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Relative Frequency of Sapovirus Among Children Under 5 Years of Age With Acute Gastroenteritis at the Aboozar Hospital, Ahvaz, Iran

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ABSTRACT

Background: Gastroenteritis is a common cause of morbidity and mortality in humans all over the world, especially in infants under 5 years of age. Many microorganisms, including viruses, have been identified as the causative agents of gastroenteritis. Sapovirus is a major causative agent of acute viral diarrhea that occurs mostly in children under 5 years of age.
Objectives: The aim of this study was to determine the prevalence of Sapovirus infection among children under 5 years of age who had gastroenteritis and were referred to Aboozar Hospital, Ahvaz, Iran.
Patients and Methods: All fecal specimens were collected from children with acute gastroenteritis, Sapovirus RNA was extracted using TRIzol and detected by reverse transcriptase-polymerase chain reaction (RT-PCR) followed by sequencing of the positive samples.
Results: Of the 200 clinical stool samples collected, 6 (3%; 5 samples from male patients and 1 from a female patient) were found to be positive for Sapovirus by the RT-PCR method. The identityof the PCR products was confirmed by sequencing. Sapoviruses belonging to genogroup II were identified as the dominant type causing gastroenteritis in children. The incidence of Sapovirus infection was the highest during the coldest months.
Conclusions: Sapovirus prevalence in children under the age of 5 years with acute gastroenteritis was 3%, and genogroup II was the dominant type.

Implication for health policy/practice/research/medical education:
Since prevalence of Sapovirus was not determined in Ahvaz, this project was undertaken to clarify the significance of this viral agent in diarrheic patients living in our area.

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1. Background

Acute gastroenteritis is a common cause of morbidity and mortality, especially in infants under the age of 5 and the elderly (1), and is reported to be associated with a high mortality rate in developing countries (2). A large number of microorganisms, including viruses, have been identified as the causative agents of gastroenteritis (3). Sapoviruses belong to the *Caliciviridae* family and are small, non-enveloped, icosahedral viruses with a linear single-strandpositivesense RNA genome (4). These viruses are genetically divided into 5 distinct genogroups on the basis of the presence or absence of the capsid gene: GI, GII, GIII, GIV, and GV. Viruses belonging to the GI, GII, GIV, and GV genogroups cause infection in humans, and

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those belonging to the GIII genogroup infect swine species (5).

Several cases of pediatric gastroenteritis caused by Sapovirus have been documented, with detection rates ranging from 1.8% to 9% and the peak of incidence being between the ages of 6 months and 2 years (6).

2. Objectives

This study was aimed at determining the prevalence of Sapovirus in Ahvaz and improving the epidemiological knowledge on acute gastroenteritis caused by Sapovirus, which in turn can help develop strategies to control infection by this virus.

3. Patients and Methods

We collected fecal samples from children who were referred to the Aboozar Hospital, Ahvaz for gastroenteritis during 2008–2009 (male patients, 114 [57%]; female patients, 86 [43%]; age range, 1–5 years; average age, approximately 8 months). After performing routine diagnostic tests to rule out bacterial and parasitic infections, 200 samples in which no bacteria or parasites were detected were stored at–80°C. Viral RNA was extracted from fecal suspensions by using TRIzol (Fermentas, Lithuania), according to the manufacturer's instructions, and eluted in 50µl of diethylpyrocarbonate (DEPC) water (Fermentas, Lithuania). Reverse transcription was performed in a final volume of 20µl: 4µl 5 × reverse transcriptase RT buffer (Fermentas, Lithuania), 1µl deoxyribonucleoside triphosphates (dNTPs; 10 mM; Fermentas, Lithuania), 1µl random hexamer (0.2 U/µl; Fermentas, Lithuania), 0.5µl ribonuclease (RNase) inhibitor (40 U/µl; Fermentas, Lithuania), 0.5µl RT enzyme (200 U/µl; Fermentas, Lithuania), 0.5µl $MgCl_2$ (50mM; CinnaGen, Iran), 6.5µl DEPC water, and 6µl extracted RNA. The reaction mixture was incubated at 42°C for 1h. The obtained cDNA was stored at–20°C until subsequent use as template in the polymerase chain reaction (PCR). The following primers were used during PCR analysis for the detection of Sapovirus:SR80F, 5′-TGG GAT TCT ACA CAA AAC CC-3′ and JV33R, 5′-GTG TAN ATG CAR TCA TCA CC-3′. These primers could amplify 320 bp from open reading frame 1 (ORF1), which encodes RNA polymerase (7). The PCR mixture had a volume of 50µl: 5 µl 10 × PCR buffer (CinnaGen, Iran), 1µl of each primer 50pmol, 1µl dNTPs (10mM; Fermentas, Lithuania), 5µl cDNA as the template, 1.5µl $MgCl_2$ (50mM CinnaGen, Iran), 0.3µl Taq DNA polymerase (5U/µl; CinnaGen, Iran), and 35.2µl DEPC water.

The conditions for PCR were as follows: 1 cycle at 94°C for 5min 35 cycles, including denaturation at 94°C for 60 s, annealing at 51°C for 55 s, and extension at 72°C for 50 s; and final extension at 72°C for 6min. The PCR product was loaded onto 2% agarose gel and horizontally electrophoresed at 100 V for about 30min. The gel was stained with ethidum bromide and observed under an ultraviolet (UV) transilluminator (Vibrant, France). All samples that showed the presence of Sapovirus were sent to MilleGen Company (France) for sequencing.

4. Results

Sapovirus was detected in 6 of the 200 stool samples tested using RT-PCR (prevalence, 3%; 5 samples from male patients and 1 from a female patient); this result was further confirmed by the sequencing method.The obtained sequences were searched against the *National Center for Biotechnology Information* (NCBI) database by using b*asic local alignment search tool* (BLAST), and the results showed 94% similarity between the PCR products and genogroup II of Sapoviruses, confirming that the Sapovirus in all 6 samples belong to genogroup II *Table* shows the seasonal distribution of this infection in addition to the age of the patients.

Table. Age and Seasonal Distribution of Sapovirus Infection

Age Group, mo	Frequency and Season	Prevalence, %
0–6	4 (3 in winter and in fall)	66.7
7–12	2 (2 in fall)	33.3
13–24	0	0.0
25–36	0	0.0
36–60	0	0.0
Total	6	100

5. Discussion

Acute gastroenteritis is a common disease in children, especially in developing countries (8). Many different viruses have been detected in stool samples of patients with gastroenteritis (9), such as group A rotaviruses, caliciviruses, astroviruses, and adenoviruses (10). Sapovirus infections occur mainly in children of age up to 5 years (11). Although sapoviral infection shows seasonal distribution, most cases of gastroenteritis caused by Sapovirus occur during winter in temperate climates and during the rainy season in tropical climates.

Our study resultsare in agreement with those of other studies (12-14). The prevalence of Sapovirus was determined to be 3% by RT-PCR. Our results also correspond with those of studies that included data from all over the world, which reported the prevalence of Sapovirus infections as being 0.3%–9% (15). In 2005, Shuvra Kali Dey *et al.* determined the incidence of Sapovirus infections in children in Bangladesh (16). They analyzed 917 stool samples using RT-PCR and found that 25 (2.7%) samples were positive. The prevalence of sapoviral gastroenteritis in different countries has been determined the RT-PCR method: 11% in Thailand (17); 2% in Malawi (18); 17.6% in Japan (19); 1.2% in Vietnam (20); and 3.3% in Hong Kong (21). Reports from some other countries show that sapoviral gastroenteritis is very common in children below the age of 1 year (22), especially 0–6-month old (23). In our study, the prevalence of Sapovirus infection in the age group of 0–6 months was 66.7%, which was higher than that in other

age groups; and in the age group of 7–12 months was 33.3%, and there were no positive samples in age group 1-5 years. We assume that the children had acquired immunity to Sapovirus before they were 1 year old, whichindicates a previous infection. According to reports, the most prevalent genogroup of Sapovirus is GII (1, 17); in our study as well, GII was the dominant type. Our study results were in agreementwith those of a study conducted in India (2). Sapovirus is one of the important causes of nonbacterial gastroenteritis especially in children below 5 years of age. In our study, the incidence of this virus was 3%.We advise awareness and improved personal hygiene for the elimination of infections due to this virus.

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All authors declare that they have no conflict of interest.

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