Detection of Astrovirus in Young Children Hospitalized with Gastroenteritis in Iran, over a Period of Seven Years, by RT-PCR

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Abstract

Human astroviruses have been increasingly identified as important agents of diarrheal disease in children. Outbreaks of diarrhea due to astrovirus have frequently been reported and astroviruses have also been associated with nosocomial infections in hospitals. The aim of this study is detection of astrovirus in hospitalized children with gastroenteritis.

In this 7 years descriptive study 2,490 gastroenteritis samples were conducted to determine the prevalence and age distribution of human astrovirus infection as well as the seasonality pattern in 5 different cities of Iran using reverse Transcriptase-polymerase chain reaction (RT-PCR).

Astrovirus was detected in 40 of 2490 specimens tested by RT-PCR, and astrovirus infection was confirmed by Southern hybridization. Detection rates were higher in winter, although astrovirus infections also occurred in summer months. The overall incidence of astrovirus was found to be 1.6%. The mean age of infected children was 14.7 months, the median age was 15 months. Majority of the infected children were less than 2 years of age making up 36 (90%) infected children, only 4 cases of infected children were more than 2 years of age (10%). The difference between the two age groups was statistically significant (P < 0.02).

Our findings provide Evidence that astrovirus can be a leading cause of viral gastroenteritis infections and highlight the need to implement astrovirus detection assays in association with rapid rotavirus and adenovirus detection enzyme immunoassays (EIAs) for the clinical diagnosis and nosocomial prevention of viral Gastroenteritis infections in pediatric departments.

Introduction

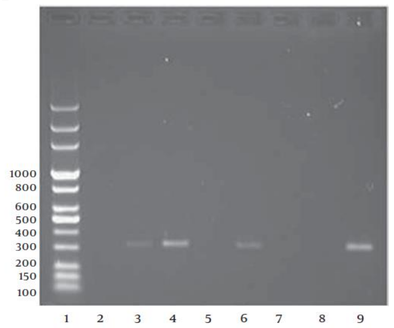
Acute gastroenteritis is an important cause of childhood morbidity and mortality, especially in children under 5 years old. Recently, it has been estimated that in developing countries, there are 450 million cases of diarrhea in children less than 5 years old annually and that 1-4% of them may die consequently. Among the viral infectious agents of acute gastroenteritis, Rotavirus, human Calicivirus, Astrovirus, and Adenovirus have been characterized. The norwalk-like and sapporo-like viruses are recently renamed Norovirus and Sapoviruses, respectively.

Human astroviruses have been increasingly identified as important agents of diarrheal disease in children and the elderly. The main symptom of infection is watery diarrhea, which is often associated with vomiting, fever, and abdominal pain. Outbreaks of diarrhea due to astrovirus have frequently been reported and astroviruses have also been associated with nosocomial infections in hospitals. They have also been detected in immunocompromised and AIDS-infected patients. Astrovirus infections occur worldwide, and their incidence in children with gastroenteritis in both developing and developed countries ranges from %2 to %9, although some studies report occurances up to 26%. In many cases, astroviruses are regarded as the second most common cause of viral gastroenteritis in children after rotavirus.

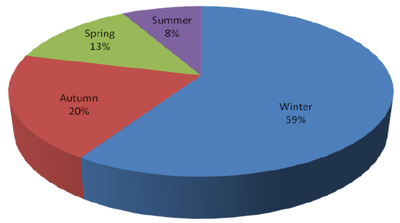
We wanted to determine the prevalence and age distribution as well as the seasonality pattern of astrovirus infections from children with gastroenteritis in 5 different cities of Iran, during a 7-year period using RT-PCR.

Results

A **PCR Products on Gel Electrophoresis**

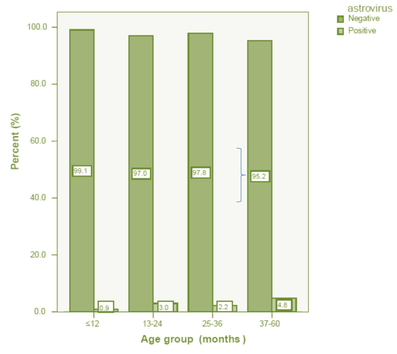


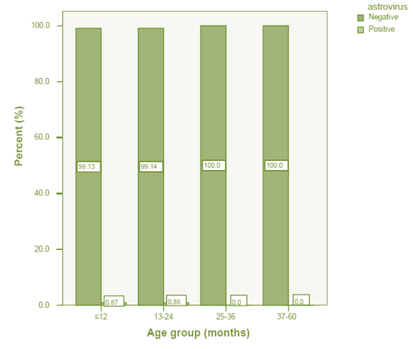
B **The seasonality pattern of astrovirus gastroenteritis in children in 5 cities of Iran.**



Age distribution of children with Astrovirus gastroenteritis in Bandar Abbas

Age distribution of children with Astrovirus gastroenteritis in Tabriz

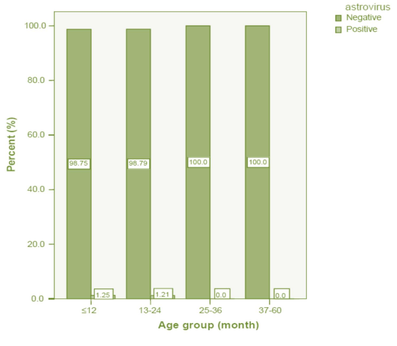




Age distribution of children with Astrovirus gastroenteritis in Tehran

Age distribution of children with Astrovirus gastroenteritis in Mashhad

E



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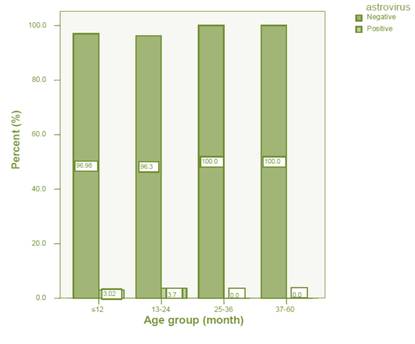


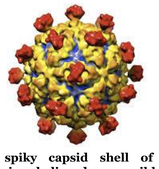
(A-D) Age distribution of children with astrovirus gastroenteritis in 4 cities of Iran. (C) Bandar Abbas, (D) Tabriz, (E) Tehran, (F) Mashhad.

Materials and Methods

2,490 fecal samples were collected from infants and children with gastroenteritis who were admitted to one of the hospitals involved in the present clinical study from 5 different cities over the period of 7 years. Studied patients had a minimum age of 30 days and a maximum of 4 years. Mean age of the studied patients was 48 months. From 2490 fecal samples, 414 were from Bandar Abbas, 394 from Tabriz, 624 from Tehran, 325 from Mashhad and 733 from Shiraz.

For astrovirus detection, stools were suspended (10%, w/v) in phosphate-buffered saline containing 2 M NaNO3, 1% bovine serum albumin; fraction V, and 0.1% Triton X-100 (pH 7.2) and pelleted at 1,000 ×g for 5 min, and the resulting supernatant was stored at -70°C for later analysis.

Astrovirus was detected by RT-PCR after extraction of its RNA and subsequently confirmed by Southern blot hybridization with an internal probe. RNA was purified from 50µl of fecal supernatant by guanidine thiocyanate extraction, as previously described (Boom et al., 1990). RT-PCR was carried out with primers Mon 340 and 348, which amplify a fragment of ORF1a (Table 1). Five microliters of the extracted RNA was heated to 99°C for 5 min and was immediately placed on ice. First-strand cDNA was synthesized at 42°C for 60 min by adding 1µM primer Mon 348 and 3 U of reverse transcriptase (Expand; Roche) in 10µl (final volume) containing 50mM Tris-HCl (pH 8.3), 40mM KCl, 5mM MgCl2, 10mM dithiothreitol, 0.5mM Tween 20, and 0.2mM concentrations of each deoxynucleoside triphosphate. Five microliters of the RT product was amplified using 0.5 U of the expand high-fidelity PCR system enzyme mix (Roche) and 0.5µM (each) primers Mon 340 and 348 in a total volume of 50µl containing 5µl of the expand high-fidelity buffer (Roche), 2mM MgCl2, and each deoxynucleoside triphosphate at 0.2mM. After a denaturation step of 3 min at 95°C, 40 cycles of amplification (94°C, 30 s; 55°C, 30 s; 72°C, 30 s) were performed followed by a final extension of 7 min at 72°C. Ten microliters of the PCR product was analyzed on a 1.5% agarose gel and detected by ethidium bromide staining. PCR products were confirmed by Southern blot hybridization with an internal digoxigenin-labeled probe under stringent conditions.



The spiky capsid shell of the astrovirus believed responsible for a form of juvenile diarrhea contains and protects single-strand RNA until it can be delivered to a cell. Image by Jinhui Dong/Rice University.

Astrovisrus in the blood.
Image from https://www.naturalpedia.com/astrovirus-infection-causes-side-effects-and-treatments-at-naturalpedia-com.html

Discussion

Astrovirus showed a winter peak in each 1-year period. RT-PCR is the most sensitive test for astrovirus detection as described previously (Guix et al., 2002). In a study by Grote et al. (2011), it was revealed that 30% of fecal samples negative for astrovirus by enzyme-linked immunosorbent assay (ELISA) were found to be positive when tested with RT-PCR. These findings emphasize the role of RT-PCR as the most sensitive test for virus detection among infected samples. Conclusively, our findings provide evidence that astrovirus can be a leading cause of viral gastroenteritis infections and highlight the need to implement astrovirus detection assays in association with rapid rotavirus and adenovirus detection enzyme immunoassays (EIAs) for the clinical diagnosis and nosocomial prevention of viral gastroenteritis infections in pediatric department. However comprehensive research is needed to estimate the exact number of infected children using accurate molecular methods.

Finally these diseases can be prevented by an easy and effective method as ***handwashing***, since it is the first line of defense against the spread of diarrheal and respiratory and many other illnesses.