Short Communication

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

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cute gastroenteritis is one of the most common disease in infants 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 important cause of 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 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.

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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 manufacturer's instruction and eluted in 50µl RNase-free sterile water. transcription was carried out in final volume 20 ul: 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 $u/\mu l$), 0.5 μl MgCl₂ (50mM), 6.5 μl (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-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 AGG CCA TAT -3', NV36R: 5'-ATA AAA GTT GGC ATG AAC A-3'. These primers amplify 470 bp. PCR

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
	59		121
180	32.8%		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 $94^{\circ}C$, 1min in $94^{\circ}C$, 1 min in $49^{\circ}C$ and 1 min in $72^{\circ}C$) and final extension at 6 min in $72^{\circ}C(8)$.

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 (32.8½). Among these positive samples there were two Sapovirus positive specimen (3.38%) and there were no samples rotavirus positive in 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 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. Coinfection 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.

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