Gastroenterology and Hepatology From Bed to Bench

ORIGINAL ARTICLE

Prevalence of sapovirus infection among infant and adult patients with acute gastroenteritis in Tehran, Iran

Sara Romani[1], Pedram Azimzadeh[1], Seyed Reza Mohebbi[1], Sajad Majidizadeh Bozorgi[1], Narges Zali[1], Farzaneh Jadali[2]

[1]Research Center for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2]Department of Pathology and Laboratory sciences, Mofid Children's Hospital, Shahid Beheshti University of Medical sciences, Tehran, Iran

ABSTRACT

Aim: This study investigated the prevalence of sapovirus infections in patient with acute gastroenteritis in Tehran, Iran.

Background: Sapovirus, a member of the family Caliciviridae is one of the major causative agents of viral gastroenteritis affecting both children and adult individuals. There isn't enough data about prevalence and genotypes of sapovirus infection in Tehran, the capital city of Iran.

Patients and methods: A total of 42 fecal samples were collected from patients with acute gastroenteritis from May to July 2009. RT nested- PCR was performed for screening. To genotype the sapovirus isolates, some positive samples were subjected to phylogenetic analysis by sequencing of fragments of viral capsid gene region.

Results: Sapovirus was detected in 5 of 42 stool specimens from patients with acute gastroenteritis. Sapovirus detected in this study was clustered into only one distinct genogroup I/2. Sapovirus GI/2 was predominant.

Conclusion: Our results show that among the studied viruses responsible for this disease, sapovirus was a major viral isolate virus.

Keywords: Sapovirus, Genogroup, Acute gastroenteritis.

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Introduction

Viral gastroenteritis is one of the most common diseases in all age groups, and continues to be a significant cause of morbidity and mortality worldwide (1, 2). Annual mortality associated with acute gastroenteritis was estimated to be 2.1 million in 2000 (3, 4). Rotavirus is the most important diarrheagenic viral agent among different viruses. Human sapovirus, formerly known as sapporo-like viruses, is a significant leading cause of gastroenteritis (5-8).

Sapoviruses contain single-stranded positive sense RNA viruses in the family Caliciviridae (Caliciviridae family). Other viruses in the family include norovirus, lagovirus, and vesivirus but norovirus and sapovirus are the only medical pathogens in the Caliciviridae family (9). The virions are composed of a single structural capsid protein, with icosahedric symmetry (10). The prototype of Sapovirus is the Sapporo virus (Hu/ Sapovirus/Sapporo virus/1977/JP) which was first

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Reprint or Correspondence: Seyed Reza Mohebbi, PhD. Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
E-mail: srmohebbi@gmail.com

detected in an outbreak of gastroenteritis in infant home in Sapporo, Japan in 1977 (11). Noroviruses are a major cause of both sporadic cases and outbreaks of gastroenteritis (3, 5-7), whereas sapoviruses have been detected in many areas in the world but only infrequently, a few outbreaks of sapovirus have been reported (8, 9). Meanwhile, the impact of the sapoviruses has not been fully established. Sapoviruses were primarily associated with cases of pediatric acute gastroenteritis (12, 13).

Most sapovirus genome contains two ORFs (ORF 1 and 2), but some of them contain three ORFs; ORF 1 encodes capsid proteins. Based on the sequence analysis of the capsid gene, sapovirus is divided into five genogroups (I, II, III, IV, and V) and currently genogroups I, II, IV, and V are currently considered as known genogroups able to infect human (7, 9, 14). Recent studies described the diversity of sapoviruses in which genogroup I and II sapoviruses could be classified into seven and five genotypes, respectively (8).

The objectives of this study were: a) to determine the incidence of sapovirus infection in patient with acute gastroenteritis in Tehran, Iran; b) to characterize the detected sapovirus according to genogroup and genotype; and c) to describe the age-related distribution of sapovirus infections.

Patients and Methods

Fecal specimens were collected from 42 patients admitted to the Shohadaye Tajrish and Mofid Children Hospital, Tehran, Iran with acute gastroenteritis during May-July 2009. All fecal specimens were determined previously to be negative for norovirus and adenovirus (15, 16). These specimens were diluted with NaCl 10%, and sedimented by centrifugation at 4,000g for 20 min. Supernatants were stored at -20 °C until use.

Viral RNA was extracted from 140 ml of the supernatant by the QIAamp Viral RNA kit according to the manufacturer's instructions (QIAGEN, Germany).

Complementary DNA (cDNA) was prepared in a 32.5 µl reaction mixture consisting 1 µl of random hexamers primers (Fermentas, Latvia), 5 µl ssRNA template (100ng), 4 µl 5 x buffer, 0.5 µl Ribolock RNase inhibitor (Fermentas, Latvia), 2 µl of dNTP mix (10 mM each of dNTP; Fermentas, Latvia), 200 u RevertAid^{TM} M-MuLV Reverse Transcriptase (Fermentas, Latvia) and 19 µ1 of RNase free water (Fermentas, Latvia). The RT assay was carried out at 42°C for 1h.

To amplify the sapovirus capsid region, PCR was carried out using primer sets SLV5317 and SLV5749 (17). Nucleotide sequences of primers are shown in Table 1.

Table 1. Nucleotide sequences of primers that were used for sapovirus typing based on capsid region

Primer Name	Primer Sequence	Primer positions
SLV5317 Forward	5'- CGGRCYTCAAAVSTACCBCCCCA -3'	5083-5105
SLV5749 Reverse	5'- CTCGCCACCTACRAWGCBTGGTT -3'	5494-5516

For the PCR, 5 µl of cDNA were added to a 20 µl PCR mix containing 2.5 µl 10× PCR buffer, 0.75 mM MgCl2, 0.2 mM of each dNTP, 10 pmols of each primer and 2 units of Taq DNA polymerase. The PCR was performed using the following conditions, 94 °C for 5 min, then 35 cycles of 94 °C for 45 s, 55 °C for 45 s and 72 °C for 1 min, followed by final extension of 7 min at 72 °C. PCR products were visualized under UV illumination after electrophoresis on a 1% agarose gel stained with ethidium bromide. The estimated amplified fragment size for sapovirus was 434 bp.

The nucleotide sequences of PCR products (DNA) positive for sapovirus were determined with the Big-Dye terminator cycle sequencing kit and an ABI 3130xl genetic analyzer (Applied Biosystems, USA). Sequence analysis was performed using aligning sequences with the

MEGA4 program and a phylogenetic tree was constructed by neighbor-joining method.

Results

Studied population

A total of 42 fecal specimens were collected from patients with acute gastroenteritis in Tehran, Iran during the period of May to July 2009. All of them were negative for norovirus or adenovirus. The lowest age was 3 month**s**, the highest was 69 years old, and the average age was 15.3 years. 79% of all patients were less than 17 years old. The percentage of male patient was 52.4%. All fecal specimens were tested for the presence of sapovirus by RT-PCR method. Of 42 fecal specimens from patient with acute gastroenteritis, five (11.9%) were found positive for sapovirus, including 3 girls and 2 boys. All of the patients with sapovirus were less than 5 years old.

Clinical data of the 5 patients infected with sapovirus are listed in Table 2. It was found that the common clinical symptoms of sapovirus infected patients were dehydration and abdominal pain (80% each), vomiting (60%). Fever was also frequently seen among sapovirus-infected patients (40%).

Table 2. Clinical features of the patients infected with sapovirus

Sign and symptoms	Number (percent)
Diarrhea	5 (100)
Dehydration	4 (80)
Vomiting	3 (60)
Abdominal pain	4 (80)
Fever	2 (40)

Phylogenetic analysis

A total of 5 sapovirus isolates associated with acute gastroenteritis were further characterized for genogroup, genotypes and genetic relationship with the reference strains. PCR products were further subjected to sequencing and the sequencing results were confirmed with NCBI BLAST online software. All of the sequenced sapoviruses were belonged to human sapovirus genogroup I. In addition, phylogenetic analysis was also performed on Iranian isolates based on nucleotide sequences of the capsid region of the sapovirus. Phylogenetic tree were constructed using comparison with the reference strains. Their partial nucleotide sequences were compared to each other as well as to those of reference sapovirus strains available in the GenBank database.

In the present study, all of the sapovirus sequences were classified into only one distinct genogroup I.2 (Figure 1) with high bootstrap values. The results indicated that sapovirus group I was a dominant genogroup in studied population.

Discussion

Gastroenteritis, especially diarrheal disease, is a leading cause of death in children throughout the world, with 3–6 million deaths estimated to occur annually in Asia, Africa, and Latin America (18). Sapovirus mainly infect infants and young children. Sapovirus associated diarrhea is milder, but severe cases can occur (19). Sapovirus is one of the global causes of viral gastroenteritis and also associated with sporadic cases and outbreaks of gastroenteritis worldwide and its prevalence was shown to range from 0.3% to 9.3% and usually much lower than norovirus (5-7, 20).

Pang et al. described the epidemiology and circulating strains of sapovirus associated with gastroenteritis outbreaks in Alberta, Canada, from 2004 to 2007. They reported sapovirus positive samples in 17.6% of outbreaks occurred in Canada (21). Sdiri-Loulizi et al. in 2011 performed a molecular genetics study and detected genogroup I sapovirus in Tunisian children suffering from acute gastroenteritis. They found 6 sapovirus positive samples (0.8%) with mean age of 11 months (22). Hansman et al. in 2004 conducted a

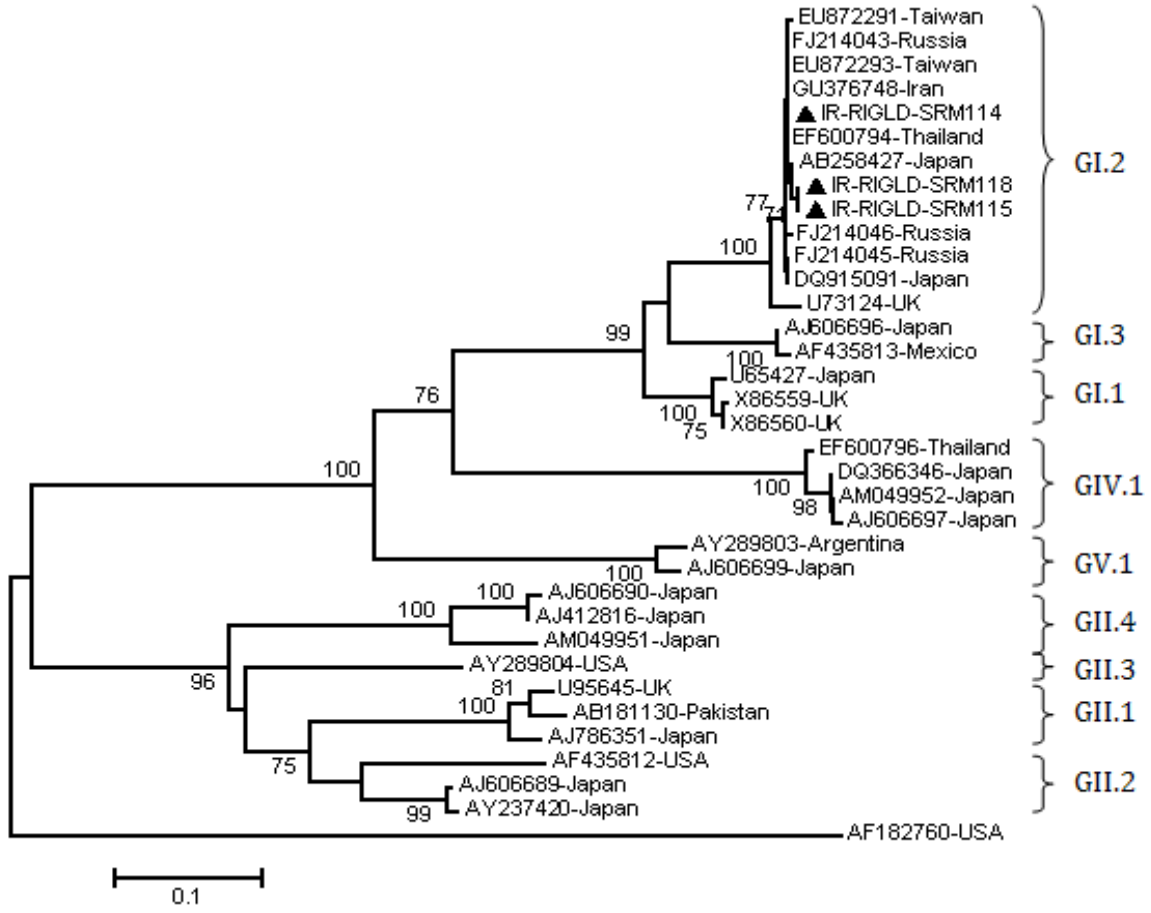

Figure 1. Neighbor-joining analysis of 3 Iranian SaV isolates (labeled with black triangles) partial capsid nucleotide sequences (293 nt) and 32 reference sequences retrieved from GenBank. Different SaV genogroups are indicated in phylogenetic tree. The numbers on each branch indicate the bootstrap values for the cluster, where values of 70 or higher were considered sufficiently significant for grouping. The scale indicates nucleotide substitution per site. Nucleotide sequence of swine SaV with accession number AF182760 (Sw/SaV/GIII.1/PEC-Cowden/1980/US) was used as out-group.

Reference sequences

GENBANK ACCESSION NUMBERS FOR THE REFERENCE ISOLATES ARE USED IN PRESENT AS FOLLOWS:

EU872291(Hu/SaV/CMH024/05/2005/THA), FJ214043 (Hu/SaV/Chelyabinsk/7365/2005/RUS), EU872293 (Hu/SaV/CMH082/05/2005/THA), GU376748 (Hu/SaV/Tehran/GE36/2008/IRN), EF600794 (Hu/SaV/CMH013/04/2004/THA), AB258427 (Hu/SaV/Chiba/041413/2004/JP), FJ214045 (Hu/SaV/Chelyabinsk/8300/2005/RUS), FJ214046 (Hu/SaV/Chelyabinsk/8320/2005/RUS), DQ915091 (SaV/Water-64), U73124 (Hu/SaV/Parkville/1994/US), AJ606696 (Hu/SaV/Chiba/010658/2001/JP), AF435813 (Hu/SaV/Mex14917/2000/USA), U65427 (Hu/SaV/Sapporo/1982/JP), X86559 (Hu/SaV/Plymouth/1995/UK), X86560 (Hu/SaV/Manchester/1997/UK), EF600796 (Hu/SaV/CMH044/03/2003/THA), DQ366346 (Hu/SaV/Ehime1596/1999/JP), AM049952 (Hu/SaV/Ehime/99-1596/1999/JP), AJ606697 (Hu/SaV/Ehime/99-1596/1999/JP), AY289803 (Hu/SaV/Argentina39/2003/AR), AJ606699 (Hu/SaV/Ehime/01-1669/2001/JP), AJ606690 (Hu/SaV/Chiba/990763/1999/JP), AJ412816 (Hu/SaV/Chiba/010004H/2001/JP), AM049951 (Hu/SaV/Ishikawa/04-721/2004/JP), AY289804 (Hu/SaV/cruise ship/2000/USA), U95645 (Hu/SaV/London/29845/1992/UK), AB181130 (Hu/SaV/Karachi/824/1991/Pak), AJ786351 (Hu/SaV/Ehime/2K-1948/2000/JP), AF435812 (Hu/SaV/Mex340/1990/USA), AJ606689 (Hu/SaV/Chiba/990727/1999/JP), AY237420 (Hu/SaV/Mc10/2003/JP), AF182760 (Sw/SaV/GIII.1/PEC-Cowden/1980/US).

study with the aim of detection of norovirus and sapovirus infection among children with gastroenteritis in Ho Chi Minh city, Vietnam. They found only one sapovirus positive sample among 448 investigated samples and no mixed infections of norovirus and sapovirus were found

(5). From the studies that have been performed in Asian countries, one study is conducted in Pakistan to detect human astrovirus, norovirus (GI, GII), and sapovirus infections in Pakistani children with diarrhea. They reported sapovirus infection in 3.2% of fecal samples (20).

Even if numerous epidemiological studies of sapovirus infection have been published worldwide, data of the illness caused by this virus in Iran are limited. There is only a study published previously on sapovirus infection among Iranian population that reported a prevalence of 2.1% (23). In this study, sapovirus was detected in 11.9% (5/42) fecal specimens collected from patients with acute gastroenteritis. The incidence of our sapovirus positive samples in infants and children population is in consistence with the previous studies. The nucleotide sequence of the sapovirus capsid gene was determined by direct sequencing with the amplified fragments. This region has been shown to be suitable for genotyping (9, 24). The results in this study showed that all Iranian sapovirus sequences belonged to only one distinct sapovirus genogroup I/2. Altogether, the results in this study suggested that sapovirus GI/2 isolates detected in infants and children in Tehran were closely homologous to each other.

In this study, we used RT-PCR for the detection of sapovirus. It is very sensitive and effective method for molecular epidemiological research. Some researchers used ELISA for detecting sapovirus. However, ELISA has lower sensitivity as compared to PCR (25).

Although the importance of viral gastroenteritis as a prime cause of morbidity and mortality in developing countries is well recognized, to our knowledge very few studies have evaluated the role of viral agents in childhood diarrhea in Iran.

In conclusion, the present findings confirmed sapovirus as one of the common viral agents responsible for gastroenteritis among infants and young children and also indicated that sapovirus G I/2 is a dominant genogroup in Iran.

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