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RESEARCH ARTICLE

DETECTION OF *Calicivirus* IN STOOL SAMPLES OF CHILDREN WITH ACUTE GASTROENTERITIS USING RT-PCR IN 5 DIFFERENT CITIES OF IRAN

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ABSTRACT

Background: Human *caliciviruses* (*HuCV*) are emerging enteric pathogens that are a common cause of diarrhea in humans worldwide. The prevalence of *HuCV* responsible for acute gastroenteritis in children under 5 years old was determined using Reverse transcription-Polymerase Chain Reaction (Rt-PCR) on purified viral nucleic acid from fecal samples.
Objectives: The aim of this study was to detect *Calicivirus* in stool samples by Rt-PCR in 5 different centers in Iran.
Methods: In this study, 3260 stool samples were collected from children less than 5 years old with acute gastroenteritis from five different cities. *Calicivirus* detection was performed through Rt-PCR. Fecal speciments were collected within 24 hours of admission. The speciments were frozen, sent to the laboratory, and then stored at -80 ^{0}C until being tested for *Calicivirus*.
Results: Rt-PCR was performed for 3260 stool samples containing 53 (1.62%) *HuCV* positive. The Rt-PCR was validated with published primers for *HuCV* (P289/P290). 84.2% of *Calicivirus*-caused gastroenteritis was found among children under 2 years old, which was statistically significant (P value < 0.05).
Conclusion: *HuCV*s, one of the most important causes of acute gastroenteritis in children under 5 years old, can be detected by Rt-PCR.

INTRODUCTION

Diarrhea, characterized by frequent liquid or loose stools, commonly results from gastroenteritis caused by infection with bacteria, parasites, or viruses. Patients with mild diarrhea do not require medical attention; the illness is typically self-limited, and disease symptoms usually resolve quickly. However, diarrheal diseases can result in severe illness and death worldwide and are the second leading cause of death around the world in children <5 years of age (1). Recently, mortality due to diarrheal disease has been reported in > 1 million human deaths annually, with young children under 5 years old comprising the most important age group affected (2). In recent years, many novel viruses responsible for acute gastroenteritis have been identified including *Rotavirus*,human *Calicivirus*, *Astrovirus*, and *Adenovirus* (3). *Caliciviruses* are a family of positive-sense, single-stranded RNA viruses that includes important human pathogenes (4). Human *caliciviruses* (*HuCV*) include the genera *Norovirus* and *Sapovirus*; in particular, *Norovirus* has been recognized as the most important cause of nonbacterial, acute gastroenteritis in humans of all age groups. Additionally, *Norovirus* is responsible for at least 50% of all gastroenteritis outbreaks globally; however, the incidence of this agent is rarely laboratory-registered in developing countries (5). The highly genetic diversity that *HuCV* exhibit has made it complicated to develop a universal system for their classification. Reverse transcription-Polymerase Chain Reaction (Rt-PCR) assays, have been extensively employed for their detection.

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Usually, the genomic region utilized to detect and genotype *HuCV* by Rt-PCR codify to the polymerase gene, which is relatively conserved in both genera (6). To identify *HuCV* in the enteric tracts of children with diarrhea, we performed Rt-PCR on purified viral nucleic acid from fecal samples obtained from children with diarrhea in 5 different cities of Iran. This work provides information on epidemiology of *HuCV* infection associated with acute diarrheal disease in children up to 5 years of age in 5different cities of Iran by Rt-PCR.

MATERIALS AND METHODS

In this study, 3260 samples of children with acute gastroenteritis, who were admitted to the pediatric hospitals in 5 cities of Iran (Tehran, Mashhad, Tabriz, Shiraz, Bandar Abbas) were collected. Written informed consent was obtained from all patients. Fecal specimens were collected within 24 hours of admission. The specimens were frozen, sent to the laboratory, and subsequently stored at -80° C until *Calicivirus* testing. Viral RNA was extracted from 30% stool suspensions in physiologic serum, then centrifuged at 6000 rpm for 20 minutes and filtered by 0.2 µm filter. Then 140 µL of the filtered samples was transferred to micro tubes and extracted by QIAamp viral RNA extraction mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The extracted samples were then stored at -20° C for a short time and further studied using Rt-PCR.
The cDNA synthesis was carried out using Rt-premix kit (Bioneer, U.S.A). The PCR products were analyzed by electrophoresis on a 1% agarose gel and visualized with UV light. TBE buffer was used as the electrophoresis gel (12). The PCR products obtained using the primer

set JV12/ JV13 was 326 bp. The primer pair sequences used in the study has been shown in Table 1.

Table 1. The Primer Pair Sequences Used in This Study

Primer Gene Location Polarity Sequence (5´- 3´)
P290 5`GATTACTCCAAGTGGGACTCCAC 3`
P289 5` TGACAATGTAATCATCACCATA 3`

RESULTS

Detection of *HuCV* in 3260 stool samples was done using Rt-PCR which was authenticated with approved primers for *Calcivirus* (P289/P290). The prevalence of *HuCV* in 5 large cities of Iran was reported to be 1.62%. There was a significant relationship between patients` age and their *Calicivirus*-caused gastroenteritis. 84.2% of *Calicivirus*-caused gastroenteritis was found among children under 2 years old (P value < 0.05). The prevalence of *Calicivirus* in different cities is studied and is shown in Table 2.

Table 2. Prevalence of *Calicivirus* in the Different Cities of Iran

City	Sample Positive	No. %
Bandar Abbas	573	9 (1.57%)
Tabriz	558	11 (1.97%)
Tehran	542	12 (2.21%)
Mashad	509	6 (1.17%)
Shiraz	1078	15 (1.39%)
Total	3260	53 (1.62%)

DISCUSSION

The aim of this study was to detect *HuCV* associated with acute diarrheal disease in children using RT-PCR as the principal molecular tool for the detection and genotyping of these viral agents from different Iran geographic areas that referred cases of acute gastroenteritis in children. To our knowledge, this is the first report indicating the frequency of *HuCV* related with acute diarrheal disease in children up to 5 years of age in Iran during the last decade. *Calicivirus* outbreaks are reported to be 7.3% in France (7), 29% in Japan (8), 9% in Brazil (9) and 8.5% in U.S.A (10) which could be explained by the ease with which *HuCV* is transmitted by water, food, direct contact, airborne droplets, and vomit, persistence in the environment as a source of contamination, the low infectious dose required to cause illness, and viral resistance to the disinfection process on surfaces, as well as to management and disease prevention in the community (11, 12). In this study, 53 of 3260 (1.62%) samples were positive for *HuCV*; the low prevalence rate in our study may be due to the limited number of samples collected. Despite the limited number of positive samples to determine seasonality, distribution of *HuCV* infection occurred mainly during the cold seasons, in contrast with Brazil, where infection has been reported along the entire year without a seasonal pattern (13). Interestingly, the age group most affected with *HuCV* infection (under 2 years old) corresponded to the group most commonly affected by rotavirus, with a median age of 10 months. Some difficulties in establishing the diagnosis of *HuCV* infection have been the lack of a rapid and sensitive diagnostic method for use in public health laboratories and hospitals; consequently, the frequency of *HuCV* infections is underestimated. However, although the RT-PCR test for diagnosis of these *HuCV* is the gold standard, it is not routinely performed on all stool specimens negative for rotavirus, despite that *HuCV* are almost a common cause of infectious gastroenteritis.

Due to that acute diarrhea remains a major public health problem in developing countries including Iran, detection of the *HuCV* infection could improve the diagnostic coverage of viral gastroenteritis in children < 5 years of age, because this is the major age group affected by this disease (2).

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