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ORIGINAL ARTICLE

Human bocavirus infections among children less than two years old in Iran during fall and winter 2012-2013

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ABSTRACT

Background and Objectives: Human bocavirus (HBoV) is a newly discovered parvovirus. It has been detected primarily in children with acute respiratory tract infections. This study was conducted to clarify the frequency and genotype circulation pattern of HBoV in Iran.

Materials and Methods: Conventional PCR was performed on throat swabs of patients less than two years of age with respiratory illnesses during fall and winter 2012-2013.

Results: HBoV virus DNA was detected in 15 of 140 samples (10.7 %). Sequencing and phylogenetic analysis on 5 samples showed that all were HBoV1. The positive samples were negative for influenza A and B viruses while co-infection with RSV was found in 2 (13.3%).

Conclusion: This study adds to the body of knowledge about the role of HBoV in acute respiratory illnesses in children in Iran.

Keywords: Human bocavirus; genotype; children; Iran

INTRODUCTION

Respiratory tract infections (RTI) are the most important causes of morbidity and mortality among children especially in developing countries (1). Human coronaviruses including middle east respiratory syndrome coronavirus (MERS-CoV), NL-63 and HKU-1, human metapneumovirus (HMPV) , a new genogroup of human rhinoviruses called type C rhinoviruses, human bocavirus (HBoV) and human polyomaviruses WU and KI have been reported recently as a cause of RTIs (2, 3).

HBoV (later named as HBoV1) was first identified in Sweden from pooled nasopharyngeal aspirate specimens by Allander et al. in 2005 and classified in the genus Bocavirus, Parvovirinae subfamily, *Parvoviridae* family. Soon after, three other genotypes (HBoV 2-4) were identified in this genus. While HBoV1 was commonly associated with RTIs, HBoV2-4 were isolated from fecal samples (4-7).

HBoV1, the fourth most prevalent respiratory virus, was detected worldwide in 2-33% of children less than two years of age with RTIs, often showing a high rate of co-infections with other respiratory viruses specially respiratory syncytial virus (RSV) (7-10). HBoV1 is responsible for the upper and lower RTIs. Bronchiolitis, pneumonia, bronchitis and asthma exacerbation are the most frequent symptoms of HBoV infection (11-12).

The aim of this study was to determine the frequency and genotype circulation pattern of HBoV in-

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fections among children younger than two years old with RTI during fall and winter 2012-2013.

MATERIALS AND METHODS

Patients. This cross-sectional study was performed in National Influenza Center, Virology Department, School of Public Health, Tehran University of Medical Sciences. During fall and winter 2012-2013, a total of 140 throat swabs were collected from hospitalized patients less than two years of age with RTIs. All samples were stored at -70 °C until used for DNA extraction and PCR.

DNA extraction and PCR. DNA was extracted from the specimens by using High Pure Viral Nucleic Acid kit (Roche, Germany). HBoV DNA detection and genotyping were performed by PCR amplification for the NP-1 and VP2 genes with products lengths of 354 and 1054 bp, respectively. PCR primers were used according to the previous published articles (8). The PCR products were analyzed by electrophoresis on agarose gel. All samples were tested at least twice and positive samples were confirmed by sequencing.

Sequencing. PCR products of VP2 genes were cloned by TA-cloning method and sequenced in both directions with the use of Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions. The obtained sequences were assembled by Staden Package-2 and edited using BioEdit Sequence Alignment Editor Version 7.0.5.3.

Phylogenetic analysis. For phylogenetic analysis, 5 Iranian HBoV sequences were included. Sequence alignment was performed by using Clustal W multiple alignment. MEGA 5.2 program phylogenetic neighbor-joining analysis was conducted. The evolutionary distances with the Kimura 2-parameter model and bootstrap analysis with 1000 replicates were computed. The sequences were deposited at GenBank under the accession numbers KJ598617-KJ598621.

Co-infections. All samples were screened for detection of influenza A and B and RSV using real-time RT-PCR and hemi nested RT-PCR respectively.

RESULTS

Study population. During the fall and winter 2012-2013, a total of 140 throat swabs were tested. Patients were from four different provinces: 68, 22, 24 and 26 individuals from Tehran, Alborz, Qom and Lorestan, respectively. All patients were hospitalized children less than two years of age with RTIs. 57% of all participants were male. This slight predominance in male participants was not statistically significant.

HBoV Frequency. HBoV DNA was detected in 15 (10.7 %) of 140 respiratory samples (95% confidence interval, 5.6-15.8). The male: female ratio was 10:5 in the HBoV-positive patients which did not differ significantly from the HBoV-negative patients by using X^2 test ($P > 0.05$).

During this 6-months study, low positive rate of 4.3% was detected in October 2012 while high positive rate of 15.1% was found in February 2013 in four provinces. The estimated prevalence of each province is given in Table 1.

Among the 15 HBoV positive samples, co-infection with RSV were found in two (13.3%) samples while all of them were influenza A and B negative.

Phylogenetic analysis. In 15 positive samples only five were available for VP2 PCR which analyzed for identification of genotypes and comparison with HBoV reference sequences. Phylogenetic tree was drawn with VP2 genes by using the neighbor-joining method. In sequencing and phylogenetic analysis all five positive samples belonged to HBoV1 (Fig.1).

DISCUSSION

HBoV is a novel parvovirus first described in 2005 by T. Allander. It has been associated with upper and lower RTIs and gastrointestinal illnesses worldwide (13).

According to the previous published articles and the current study, HBoV circulates in Iranian children. The prevalence rate obtained in this research was higher than two previous studies (10.7% versus 6.7% and 8%) (14,15). This rate of HBoV detection is similar to the previous reports for pediatric patients

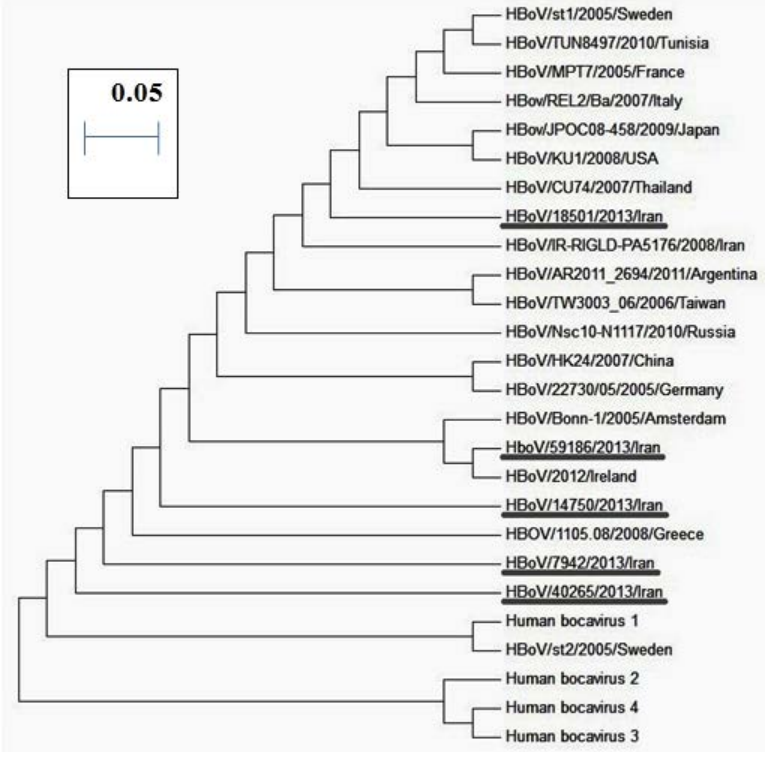

Fig. 1. Human bocavirus VP2 gene sequences obtained in this study was compared with the HBoV reference sequences. A phylogenetic tree was drawn with VP2 genes by using the neighbor-joining method. Genotypes are shown by Virus Name / Number of sample / year / country, respectively (from left to right). Five samples of this study are underlined.

Table 1. Demographic characteristics of children with or without HBoV infection.

Variables		Total	HBoV positive	%
Sex	Male	79	10	12.7
	Female	57	5	8.8
	Missing	4	0	0
	Total	140	15	10.7
Month	October	23	1	4.3
	November	19	2	10.5
	December	26	2	7.6
	January	23	3	13
	February	33	5	15.1
	March	16	2	12.5
	Total	140	15	10.7
Province	Tehran	68	7	10.3
	Alborz	22	2	9
	Qom	24	3	12.5
	Lorestan	26	3	11.5
	Total	140	15	10.7

worldwide. Generally HBoV1 DNA prevalence in young children with RTIs is about 10% but in some studies reported up to 33% (13). The diversity of reported values may be because of sampling techniques, study populations and the sensitivity of the detection assays (1).

Seasonal peak of HBoV infection is different among countries and regions because of climate and other factors. Many previous studies showed a higher detection rate in winter (16). In the current study the highest prevalence was in February (Table 1).

HBoV DNA-positive respiratory samples have a high co-infection rate reached up to 83 % (10,12,15,17). All positive samples in this research were negative for influenza A and B viruses and just two samples (13.3%) were positive for RSV (RSV/A). According to the HBoV noticeable proportion of co-infections, further studies are needed to find co-infections with the other respiratory viruses.

Further types of HBoV including HBoV2-4 were described in 2009-2010 (4-6) indicating diversity within this group of viruses. As previously described HBoV3 is the result of HBoV1 and HBoV2 recombination and HBoV4 derived from recombination between HBoV2 and HBoV3. Both recombinations were happened in the entrance area near the NP1 / VP1 junction (5). Some of the nucleotide changes in sequences obtained in this study are similar to other studies, like C168 T and several changes of A to G.

Based on HBoV frequency in this study, it can be concluded that it is a common circulating virus in the community. This virus might play a significant role as a causative pathogen in RTIs in young children.

This study had some limitations. First, we did not have more information about the patient's signs and symptoms. Second, we did not have enough clinical samples for doing VP2 PCR on all 15 positive specimens. Because of the special importance of RTIs in infants and young children and for a correct understanding of HBoV frequency and its circulating genotypes, it is recommended to do more molecular studies over several years. In addition, further studies should be conducted to detect HBoV within healthy individuals as a control group to find more about the virus infectivity and pathogenicity.

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