

Hospital based study of prevalence and genotyping of Noroviruses and Sapoviruses isolated from children with acute gastroenteritis referred to Masih Daneshvari hospital

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ABSTRACT

Aim: The aim of this study is to determine the prevalence and genotype of human caliciviruses among children suffering from acute gastroenteritis referred to Masih Daneshvari Hospital.

Background: Human caliciviruses have been recognized as the major viral cause of acute gastroenteritis in all age groups worldwide. Studies revealed that the noroviruses and sapoviruses could be divided into five genogroups.

Patients and methods: A total of 47 fecal samples were collected from children up to 17 years of age, with acute gastroenteritis from 2006 to 2008. RT nested-PCR was performed for screening. To genotype the norovirus and sapovirus isolates, some positive samples were subjected to phylogenetic analysis by sequencing of fragments of viral capsid gene region.

Results: The noroviruses and sapoviruses were detected in 21.3% and 2.1% of samples, respectively. The phylogenetic analysis revealed that isolates belong to genotype GII.4 and GII.3. The sole isolate of sapovirus belongs to GI/2 genogroup.

Conclusion: Our results show that calciviruses are indeed a major cause of acute gastroenteritis in children.

Keywords: *Children, Acute gastroenteritis, Norovirus, Sapovirus, Genogroup.*

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INTRODUCTION

Viral gastroenteritis is one of the most common illnesses in all age groups worldwide, and an important cause of morbidity and mortality. Viruses are found to be that could cause such outbreaks, with the initial descriptions of

rotaviruses, adenoviruses, caliciviruses and astroviruses (1-5).

The members of the genera Sapovirus (SaV) and Norovirus (NoV) in the family *Caliciviridae* (6, 7) are the important cause of gastroenteritis in human (8, 9) and animals (10, 11). Caliciviruses are non-enveloped viruses, 27 to 35 nm in diameter with single-stranded RNA positive strand genomes of 7 to 8 kb. The genome encodes three open reading frames (ORFs). The ORF1 encodes a

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polypeptide with regions of similarity to helicase, cysteine proteinase and RNA-dependent RNA-polymerase. ORF2 encodes a viral capsid protein, and ORF3 encodes a small protein of unknown function (6, 7).

The illnesses caused by SaVs and NoVs differ in epidemiological features and symptoms. While NoVs are the major cause of outbreaks of nonbacterial gastroenteritis in all age groups, SaVs have been primarily associated with paediatric gastroenteritis (6, 12, 13).

Based on sequence information obtained from the POL gene (ORF1) or VP1 gene (ORF2), NoVs can be subdivided into five separate genogroups (GI, GII, GIII, GIV and GV) of which viruses of genogroup III have so far only been found in cattle, while GV was found in mice (6, 14).

SaV can be divided into five genogroups (GI–GV), among which GI, GII, GIV and GV are known to infect humans, whereas SaV GIII infects porcine species (6, 7).

The aim of this study is to determine the prevalence and genogroup patterns of caliciviruses which were isolated among children less than 17 years old whom suffering from acute gastroenteritis and referred to Masih-Daneshvari hospital.

PATIENTS and METHODS

Stool samples were collected from 47 individuals with acute gastroenteritis between February 2006 and October 2008. The specimens were collected from children less than 17-years-old who were admitted to Masih Daneshvari Hospital, National Research Institute for Tuberculosis and Lung Disease (NRITLD), Tehran, Iran. The specimens were immediately transported in cold boxes (2 to 8 °C) to the virology laboratory at Virology Research Center, NRITLD stored at -70 °C before use. A 10% (w/v) stool suspension of total volume (10 ml) was made in 1XPBS (pH 7.0), shaken well and centrifuged

for 10 min at 2500 rpm. The clarified supernatant was collected and stored at -70 °C.

RNA Extraction and RT-PCR: Viral RNA was extracted from 100 µl of the 10% stool suspension with the RNX^{plus} RNA extraction kit (Sinnagen, Iran) according to the manufacturer's instructions. The RNA was eluted with 50 µl of diethyl pyrocarbonate-treated water and kept at -70 °C until use. cDNA was synthesized by cDNA synthesis kit (Fermentas) according to the manufacturer's instructions. Screening and genotyping: To screen for NoV genome, nested PCR was performed using primers in the RNA polymerase encoding region as described previously (15). To genotype the norovirus isolates, the positive samples were re-amplified by using the VP1 (D region) gene region primer sets (genogroup specific for GI and GII) (14) and positive samples were subjected to sequencing. Detection of SaVs was done by applying a nested PCR directed to viral capsid protein gene and was based on a previously published article (7, 16). Genotyping was conducted by direct sequencing of screening PCR products for SaV. The DNA sequence was determined with the Big-Dye terminator cycle sequence kit and an ABI 377A sequencer (Applied Biosystems Inc.). The sequences were edited with the BioEdit software program version 7.0.5.2 and then phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1. The neighbor-joining method was used for phylogenetic reconstructions that were implemented in the MEGA3.1 program.

Nucleotide sequence accession number: All sequences determined by this study have been deposited in the Genbank database under accession numbers GU139361- GU139368 for NoV isolates and GU376748 for SaV isolate.

Statistical data processing: Data were processed by SPSS statistical software program version 16.0. The correlations were subjected to χ^2 (Pearson Chi-Square) and Fisher's Exact test.

Statistical significance was set as a *P* value less than 0.05.

RESULTS

Between February 2006 and October 2008, clinical samples were collected from children less than 17-years of age. All of the clinical samples were provided by the Department of Pediatrics at Masih Daneshvari Hospital, NRITLD, Iran. The characteristics of study subjects, including age, gender and calicivirus RNA are shown in Table 1.

Table 1. The characteristics of study subjects and Calicivirus status

Parameter	Gastroenteritis (n=47)
Age (month ±SD)	30.26±28.61
Gender	
female	19(40.4)*
Male	28(59.6)
Season	
Spring	15(31.9)
Summer	6(12.8)
Autumn	15(31.9)
Winter	11(23.4)
NoVs	
Positive	10 (21.3)
Negative	37 (78.7)
SaVs	
Positive	1(2.1)
Negative	46(97.9)

* Numbers in parentheses are percentages.

A Total of 47 children with acute gastroenteritis, were recruited into this study. The mean age of studied subjects was 30.26 ± 28.61 (SD) (range 3-120) months. The gender ratios (male: female) was 1.47. NoVs and SaVs were detected in 21.3% and 2.1% of studied cases (table 1).

In table 2, frequency of NoVs and SaVs is stratified by age group, sex and season of sample collection. Statistical differences were not observed in gender, age group and seasonal distribution of NoVs and SaVs in gastroenteritis cases in this study. 8 norovirus positive samples were selected for genotyping. All studied NoV

isolates were identified as genogroup GII. The phylogenetic analysis revealed that 7 samples belong to genotype GII.4 and only 1 sample belongs to genotype GII.3 (Figure 1). The sole isolate of SaV belongs to GI/2 genogroup (Figure 2).

Table 2. Calicivirus status according to characteristics of study subjects*

	Positive (%)	Number	Positive (%)	Number
Age(year)				
≤3	6 (16.7)	36	0	36
4-6	2 (40)	5	1 (20)	5
≥7	2 (33.3)	6	0	6
Gender				
female	4 (21.1)	19	0	19
Male	6 (21.4)	28	1 (3.6)	28
Season				
Spring	5(33.3)	15	1 (6.7)	15
Summer	0	6	0	6
Autumn	3(20)	15	0	15
Winter	2(18.2)	11	0	11

* There were no significant differences between two groups for mentioned characteristics

DISCUSSION

To our knowledge, this is the first study in which genotypes of NoV and SaV have been investigated as etiologic agents of sporadic gastroenteritis cases in Iranian children. NoVs are the most common cause of outbreaks of nonbacterial gastroenteritis and it is estimated that they are responsible for 68–80% of all outbreaks of gastroenteritis in industrialized countries (17). Studies all over the world have demonstrated the high incidence of NoV disease in people of all age groups (17, 18). In a study conducted on 15 hospitals in the UK monitored the occurrence of nosocomial outbreaks of gastroenteritis, many of which were caused by NoVs (19).

In our study, Caliciviruses were found in 23.4% of gastroenteritis in children whom referred to Masih Daneshvari Hospital (Table 1). These viruses, after Rotaviruses with the prevalence of

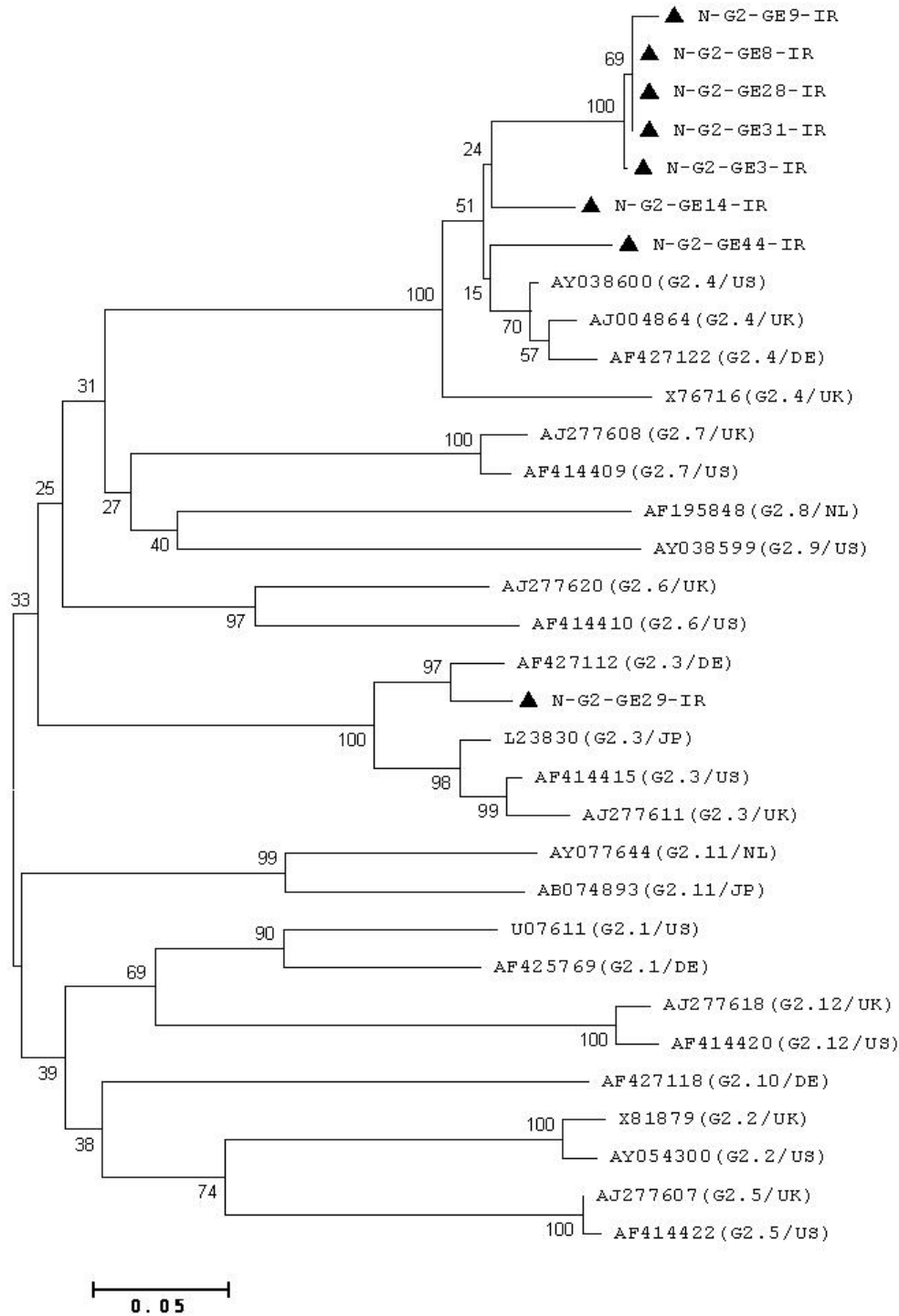


Figure 1. Phylogenetic analysis of VP1 gene sequences in NoV isolates. The tree was constructed by neighbor-joining method by using MEGA 3.1.

48.9%, were more prevalent than other enteric viruses (Data not shown). 21.3% of viral gastroenteritis was due to NoVs (Table 1). Some studies reported the highest incidence of NoV

infection in elderly women (20, 21) but others didn't find any relation with sex (22-24). Our study reports no association between sex and presence of NoVs (Table 2).

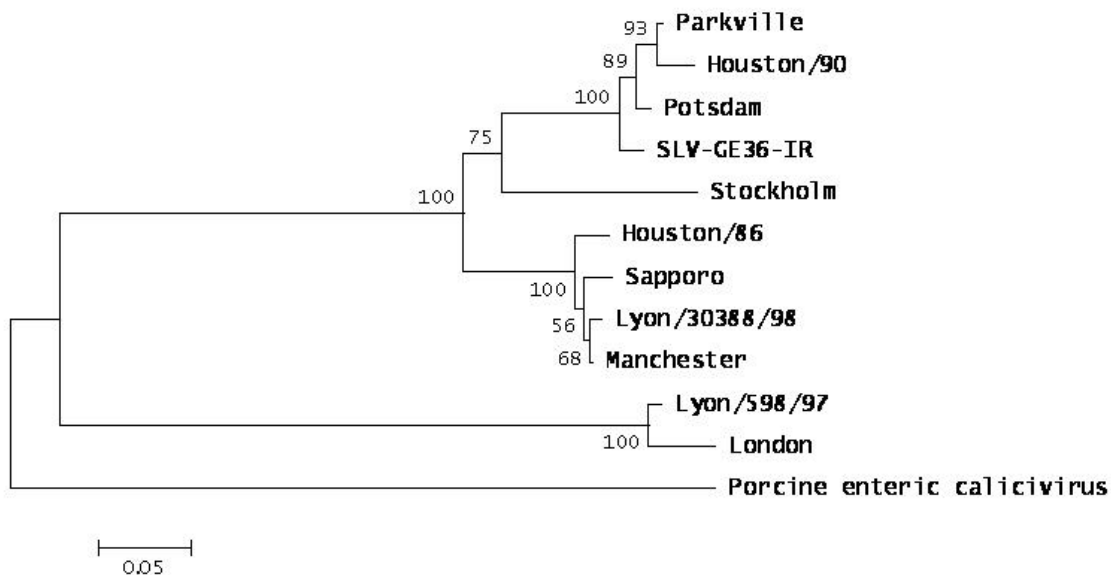


Figure 2. Phylogenetic analysis of VP1 gene sequences in SaV isolate. The tree was constructed by neighbor-joining method by using MEGA 3.1.

The seasonal periodicity of different NoV strains has been demonstrated indicating a marked seasonal periodicity with significant peaks in the late spring/early summer periods (20, 21). Our study didn't show any significant relation to seasons; however most of the NoV isolates were detected in spring and autumn (Table 2). Due to small sample size, additional studies with large samples and following through the seasons will be needed.

Our study indicated low prevalence for SaV (Table 1). This result confirms with previously published papers on SaV epidemiology worldwide, in which SaV prevalence was shown to range from 0.3 to 9.3% and far below the prevalence of NoVs (25- 28).

Mainly genogroup GII of the NoVs are indeed a major cause of both outbreaks and sporadic cases of acute gastroenteritis in infants and young children worldwide (29-32). The finding showed that NoV GI Strains are less common than NoV GII. Our study indicated that NoV GII strains were dominant in Iranian children with gastroenteritis (Figure 1). For SaV infection, SaV genogroup GI has been reported worldwide as the

most predominant strain (9, 16) and our finding is in agreement with these studies (Figure 2).

In conclusion, our results show that caliciviruses, after rotaviruses, are indeed a major cause of acute gastroenteritis in children in Iran and genogroup GII, mainly GII.4 genotype, in NoVs and GI/2 in SaVs are the most common types.

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