Short Communication

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

 Parsa-Nahad M¹, Samarbaf-Zadeh AR¹, Makvandi M¹, Jalilian Sh¹, Kalvandi Gh¹, Sheikholeslami F², Pirmoradi R¹, Ajorloo M², Mozhgani SHR²

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.

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 MgCl₂ (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: 5'-TGG GAT TC T ACA CAA AAC CC-3', JV33R: 5'- GTG TAN ATG CAR TCA TCA CC CC-3'; The amplicon size of this primer was 320 bp. PCR condition was as follow: 35 cycles of amplification (5min in 94°C , 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 AAG CCA TAT -3’, NV36R: 5’-ATA AAA GTT GGC ATG AAC A-3’.

These primers amplify 470 bp. PCR
Determination of Rotavirus, Sapovirus and Norovirus Co-Infection among Children …

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.

<table>
<thead>
<tr>
<th>Total Number of Specimens</th>
<th>Rotavirus Positive</th>
<th>Rotavirus Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>59</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>32.8%</td>
<td>67.2%</td>
</tr>
<tr>
<td>180</td>
<td>Sapovirus &amp; Rotavirus Co-Infection</td>
<td>Norovirus &amp; Rotavirus Co-Infection</td>
</tr>
<tr>
<td></td>
<td>3.38%</td>
<td>0</td>
</tr>
</tbody>
</table>

condition was as follow: 35 cycles of amplification (5 min in 94°C, 1 min in 94°C, 1 min in 49°C and 1 min in 72°C) and final extension at 6 min in 72°C. 10μl of the final PCR product was subjected to electrophoresis in 2% agarose gel. Stained with ethidium 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 norovirus were tested by nested PCR using one pairs of internal primers: NV51F: (5’-GTG 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 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