Iranian Journal of Virology 2011;5(4): 29-31

Short Communication

Determination of Rotavirus, Sapovirus and Norovirus Co-Infection among Children Suffering from Gastroenteritis Referred to Ahvaz Abuzar Hospital, Southern Iran

Parsa-Nahad M[1], Samarbaf-Zadeh AR[1], Makvandi M[1], Jalilian Sh[1], Kalvandi Gh[1], Sheikholeslami F[2], Pirmoradi R[1], Ajorloo M[2], Mozhgani SHR[2*]

1. Department of Medical Microbiology, School of Medicine and Infectious and Tropical Disease Research Center, Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran.
2. Departments of Viral Vaccine Production, Production and Research Complex of Pasteur Institute of Iran, Karaj, Iran.

Acute gastroenteritis is one of the most common disease in infants and children both in developing and developed countries. Almost 80% of acute gastroenteritis is due to viruses (1). Between various kinds of diarrheal viruses, rotavirus is the most important cause of severe gastroenteritis in infants and children in the world (2). Norovirus and Sapovirus however are also considered to be significant global cause of gastroenteritis (3, 4). Transmission of these viruses occurs through fecal-oral route and might be other unknown modes of transmission (5, 6). The aim of this study was to determine co-infection of Rotavirus, Norovirus and Sapovirus in fecal specimens of children suffering from gastroenteritis referred to Ahvaz Abuzar Hospital.

One hundred eighty fecal specimens were collected from children up to 5 years old suffering from acute gastroenteritis. For determination of viral co-infection we used two methods. First we used an ELISA kit for detection of rotaviruses and then the positive and negative samples were tested by the RT-PCR method to detection of Noroviruses and Sapoviruses RNA.

***Corresponding author:** Seyed Hamid Reza Mozhgani, MSc, Departments of Viral Vaccine production, Production and Research complex of Pasteur institute of Iran, Karaj, Iran.
Tel: (+98) 912 66 18 743 Fax: (+98) 263 61 02 900
Email: hamidrezaMozhgani@yahoo.com

After suspension of about 5 grams of samples in ELISA buffer, ELISA test was done according to manufacture instructions. All tests carried out in duplicate.

Viral RNA was extracted by Fermentas extraction kit (Lithuania) according to manufacturer's instruction and eluted in 50µl of RNase-free sterile water. Reverse transcription was carried out in final volume 20 µl: 4µl 5x RT buffer , 1µl dNTPs (10 mM), 1µl Random hexamer (0.2 u/µl), 0.5µl RNase inhibitor (40 u/µl), 0.5µl RT enzyme (200 u/µl), 0.5µl $MgCl_2$ (50mM), 6.5µl DEPC (RNase free water) and 6µl extracted RNA. The tubes were incubated at 42˚C for 1 hour. Then, the cDNA was stored at -20˚C until subsequent use as template in PCR reaction. 5µl of these cDNA was used as template for PCR by using specific primers.

The primer of PCR reaction for detection of sapovirus was as follows:SR80F: 5́-TGG GAT TC T ACA CAA AAC CC-3́, JV33R: 5́- GTG TAN ATG CAR TCA TCA CC-3́; The amplicon size of this primer was 320 bp. PCR condition was as follow: 35 cycles of amplification (5min in 94^0C , 1min in 94˚C , 55 seconds in 51˚C and 50 seconds in 72 ˚C) and final extension at 6 min in 72˚C(7).The primer of PCR reaction for detection of Norovirus was as follow: NV35F: 5'-CTT GTT GGT TTG AGG CCA TAT -3', NV36R: 5'-ATA AAA GTT GGC ATG AAC A-3'. These primers amplify 470 bp. PCR

[DOI: 10.21859/isv.5.4.29]

Table 1. **The relative frequency of viruses in whole specimens.** According to our collected samples, 32.8% of specimens were belonged to Rotavirus group. These positive Rotavirus samples had 3.38% co-infection with Sapovirus but there wasn't any co-infection between Rotavirus and Norovirus. About 67.2% of whole samples were Rotavirus negative and only 0.8% co-infection was existed between Norovirus and Sapovirus.

Total Number of Specimens	**Rotavirus Positive**		**Rotavirus Negative**
180	59 32.8%		121 67.2%
	Sapovirus & Rotavirus Co-Infection	**Norovirus & Rotavirus Co-Infection**	**Norovirus & Sapovirus Co-Infection**
	3.38%	0	0.8%

condition was as follow: 35 cycles of amplification (5min in 94˚C , 1min in 94˚C , 1 min in 49˚C and 1 min in 72˚C) and final extension at 6 min in 72˚C(8).

10µl of the final PCR product was subjected to electrophoresis in 2٪ agarose gel. Stained with etidium bromide and then visualized by UV transiluminater (Vilber Lourmat, France). For confirmation sapovirus positive samples were sequenced by milogen company (France) and the positive samples of noroviruses were tested by nested PCR using one pairs of internal primers: NV51F: (5'-GTT GAC ACA ATC TCA TCA TC-3') and NV3R: (5'-GCA CCA TCT GA GAT GGA TGT- 3') (9).

59 of 180 samples were found to be positive for rotavirus infection (32.8٪). Among these positive samples there were two Sapovirus positive specimen (3.38٪) and there were no positive samples in rotavirus positive specimens for Norovirus. After doing RT-PCR on 121 negative rotavirus samples there was one Sapovirus samples that also were positive for Norovirus infection. So there were 0.8 ٪ positive specimens for Sapovirus and Norovirus in rotavirus negative samples.

Gastroenteritis is a major cause of morbidity and mortality in the entire world (2). In this study we could found co-infection of Rotaviruses and Noroviruses and Noroviruses with Sapoviruses, because these infections transmit by contaminated food or water. Co-infection with these viruses show high contamination of food and drinking water in their area so for reduction of the incidence of these viral infections in children and adult education and improved personal hygiene is advised.

References

1. Domiguez A, Torner N, Ruiz L. Etiology and epidemiology of viral gastroenteritis outbreaks in Catalonia (Spain) in 2004-2005. J Clin Virol. 2008;43:126-131.

2. Parashar UD, Hummelman EG, Miller MA. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis. 2003;9:565–572.

3. Bon F, Fascia P, Dauvergne M. Prevalence of group A rotavirus, human calicivirus, astrovirus, and adenovirustype 40 and 41 infections among children with acute gastroenteritis in Dijon, France. J Clin Microbio. 1999; l(37):3055–3058.

4. Carter MJ, Willcocks MM. The molecular biology of astroviruses. Arch Virol. 1996;12:277–285.

5. Oh DY, Gerhard G, Eckart S. Viral agents of acute gastroenteritis in German children: prevalence and molecular diversity. J Med Virol. 2003;71:82–93.

6. Lopman BA, Brown DW, Koopmans M . Human caliciviruses in Europe. J Clin Virol. 2002;24:137–160.

7. Ike AC, Hartelt K, Oehme RM, Brockmann SO. Detection and characterization of sapovirus in outebreak of gastroenteritis in Southwest Germany. J Clin Virol. 2008;43(1):37-41.

[DOI: 10.21859/isv.5.4.29]

8. Wang J, Jiang X, Madore HP. Sequence diversity of small round-structured viruses in the Norwalk virus group. J Virol. 1994;68:5982-5990.
9. Moe C, Gentsch J, Ando T. Application of PCR to detect Norwalk virus in fecal specimens from outbreaks of gastroenteritis. J Clin Microbiol. 1994;32:642-648.

[DOI: 10.21859/isv.5.4.29]