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Astrovirus and Rotavirus Co-Infections in Children With Gastroenteritis who Were Referred to Ahvaz Aboozar Hospital, Southern Iran

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ABSTRACT

Background: Acute gastroenteritis, which is one of the most common diseases in humans,is responsible for many illnesses in both children and adults. Group A rotaviruses are considered the main agents of gastroenteritis, and these are followed by calciviruses, adenoviruses, and astroviruses.

Objectives: The aim of this study was to determine the rate of astrovirus and rotavirus coinfection among children up to 5 years of age who had gastroenteritis and who were referred to Ahvaz Aboozar Hospital.

Patients and Methods: A total of 180 stool specimens, which were collected from children with gastroenteritis who were less than 5 years old and who were referred to Ahvaz Aboozar Hospital, were tested by enzyme-linked immunosorbent assay methods for the detection of rotavirus infections. Detection of astroviruses in positive rotavirus stool specimens was performed by reverse transcriptase-polymerase chain reaction (RT-PCR) methods.

Results: Fifty-nine of the 180 samples were positive for rotavirus infection. These positive samples were subjected to RT-PCR to test forastrovirus. After RT-PCR with specific astrovirus primer sets, 8 samples were positive for astrovirus as well. Therefore, 13% of rotavirus-positive samples were also positive for astrovirus.

Conclusions: Group A rotaviruses, in addition tocalciviruses, adenoviruses, and astroviruses, can cause acute gastroenteritis. Studies have shown that 2.5 million deaths occur every year from gastroenteritis. In this study, we found that the prevalence of rotavirus infections was very high and that of coinfections of rotavirus and astrovirus were considerable. In order to reduce the risk of infections and to eliminate viral gastroenteritis in this zone of the region, education, vaccinations, and improved personal hygiene must be improved.

Implication for health policy/practice/research/medical education:
This study was performed to improve our epidemiological knowledge about the prevalence of these viruses and their proper managementin order to control them.

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1. Background

Acute gastroenteritis, which is one of the most common diseases in humans, is responsible for illnesses in both children and adults (1). Annually, 2.5 million deaths are attributed to gastroenteritis, and these illnesses re-

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sult in a great impact on the health statuses of children under 5 years of age (2). Group A rotaviruses are considered the main agent of gastroenteritis, and these are followed by calciviruses, adenoviruses, and astroviruses. A number of studies have shown that the prevalences of these viruses vary from 20–60%, 3.5–29.3%, 1–31%, and 1.8–16% among children suffering from gastroenteritis (3-5). The clinical signs of gastroenteritis are vomiting, fever, and abdominal pain, which are followed by watery diarrhea that may last 5–7 days (6).

The genome of rotavirus is comprised of 11 segments of double-stranded RNA, which are compacted within core proteins in the middle of the viral capsid, and the capsid of the virion contains 2 neutralization antigens, vp7 and vp4 (7). astroviruses are icosahedral and nonenveloped positive ssRNA viruses. The lengths of their genomes are 6.8 to 7.2 Kb with 3 open reading frames (ORFs) named ORF1a, ORF1b, and ORF2. ORF1a and ORF1b encode nonstructural proteins, and ORF2 encodesa capsid protein (8).

2. Objectives

This study was conducted in order to determine the prevalence of rotavirus infections and of coinfections of rotaviruses and astroviruses in fecal specimens of children with gastroenteritis. The collected data will be useful for health policymakers and help themplan for the control of these infectious agents more efficiently.

3. Patients and Methods

Stool specimens were collected from children less than 5 years old who had gastroenteritis and who were referred to Ahvaz Aboozar Hospital from 2008 to 2009. Fecal samples were examined by microscope in order to evaluate the white blood cell status of the specimens. Following bacteriological tests, negative specimens were saved at -80°C. A total of 180 negative samples were collected in order to detect viral coinfection. In order to determine viral coinfections, we examined these specimens with the following 2 methods:the enzyme-linked immunosorbent assay (ELISA) test for the detection of rotavirus infection and thereverse transcriptase-polymerase chain reaction (RT-PCR) method for the investigation of astrovirus RNA among rotavirus-positive samples.

3.1. ELISA

Following the suspension of about 5g of the stool sample in the buffer of the ELISA kit (Diaplus, Inc., North York, Ontario, Canada), the sandwich ELISA test was conducted according to the manufacturer's instructions. All testswere carried out in duplicate, and rotavirus-positive samples were then subjected to RT-PCR tests.

3.2. RT-PCR

Viral RNA was extracted from fecal suspensions using TRIzol (Fermentas UAB, Vilnius, Lithuania) according to the manufacturer's instructions. All of the rotavirus-positive samples were tested by RT-PCR using the following astrovirus-specific primers: Mon348 (5′-ACA TGT GCT GCTG TTA CTA TG-3′) and Mon340 (5′-CGT CAT TAT TTG TTG TCA TAC T-3′) for ORF1a. The size of the amplicon was 289 bp (9). Prior to RT-PCR and after extraction, denaturation of the viral RNA was conducted at 70°C for 5min, which was followed by a 2-min incubation on ice. The tubes in the reverse transcription reaction contained the following: 4-μL RT buffer (10×) (Fermentas UAB),1-μL DNTP (10mM) (Fermentas UAB), 1-μrandom hexamer (Fermentas UAB), 0.5-μL RN aseinhibitor (Fermentas UAB), 0.5-μL RT enzyme (200u/μL) (Fermentas UAB), 6.5-μL H_2O, 0.5-μL $MgCl_2$ (50mM) (CinnaGen, Inc., Tehran Iran), and 6-μL extracted RNA, which was incubated at 42°C for 1h. Five microliters of constructed cDNA was used as a template of the PCR reaction. Each PCR tube contained the following: 5-μL PCR buffer (10×) (CinnaGen, Inc.), 0.5-μL primer P1 (100pmol/μL), 0.5-μL primer P2 (100pmol/μL), 1-μL DNTP (10mM) (Fermentas UAB), 0.3-μL Taq DNA polymerase (500μ/μL) (CinnaGen, Inc.), 0.5-μL $MgCl_2$ (50mM) (CinnaGen, Inc.), and 37.2-μL H_2O. The PCR conditions were as follow: 35 cycles of amplification (94°C for 30s, 50°C for 30 s, and 72°C for 1min) and a final extension at 72°C for 10 min; 10μL of the final PCR product was subjected to electrophoresis in a 2% agar gel, stained with ethidium bromide, and then visualized with an UV Transilluminator (VilberLourmat, Marne-la-Vallee, France). All positive samples were sequenced by the MiileGen, Immeuble, BIOSTEP – Batiment A Cedex (France) for confirmation of the results of astrovirus RT-PCR and genotyping.

4. Results

A total of 59 of the 180 samples were positive for rotavirus infections (32.7%.). Sixteen samples of these positive samples belonged to children between 0–6 months of age (27.1%). The peak of the rotavirus infections was in fall (32.2%). The 59 positive samples for rotavirus were subjected to RT-PCR. Eight (13.6%) patients were coinfected with astrovirus. The authenticity of the RT-PCR stage was confirmed by sequencing.

5. Discussion

Morbidity of children due to diarrhea is commonthroughout the world (10). There are 4 major viral pathogens that are responsible for gastroenteritis (rotaviruses, Sapoviruses, astroviruses, and enteric adenoviruses) (11). In France, Bon *et al.* demonstratedin 1999 that rotavirus group 1 was recognized in 61% of the cases of gastroenteritis, calciviruseswere recognized in 14%, astroviruseswere recognized in 6%, and enteric adenoviruses were recognized in 3% (12). These viruses sometimes can cause coinfections with each other. In the US, Simpson *et al.* showed that rotavirus was detected in 28% of the cases of gastroenteritis. Norovirus was responsible for 13%, Sapo-

virus was found in 1% of the cases of gastroenteritis, and 9% were cases of coinfection with each other (13).

In Korea, Hong *et al.* (14) showed that totaviruses were detected in 41.3% of the cases of gastroenteritis, noroviruses were detected in 36.2%, enteric adenoviruses were detected in 7.1%, and astroviruses were detected in 0.6%. In their report, 31.7% were cases of coinfection of rotaviruses with noroviruses and adenoviruses. In norovirus-positive samples, 41.1% had coinfection with rotaviruses, adenoviruses, and *Salmonella*. In addition, 12.9% of adenoviruses were coinfected with rotaviruses and noroviruses. In this study, we found that 34% of the cases were positive for rotavirus infection, which was in concordance with other studies (10, 12). Among the positive samples, we detected that 8 were positive for astrovirus coinfection (13%). Monoinfection or coinfection with this virusis shown with contamination of food and drink water in this zone of the region. Improvements in sanitation and in vaccinations might reduce the incidences of infection with this virus. Recently, 2 new rotavirus vaccines were licensed (Rotateq from Merck & Co., Inc. and Rotarix from GlaxoSmithKlineplc). These vaccines show promise against rotaviral infections, and intussusceptions have not been reported following their uptake(15). rotavirus vaccination will reduce the incidence of infection with this virus.

Usually, most viral infections occur during the cold season of the year (16). In our study, the peak of the rotavirus infection was in fall (32.2%), and this was in concordance with another study (16). In this study, we demonstratedcoinfections of rotavirus and astrovirus in the Ahvaz Southwest of Iran. According to this study,the rate of coinfections of rotavirus and astrovirus were considerable in our area. Because prevention methods for both virus particles are similar, improvements in sanitation, especially that of hand washing, and vaccinations for rotaviruses will reduce the infections with these viruses.We hope the reported data are useful for the relevant authorities who are responsible for the sanitation of our society.

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