2/46)	
	HBoV + HCoV -229E	2.2 (1/46)	
	HBoV + ADV+ HRV	4.3 (2/46)	
Triple pathogens	HBoV + HRV+ PIV-1	2.2 (1/46)	
	HBoV+ RSV+ INF-A H1N1	2.2 (1/46)	

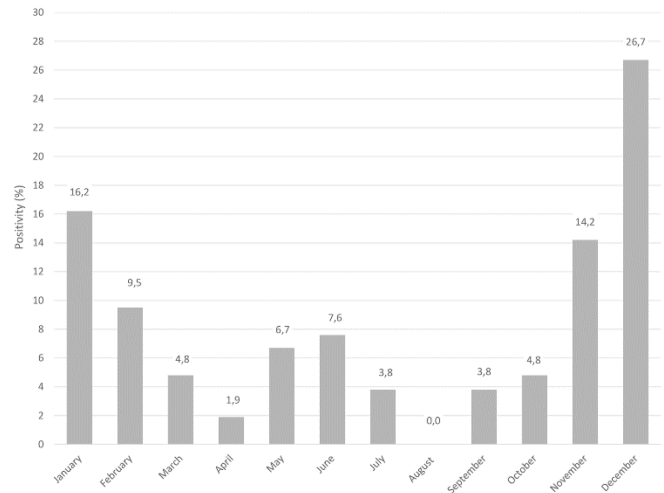
HRV: Human rhinovirus; INF-A: Influenza A virus; H1N1: Pandemic influenza A H1N1 virus; hMPV: Human metapneumovirus; HBoV: Human bocavirus; HCoV: Human coronavirus; PIV-1: Parainfluenza virus tip 1; PIV-4: Parainfluenza virus tip 4; ADV: Adenovirus; * Mann-Whitney U test and One Sample Chi-Square test respectively; IQR: interquartile range.

Majority of the studies on this subject include the pediatric age group [16]. In this study, HBoV was detected in 4.5% (105/2310) of nasopharyngeal swab samples of patients in the age group between 0-18. In studies on the incidence of HBoV in pediatric patients in our country, the rates were 2.5% (5/76) and 6.7% (8/120) in Istanbul [17,18] and 6.5% in Ankara [19]. The results of this study with high number of patients were almost similar to those of other studies performed in Turkey.

In several studies, median age of children infected with HBoV was about 12 months [4]. In this study, median age of the patients with HBoV infection was 1.6 years and compliant with the results of similar studies [1,20,21]. When the distribution of HBoV according to age groups was evaluated HBoV was found lowest in patients under the age of 1 (3.9%), and highest in patients between the ages of 1-5 (7.7%) (p < 0.001). In a seroepidemiologic study, while maternal antibody level was observed at a rate of 90.5% in children with the age of three months this rate decreased to 5.6% in children between the ages of six and eight months. The high rate of seropositivity in infants under the age of 1, especially in infants younger than six months old, was thought to be due to the presence of maternal antibodies [22].

In respiratory tract samples, HBoV was commonly accompanied by other viral pathogens. There are studies reporting that co-infection rates are as high as 83% [6]. These high rates have been explained with the fact that HBoV viral release in asymptomatic patients could last for a long term and be persistent [23]. In a study in Ankara, the incidence rate of mixed infection accompanying HBoV was 38.7% [19]. HBoV was reported to cause mixed infections with viruses such as HRV, RSV and hMPV [24]. In a study in Thailand,

Figure 2. Distribution of human bocavirus incidence rates according to months.



Distribution of HBoV according to months.

HBoV was found in 4.5% of patients diagnosed with pneumonia. HBoV was found in 83% of patients under the age of 5 whereas only 1% was HBoV positive in the asymptomatic control group. The most commonly observed viruses accompanying HBoV were HRV, RSV and PIV. Viral load detected in children by PCR was higher in patients with only HBoV than in patients with mixed infection [25]. In Spain, 123 of out of 917 (13.4%) nasopharyngeal aspirate samples belonging to 730 patients under the age of 14 were HBoV positive. In 74 (60%) of HBoV positive samples, coinfection with other respiratory viruses was detected. HBoV was found in 20 samples (27%) with ADV, 17 (23%) with RSV and 12 (16%) with HRV. In 18 cases (24%), three or more viruses were detected [26].

Table 2. Pathological auscultation findings of the patients, and comorbid diseases.

	Mono HBoV infection	Mixed infection	p* values
Outpatients % (n)	57.6 (34/59)	76.1 (35/46)	< 0.05
Inpatients % (n)	42.4 (25/59)	23.9 (11/46)	
LRTI findings	35.6 (21/59)	26.1 (12/46)	0.30
Comorbid disease	40.7 (24/59)	28.3 (13/46)	0.86
CPD	6.8 (4/59)	6.5 (3/46)	
Cardiac disease	6.8 (4/59)	2.2 (1/46)	
CND	6.8 (4/59)	4.3 (2/46)	
HMD	6.8 (4/59)	2.2 (1/46)	
Renal disease	3.4 (2/59)	2.2 (1/46)	
Down syndrome	0	2.2 (1/46)	
Malignity	3.4 (2/59)	2.2 (1/46)	
Others	6.8 (4/59)	6.5 (3/36)	

LRTI: Lower respiratory tract infection; CPD: Chronic pulmonary diseases: asthma, cystic fibrosis; CND: Chronic neurological disease; HMD: Hereditary metabolic disease; Others: pneumothorax, tuberculosis peritonitis, idiopathic neutropenia, hereditary skin and connective tissue disease, trisomy 18; * One Sample Chi-Square test.

It was suggested that HBoV may be the etiological agent responsible for high viral load in respiratory tract specimens of children with HBoV [7,27,28]. In a variety of prevalence studies, HBoV is isolated from respiratory tract specimens at a rate of 1.5%-21.5% as a single pathogenic agent and at a rate of 30%-80% with other viruses [29,30]. In this study, the incidence rate of mono infection was 56% (59/105) and the rate of mixed infection was 44% (46/105). It was found that the most common viral pathogens accompanying HBoV were RSV, hMPV, HRV, and ADV, which is similar to the findings in other studies [26]. In addition, HBoV was qualitatively evaluated with multiplex PCR method in this study and since viral load amount could not be evaluated its importance in respiratory tract disease could not be completely determined.

In this study, abnormal findings on auscultation in patients with HBoV were detected more commonly in inpatients than in outpatients. Abnormal findings on auscultation were detected in 21 out of 59 patients with HBoV mono-infection. Although this finding is important in supporting HBoV as a respiratory pathogen it has not been proven to be a pathogen responsible for LRT infection since the viral load of HBoV is qualitatively assessed in this study. It is important to determine the viral load of HBoV when it is detected alone especially in patients with a comorbid disease [30].

The distribution of HBoV-positive samples according to months shows an epidemiological profile similar to those of ADV and HRV [26]. While HBoV was isolated all year long it was isolated most commonly during winter and autumn [28]. In a study in Korea, while HBoV infections were commonly seen in March and December it peaked in June [20]. However, it was seen all year round without a seasonal difference in Canada [31]. In a study in Ankara, it was reported that HBoV in 61.3% of patients was seen in November and March [19]. In this study, while HBoV was seen throughout the whole year it was interestingly not seen in August. The months when HBoV was most common were the ones between November and February.

This study has some limitations. Since especially RSV infections are among the most important agents of childhood viral pneumonias it is important to compare them with HBoV. As this is a retrospective study other virus infections accompanying HBoV could not be compared in terms of patient characteristics (clinical findings, radiological findings, comorbid diseases, etc.). Furthermore, quantitation of viral load in detecting HBoV and other viruses could not be performed in this study. Therefore, the relationship of

HBoV with the severity of disease could not be investigated in patients either with HBoV alone or with co-infections.

Conclusion

This study is important as it is one of the rare studies performed on the incidence of HBoV in the Marmara region. In pediatric age group, the incidence of HBoV was found 4.5%. The incidence rate of HBoV in this study was similar to those in studies around the world, but close to low rates. The incidence of HBoV was found higher especially among children between the ages of 1-5 in this study. In addition to the incidence of HBoV, accompanying co-infections in the pediatric age group were also investigated in this study. Since concurrence of RSV, HRV and hMPV with HBoV was the most common it must be considered that there may be more than one agents in patients with symptoms of respiratory tract infection.

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