Human bocavirus single infections and co-infections with Respiratory Syncytial Virus and Rotavirus in children with acute respiratory or gastrointestinal infections

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Summary

Human bocavirus (HBoV) has been reported in the respiratory and stool samples in pediatric infections. The aims of study were to find the correlation between of HBoV single and co-infections in respiratory and gastrointestinal cases. This report was in the period 2017–2018 that involved children less than 3 years old, who admitted to Mofid Children's Hospital in the center of Iran in Tehran. At first, the respiratory and stool samples tested for the RSV and Rotavirus by RT-PCR respectively and then all samples tested for the NP-1 gene of HBoV by PCR. The 67 out

of 500 respiratory samples (13.4%) and 72 out of 500 stool samples (14.4%) were positive of HBoV. The 44 (65.6%) of respiratory samples have co-infection with RSV and 45 (62.5%) of stool samples have co-infection with Rotavirus. The HBoV infections emerged often as co-infections with other viral agents.

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Key words
human bocavirus; viral infections; co-infections

Introduction

Acute respiratory tract infection (ARTI) and Acute gastroenteritis (AGE) are two important causes of morbidity and mortality with a worldwide disease burden.[1,2] Human Bocavirus (HBoV) determined in 2005, in Sweden by Allander by a molecular technique in respiratory specimens of children.[3] The HBoV is a member of the genus Boca parvovirus, subfamily Parvoviridae, family Parvoviridae.[4] They are small non-enveloped viruses. The linear negative or positive-sense single-stranded DNA with a length of about 5.3 kb packed in an icosahedral nucleocapsid with a diameter of 25 nm.[4,5] The genome of HBoV organized in three open reading frames (ORFs) that encoding NS1, NP1, VP1, and VP2 proteins. The NP1, the protein that is unique to bocavirus, represents no similarity to other parvovirus proteins.[6] There were four viral species of this virus that including of HBoV-1, HBoV-2, HBoV-3, and HBoV-4, which were identified by genomic analysis of the structural (VP1/VP2) and non-structural (NS1 and NP1) regions of the HBoV.[4-6] HBoV-1 isolated mainly in respiratory samples.[4] The other HBoV subspecies were then isolated in human stool samples.[4] HBoV detected in various clinical samples, including nasopharyngeal aspirate, serum, feces, and urine.[4,5] The average prevalence of HBoV in respiratory tract samples extended from 1.0% to 56.8% and in stool specimens from 1.3% to 63%. This distribution of infections is depending on the geographical features.[4,7,8] The HBoV infections extension is worldwide; its transmission and infection occurs throughout the year but is predominant during cold seasons as like as winter and spring months.[4,5] The transmission routes of human bocavirus are unknown.[5] To clinically diagnose an HBoV infection, it is necessary to screen respiratory tract or stool samples based on PCR. In most laboratories use PCR assays targeting the NP-1, NS-1 or VP1/2 gene.[9] A variety of symptoms and diseases described in HBoV positive-patients, such as rhinitis, pharyngitis, cough, dyspnea, wheezing, pneumonia, acute otitis media, fever, nausea.[4,5] The co-infection of HBoV with other viruses is very common.[4,10] There wasn't any comprehensive study of HBoV infections and Co-infections with other viral agents in Middle-east, by notice of this issue, this paper reports a cross-sectional study involving children less than 3 years old during 2016-2017.

Material and Methods

The study was part of a cross-sectional study in the period 2017–2018 that involved children less than 3 years old, who admitted to Mofid Children's Hospital in center of Iran in Tehran city. The respiratory tract infection group included 500 nasopharyngeal swabs (NPS) obtained from pediatric patients who visited the hospital while presenting with respiratory tract infection symptoms, including fever, wheezing, coughing, and sputum, hypoxia, dyspnea, rhinorrhea during the same period. The second group is the gastroente-

ritis patients: 500 stool samples from patients with acute gastroenteritis symptoms (include diarrhea, fever, abdominal pain, dehydration and vomiting without bloody samples) in the same period.

Initially, RSV RNA was extracted from nasopharyngeal swabs samples by using high pure RNA nucleic acid kit (Roche), followed by cDNA preparation for each specimen. The nested RT-PCR was done.[11] For Rotavirus Detections, nucleic acid was extracted from 10% (w/v) stool suspension with carrier RNA using QIAamp Viral RNA mini kit (QIAGEN®, Hilden, Germany). For detection of rotaviruses, a reverse transcription (RT)-PCR method based on amplification of a VP6 fragment was performed using the (QIAGEN® One-step RT-PCR Kit).[12] To screen for the HBoV genome, a polymerase chain reaction PCR) was performed using primers in the NP-1 coding region.[13] All statistical analyses were performed using SPSS 13.0 software (SPSS, IBM, Armonk, NY). The correlations were subjected to the Pearson Chi-square. A p-value below 0.05 was considered significant.

Results

1-The part of HBoV and RSV infections:

In our 500 respiratory samples, 128 (25.6 %) specimens were positive for RSV that 70 of 128 RSV-positive patients (54.6%) were in between the age group of one to two years' category (P=0.035) and most cases presented during the winter (69.5%). The 67 out of 500 respiratory samples (13.4%) were positive for HBoV. The 44 (65.6%) of HBoV positive samples have a co-infection with RSV. Fifty cases (74,6%) were presented in winter. There was meaningful statistically significant in the HBoV respiratory infection and season (P=0.035) (Figure 1). Among the acute respiratory infection, the clinical symptoms in single infections of HBoV were cough (89.5%), fever with a temperature above 38°C (49.2%), wheezing (70%), rhinorrhea (70%), hypoxia (44%) and dyspnea (44 %). There was a meaningful relation between HBoV single ARTI and cough (P=0.011), wheezing (P=0.010) and rhinorrhea (P=0.022) (Table 1). The clinical signs of fever (79.5%), wheezing (88.6%) and rhinorrhea (95.4%) had been worsen in co-infection form compared with single forms of HBoV and RSV infection (Table 1).

2-The part of HBoV and Rotavirus infections:

Of the 500 stool samples, 169 (33.8 %) were positive for Rotavirus. Seventy-one of 169 Rotavirus positive patients, (42%) were in between the age group of one to two years' category (P=0.028). Acute gastrointestinal infection by rotavirus had a major peak in winter, as 99 cases of 169 (58,5%) were presented in this season. Of the 500 fecal specimens, 72 (14.4%) were positive for HBoV. Among the acute gastroenteritis samples, 45 (62.5%) from all HBoV positive samples had a co-infection with rotavirus. In seasonal distribution, 52 specimens (72.2%) were presented in winter. There was a meaningful statistically significant of the HBoV gastrointestinal infection and season (Figure 2).

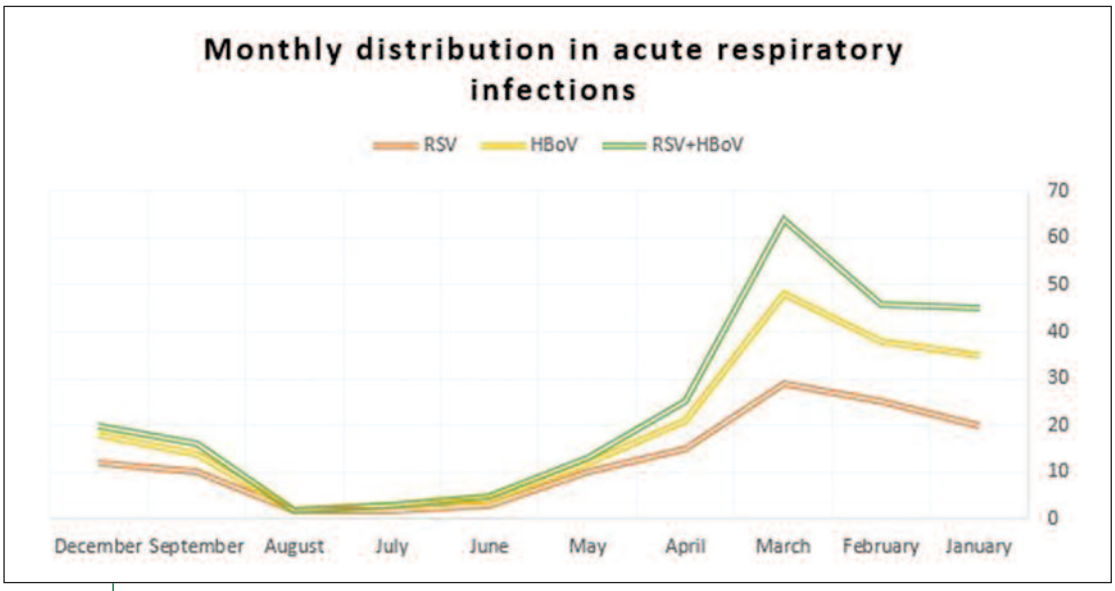

Figure 1 The monthly distribution of HBoV and RSV infections and co-infections in acute respiratory infection.

Table 1 Clinical characteristics associated with infections caused by human bocavirus (HBoV) single infections, respiratory syncytial virus (RSV) single infections and HBoV plus RSV dual infections.

P-value	RSV+HBoV (n=44)	P-value	HBoV(n=67)	P-value	RSV (n=128)	Clinical features
0.061	21 (47.7%)		35 (55.2%)	0.562	72 (56.25%)	Male
	23 (52.3%)	0.061	32 (47.8%)		56 (43.75%)	Female
0.029	35 (79.5%)*	0.721	33 (49.2)	**0.0119**	110 (85.9%)*	Fever > 37.9
0.002	39 (88.6%)*	**0.010**	47 (70%)*	**0.0119**	64(50%)*	Wheezing
0.021	36 (81.8%)*	**0.011**	60 (89.5%)*	**0.021**	96 (75%)*	Cough
0.207	42(95.4%)*	**0.022**	47 (70%)*	**0.031**	80 (62.5%)*	Rhinorrhea
0.605	14 (31.8%)	0.659	30 (44%)	0.721	35 (27.3%)	Hypoxia
0.748	10 (22.7%)	0.632	30 (44%)	0.719	25 (19.5%)	Dyspnea
0.031	33 (75%)*	**0.041**	33 (49%)	**0.035**	70 (54.6%)*	Age 1 y to 2 y

Among the acute gastroenteritis clinical symptoms in single infections of HBoV, diarrhea (83.3%), fever with a temperature above 38°C (50%), abdominal pain (81.9%), vomiting (83.3%) and dehydration (34.7%), were common findings. There was a meaningful relation between HBoV infection diarrhea, abdominal pain and vomiting (Table 2). The clinical symptoms of vomiting (97.7%) and dehydration (51.1%) worsened in co-infection form compared with single forms of HBoV and rotavirus infection (Table 2).

Discussion

The acute respiratory infections and acute gastrointestinal infections are major causes of mortality and morbidity in children aged under five years all over the world. After RSV, rhinovirus, and adenovirus, HBoV is the fourth most common virus detected in viral respiratory infections.[1,2] This study is the one of comprehensive studies on HBoV infections and co-infections in Middle-east and Iran within children with acute respiratory and gastrointestinal infections. This study shows the frequency of acute respiratory and gastrointestinal HBoV infections in patients with less than a three-year age during the spring to winter of 2017-2018.

In many reports, HBoV is the most common infectious cause and after major viruses can make an acute respiratory infection in children under three years old.[4,14,15] In Calvo et al study, HBoV detected in 9.9% as a distinctive pathogen, and 75% as a co-infection with other viral agents.[16] As like as our study RSV is a fundamental viral isolated agent in acute respiratory infections in children. We showed in agreement with this report that HBoV co-infection with RSV was common (65.6%) in HBoV-positive patients in our study and we found a potential relation between RSV and HBoV infections. In our study, a higher frequency of HBoV in ARTI observed in winter and between January and April with a peak in February. This issue was related to an activation of HBoV in cold season beside RSV. In our report, we showed that, in clinical manifestations, wheezing and rhinorrhea has been heightening at co-infection form.

In another report, Calvo et al observed that HBoV and RSV co-infection form were 74% and the average of age was under two years old. They also reported that in co-infection form the clinical manifestations are equal (44-46%).[17] In our study we showed that the clinical manifestations were not equal in co-infection form. More in details, wheezing and rhinorrhea symptoms were worse in dual infection (Table 1).

There were a few comprehensive reports on HBoV and rotavirus single and dual infections in acute viral gastroenteritis.[18-24] In an excellent report by De R and et al. about the risk of acute gastroenteritis associated with HBoV infection in children, that including 36 studies all over the world,[24] the HBoV prevalence was 6.9%. In this study, authors showed the global distri-

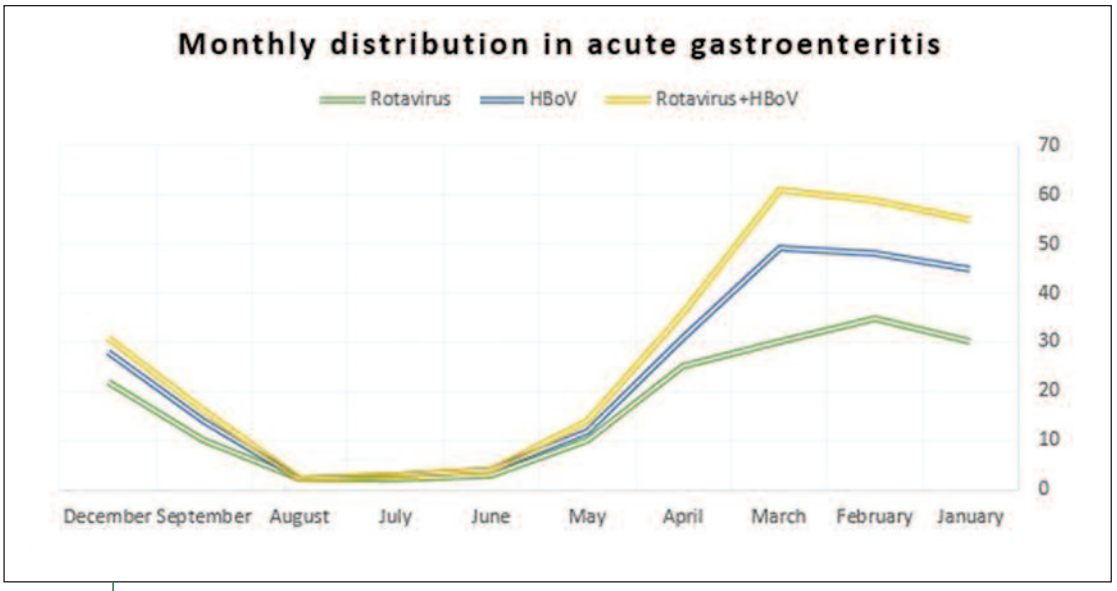

Figure 2 The monthly distribution of HBoV and rotavirus infections and co-infections in acute gastrointestinal infection

Table 2 Clinical characteristics associated with infections caused by human bocavirus (HBoV) single infections, rotavirus (RV) single infections and HBoV plus rotavirus dual infections.

P-value	Rotavirus +HBoV (n=45)	P-value	HBoV(n=72)	P-value	Rotavirus (n=169)	Clinical features
0.612	26 (57.7)	0.059	36 (50%)	0.692	72 (56.25%)	Male
	19 (42.3%)		36 (50%)		56 (43.75%)	Female
0.049	36 (80%)	0.514	36 (50%)	**0.0119**	110 (85.9%)*	Fever > 37.9
0.012	36 (80%)*	**0.018**	60 (83.3%)*	0.512	64(50%)	diarrhea
0.021	36 (80%)*	**0.016**	59 (81.9%)*	**0.033**	96 (75%)*	Abdominal pain
0.0011	44 (97.7%)*	**0.112**	60 (83.3%)*	**0.0211**	80 (62.5%)*	vomiting
0.705	23 (51.1%)	0.662	25 (34.7%)	0.719	35 (27.3%)	dehydration
0.041*	35 (77.7%)*	**0.035**	30 (41.6%)	**0.028**	71 (42%)*	Age 1 y to 2 y

bution of the HBoV incidences in pediatric patients under five years old with acute gastroenteritis reported from 18 countries from 2005 to 2016. In conclusion, current evidence suggested that HBoV may be considered as a probably causes of acute gastroenteritis. In our report, the finding of HBoV and rotavirus co-infection showed the main HBoV pathologic role in acute gastroenteritis in children.

In acute gastrointestinal infection by HBoV, a study from Pakistan in 2015 showed that all positive samples for HBoV, 98% were found to be co-infected with rotavirus.[22] Amongst the clinical features, fever, and vomiting defined symptoms in 89% and 87% children, respectively. In agreement with this report, we found that the HBoV acute gastroenteritis infection was 14.4% but coinfection form with Rotavirus was 62.5%. The clinical symptoms, vomiting, and dehydration were worse in dual infection form (Table2).

One study from Albania in 2016, showed that HBoV was detected in 9.1% from stool speciments.[21] All HBoV-positive cases were co-infected with other enteric viral agents. As like as our study, HBoV gastrointestinal infection revealed in co-infection forms with rotavirus. We showed in agreement with earlier studies that co-infection with rotavirus was common (62.5%) in HBoV-positive patients and we found a potential relation between rotavirus and HBoV infections. The single infections characterized by the presence of fever, diarrhea, vomiting, abdominal pain and dehydration. Seasonal peaks of HBoV infection vary among different countries because of climate and geographic issues. Previous studies suggested that HBoV infection had a higher detection rate in winter.[15,17,19,23-26] In our study, a higher frequency of HBoV in AGE and ARTI observed between January and April with a peak in February. Our results show that HBoV is mostly detected in respiratory samples and fecal samples of young children with ARTI, in agreement with earlier reports.

In conclusion, HBoV was a main cause of acute respiratory infections and co-infections of other viral causes must investigate and explain their roles in dual infections, but in gastrointestinal infections of HBoV, we need extended studies in our country and other geographical areas.

Abbreviations:

AGE: acute gastroenteritis, ARTI: acute respiratory tract infection, HBoV: human bocavirus,

RSV: respiratory syncytial virus, PCR: a polymerase chain reaction. NP1: nucleoprotein 1, ELISA: Enzyme-linked immunosorbent assay

Acknowledgments:

Not applicable.

Funding:

This study was not funded.

Ethics approval and consent to participate:

This study was approved by two ethical committees: Shahid Beheshti University of Medical Sciences, Tehran, IR Iran, Pediatric Infections Research Center, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran. Adult subjects or parents of the child subjects signed the consent form for participation in the study.

Conflict of interest:

The author has no conflicts of interest to declare for this study.

Περίληψη

Λοιμώξεις από ανθρώπινο bocavirus (human bocavirus) και συν-λοιμώξεις με αναπνευστικό συγκυτιακό ιό (Respiratory Syncytial Virus) και ροταϊό (Rotavirus) σε παιδιά με οξεία λοίμωξη αναπνευστικού ή γαστρεντερικού συστήματος

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Ο ανθρώπινος bocavirus (human bocavirus, HBoV) είναι υπεύθυνος για οξείες αναπνευστικές και γαστρεντερικές παιδιατρικές λοιμώξεις. Σκοπός της παρούσας εργασίας είναι η εύρεση του ιού τόσο μόνο του, όσο και μαζί με άλλους ιούς σε περιπτώσεις οξείας παιδιατρικής λοίμωξης αναπνευστικού ή γαστρεντερικού συστήματος. Η μελέτη αφορά την περίοδο 2017-2018 και περιλαμβάνει παιδιά ηλικίας μικρότερης των 3 ετών τα οποία προσήλθαν στο Mofid Children's Hospital, της Τεχεράνης του Ιράν με αντίστοιχη κλινική εικόνα. Αρχικά τα δείγματα του ανώτερου αναπνευστικού (ρινοφαρυγγικό επίχρισμα) και δείγματα κοπράνων μελετήθηκαν για αναπνευστικό συγκυτιακό ιό (Respiratory Syncytial Virus, RSV) και ροταϊό (Rotavirus) με RT-PCR και στη συνέχεια για HBoV με ανίχνευση του NP-1 γονιδίου του ιού με PCR. Σύμφωνα με τα αποτελέσματα, 67 από τα 500 δείγματα αναπνευστικού (13.4%) και 72 από τα 500 δείγματα κοπράνων (14.4%) βρέθηκαν θετικά για HBoV. Από τα θετικά HBoV δείγματα, 44 (65.6%) των αναπνευστικών δειγμάτων βρέθηκαν θετικά για RSV και 45 (62.5%) των κοπράνων για ροταϊό. Συμπερασματικά η HBoV λοίμωξη παρουσιάζεται συχνά ως συν-λοίμωξη με άλλους ιούς και ειδικά RSV και ροταϊούς.

Λέξεις κλειδιά
ανθρώπινος bocavirus, ιογενείς λοιμώξεις, συν-λοιμώξεις

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