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Original Article

Investigation of Rotavirus Genotypes in Children Under five Years With Acute Gastroenteritis in Kermanshah, Iran

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Abstract

Background and Aims: Rotavirus is one of the most important causes of acute diarrhea in children under five years. Therefore, the diarrhea samples examination in children is necessary to diagnose the prevalence of rotavirus and determine the predominant genotype. The focus of this study was to investigate the prevalence of rotavirus and its predominant genotype in the population of children under five years with acute gastroenteritis in Kermanshah, Iran.

Materials and Methods: Between October to March 2017, 100 samples were collected and the rotavirus positivity was evaluated by VP6 expression. Then, the dominant genotype in the positive samples was examined by Multiplex seminested PCR with specially designed primers.

Results: 100 stool samples of children under 5 years with gastroenteritis were taken, 21 sample positive for rotavirus. The highest frequency of genotype was G1P8 (38.1%) and the lowest was G2P4 (14.3%). The most positive rotavirus samples were in the age group of 1-2 years and no rotavirus positive samples were found in the 4-5 age group.

Conclusion: Rotavirus should be considered as an important agent in diarrhea of children, especially children under 1 year, and inappropriate use of antibiotics should be avoided. It is also recommended to determine the dominant genotype to provide a suitable situation for immunization of children against rotavirus in Iran.

Keywords: Genotyping, Acute gastroenteritis, Rotavirus

Introduction

Rotavirus was first introduced in the duodenal epithelial cells of a child with diarrhea by electron microscopy in 1973 and is recognized as a major cause of diarrhea in children worldwide (1). Rotavirus is a cause of diarrhea, hospitalizations, and deaths in children under 5 years (2) Rotavirus is a member of Reoviridae family and their diameter is 60 to 80 nm. Rotavirus is non enveloped and has 132 capsomer three layers capsid (3). Rotavirus is divided into seven groups A-G, and group A is the main cause gastroenteritis in humans. But other rotaviruses that have distinct antigenic properties also cause diarrhea in children and adults (4).

Molecular epidemiological studies on rotaviruses and based on differences in motion have identified eleven fragments of the double-stranded RNA genome (5). The viral genome encodes structural proteins (VP) and non-structural proteins (NSPs). So far, three major groups of antigens have been identified in rotavirus and two of them are major and they contain VP4 and VP7 as the foreign capsid proteins with epitopes that are important in the virus neutralization reaction (6). Rotavirus is divided into more than twenty different genotypes based on P (VP4) type and G (VP7) type, respectively. Rotavirus with genotypes G1-G4 and P (P8) is one of the most common genotypes reported in the worldwide.

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Various reports have suggested that G1P (8), G4P (8), and G2P (4) are primarily responsible for causing diarrhea in children and infants (7). Thus, the focus of this study was to investigated prevalence of rotavirus genotype pattern in children with diarrhea in Kermanshah, Iran.

Methods and Methods

Sampling. The 100 samples of watery and non-bloody diarrhea of children under 5 years with gastroenteritis were transferred from the laboratory of Dr. Mohammad Kermanshahi Hospital in Kermanshah, Iran. The present study was ethically approved by the Institutional Review Board of Kermanshah University of Medical Sciences (IR.KUMS.REC. 1396.336). Between October to March 2017 to the research laboratory of the Department of Microbiology. The samples were stored at -20 °C until starting the diagnostic tests. The samples were mixed with PBS buffer in a ratio of 1.5 after receiving the stool samples and then centrifuged at 2500 rpm for 5 minutes. The supernatant was used to extract the double-stranded RNA of the virus and stored in a freezer at -70 ° C.

RNA Extraction and cDNA Synthesis

RNA extraction from clinical specimens was performed by RNA extraction ki (Bio Basic Inc., Canada) according to the manufacturer's manual.

The purity of the RNA was determined by the Nano drop ND-1000 spectrophotometer (Nano drop Technologies, Inc., Wilmington, DE, USA). The RNA samples were stored at −70 °C until further analysis.

In the next step, Complementary DNA (cDNA) was synthesized using BioFact RT-PCR Pre Mix Synthesis Kit (BioFACT™, Daejeon, Korea). Then to the cDNA synthesis kit, which was a ready-made kit with 15 µL microtubes, 5 µL were added from the RNA extraction product and then the microtube was incubated for 15 minutes at 55° C for cDNA synthesis.

Performing the Genotyping and PCR

Two stages of PCR were performed for genotyping, which includes P-typing and G-typing. For the first stage, G-typing of VP7 gene amplification and in the second stage, the product of the first step to identify the typing was used by multiplex -PCR method. And about the P-typing also the first stage was VP4 amplification and the second stage of type was performed by using the product of the first step.

The primers and the temperature conditions are shown in Table 1. For G and P typing, the final volume of contents in PCR microtubules was 25 µl, which included 12.5 µl of master mixer, 1 µl of forward primer, 1 µl of reverse primer, 5 µl of cDNA and 5.5 µl of distilled water.

For Multiplex seminested PCR for VP7, the final volume of contents in the PCR microtube was 25 µl, which is including 12.5 µl of Master Mixer, 4 µl of forward primer, 0.5 µl of reverse primer, 2.5 µl of first run product of PCR and 5.5 µl of distilled water. Also, to perform Multiplex seminested PCR for VP4, the final volume of contents in PCR microtubule was 25 microliters, including 12.5 µl of master mixer, 0.5 µl of forward primer, 2.5 µl of reverse primer, 2.5 µl of first run of PCR product and 7 µl of distilled water. The electrophoresis was performed for final product of PCR and then was observed on 1% agarose gel by ethidium bromide staining. Finally, the results of this study were analyzed by SPSS software.

Results

Prevalence of Rotavirus in the Samples Based on the Sex

Out of 100 stool samples 60 samples were from males and 40 samples were from females. All samples were collected randomly.

Finally, 21 out of 100 samples were positive for rotavirus, of and 14 samples were male and 7 samples were female. More details are shown in Table 2.

Frequency of Rotavirus Genotypes

As shown in Table 3, the highest frequency of genotype was G1P8 (38.1%) and the lowest was G2P4 (14.3%). Electrophoresis gel images related to G and P genotyping are shown in Fig 1.

Table 1: The sequence of primers and temperature conditions for PCR

Primer	Sequence	Amplicon	PCR condition
VP6-F VP6-R	GACGGVGCRACTACATGGT GTCCAATTCATNCCTGGTG	382	94°C (3 min) 55°C (1 min) 72°C (8 min) 15°C (Hold)
G-typing(a) 1st round			
VP7-F VP7-R	ATGTATGGTATTGAATATACCAC AACTTGCCACCATTTTTTCC	881	94°C (3 min) 50°C (1 min) 72°C (8 min) 15°C (Hold)
G1	CAAGTACTCAAATCAATGATG	618	94°C (3 min) 50°C (1 min) 72°C (8 min) 15°C (Hold)
G2	CAATGATATTAACACATTTTCTGTG	521	
G3	ACGAACTCAACACGAGAGG	682	
G4	CGTTTCTGGTGAGGAGTTG	452	
G8	TTRTCGCACCATTTGTGAAAT	756	
G9	CTTGATGTGACTAYAAATAC	179	
G10	ATGTCAGACTACARATACTGG	266	
G12	GGTTATGTAATCCGATGGACG	396	
P-typing(a) 1st round			
VP4-F VP4-R	TATGCTCCAGTNAATTGG ATTGCATTTCTTTCCATAATG	663	94°C (3 min) 50°C (2 min) 72°C (8 min) 15°C (Hold)
P4	CTATTGTTAGAGGTTAGAGTC	483	94°C (5 min) 45°C (2 min) 72°C (8 min) 15°C (Hold)
P6	TGTTGATTAGTTGGATTCAA	267	
P8	TCTACTGGRTTRACNTGC	345	
P9	TGAGACATGCAATTGGAC	391	
910	ATCATAGTTAGTAGTCGG	582	

Table 2: Prevalence of rotavirus in the studied samples by sex

Gender	All studied samples	No rotavirus observation (number/ percentage)	Rotavirus observation (number / percentage)
Male	60 (60%)	46 (76.6%)	14 (23.3%)
Female	40 (40%)	33 (82.5%)	7 (17.5%)

Fig 1: Rotavirus genotypes on 1% agarose gel

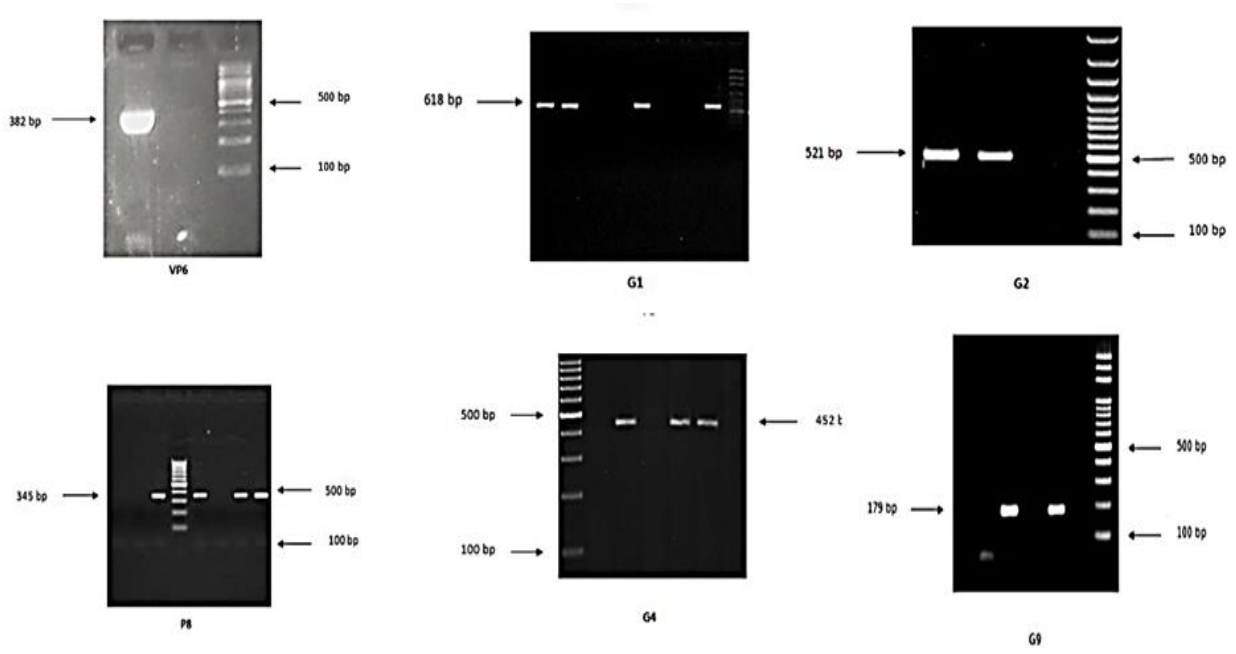


Table 3: Frequency of rotavirus genotypes in the study population

Genotype	G1P8	G9P8	G2P4	G4P8	Total
Number	8	5	3	5	100
percentage	38.1%	23.8%	14.3%	23.8%	100%

Table 4: Frequency of rotavirus genotypes based on the age groups

Genotype (number/ percentage)/ age (year)	G1P8	G9P8	G2P4	G4P8	Total
0-1	1 (12.5%)	1 (20%)	1 (3.3%)	2 (40%)	5 (23.8%)
1-2	5 (62.5%)	3 (60%)	1 (33%)	2 (40%)	11 (54.5%)
2-3	1 (12.5%)	1 (20%)	0	1 (20%)	3 (14.3%)
3-4	1 (12.5%)	0	1 (33%)	0	2 (9.5%)
4-5	0	0	0	0	0

Frequency of Rotavirus Genotypes in the Age Groups of the Study Population

The frequency of rotavirus samples was in the age group of 1-2 years and no rotavirus positive samples were found in the 4-5 age group. The highest rate of G1P8 genotype was in the age group of 1-2 years (62.5%). The highest rate of G9P8 genotype was also in the age group of 1-2 years (60%). The results of frequency analysis of rotavirus genotypes in age groups are shown in Table 4.

Discussion

Rotavirus is one of the most important causes of acute diarrhea in children and infants, by causing gastroenteritis in the worldwide (8). The prevalence of rotavirus types in different countries highlights the importance of regional and temporary changes in the G type and the P type. The prevalence of unusual genotypes in a particular region is likely to be due to gene rearrangements between animal and human rotaviruses.

Determining the rotavirus genotypes at any given time in an area greatly contributes to the vaccination strategies. In the current study, out of 100 gastroenteritis samples in children under 5 years, rotavirus was detected in 21% of the samples. In similar studies, the prevalence rate was 11.4% in Shiraz (9), 15.3% in Tehran (10), 13.5% in Karaj (11), 31.5% in Zahedan (12), 54% in Zanjan (13), 24% in Borazjan (14) and (28%) in Yasuj (14). The number of studies in Iran is limited according to the importance of rotavirus as a cause of diarrhea in children. Also, the mean prevalence of

rotavirus in Iran was 39.9%, which showed that 50% of Iranian cities were afflicted (Monavari et al. 2017). Also, the percentage of the virus in studies in Iraq and other countries were reported as 37% (15), in Nigeria 27%, (16) in Turkey 33.1% (17), in the United States (44%) (18), in India 23.5% (19), and in Spain 32% (20) which indicate and confirm the importance of rotavirus for gastroenteritis in the worldwide. In terms of genotype analysis, among the positive samples of rotavirus in this study, G1P8 had the highest frequency (38.1%) and then G9P8 and G4P8 (23.8%) and finally G2P4 (14.3%) had the lowest frequency.

In the study of Ahmed et al., as in the results of the current study, G1P8 had the highest frequency (33%) and the lowest frequency in this study belonged to the G1P8 and G9P8 genotype (11%) (15). In addition, in the study of Hakan Aydin as well as the study of Isidore J.O. Bonkoungou, the highest genotypic frequency belonged to G1P8 and the lowest frequency belonged to G2P4 (17,21) which were similar to our findings.

In addition, the results of the present study showed that 60% of rotavirus positive samples belonged to males and 40% to females. In the study of Kargar et al., 46% of positive cases belonged to males and 36% to females (14). Also, in the study of Motamedifar et al., 59.5% of the positive samples belonged to males and 40.5% belonged to females (22). Rotavirus infection may have a sex-linked pattern which involves more males than females. The most prevalent genotype of pediatric rotavirus samples in Kermanshah and other previous studies in Iran are consistent with common genotypes in the world.

Conclusion

According to the results, Rotavirus should be considered as a causative agent of diarrhea of children, and inappropriate use of antibiotics should be avoided. It is also recommended to determine the dominant genotype to provide a suitable situation for immunization of children against rotavirus in Iran.

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Conflict of Interest

No conflict of interest is declared.

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