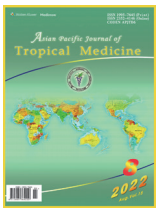

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Human bocavirus infection in children hospitalized with lower respiratory tract infections: Does viral load affect disease course?

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ABSTRACT

Objective: To examine the effects of human bocavirus type 1 (HBoV1) on the course of lower respiratory tract infections in cases of monoinfection and coinfection, and the effects of HBoV1 viral load on the disease in children under six years old hospitalized with a diagnosis of HBoV1-associated lower respiratory tract infections.

Methods: Children under six years of age, who were hospitalized with the diagnosis of lower respiratory tract infection due to HBoV1 between 1 January 2021 and 1 January 2022 were included in the study. Laboratory confirmation of the respiratory pathogens was performed using polymerase chain reaction (PCR).

Results: Fifty-four (16.4%) children with HBoV1 among 329 children whose PCR was positive with bacterial/viral agent in nasopharyngeal swab samples were included in the study. There were 28 (51.9%) males and 26 (48.1%) females with a median age 23.4 months [interquartile range (IQR): 13.2, 30.0 months] (min-max:1 month-68 months). HBoV1 was detected as a monoinfecton in 26 (48.1%) children, and as a coinfection with other respiratory agents in 28 children (51.9%). In multiple regression analysis, coinfection (P=0.032) was associated with the length of hospitalization (P<0.001; R^2=0.166). There was a negative correlation (r= 0.281, P=0.040) between cough and cycle threshold. Fever was found to be positively correlated with C-reactive protein (r=0.568, P<0.001) and procalcitonin (r=0.472; P=0.001).

Conclusions: Although we found a higher HBoV1 viral load in children with more cough symptoms in our study, it had no effect on the severity of the disease, such as length of hospital stay and need for intensive care. Coinfection was found to affect the length of hospitalization.

KEYWORDS: Human bocavirus; Lower respiratory tract infection; Children; Viral load

1. Introduction

Respiratory tract infections (RTI) are among the most common causes of infection in children. Although lower respiratory tract infections (LRTI) are a significant cause of death particularly in children under the age of five, it has been determined as the sixth cause of death in all age groups. It was reported that 2.38 million cases of LRTI died in 2016[1]. Viruses are the primary etiology and the main viral agents in children include human respiratory syncytial virus, influenza A and B viruses (IAV, IBV), human adenoviruses (HAdVs), human parainfluenza viruses, human bocavirus (HBoV) and human coronaviruses (HCoVs) including SARS-CoV-2 which was identified in the early 2020s[2]. HBoV is a DNA virus that belongs to the Parvoviridae family and the genus *Bocavirus*. It was first detected in the upper respiratory tract secretions of a

Significance

It is known that lower respiratory tract infections caused by HBoV1 in children generally have a benign course. However, there are few studies on viral load. Although no correlation was found between viral load and disease severity in this study, the presence of coinfection prolongs the length of hospital stay.

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Swedish pediatric patient in 2005[3]. There are four types of HBoV: HBoV type 1 is associated with RTIs, while HBoV types 2-4 have been associated with gastroenteritis[3,4]. Although it varies across countries, the mean detection prevalence of HBoV in respiratory tract isolates was between 1.0% and 56.8%; the overall prevalence of detection of RTIs worldwide is estimated to be 6.3%[2,5]. HBoV can infect all age groups, but it is especially common in young children[5]. While an asymptomatic course can be observed if the HBoV viral load is low in children, symptoms related to RTIs can be observed in cases with high viral load[6]. The main respiratory symptoms of HBoV1 infection include runny nose, fever, cough, and lower respiratory tract infections[2,3]. Apart from RTI, which is the most common clinical picture, it may also present with findings such as diarrhea, conjunctivitis and even encephalitis, which shows the importance of the virus[7,8].

Since HBoV is a novel virus that was identified in the 2000s, data on HBoV-related LRTIs in children are still limited. In the present study, we aimed to examine the effects of HBoV1 on the course of the disease in cases of monoinfection and coinfection, and the effects of HBoV1 viral load on the disease in children under six years old hospitalized with a diagnosis of HBoV1-associated LRTI.

2. Subjects and methods

2.1. Study design

This retrospective descriptive study was conducted in a tertiary city hospital in Istanbul, Turkey, from January 2021 to January 2022.

2.2. Data collection

Participants were identified through the department's patient files archive. The age, sex, clinical findings, laboratory findings [white blood cell (WBC), the absolute neutrophil count (ANC), C-reactive protein (CRP), procalcitonin (PCT)], microbiological findings (PCR for viral and bacterial respiratory agents), radiological findings (chest X-ray), intensive care unit stay, and stay of hospital were obtained from patient records.

2.3. Definition

LRTI is defined as a clinical picture in which fever, respiratory symptoms and parenchymal involvement are identified on physical examination and/or chest radiography findings[9,10]. All of our patients had a chest X-ray, and advanced imaging methods such as computed tomography of thorax were applied to patients with clinical worsening and when necessary.

Nasopharyngeal swab samples of patients were collected using Dacron or Polyester swabs in Bio-Speedy® vNAT® Viral Transfer Tubes (Cat No:BS-NA-513-100). Proper vigorous vortexing of samples in vNAT enables viral nucleic acid extraction from the nasopharyngeal swabs while vNAT reagent breaks down the virus and releases the nucleic acids[11].

Samples were tested with Bio-Speedy®Respiratory RT-qPCR MX-24S Panel. This kit is performed by one-step reverse transcription and real-time PCR (qPCR) (RT-qPCR), targeting genomic RNA and DNA regions specific to the target agent. PCR reactions are performed in the Bio-Rad CFX 96 instrument. LoD values of the kit is given as 83 copies/mL for human bocavirus, sensitivity and specificity values are 98.95% and 99.13%, respectively. Screened pathogens (18 viruses and 6 bacteria) were SARS-CoV-2, influenza A, influenza B, human coronavirus 229E, human coronavirus OC43, human coronavirus NL63, human coronavirus HKU1, parainfluenza 1, parainfluenza 2, parainfluenza 3, parainfluenza 4, metapneumovirus (MPV), respiratory syncytial virus (RSV) A/B, enterovirus (HEV), adenovirus (AV), HBoV, human parechovirus (HPeV), rhinovirus (HRV), *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Haemophilus (H.) influenzae*, *Bordetella pertussis* and *Streptococcus (S.) pneumoniae*.

According to the kit protocol, 5 µL patient samples with vNAT were added to a 10 µL Prime Script Mix (DNA polymerase, dNTP miks, reverse transcriptase enzyme, ribonuclease inhibitor and reaction buffer) to achieve 15 µL PCR mixture in total. The human RNaseP oligo set was used to check for RNA stability, nucleic acid extraction, and inhibition of both qPCR and reverse transcription. Negative and positive control templates were also added to test for contamination and qPCR reagent stability. The real-time PCR program was 52 ℃ for 5 min and 95 ℃ for 10 s, followed by 40 cycles of 95 ℃ for 1 s and 55 ℃ for 10 s. Test interpretation was performed as per the manufacturers' protocol. Cycle threshold (Cq) and relative fluorescence units (RFU) results values were evaluated. Threshold level to calculate the number of threshold cycles was 200 RFU for CFX96 Touch™ instrument. If Cq of the gene targets are ≤38, it is concluded as positive. If Cq of the gene targets are >38, it is concluded as negative. If the final result was positive, it was interpreted as follows: 32 Cq 38=low positive, 18 Cq<32=positive and Cq<18=very high positive[12].

2.4. Ethical approval

The Medical Research Ethics Committee of our institution approved this study (Report Number: 2022/514/226/9).

2.5. Statistical analysis

Normally distributed quantitative variables are expressed as mean±standard deviation (SD) whereas non-normally distributed

quantitative variables are expressed as median with interquartile ranges (IQR). The *Chi*-square test or Fisher exact test was used for comparing categorical variables. The Mann-Whitney *U* test was used for comparing non-normally distributed quantitative variables. Multiple regression analysis was done to assess confounding factors. All analyses were conducted using SPSS version 25 software (IBM SPSS Statistics, New York) and $P<0.05$ was used to indicate a statistically significant difference.

3. Results

3.1. Sociodemographic, clinical and laboratory characteristics

Fifty-four (16.4%) children with HBoV1 among 329 children whose PCR was positive with bacterial/viral agent in nasopharyngeal swab samples were included in the study. There were 28 (51.9%) males and 26 (48.1%) females with a median age 23.4 months (IQR: 13.2, 30.0 months) (min-max: 1-68 months). LRTI-associated HBoV1 was detected as a monoinfecton in 26 (48.1%) chilren and as a coinfection with other viruses in 28 children (51.9%) (Table 1).

The most common presenting symptoms were cough (85.2%), fever (63.0%), and rhinorrhea (27.8%). The other clinical symptoms were rash (7.4%) and seizures (5.6%). All participants had parenchymal involvement on radiological imaging confirming the LRTI. Eight (14.8%) children needed intensive care unit (ICU) admission.

The median WBC count was 13 925/mm^3 (IQR: 11 275, 19 637) (min-max: 3 800-35 300/mm^3), median ANC was 9 450/mm^3 (IQR: 4 675, 14 925) (min-max: 1 000-27 700/mm^3); the median CRP was 18.00 mg/dL (IQR: 4.20, 44.50) (min-max: 1-244) and the median PCT was 0.39 mg/dL (IQR: 0.13, 1.50) (min-max: 0.056-83). The median RFU was 22 286.5 (IQR: 6 339.0, 31 546.0) (min-max: 488-40 609) and the median Cq was 25.65 (IQR: 21.00, 31.60) (min-max: 15.12-36.50). The median length of hospitalization was 8 (IQR: 6, 10) days (min-max: 3-17 days) (Table 2). Fever was found to be positive correlated with CRP ($r=0.568$, $P<0.001$) and PCT ($r=0.472$, $P=0.001$). Rhinorrhea was found to be negative correlated with CRP ($r=-0.357$, $P=0.008$) and PCT ($r=-0.309$, $P=0.032$). Seizures was found to be negative correlated with WBC ($r=-0.332$, $P=0.014$) and ANC ($r=-0.329$, $P=0.015$) (Table 3).

The monthly distribution of HBoV1 infection is shown in Figure 1. The peak incidence of HBoV1 infection appeared in December (27.8%), November (25.9%) and October (22.2%).

Table 1. Overview of the general information and clinical findings of children with HBoV1 monoinfection and HBoV1 coinfection.

Variable		All children (n=54)	HBoV1 monoinfection (n=26, 48.1%)	HBoV1 coinfection (n=28, 51.9%)	χ^2/Z	P value
Age, months		23.4 (13.2, 30.0)	27.0 (17.4, 33.6)	21.6 (9.6, 30.0)	-0.14	0.480[a]
Age groups, years, *n* (%)	1 month-<1 year	10 (18.5)	2 (7.7)	8 (28.6)	4.47	0.107[b]
	1-<5 years	41 (75.9)	23 (88.5)	18 (64.3)		
	5-<18 years	3 (5.6)	1 (3.8)	2 (7.1)		
Sex, *n* (%)	Female	26 (48.1)	12 (46.2)	14 (50.0)	0.08	0.777[b]
	Male	28 (51.9)	14 (53.8)	14 (50.0)		
Clinical findings, *n* (%)	Fever	34 (63.0)	15 (57.7)	19 (67.9)	0.59	0.440[b]
	Cough	46 (85.2)	23 (88.5)	23 (82.1)	-	0.706[c]
	Rhinorrhea	15 (27.8)	8 (30.8)	7 (25.0)	0.22	0.636[b]
	Rash	4 (7.4)	0 (0.0)	4 (14.3)	-	0.112[c]
	Seizuresc	3 (5.6)	1 (3.8)	2 (7.1)	-	1.000[c]
ICU stay, *n* (%)		8 (14.8)	6 (23.1)	2 (7.1)	1.87	0.100[b]
Length of hospitalization, days		8 (6, 10)	7 (6, 8)	8 (7, 10)	-1.82	0.069[a]

ICU: intensive care unit, *CI*: confidence interval. Categorical data are presented as number (percentage) and continuous variables as median (interquartile range). [a]*P* value by Mann-Whitney *U* test; [b]*P* value by *Chi*-square; [c]*P* value by Fisher exact test; $P<0.05$ is statistically significant.

Table 2. Overview of the laboratory findings of children with HBoV1 monoinfection and HBoV1 coinfection.

Variable	All children (n=54)	HBoV1 monoinfection (n=26, 48.1%)	HBoV1 coinfection (n=28, 51.9%)	χ^2/Z	P value
WBC, /mm^3	13 925 (11 275, 19 637)	13 200 (9 650, 16 150)	16 300 (11 675, 21 425)	-1.84	0.065[a]
ANC, /mm^3	9 450 (4 675, 14 925)	10 000 (4 675, 13 025)	8 250 (4 325, 15 800)	-0.32	0.749[a]
CRP, mg/dL	18.00 (4.2, 44.5)	18.00 (5.00, 33.20)	12.50 (2.25, 63.00)	-0.19	0.849[a]
PCT, μg/L	0.39 (0.13, 1.51)	0.39 (0.10, 1.30)	0.40 (0.10, 1.80)	-0.67	0.502[a]
RFU	22 286.5 (6 339.0, 31 546.0)	22 286.2 (12 422.0, 28 683.0)	23 255.0 (4 470.0, 32 516.7)	-0.04	0.972[a]
Cq, *n* (%)	25.65 (21.00, 31.60)	26.30 (21.60, 29.10)	24.14 (20.20, 33.90)	-0.14	0.890[a]
Cq<18	5 (9.3)	3 (11.5)	3 (7.1)	3.35	0.185[b]
18≤Cq<32	37 (68.5)	20 (76.9)	17 (60.7)		
32≤Cq≤38	12 (22.2)	3 (11.5)	9 (32.1)		

WBC: white blood cell, ANC: absolute neutrophil count, CRP: C-reactive protein, PCT: procalcitonin, *CI*: confidence interval, RFU: relative fluorescence units, Cq: cycle threshold. Categorical data are presented as number (percentage) and continuous variables as median (interquartile range). [a]*P* value by Mann-Whitney *U* test (for continuous variables non-normally distributed). [b]*P* value by *Chi*-square (for categorical variables); $P<0.05$ is statistically significant.

Table 3. Correlation analysis between clinical and laboratory findings.

Variable	RFU		Cq		WBC		ANC		CRP		PCT	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Fever	-0.025	0.860	0.054	0.697	0.100	0.473	0.192	0.164	0.568**	<0.001	0.472**	0.001
Cough	0.268	0.051	-0.281*	0.040	0.227	0.098	0.216	0.117	0.089	0.523	0.123	0.405
Rash	-0.290*	0.033	0.336*	0.013	0.041	0.769	-0.095	0.493	-0.057	0.683	-0.065	0.659
Rhinorrhea	0.160	0.246	-0.203	0.141	0.021	0.879	0.008	0.954	-0.357**	0.008	-0.309*	0.032
Seizures	0.122	0.380	-0.122	0.380	-0.332*	0.014	-0.329*	0.015	-0.216	0.117	-0.168	0.252

WBC: white blood cell, ANC: absolute neutrophil count, CRP: C-reactive protein, PCT: procalcitonin, *CI*: confidence interval, RFU: relative fluorescence units, Cq: cycle threshold. *Correlation is significant at the 0.05 level, **correlation is significant at the 0.01 level.

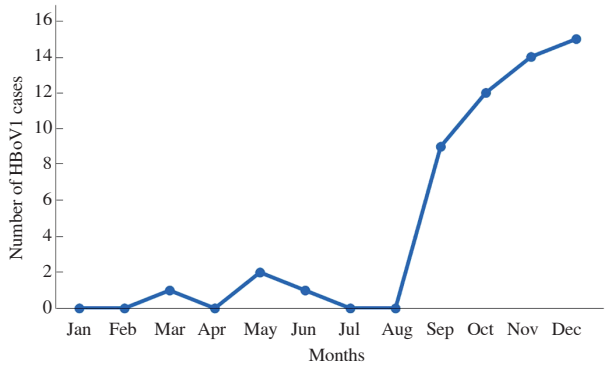

Figure 1. The monthly distribution of HBoV1 infection.

3.2. Comparison results of monoinfection and co–infection cases

Coinfection was detected in 28 (51.9%) children: bilateral coinfection in 23 (82.1%) children and triple coinfection in 5 (17.9%) children. *S. pneumoniae* (32.1%), RSV (21.4%), rhinovirus/enterovirus (17.9%), parainfluenza type 3 (14.3%), SARS-CoV-2 (10.7%), Coronavirus OC 43 (7.1%), *H. influenza* (7.1%) and parainfluenza type 1 (3.6%) were responsible for coinfection.

There was no difference in age, length of hospitalization, admission to the ICU, laboratory findings, RFU and Cq between patients with HBoV1 monoinfection and patients who were co-infected with other agents. Clinical, epidemiological and laboratory findings of participants with LRTI with HBoV1 monoinfection *versus* HBoV1 coinfection is shown in Table 1 and 2.

Multiple regression analysis was done using the length of hospitalization as the dependent variable and WBC, CRP, PCT, ANC and coinfection as the independent variables. From this, only coinfection (P=0.032) was found to affect the length of hospitalization (P<0.001, R^2=0.166) (Table 4).

3.3. Results on HBoV1 viral load

There was a negative correlation between the occurrence of a rash and the RFU value (r=0.290, P=0.033), and a positive correlation with the Cq value (r=0.336, P=0.013). There was a negative correlation (r=0.281, P=0.040) between cough and Cq (Table 3). None of the patients died and a clinical cure was achieved in all patients.

4. Discussion

A common feature of HBoV-associated LRTIs is the high rate of coinfection[2,13,14]. This phenomenon raises the question of whether HBoV is the real agent or it is detected as a passenger host because it can remain in the host for a long time like other Parvoviridae members[15]. However, there are studies that support the association of HBoV with infection in many regions of the world[2,13,16],17]. Several other studies equally showed that patients are more symptomatic, in particular with fever, cough, and lung involvement on radiological imaging, as the viral load increases in HBoV-associated LRTIs[16,18,19]. Consistent with the literature, we observed fever and cough as the most common findings in our patients[7,17]. Moreover, a negative correlation was found between cough and Cq, suggesting that cough is more common in patients with a high HBoV1 viral load. We suppose that HBoV1 is a real RTI agent, since all of the symptomatic patients in our study had indications for hospitalization due to LRTI and all had high detection rates of monoinfection. In our study, the incidence of rash was more common in co-infected patients than in patients with HBoV1 monoinfection. In accordance with this, there was a negative correlation between rash and the RFU value, and a positive correlation with the Cq value.

Table 4. Evaluation of the effect of variables on length of hospital stay with linear regression analysis.

Variable	B	Std.Error	β	*t*	*P* value	95% *CI*	
						Lower	Upper
Length of hospitalization (Constant)	8.247	1.209		6.820	<0.001	5.807	10.688
WBC	-9.935E-5	0.000	-0.242	-0.617	0.541	0.000	0.000
CRP	-0.012	0.010	-0.251	-1.205	0.235	-0.033	0.008
PCT	0.083	0.045	0.334	1.833	0.074	-0.008	0.174
ANC	6.603E-5	0.000	0.151	0.404	0.688	0.000	0.000
Coinfection	2.207	0.994	0.370	2.221	0.032	0.202	4.212

R^2=0.166, WBC: white blood cell, ANC: absolute neutrophil count, CRP: C-reactive protein, PCT: procalcitonin, *CI*: confidence interval.

This strongly suggests that the cause of the rash is associated with other respiratory agents rather than HBoV1.

In studies of pediatric patients in our country, the incidence of HBoV monoinfection ranges from 2.5%-10%, and coinfection rates range from 38.7%-43.8%[14,17,20–22]. In the meta-analysis of Falahi *et al*, which examined 22 studies and 6 751 cases, the prevalence of LRTI-associated HBoV in children under the age of 2 was 13%. In the same study, the detection rates of HBoV as a single agent and as coinfection were 4% and 9%, respectively[23]. In addition, studies in Belgium and Italy in pediatric patients with RTIs and HBoV, found high coinfection rates at 85.1% and 51.7%, respectively[2,13]. Others consider it controversial to accept HBoV as a true pathogen because of its high detection rate as a coinfection with other viruses[5,15]. In the study by Xu *et al*. in Finland, it was reported that HBoV persisted in the respiratory tract, palatine and adenoid tonsils, which may be associated with the high frequency of its coinfection[24]. In our study, the rate of detection of HBoV1 as a single agent was 48.1%, while the rate of detection as a coinfection was 51.9%. We found both monoinfection and coinfection rates in our study to be high, similar to the studies conducted in Belgium and Italy, and we suppose that it would be more accurate to consider that HBoV1 was the real causative agent in our patients given that they had a diagnosis of LRTI requiring hospitalization. Moreover, in our study, HBoV1 viral load was found to be lower in the co-infected patient group, albeit not statistically significant. Not with standing, it is difficult to say which of the determined factors is the dominant factor in cases of coinfection; however, we presume that the high viral load of the detected agent in particular will increase the probability of it being a possible factor. RSV was found to be the most frequently co-detected virus with HBoV in different studies[13,14,25]. The most common coinfection agent in our study was *S. pneumoniae* followed by RSV, consistent with the literature. In a study conducted by Zhang *et al*. in China, *S. pneumoniae* was detected as a coinfection agent with a rate of 13.3%, similar to the findings in our study[26]. On the other hand, it should not be forgotten that *S. pneumoniae* detected in nasopharyngeal swab with RT-PCR is not always a coinfection, it may also be detected as a result of nasopharyngeal colonization.

In a study conducted in the United States, it was reported that the frequency of HBoV was highest in the months of spring, whereas, in two different studies conducted in Hunan (Changsha) and Zhejiang (Ningbo) in China, the frequency of HBoV was found to be highest in summer and winter, respectively[7,16,26]. HBoV1 had a peak during autumn and winter seasons (September-December) in our study. Similarly, in the studies conducted in our country, it was observed that the detection rates of HBoV are higher in November, December and January[14,17]. The different geographical regions and with different population groups may have contributed to the differences in the detection seasons of HBoV across studies.

Data on the detection rates of HBoV in pediatric patients with LRTI during the pandemic is quite limited. In a study comparing the causative agents detected in 2018 and 2019 in pediatric patients with RTI in China during the pandemic, there was no statistical change in the frequency of HBoV[27]. In a study conducted between May 2017 and March 2021 in pediatric patients with RTIs in Croatia, which partially included the pandemic period, the rate of HBoV as the sole agent was 7.6%, while the rate of detection as a coinfection was 82.2%[28]. Our study covers a section of the pandemic and we found HBoV1 as the only factor with a rate of 48.1% in pediatric patients with LRTI, which is quite high. Since the data on this subject is presently limited, it is difficult to conclude whether HBoV is seen at increasing rates during pandemics; new studies will provide more insight on this issue.

In a study conducted by Eşki *et al*. in our country, in which they investigated viral agents and coinfections in pediatric patients with LRTIs for a period of 10 years, HBoV coinfection increased the severity of LRTI[21]. In a study conducted by Sun *et al*. in pediatric patients in China, it was shown that, HBoV coinfection increases HBoV infection severity[29]. On the other hand, there was no difference in clinical and radiological findings between pediatric patients with acute RTI with HBoV coinfection or monoinfection in Rome in the study of Petrarca *et al*[13]. In our study, coinfection was found to affect the length of hospitalization.

In the study of Zhou *et al*. in pediatric patients with HBoV-associated LRTI in China, it was reported that the clinical characteristics of the patients were not correlated with the HBoV viral load[16]. On the other hand, in the study conducted by Sun *et al*. in pediatric patients with acute RTI between 2012 and 2014, HBoV DNA copy numbers were found to be positively correlated with the length of hospitalization[29]. Moreover, in a study conducted in three different centers in Belgium investigating HBoV in RTIs in children, it was shown that increased HBoV viral load was associated with wheezing[2]. Similarly, in the study of Jiang *et al*. in which patients diagnosed with HBoV-related LRTI were examined, HBoV viral load was higher in younger patients and patients with wheezing and tachypnea/dyspnea. In the same study, it was shown that coinfection was detected more frequently among patients with a low viral load[30]. In a study by Ding *et al*. in China, although it was shown that the HBoV viral load was higher in children with wheezing, it was reported that there was no significant relationship between the severity of the LRTI and the HBoV viral load[31]. LRTI's pose a serious problem for intensive care units with their increasing incidence and mortality rates[32]. Although we found a higher

HBoV1 viral load in patients with more cough symptoms in our study, we found that it had no effect on the severity of the disease, such as length of hospital stay and need for intensive care.

There were several limitations in our study. First, this retrospective study was conducted in a single center for one year. We think that the findings of multicenter studies covering a wider geographical area and period will provide more accurate results. Second, a part of our study coincided with the pandemic period. In this period, the use of masks, less social activity than normal, and social distance may have reduced the incidence of RTIs or have altered the results. However, we believe that more studies are needed to make clearer comments on this issue.

In conclusion, in the present study, we found that HBoV1 is a common and important factor in the etiology of LRTI. Although coinfection of HBoV1 with other respiratory factors did not have an effect on the course of the disease, such as hospitalization in the ICU and mortality, we found that it prolonged the length of hospital stay. In addition, only cough was detected more in those with a high HBoV1 viral load, no correlation was found between clinical and laboratory findings other than this. Since HBoV1 is a novel virus detected in the 2000s, it is still a subject of research.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Authors' contributions

AK and CÇ contributed to the study conception and design. AK, CÇ and SDT implemented the study. AK, CÇ, MTK, SDT, UY and YA analyzed and interpreted the data. AK and CÇ revised the work critically for intellectual content and granted final approval for publishing. All authors have reviewed the manuscript and consent was given to publish.

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