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Prevalence and molecular characterization of rotaviruses as causes of nosocomial diarrhea in children

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Rotaviruses have been confirmed as causative agents of nosocomial gastroenteritis in children, but limited data exist concerning the epidemiology of nosocomial rotavirus gastroenteroentritis in Iran. The aim of this study was to determine the prevalence and molecular characteristics of rotavirus in children less than five years old with nosocomial diarrhea in Shahrekord (southwest of Iran).This cross-sectional study was conducted between December 2010 and October 2011. The study population consisted of children aged 6 to 60 months who were hospitalized in the pediatric ward of Hajar Hospital in Shahrekord, Iran, due to diseases other than diarrhea. Nosocomial diarrhea was defined as that occurring more than 48 hours after admission to the hospital for non-diarrheal causes. Rotavirus and G genotypes were determined by seminested reverse transcriptase polymerase chain reaction in 100 stool samples. In these 100 samples, the prevalence of rotavirus infection was 30%; the most common genotyes were G1 (20%) and G9 (20%). According to the findings of the study, genotyping of rotavirus is necessary to monitor changes in strain prevalence. Identifying strains over time could affect future vaccine strategies and detect any regional differences of genotype prevalence.

Key words: genotype, nosocomial diarrhea, rotavirus.

Diarrhea is one of the most common diseases in infants and young children in developed and developing countries. The incidence of diarrhea in African, Latin American and Asian countries has been estimated above one billion, with approximately 3.3 million deaths per year in children under 5 years old[1-3].

In contrast to adults, in whom the most common etiologic agent is *C. difficile*[4], nosocomial diarrhea in children is usually due to viruses circulating in the community, such as rotavirus, enteric adenovirus, astrovirus, Norwalk virus, calicivirus[5] and torovirus[6].

Rotavirus is considered the most frequent etiological agent of nosocomial diarrhea[7-9], but prevalence varies from year-to-year and depends on geographic location. Alrifai et al.[10]carried out a cross sectional study at Tikrit teaching hospital in Iraq to identify the prevalence and etiology of nosocomial diarrhea among children under 5 years of age. In this study, rotavirus was responsible for 18.5% of nosocomial diarrhea. Sherchan et al.[11] found that the prevalence of nosocomial infection due to rotavirus in Nepal was 30.2%. Ogilvie et al.[12], in a review of 76 studies from 16 countries, found rotavirus to be responsible for 47-69% of all nosocomial diarrhea. In an epidemiological study by Zeng et al.[13], rotavirus was responsible for 54.8% of nosocomial diarrhea in children. Frühwirth et al.[14] compared the characteristics of nosocomially acquired rotavirus disease in Austria, Germany and Switzerland. Rotavirus was detected in 57%, 69% and 49% of children with nosocomial gastroenteritis in Austria, Germany and Switzerland, respectively.

Nosocomial enteric infections are defined as those occurring more than 48-72 hours after admission to the hospital for non-diarrheal causes, or shortly after discharge. Nosocomial

diarrhea, which in developed countries accounts for up to 35% of all pediatric hospital-acquired infections[15], is an important cause of childhood morbidity and mortality. Rotavirus has been recognized as the most important cause of nosocomial gastroenteritis, particularly in infants during the winter months[15].

The virus is mainly transmitted by the fecal-oral route or by direct contact, but it can occasionally transmit through droplets. Since the virus is stable in the environment, transmission can occur through the ingestion of contaminated water and food, and through contact with contaminated surfaces and objects. Cross-infection through contamination of the hands is probably the most common transmission route in healthcare settings. Rotavirus acute gastroenteritis (RV AGE) has an incubation period of 1-3 days, which is followed by the sudden onset of watery diarrhea, with possible dehydration, vomiting and fever lasting from 4 to 7 days[15].

Rotavirus affects 95% of children worldwide by the age of 5 years and is the leading cause of severe dehydrating diarrhea[12]. The rotavirus genome consists of eleven segments of double-stranded RNA that code for the triple capsid and non-structural proteins. Based on the VP6 protein of the internal capsid, seven distinct groups are distinguished (A to G). Rotavirus groups A, B and C can infect humans and animals, with group A having been widely recognized as the cause of severe diarrhea in infants[16]. In neutralization assays, different serotypes of the rotavirus group A were determined by using antibodies of high neutralizing activity against the proteins VP7 and VP4. Based on the genetic and antigenic diversity of these external capsid proteins, the rotaviruses have been classified into types G and P, respectively[16].

The predominance of a particular rotavirus genotype combination during a rotavirus gastroenteritis (RVGE) season may vary between geographical areas and from one season to the next[17].

However, few studies have addressed the molecular epidemiology of nosocomial rotavirus in Iran. This article describes the prevalence and molecular characteristics of nosocomial diarrhea among pediatric inpatients at Hajar Hospital in Iran.

Material and Methods

Ethical considerations

The study protocol was approved by the Ethics Committee of Shahrekord University of Medical Sciences, Iran. Informed consent was obtained from parents of the study participants.

Sample collection

This cross-sectional study was conducted between December 2010 and October 2011. The study population comprised all children aged 6-60 months who had been hospitalized in the pediatric ward of Hajar Hospital because of diseases other than diarrhea. The same pediatrician visited the patients daily. Nosocomial diarrhea was defined as that occurring more than 48 hours after admission to hospital for non-diarrheal causes.

Fecal specimens were collected from a total of 100 children with signs of nosocomial diarrhea and transported daily to the Shahrekord University of Medical Sciences Cellular and Molecular Research Center in refrigerated boxes. Each specimen was stored at –70°C for further use.

RNA extraction and cDNA synthesis

Viral RNA was extracted from each stool specimen using the QIAamp Viral RNA Mini Kit (QIAGEN, Crawley, UK) according to the manufacturer's protocol. Extracted RNA was immediately used or stored at –70°C until needed. The total viral RNA was measured at 260 nm optical density, according to the method described by Sambrook et al[18].Viral RNA was reverse transcribed to cDNA with a First Strand cDNA Synthesis Kit (Fermentas, Germany) according to the manufacturer's instructions and was either stored at -20°C or used immediately for genotyping.

Rotavirus G-typing

In the present study, the seminested RT-PCR technique, which included two rounds of PCR, were used for the genotyping of rotavirus group A strains. In the first round, the cDNA of each sample was used as template DNA, and oligonucleotide primers

Beg9 (5'-GGCTTTAAAAGAGAGAATTTCCGT

CTGG-3') and End9 (5'-

GGTCACATCATACAATTCTAATCTAAG-3'), described by Gouvea et al.[19], were used

to generate the 1062-bp DNA fragment corresponding to the entire length of segment 9. Then, 2 μL of each product was used as template DNA for the second round of RT-PCR. The oligonucleotide primers reported by Gouvea et al.[19] were used for detection of the aBT1 (G1), aCT2 (G2), aET3 (G3), aDT4 (G4), aAT8 (G8), and aFT9 (G9) genotypes of rotavirus group A strains in the second round of PCR[20] (Table I). End9 was used as a reverse primer in all reactions; forward primers were different for the detection of each genotype. The sequences of each primer are given in Table I.

Two sets of amplification programs were carried out in 25 μL total reaction volumes, each containing 100 ng of template DNA, 0.2 pmol of each primer, 2.5 μL of 10X PCR buffer, 1.5 mM $MgCl_2$, 200 microMdNTPs and 1 unit of Taq DNA polymerase (Roche Applied Science, Germany). The amplification reaction for the first round of PCR consisted of 5 min of pre-denaturing at 94°C, followed by 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 58°C and a 1 min extension at 72°C, then by a final extension at 72°C for 5 min; amplified samples were held at 4°C. The specimens were amplified in a Gradient Palm Cycler (Corbett Research, Australia). The second round of PCR was performed with genotype-specific inner oligonucleotide primers for 25 cycles with the same reagent concentrations and temperature conditions.

Analysis of PCR products

The amplified products were detected on 1% agarose gel electrophoresis. The electrode buffer was TBE (TRIS Base – 10.8 g; boric acid – 5.5 g; 4 ml of 0.5M EDTA, pH=8.0). Aliquots of 10 μL of the PCR products were applied to the gel. Constant voltage of 80 V for 30 min was used for product separation. After electrophoresis, the gel was stained with ethidium bromide and images were obtained on UVIdoc gel documentation systems.

Statistical analysis

Analysis of the data and investigation of the rotavirus G-typing were performed using SPSS version 16.0 computer software (SPSS, Chicago, IL) and the chi-square test ($\chi 2$).

Results

The mean age of the patients was 11.8±15.3 months (range 6 to 60 months); 47% were female. The average weight of the patients was 9±2.7 kg. Most of the patients were on antibiotic therapy, with the most common antibiotic being ceftriaxone (93%). The average length of hospitalization was 4±5.2 days and the average time of diarrhea onset was 3±4.3 days. The patients were admitted with diagnoses of flu-like illness (15%), bronchiolitis (30%), pneumonia (15%), febrile convulsion (12%), urinary tract infection (18%) and meningitis (10%).

Viral DNA was isolated from patients' stool specimens. In this study, the size of the seminested RT-PCR products was used for genotyping of the rotavirus group. A strains revealed 885, 749, 625, 583, 374, and 306 bp(s)

Table I. Primers Used for Detection of Genotypes of Rotavirus Group A Strains Isolated from Stool Specimens of Patients

Primer	Sequences	Location
Beg9	5'-GGCTTTAAAAGAGAGAATTTCCGTCTGG-3'	1-28
End9*	5'-GGTCACATCATACAATTCTAATCTAAG-3'	1036-1062
RVG9	5'-GGTCACATCATACAATTCT-3'	1044-1062
aAT8	5'-GTCACACCATTTGTAAATTCG-3'	178-198
aBT1	5'-CAAGTACTCAAATCAATGATGG-3'	314-335
aCT2	5'-CAATGATATTAACACATTTTCTGTG-3'	411-435
aDT4	5'-CGTTTCTGGTGAGGAGTTG-3'	480-498
aET3	5'-CGTTTGAAGAAGTTGCAACAG-3'	689-709
aFT9	5'-CTAGATGTAACTACAACTAC-3'	757-776

*End9 is used as a reverse primer.

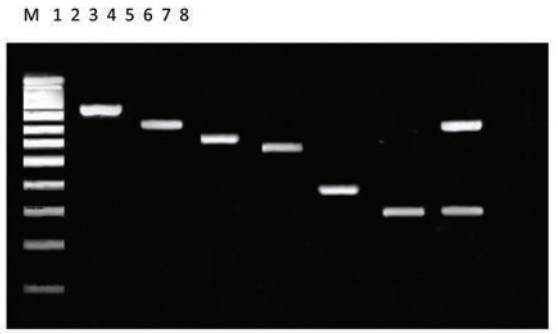

Fig. 1. Gel electrophoresis of seminested RT-PCR for detection of rotavirus genotypes isolated from children's stool specimens

for the G8, G1, G2, G4, G3 and G9 rotavirus genotypes, respectively (Fig. 1) (line M: 100 bp DNA ladder (Fermentas, Germany); lines 1-6: G1, G2, G3, G4, G8 and G9 rotavirus genotypes, respectively; line 7: G1+G9; and line 8: negative control).

In a total of 100 stool specimens from children with nosocomial diarrhea, 30 samples (30%) were positive for rotavirus, with the most frequent single genotypes being G1 (20%) and G9 (20%). Some specimens contained mixed genotypes: G1+G9 (13.3%), G1+G4 (6.7%), G1+G3 (3.3%), and G1+G8 (3.3%) (Table II).

Discussion

In the present study, rotavirus was identified in 30% of samples; there was no significant difference in age or sex in positive and negative samples. The prevalence of rotavirus diarrhea reported in different studies varies, but there has been limited study in Iran concerning the prevalence of nosocomial diarrhea and, especially, the molecular characteristics of rotaviruses. In a study by Sadeghian et al.[21], the incidence of rotavirus diarrhea in children less than 6 years of age referred to the Pediatric Emergency and Clinic of Ghaem Hospital in Mashhad, Iran,[1] was 28.8%. A study by Modarres et al.[22] in Tehran isolated rotaviruses in 19% of samples from children, with the predominant genotype being G1 (76.3%), followed by G4 (11.5%), G8 (0.8%), P [4] (9.2%) and P [8] (66.4%).

Kargar et al.[23] conducted a study in a children's hospital to determine the prevalent rotavirus genotypes, using the RT-PCR method. In this study, from a total of 163 collected samples, 75 were positive for rotavirus by ELISA. The frequency of G1, G2, G3, G4, G9 and mixed genotypes was 17.33%, 13.34%, 2.67%, 30.66%, 2.67% and 2.67% respectively.

Most studies from European countries have indicated that rotavirus is responsible for 31% to 87% of pediatric nosocomial diarrhea[24]. Kamalaratnam et al.[25] showed that one in five children was at risk of developing a nosocomial gastrointestinal infection, and rotavirus was the most common etiological agent. Lam et al.[26] found that in a pediatric ward in Hong Kong, nosocomial rotavirus gastroenteritis was 3.4 times more frequent than nosocomial gastroenteritis due to bacterial pathogens. In our study, 30% of the cases studied had nosocomial infection due to rotavirus; this prevalence was 27.7 % in a study conducted in Italy [27] and 5% in a study in Spain[7].

Additionally, in the present study, in a total of 30 positive stool specimens from children with rotavirus nosocomial diarrhea, the most frequent single genotypes were G1 (20%) and G9 (20%). A number of specimens contained mixed genotypes: G1+G9 (13.3%), G1+G4 (6.7%), G1+G3 (3.3%) and G1+G8 (3.3%).

The genetic mechanisms underlying the diversity of cocirculating rotavirus (RV) wild-type (wt) strains can be summarized as follows[15,28]: (1) accumulation of point mutations that can lead to antigenic changes and failure to serotype or genotype[29]; (2) ability of cocirculating human RVs to reassort[32], reassortment not

Table II. Frequency of Rotavirus Genotypes Isolated from 100 Stool Specimens of Children after Seminested RT-PCR Assay

Genotypes	G1+G9	G1+G8	G1+G4	G1+G3	G9	G8	G4	G3	G2	G1	Total
n	4	1	2	1	6	3	4	2	١	6	30
%	13.3%	3.3%	6.7%	3.3%	20%	10%	13.3%	6.7%	3.3%	20%	100%

Line M: 100 bp DNA ladder (Fermentas, Germany); lines 1-6: G1, G2, G3, G4, G8 and G9 rotavirus genotypes, respectively; line 7: G1+G9; line: 8 negative control

being restricted to the VP4, VP7 and VP6 genes[32]; (3) ability of cocirculating animal RV strains to reassort with human strains and thus to introduce animal RV genes into strains circulating in humans[30]; (4) ability of animal RVs to infect humans and to "emerge" by circulating in humans[30,31]; and (5) ability of RVs to rearrange their genomes.

In a hospital-based study conducted by Zeng et al.[13] in Shanghai, rotavirus was responsible for 54.8% of nosocomial diarrhea in children, with G3P [8] (56.8%) being the most prevalent genotype, followed by G1P [8] (15.8%), G2P [4] (3.0%) and G9P [8] (2.3%).

Gimeno et al.[32] found that in 35 of 59 children with nosocomial diarrhea, rotavirus was present in 59%, and the genotype most commonly found was G9P[8] (23 cases, 66%), followed by G1P[8] (4 cases, 11%).

In temperate zones of the planet, the virus has seasonal peaks (November to March in the Northern Hemisphere), whereas in tropical regions RV infections occur all year round[15].

In a study by Táborská[33], nosocomial infection with rotaviruses was detected in 4% of patients with diarrhea. Kang et al.[34] showed that the positive rate of rotavirus antigen among hospitalized children with gastroenteritis was 27%, with nosocomial infection in 22.3% of children. The varying prevalences that have been reported may be due to different methods of study or different laboratory methods, or to epidemiologic issues in different hospitals, such as isolation of patients with diarrhea or the way infection control strategies are followed.

Most studies in this field have focused on patients who were admitted to a hospital with the chief complaint of diarrhea and did not study nosocomial diarrhea. The present study was designed to determine the molecular characteristics of nosocomial rotavirus diarrhea. Therefore, all of our patients were those who developed diarrhea after 3 days of admission.

Limitations

This study was conducted in only one hospital, but in view of the fact that this is the main referral hospital in the province, the study subjects may be considered a good representative sample.

Conclusion

The study showed a high frequency of nosocomial infection due to rotavirus, emphasizing the importance of nosocomial infections in pediatric wards, which, in turn, result in considerable costs for hospitalization and treatment.

We suggest that infection control strategies should be followed more strictly in all pediatric wards. In view of the high risk of nosocomial infection, it is suggested that children with acute diarrhea be isolated, and that some simple health precautions that can be helpful in decreasing the prevalence of nosocomial infection, such as handwashing before and after examination of each patient and cleaning examination instruments after each use, be put in place.

At the same time, continued and nationwide genotype surveillance of rotavirus is needed to monitor changes in strain prevalence, to identify the emergence over time of new strains that could affect future vaccine strategies and to identify any regional differences in genotype prevalence in Iran.

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REFERENCES

1. Bern C, Martines J, de Zoysa I, Glass RI. The magnitude of the global problem of diarrhoeal disease: a ten-year update. Bull World Health Organ 1992; 70: 705-714.
2. Jain V, Parashar UD, Glass RI, Bhan MK. Epidemiology of rotavirus in India. Indian J Pediatr 2001; 68: 855-862.
3. Urbina D, Arzuza O, Young G, Parra E, Castro R, Puello M. Rotavirus type A and other enteric pathogens in stool samples from children with acute diarrhea on the Colombian northern coast. Int Microbiol 2003; 6: 27-32.
4. Johnson S, Gerding DN. Clostridium difficile-associated diarrhea. Clin Infect Dis 1998; 26: 1027-1034.
5. Kapikian A. Overview of viral gastroenteritis. Arch Virol Suppl 1996; 12: 7-19.
6. Jamieson FB, Wang EE, Bain C, Good J, Duckmanton L, Petric M. Human torovirus: a new nosocomial gastrointestinal pathogen. J Infect Dis 1998; 178: 1263-1269.

7. Román Riechmann E, Wilhelmi de Cal I, Cilleruelo Pascual ML, Calvo Rey C, García García ML, Sánchez-Fauquier A. [Nosocomial gastroenteritis and asymptomatic rotavirus and astrovirus infection in hospitalized children]. [Article in Spanish] An Pediatr (Barc) 2004; 60: 337-343.
8. Lopman BA, Reacher MH, Vipond IB, et al. Epidemiology and cost of nosocomial gastroenteritis, Avon, England, 2002-2003. Emerg Infect Dis 2004; 10: 1827-1834.
9. Korycka M. [Epidemiology of rotaviral infections among children in Poland]. [Article in Polish] Przegl Epidemiol 2004; 58: 649-653.
10. Alrifai SB, Alsaadi A, Mahmood YA, Ali AA, Al-Kaisi LA. Prevalence and etiology of nosocomial diarrhoea in children < 5 years in Tikrit teaching hospital. East Mediterr Health J 2009; 15: 1111-1118.
11. Sherchan JB, Ohara H, Sherpa K, et al. Rotavirus nosocomial infection in children under 5 years of age: a preliminary study in Nepal. J Nepal Paediatr Soc 2011; 31: 30-34.
12. Ogilvie I, Khoury H, Goetghebeur MM, El Khoury AC, Giaquinto C. Burden of community-acquired and nosocomial rotavirus gastroenteritis in the pediatric population of Western Europe: a scoping review. BMC Infect Dis 2012; 12: 62.
13. Zeng M, Zhang Y, Zhu Q, Wang X, Yu H. Clinical and molecular epidemiology of rotavirus in children with community-acquired and hospital-acquired diarrhea in Shanghai, China. Pediatr Infect Dis J 2010; 29: 177-180.
14. Frühwirth M, Heininger U, Ehlken B, et al. International variation in disease burden of rotavirus gastroenteritis in children with community- and nosocomially acquired infection. Pediatr Infect Dis J 2001; 20: 784-791.
15. Ford-Jones EL, Mindorff CM, Langley JM, et al. Epidemiologic study of 4684 hospital-acquired infections in pediatric patients. Pediatr Infect Dis J 1989; 8: 668-675.
16. da Silva Domingues AL, da Silva Vaz MG, Moreno M, Câmara FP. Molecular epidemiology of group A rotavirus causing acute diarrhea in infants and young children hospitalized in Rio de Janeiro, Brazil, 1995-1996. Braz J Infect Dis 2000; 4: 119-125.
17. Iturriza-Gómara M, Green J, Brown DW, Ramsay M, Desselberger U, Gray JJ. Molecular epidemiology of human group A rotavirus infections in the United Kingdom between 1995 and 1998. J Clin Microbiol 2000; 38: 4394-4401.
18. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual (2nd ed). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1989.
19. Gouvea V, Glass RI, Woods P, et al. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol 1990; 28: 276-282.
20. Gouvea V, de Castro L, Timenetsky MC, Greenberg H, Santos N. Rotavirus serotype G5 associated with diarrhea in Brazilian children. J Clin Microbiol 1994; 32: 1408-1409.
21. Sadeghian A, Hamedi A, Sadeghian M, Sadeghian H. Incidence of rotavirus diarrhea in children under 6 years referred to the Pediatric Emergency and Clinic of Ghaem Hospital, Mashhad, Iran. Acta Med Iran 2010; 48: 263-265.
22. Modaress S, Rahbarimanesh AA, Edalat R, et al. Human rotavirus genotypes detection among hospitalized children, a study in Tehran, Iran. Arch Iran Med 2011; 14: 39-45.
23. Kargar M, Akbarizadeh A, Yaghobi R. Molecular and serological characterization of group A rotavirus isolates obtained from hospitalized children in Jahrom. J Shahrekord Univ Med Sci 2010; 12: 15-21.
24. Gleizes O, Desselberger U, Tatochenko V, et al. Nosocomial rotavirus infection in European countries: a review of the epidemiology, severity and economic burden of hospital-acquired rotavirus disease. Pediatr Infect Dis J 2006; 25: S12-S21.
25. Kamalaratnam CN, Kang G, Kirubakaran C, et al. A prospective study of nosocomial enteric pathogen acquisition in hospitalized children in South India. J Trop Pediatr 2001; 47: 46-49.
26. Lam B, Tam J, Ng MH, Yeung CY. Nosocomial gastroenteritis in paediatric patients. J Hosp Infect 1989; 14: 351-355.
27. Gianino P, Mastretta E, Longo P, et al. Incidence of nosocomial rotavirus infections, symptomatic and asymptomatic, in breast-fed and non-breast-fed infants. J Hosp Infect 2002; 50: 13-17.
28. Gentsch JR, Woods PA, Ramachandran M, et al. Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. J Infect Dis 1996; 174: S30-S36.
29. Martella V, Terio V, Arista S, et al. Nucleotide variation in the VP7 gene affects PCR genotyping of G9 rotaviruses identified in Italy. J Med Virol 2004; 72: 143-148.
30. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Rev Med Virol 2005; 15: 29-56.
31. Das S, Varghese V, Chaudhury S, et al. Emergence of novel human group A rotavirus G12 strains in India. J Clin Microbiol 2003; 41: 2760-2762.
32. Gutiérrez-Gimeno MV, Martin-Moreno JM, Díez-Domingo J, et al. Nosocomial rotavirus gastroenteritis in Spain: a multicenter prospective study. Pediatr Infect Dis J 2010; 29: 23-27.
33. Táborská J, Pazdiora P. [Nosocomial rotavirus infections in children]. [Article in Czech] Cas Lek Cesk 1996; 135: 516-520.
34. Kang JO, Kim CR, Kilgore PE, Choi TY. G and P genotyping of human rotavirus isolated in a university hospital in Korea: implications for nosocomial infections. J Korean Med Sci 2006; 21: 983-988.