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Human *Bocavirus* in Iranian children with acute gastroenteritis

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Abstract

Background: Human *Bocavirus* **(**HBoV) infection is of worldwide distribution. There is increasing evidence that HBoV is pathogenic for the human gastroenteric tract. However, less data are available on the role of HBoV in gastroenteritis. The present study was aimed to determine the prevalence of HBoV in children with gastroenteritis.

Methods*:* Real-time PCR TaqMan was used to screen 200 stool specimens that had been referred to the virology laboratory for HBoV evaluation. All of samples were collected on viral transport media.

Results: Of the 200 stool samples analyzed, 16 (8%) were positive for HBoV. Human *Bocavirus* positive samples from patients aged between 1 to 5 years with acute gastroenteritis infection suggest a minor role of HBoV in gastroenteritis (p=0.0001).

Conclusion*:* The study showed a high prevalence of human *Bocavirus* in young children with acute gastroenteritis diseases in Iran, suggesting that HBoV play a role in the pathogenesis of gastroenteritis.

Keywords*:* Gastroenteritis, Child, Human *Bocavirus*, Real-time PCR.

Introduction

The human Bocavirus (HBoV), a member of the Parvoviridae family, has been cloned from nasopharyngeal aspirates of children with acute respiratory infection (ARI) in Sweden (1). Phylogenetic analyses of the complete genome of HBoV revealed that the virus is most closely related to canine minute virus and bovine parvovirus, and thus, it was called "human *Bocavirus*" (bo for bovine and ca for canine) (2, 3). Human *Bocavirus* has been detected in urine, fecal, and blood samples of children with gastrointestinal tract infections (4). Acute gastroenteritis (AGE) known by vomiting, diarrhea, and dehydration, is one of the most common causes of morbidity and mortality in infants and children (5).

Many viruses such as N*orovirus, Rotavirus, Astrovirus, Sapovirus*, and *Adenovirus* have been known to associate with these diseases and recently, human *Bocavirus* has been considered as agent associated with diarrhea in humans (6). The association of HBoV with gastroenteritis is suspected since the relation between bovine parvovirus (BPV) enteritis in cattle and gastroenteritis symptoms were found(7). Initial studies showed that HBoV is a common cause of acute upper and lower respiratory tract infection in children. It has been detected in 1.5%–11.3% of individuals with ARI in Europe, United States, Canada, Asia, South Africa, and Australia (8,9).

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The aim of this study was to determine the prevalence of *Bocavirus* in fecal specimens collected from gastroenteritis cases in the Hazrat-e-Rasool Akram Hospital of Tehran, Iran and to evaluate its contribution as a cause of acute gastroenteritis diseases.

Methods

This cross-sectional study has been done in the Hazrat-e-Rasool Akram Hospital affiliated to Tehran University of Medical Sciences from November 2010 to November 2011. It has been approved by the Ethics Committee of the university. Stool samples (n = 200) were collected from patients with acute gastroenteritis, presented at least with two of the following symptoms: vomiting, diarrhea, abdominal pain, cough, exanthematic disease or skin rash. Moreover, 200 fecal samples were collected from controls without acute gastroenteritis within the same age group.

The samples were diluted 1:10 in PBS (Peripheral Blood Smear) and then were vortexed and centrifuged. Supernatants were collected and were frozen at -70˚C until use. DNA was extracted with High Pure Viral Nucleic Acid kit (Roche Diagnostics GmbH, Mannheim, Germany). Extracted DNA stored at -20°C until use. Real-time PCR assay (Rotor Gene 6000; Corbett Research, Australia) with Hydrolyses TaqMan probe was utilized to detect HBoV DNA. The amplification reaction mixture was contained 5 µL of extracted sample, 10 µL TaqMan universal PCR master mix (PE Applied Biosystems), 0.1 µL bovine serum albumin (20 mg/ml), 300 nmol/liter of each primer (Boca-forward, 5'-AGAGGCTCGGGCTC ATATCA-3'; Boca-reverse, 5'-CACTTGG TCTGAGGTCTTCGAA-3'), and 150 nmol/ liter of the Boca probe (5'-FAM-AGGACCACCCAATCARCCACCTATCG TCT-TAMRA-3', where FAM is 6-carboxyfluorescein and TAMRA is 6-carboxytetramethylrhodamine). Amplification was performed under standard amplification conditions with the following settings: 95°C for 10 min; 42 cycles of 95°C for 15 seconds and 60°C for 1 min (10). HBoV positive samples were confirmed in a second run including dilutions of the samples. Also the HBoV negative samples were retested and confirmed.

The medical histories of the children were re-evaluated 5 to 7 days after enrollment and until the resolution of their illness by means of interviews and clinical examinations by trained investigators using standardized questionnaires. During this evaluation, information was also obtained regarding acute illnesses and related morbidity in their households. All the data were verified from medical records.

Statistical analysis: The data were analyzed using SPSS v.13 (SPSS, Chicago, IL). P-value of <0.05 was considered statistically significant. Parametric data were compared by analysis of variance (ANOVA). Skewed distributed or nonparametric data were analyzed using the Kruskal-Wallis test. The categorical data were analyzed by means of contingency analysis and the chi-square or Fisher's Exact test.

Results

Patients Characteristics: Patients' ages ranged from 2 to 3.5 years old, and included 144 (72%) male. Mean age was 3.08 ± 1.13 years (mean ± standard deviation) Clinical characteristics of the patients were recorded for further analysis. Twenty (10%) had evidence of acute respiratory tract infection with cough, 140 (70%) had vomiting, 92 (46%) had fever without any source, and 116 (58%) had exanthematic disease or skin rash; 152 (76%) had abdominal pain. Among the studied cases, 16 (8%) had evidence of chronic underlying diseases, 4 (2%) had chronic asthma, 8 (4%) had chronic kidney insufficiency, and 4 (2%) had chronic diarrhea.

Human Bocavirus Prevalence: HBoV DNA positive samples were detected in 16 patients (from a total of 200) with a total positive rate of 8%. In the control group none of samples were positive for HBoV

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DNA. HBoV DNA positive samples were also tested for 5 other potential pathogens, including: Rota virus, Norwalk, Adenovirus, Escherichia coli and helicobacter pylori by TaqMan real-time PCR, (Guangzhou HuYanSuo Medical Technology Co., Ltd). Samples were all negative for these pathogens.

Discussion

Viral gastroenteritis is one of the most common illnesses in humans worldwide. However, different viral agents have caused the disease and the clinical presentation of patients with acute gastroenteritis symptoms is not generally indicative of a specific pathogen (11, 12). Acute gastroenteritis is one of the causes of morbidity and mortality during childhood in developing countries. Human *Bocavirus* has been recognized as an important cause of severe gastroenteritis in childhood and has been detected in hospitalized children (10, 13).

The present work is a prospective study to find one of the etiologies of community-acquired gastroenteritis in symptomatic small children in Iran. This study demonstrated the presence of HBoV genomic DNA in stool samples from a group of children with acute gastroenteritis. The study is in line with previous studies in China, Australia, USA, and Brazil (14,15). The frequency of HBoV in the present study (8%) was higher than the frequency reported in the previous studies (Canada 1.5%, Sweden 3.1%, Australia 5.6%, and Japan 5.7%, Germany 10.3% and Korea 11.3%) (23). An increasing evidence suggests a relationship between HBoV and the disease. High titers of DNA in some samples suggest that the virus replicates in the human gut and none of the HBoV-positive patients showed the respiratory symptoms. The low HBoV-DNA titers in other stool samples could be explained by swallowed virus from the respiratory tract; but HBoV cannot be ruled out as a cause for single gastroenteritis cases. Control groups were used to support evidence demonstrating an association between HBoV infection and gastroenteritis (16,17). HBoV is an important agent for outbreaks of gastroenteritis in day care facilities for children (18, 19).

In a study done in Hong Kong, diarrhea was found in 11% out of 79 patients with respiratory symptoms. In that study, human *Bocavirus* was identified is fecal samples of 25 children with gastroenteritis. Of these children, 16% had blood in the stool, 8% had mucus in the stool, 32% had vomiting, and 68% had fever. The following respiratory findings were found in these children with gastroenteritis: coryza (56%), acute bronchitis (16%), and pneumonia (12%). Co-pathogens were identified in 56 % of the children: rotavirus (36%), Salmonella spp. (8%), Campylobacter spp. (4%), Staphylococcus aureus (4%), and Clostridium difficile (4%) (20-22). In a study from Korea, viral agents were found in 44.4% of the stool specimens (n=962) from children with acute gastroenteritis. The viral agents included rotavirus (25.7%), *Norovirus* (13.7%), adenovirus (3.0%), *Astrovirus* (1.1%), and human *Bocavirus* (0.8%). In another study from Brazil, stool samples of 2 % of 705 cases with diarrhea were PCR positive for human *Bocavirus*. In studies of respiratory illness associated with human *Bocavirus* infection, diarrhea has been reported in 9-38% of patients.

Previous studies have been utilized conventional nested PCR assays with agarose gel electrophoresis. To improve the diagnostic methods for HBoV detection, a real-time PCR assay was applied in this study. This assay was sensitive, specific, and reliable for HBoV DNA amplification. Obviously, this real-time PCR is simple and less time consuming. It is truly a cost-effective laboratory method to detect different viruses (24, 25). The designed primers used in this novel real-time PCR were designed based on virus sequences circulating recently and are appropriate for screening *Bocavirus* in clinical specimens from infants and children with acute gastroenteritis to detect recent strains circulating in patients (26).

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Conclusion

The study showed a high prevalence of human *Bocavirus* in young children with acute gastroenteritis in Iran, suggesting that HBoV plays an important role in the pathogenesis of gastroenteritis.

This was a small study, and these observations must be confirmed in a larger sample of patients with more analysis works.

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