

Predominance of rotavirus G8 genotype in hospitalized children with acute gastroenteritis in Yasuj, Iran

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Abstract. – BACKGROUND: Rotavirus infection is the most common cause of gastroenteritis among children worldwide. In this study, we were evaluated the occurrence of rotavirus infection and genotypes causing gastroenteritis among children under 5 years in Yasuj, Iran.

MATERIALS AND METHODS: This cross sectional-descriptive study was done on 184 fecal samples collected from children aged < 5 years old with acute gastroenteritis. All the stool specimens were tested for group A rotavirus with enzyme immunoassays (EIA). Rotavirus-positive specimens were genotyped by the Nested reverse transcription polymerase chain reaction (RT-PCR) with using different type of specific primers.

RESULTS: The prevalence of rotavirus was 28.26% and predominant genotypes were G1 (1.92%), G2 (7.69%), G4 (1.92%), G8 (46.16%), N.T.A (40.39%), mixed infections (1.92%). Predominance of rotavirus G8 genotype in the current survey was reported for the first time in Iran. The highest prevalence of rotavirus infection was observed in autumn (48.08%), and the lowest in spring (5.77%).

CONCLUSIONS: The result of study showed that prevalence of rotavirus genotypes in this region of the country is different from that of other regions. The continuous stability of rotavirus in Iran, regarding to the regional differentiates and prevalence of circulating strains, can be useful indication in order to supply and effective vaccine against the rotavirus infections.

Key Words:

Rotavirus, Gastroenteritis, Children, Genotype, Nested RT-PCR.

children in both developed and developing countries¹. Numerous epidemiologic studies in these countries have shown that 15-40% of all cases of gastroenteritis caused by rotavirus². Group A rotaviruses are member of the *Reoviridae* family which their genome consists of 11 segments of double-stranded RNA (dsRNA). Glycoprotein VP7 and protein protease-sensitive VP4, two outer capsid proteins, have been classified rotaviruses into G and P genotypes, respectively³. Surveillance of rotavirus strains has demonstrated G1 to G4 and G9 types to be the most commonly circulating rotavirus genotypes in worldwide⁴. The objective of this study was to monitor the disease burden associated with rotavirus infection and determine the G genotypes of rotaviruses circulating among children aged < 5 years old who were hospitalized for acute gastroenteritis in Yasuj, Iran.

Material and Methods

From September 2010 to August 2011, a total of 184 stool specimens were collected from children aged < 5 years old who hospitalized with acute gastroenteritis. All samples were stored immediately after collection at -20°C until use for analyses. Demographic and clinical data such as age, sex, clinical symptoms for each child were recorded. The stool samples were screened for group A rotavirus antigen by enzyme immunoassay using a commercial ELISA kit (Generic Assays kit, Dahlewitz, Germany), according to the manufacturer's guidelines. The optical density (OD) was read at 450 nm and specimens having OD values above the cut off value (0.2 + OD of the negative control) were considered positive for rotavirus antigen. The viral dsRNA was extracted

Introduction

Rotaviruses have been recognized as one of the leading etiologic agents of severe diarrhea in

from 10% stool suspension by using the RNX-Plus kit (CinnaGen, Tehran, Iran), according to protocol provided by the manufacturers. Briefly, 5 μ l of dsRNA was added with mix of dimethylsulfoxide (DMSO), 5X RT buffer, dNTPs (deoxynucleotide triphosphatases), primers Beg9, End9, DW, denatured at 97°C for 5 min, then followed by addition of RT enzyme and RNase inhibitor to a final volume of 20 μ l. The RT-PCR reaction was carried out for 60 min at 42°C to produce the complementary (cDNA). Then, the cDNA product was used as template for PCR VP7-amplification with the same Beg9/End9 pair of primers. For PCR amplification of the VP7 gene, a 1062 bp fragment was generated using Beg9 (forward) and End9 (reverse) primers were performed as previously described by Gouvea et al (1990). 5 l of the resulting amplicons of 1062 bp were then used as a template in the second round of PCR, provided by World Health Organization. All PCR products were analyzed by electrophoresis in 2% agarose gel that contained ethidium bromide (10 μ g/ml) and visualized under UV illumination⁵.

Statistical Analysis

The Chi-square test or Fisher's exact test was used for statistical analysis when appropriate using SPSS 16 (SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered statistically significant.

Results

Of the 184 fecal samples were analyzed, 52 tested positive for rotavirus by EIA (enzyme immunoassay). Rotavirus was detected in boys (12.50%) and in girls (15.76%). A significant difference was found in the sex distribution between these populations ($p = 0.049$). The seasonal distribution of rotavirus infection showed that the relationship between the prevalence of rotavirus and seasons is significant ($p = 0.001$). The highest prevalence of rotavirus infection was observed in autumn (48.08%), followed by winter (26.92%), summer (19.23%) and spring (5.77%) respectively. Statistical analysis showed a significant relationship between the incidence of rotavirus and year month ($p = 0.026$). The highest rate of rotavirus infection was found in November (21.16%) and the lowest in the April and August that no rotavirus infection was detected. Rotavirus was detected most frequently in September- November. The presence of *Rotavirus* re-

mained low in April and August in which no G type was detected ($p < 0.01$). Genotyping was performed on 52 rotavirus positive cases by using Nested reverse transcription polymerase chain reaction (RT-PCR) (Figure 1). The most common circulating genotype in the population under surveillance was G8, that being identified in 24 strains out of 52 positive samples (46.16%), followed by non-typeable (40.39%), G2 (7.69%), respectively. Prevalence of G1, G4 and mixed genotypes were equal (1.92%). In the present study, G3, G9, G10 and G12 types were not detected. The most prevalent genotype detected in female was G8 (30.77%) and G8 and non-typeable in males (15.39%) but this difference was not statistically significant ($p = 0.067$). The most prevalence of rotavirus genotype reported was G8 in the autumn (28.85%) and summer (13.47%) and in the spring and winter non-typeable ($p = 0.093$).

Discussion

Rotavirus is the major cause of non-bacterial gastroenteritis in both developed and developing countries and have been estimated to be account for 527,000 deaths in children < 5 years of age annually^{1,2}. Numerous molecular epidemiological studies have indicated that G1 is the most common circulating G type around the world⁶. However the G1 type was observed only in 1.92% of all episodes of gastroenteritis. We detected the G2 type in the specimens of 7.69% of patients with severe diarrhea, which is in contrast with

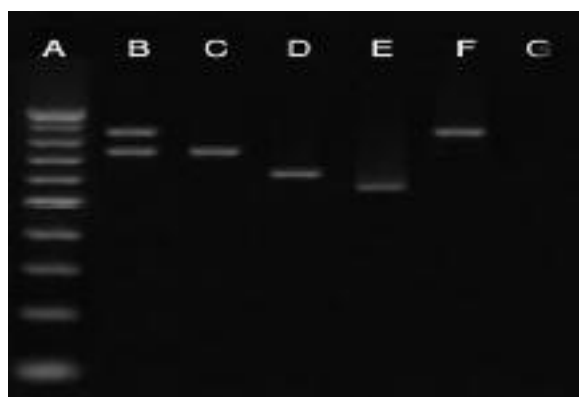


Figure 1. G types of rotavirus determined by RT-PCR using specific primers. A: 100 bp molecular weight marker, B: Mixed genotype (G1+G8), C:G1 genotype, D: G2 genotype, E: G4 genotype, F: G8 genotype and G represents the negative control.

studies conducted in China⁷, Tunisia⁸ where the G2 type was one of the most prevalent rotavirus types. In the current study, G3 genotype was not detected. This finding is distinct from those results observed in Tunisia⁸ and China⁹ where G3 genotype has been described as the most common type in children. G4 genotype was presented in 1.92% of the evaluated samples. However, this G type has been detected at high frequency in some countries such as Germany¹⁰, South Korea¹¹ and some area from Iran^{12,2}. G8 genotype is major bovine rotavirus genotype, which causes sporadic cases in humans in some parts of the world. This G type has been identified in some of African countries such as Malawi with detection rate 34.8%¹³. According to conducted studies in Iran, G8 genotype has been reported as part of mixed genotypes¹⁴. In the present work, G8 genotype was isolated as the dominant genotype for the first time in Iran. The emergence of G9 type has subsequently been demonstrated in many countries around the world^{10,15}. However, the G9 genotype was not observed in this study. In recent years there has been an increase in research of the importance of unusual types (G10, G12) in many countries including several Asian countries such as Nepal¹⁶, Vietnam¹⁷ and Europe¹⁸ in despite of using specific primers to detect these unusual strains but neither the G10 nor the G12 genotypes were detected during the study. The proportion of mixed infections with two different rotavirus genotypes (1.92%) detected among children with acute gastroenteritis was similar with those identified in other investigations from Iran¹⁹ and China²⁰. In our report, G untypeable were identified as the second most common genotype present in, 40.39% of the evaluated samples. Non typeable strains might be related to the presence of novel strains that were not investigated in this survey; for example, rare genotypes such as G5, G6, G11 and also failure in RT-PCR technique⁵. Seasonal pattern of rotavirus infection in tropical countries is classically described as occurring year round²¹. While the peak of rotavirus diarrhea in temperate climate occurs in cold and dry months of the year²². In the current study the peak appearance of rotavirus more occur in the cool and dry seasons, that follows the seasonal pattern in temperate regions²³, and there was a significant difference between the seasonal distribution and virus isolation. The highest prevalence of rotavirus gastroenteritis occurred in autumn and winter seasons with a seasonal peak observed in the months

of September to February. These findings are similar to those reported in countries with temperate climates such as Iran²³ and Spain²⁴.

Conclusions

This report provides information on the epidemiology and the distribution of rotavirus genotypes circulating among Iranian children with acute gastroenteritis. Our data show that infection caused by rotavirus in the country is an important health problem, in children aged < 5 years and during the cold season. Also accurate determination of rotavirus strains circulating in other parts of Iran can be helpful in development of immunization and vaccination programs for children at high risk, and also avoids the high cost of clinical care.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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