Characterization of human rotavirus subgroups and serotypes in children under five with acute gastroenteritis in a Saudi Hospital

Obeid E. Obeid
Department of Microbiology, College of Medicine, University of Dammam, Kingdom of Saudi Arabia

Address for correspondence: Dr. Obeid E. Obeid, Department of Microbiology, College of Medicine, University of Dammam, P.O. Box 2114, Dammam 31451, Kingdom of Saudi Arabia. E-mail: oobeid@yahoo.com

Objectives: Rotavirus is the most common cause of severe diarrhea in children. Currently, there is no published data on the prevalence of subgroups and serotypes of rotavirus in the Eastern province of Saudi Arabia. The objectives of the present study were to assess the rotavirus infection in children with acute gastroenteritis and to assess the subgroups and serotypes of rotavirus in the Children and Maternity Hospital in Dammam, Eastern Saudi Arabia. Materials and Methods: Children under 5 years of age with gastroenteritis attending the emergency rooms, or hospitalized in the pediatric wards of the Children and Maternity Hospital in Dammam were included in the study (N=156). Laboratory diagnosis of rotavirus shedding was established using the novel rotavirus STAT-PAK immunochromatographical test. Subgroup and G-serotype of the positive stool specimens were analyzed by the ELISA method. Results and Conclusions: Using the novel immunochromatographic assay, 37 samples were shown to be positive for rotavirus (23.7%). Subgroup I (serotype 2) was found to constitute 5.4% of the isolates and subgroup II (serotypes 1, 3 and 4) was found to constitute 56.7% of the isolates, whereas 37.8% were non-typeable. A survey of serotypes of rotavirus in the whole region as well as in the whole of Saudi Arabia will provide important information about the subgroups and groups of rotavirus in the community and may help in assessing the success of the rotavirus vaccine in the future.

Key words: Immunochromatography, rotavirus, subgroup, serotypes

INTRODUCTION

Rotavirus is the most common cause of severe diarrhea in children, resulting in the hospitalization of approximately 55,000 children each year in the United States. In the developing world rotavirus may account for 1 million childhood deaths as well as significant morbidity each year.

Rotavirus is a non-enveloped double-stranded RNA virus. The incubation period for rotavirus disease is approximately 2 days. The disease is characterized by vomiting and watery diarrhea for 3-8 days, with fever and abdominal pain occurring frequently. Rotaviruses are the leading cause of severe dehydrating diarrhea in infants and young children and some adult diarrheal disease. Infection results in life-long protection from the acute disease although subclinical infections may occur and protection wane in the elderly.

There are three rotavirus groups, A, B and C, which are both antigenically and genetically distinct. The inner capsid protein, VP6 is the common or group-specific antigen. Group A rotaviruses are the predominant cause of rotavirus disease throughout the world. More than 95% of all children have been infected by group A rotaviruses by the age of 4 and in developing countries infection occurs primarily between 4 months and 2 years of age. Group A rotaviruses can be subtyped by monoclonal antibodies based on VP6-specific antibodies. There are 10 rotavirus serotypes at this point, but only serotypes 1-4 are epidemiologically important for human disease throughout the world. All human subgroup I rotaviruses...
are serotype 2. Subgroup II rotaviruses are serotypes 1, 3 and 4.\cite{9}

Currently, there is no published data on the prevalence of subgroups and serotypes of rotavirus in the Eastern province of Saudi Arabia. The aim of the present study was to assess the rotavirus infection in children with acute gastroenteritis, and to identify the subgroups and serotypes of rotavirus in the Children and Maternity Hospital in Dammam, Eastern Saudi Arabia.

**MATERIALS AND METHODS**

**Study population and period of study**

All children under 5 years of age with gastroenteritis attending the emergency rooms and in the pediatric wards of the Children and Maternity Hospital in Dammam were included in the study (N=156). The period of the study was 1 year (2007-2008).

**Techniques used for data collection**

Stool specimens (N=156) were collected from the subjects by the conventional techniques. Samples were transported in buffered media. The initial laboratory diagnosis of rotavirus shedding was established using the novel Rotavirus STAT-PAK immunochromatographic test (Chembio diagnostic systems Inc., Medford, NY). Subgroup and G-serotype of the positive stool specimens were analyzed by the ELISA method, using monoclonal antibodies specific for G-serotypes 1, 2, 3 and 4, and subgroups I and II, which were produced in Sapporo, Japan.\cite{12-14} The ELISA used in the present study has been described previously.

**RESULTS**

A total of 156 stool specimens were collected from the children with diarrhea. The mean age of patients was 2.4 years of age, 69 of whom were males and 87 females. The average duration of illness was 2 days.

Using the novel immunochromatographical assay, 37 samples were shown to be positive for rotavirus (23.7%). There was no significantly different seasonal variation in prevalence of rotavirus in our study. The causative organisms for diarrhea in the rest of the samples were mainly Gram-negative bacteria (*Escherichia coli, Salmonella spp.*, *Shigella spp.*, *Campylobacter spp.*, etc).

Of the 37 rotavirus-positive specimens serotyped with an ELISA, five (13.5%) were found to be serotype 1 (subgroup II), 4 (10.8%) were serotype 3 (subgroup II), four were serotype 4 (10.8%) (subgroup II), two (5.4%) were serotype 2 (subgroup I) and eight (21.6%) were mixed (recognized by both serotype 1- and serotype 4-specific monoclonal antibodies). Hence, subgroup I was found to constitute 5.4% of the isolates and subgroup II constituted 56.7% of the isolates. The remaining 14 specimens (37.8%) were non-typeable with the monoclonal antibodies used in the present study.

**DISCUSSION**

Rotavirus is a major cause of diarrhea in children. A live-attenuated vaccine against rotavirus infection (Rotarix and RotaTeq) was recently approved by the FDA and CDC. Identification of the infection rate by rotavirus and the subgroups and serotypes of rotavirus will help to assess the efficacy of the vaccine.

Electron microscopy, ELISA, RNA electrophoresis, nucleic acid hybridization, PCR and direct cultivation have all been used to diagnose rotaviruses in clinical samples. However, diagnosis by rapid antigen detection of rotavirus in stool specimens is widely used.\cite{15}

In this study, a novel immunochromatographical method was used. The advantage of this technique is that it is sensitive, specific, fast and can be applied as a point-of-care test. However, commercial immunoassays for rotaviruses detect only Group A rotaviruses since they are the most common cause of severe watery diarrhea in infancy and early childhood.\cite{2}

The prevalence of rotavirus infection has been reported in some regions of Saudi Arabia\cite{16-25} [Table 1]. El-Sheikh et al\cite{16} assessed the prevalence of viral, bacterial and parasitic pathogens in children in Jeddah, Saudi Arabia. Rotavirus was detected in 34.6% of the specimens of the hospitalized patients and in 5.9% of the specimens of the outpatients. In the Milaat et al\cite{19} analysis of the causative agents of diarrheal diseases in two referral hospitals in Jeddah, Saudi Arabia, 41.3% of the cases were found to be due to rotavirus (RVGE) while 53.1% cases showed no causative pathogens. In the Al-Freihi et al\cite{21} prospective study of acute diarrheal diseases in the Eastern Province of Saudi Arabia, rotaviruses were responsible for only 11.5% of the cases of diarrheal diseases in children. None of the above studies used the immunochromatographical assay used this study.

The El-Assouli et al\cite{17} report of the subgroups, serotypes of rotavirus-positive stool specimens in Jeddah, Saudi Arabia indicated that 14.3% were of subgroup I, 82.1% were of subgroup II and 3.6% were a mixture of subgroup I and...
II. All subgroup I were of serotype 2 and all subgroup II were of serotype 1, 3 or 4. In the current study, 5.4% of rotavirus isolates were found to be subgroup I (serotype 2), 56.7% were subgroup II (serotypes 1, 3 and 4) and 21.6% were a mixture of subgroup I and II. In addition, 37.1% of the rotavirus isolates were non-typeable. The differences in the serotypes between the two studies could be explained by the differences in the monoclonal antibodies used. Alternatively, these differences could be due to different serotypes circulating in these localities.

The success rate of serotyping of rotavirus in stools with monoclonal antibodies in an ELISA system has been extremely variable, ranging from 100% in Sweden to only 29% in Bangladesh.[13] Likewise, 75-90% of rotavirus-positive specimens from inpatients at Royal Children’s Hospital in Victoria, Australia, were typeable, compared to only 40-50% of specimens from overseas sources (mainly Indonesia).[14] Inability to type 37.1% of the rotavirus-positive specimens in the present study, may be due to the absence of epitopes recognizable by the particular monoclonal antibodies used because of strain variations or the presence of inhibitory factors in stools.[13] On the other hand, coinfection with two serotypes may explain mixed infections.

A survey of serotypes of rotavirus in the whole region as well as in the whole of Saudi Arabia will provide important information about the subgroups and serotypes of rotavirus in the community and may help to determine the subgroups and subtypes that should be included in the rotavirus vaccine to be used in Saudi Arabia.

ACKNOWLEDGMENT

The author is thankful to Dr Hatim Alhani and Mr. Larry Bartholomew. This work was supported by a grant from The Deanship of Scientific Research, King Faisal University (grant number 6110).

REFERENCES

1. Widdowson MA, Bresee JS, Gentsch JR, Glass RI. Rotavirus disease and its prevention. Curr Opin Gastroenterol 2005;21:26-31.
2. Bines JE. Rotavirus vaccines and intussusception risk. Curr Opin Gastroenterol 2005;21:20-5.
3. Kane EM, Turcio RM, Arvey ML, Garcia S, Bresee JS, Glass RI. The epidemiology of rotavirus diarrhea in Latin America. Anticipating rotavirus vaccines. Rev Panam Salud Publica 2004;16:371-7.
4. Denneyh PH. Rotavirus vaccines: An update. Curr Opin Pediatr 2005;17:98-92.
5. Patton JT, Vasquez-Del Carpio R, Spence R. Replication and transcription of the rotavirus genome. Curr Pharm Des 2004;10:3769-77.
6. 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.
7. Ramig RF. Pathogenesis of intestinal and systemic rotavirus infection. J Virol 2004;78:10213-20.
8. Clark B, McKendrick M. A review of viral gastroenteritis. Curr Opin Infect Dis 2004;17:461-9.
9. American Academy of Pediatrics. Rotavirus Infections. In: Peter G, editor. 1997 Red Book: Report of the Committee on Infectious Diseases. 24th ed. Elk Grove Village, IL: American Academy of Pediatrics; 1997. p. 454.
10. Kapikian AZ, Chanock RM. Rotaviruses. In: Fields BN, Knipe DM, Howley PM, editors. Fields Virology. 3rd ed. Philadelphia: Lippincott-Raven; 1995. p. 1657-708.
11. Kapikian AZ. Viral Gastroenteritis. In: Evans A, Kaslow R, editors. Viral Infections in Humans: Epidemiology and control. 4th ed. New York: Plenum; 1997. p. 285-344.
12. Radwan SF, Gabr MK, Elmarghi S, Elsafi AF. Serotyping of Group A Rotaviruses in Egyptian Neonates and Infants Less than 1 Year Old with Acute Diarrhea. J Clin Microbiol 1997;35:2996-8.
13. Coulson BS, Unicomb LE, Pitson GA, Bishop RF. Simple and specific enzyme immunoassay using monoclonal antibodies for serotyping human rotavirus. J Clin Microbiol 1987;25:509-15.
14. Nakata S, Gathuru Z, Ukae S, Adachi N, Kobayashi N, Honma S, et al. Epidemiological study of the G serotype distribution of group A rotaviruses in Kenya from 1991 to 1994. J Med Virol 1999;58:296-303.
15. Gimenez-Sanchez F, Delgado-Rubio A, Martinon-Torres F, Bernaola-Ibarbe E. Multicenter prospective study analysing the role of rotavirus on acute gastroenteritis in Spain. Acta Paediatr 2010;99:738-42.
16. el-Sheikh SM, el-Assouli SM. Prevalence of viral, bacterial and parasitic enteropathogens among young children with acute diarrhoea in Jeddah, Saudi Arabia. J Health Popul Nutr 2001;19:25-30.
17. el-Assouli SM. Inter-relationships among subgroups, serotypes, and electropherotypes of rotaviruses isolated from humans. J Diarrheal Dis Res 1996;14:201-6.
18. el-Assouli SM, Banjar ZM, Mohammed KA, Milaat WA, el-Assouli MZ. Genetic and antigenic analysis of human rotavirus prevalent in Al-Taif, Saudi Arabia. J Trop Pediatr 1996;42:211-9.
19. Milaat WA, Elassouli SM. Epidemiology of diarrhoea in two major cities in Saudi Arabia. J Commun Dis 1995;27:84-91.
20. el Assouli SM, Mohammed KA, Banjar ZM. Human rotavirus genomic RNA electropherotypes in Jeddah, Saudi Arabia from 1988 to 1992. Ann Trop Paediatr 1995;15:45-53.
21. Akhter J, Sikotra S, Qadri SM, Myint SH. Comparison of paediatric viral gastroenteritis at large medical centres in Saudi Arabia and the United Kingdom. J Diarrhoeal Dis Res 1994;12:257-60.
22. Mohammed KA, el Assouli SM, Banjar ZM. Human rotavirus
subgroups and serotypes in children with acute gastroenteritis in Saudi Arabia from 1988 to 1992. J Med Virol 1994;44:237-42.
23. al-Freihi H, Twum-Danso K, Sohaibani M, Bella H, el-Mouzan M, Sama K. The microbiology of acute diarrhoeal disease in the eastern province of Saudi Arabia. East Afr Med J 1993;70:267-9.
24. Al-Frayh AR, Ramia S, Bakir TM, Zaidi MA. Rotavirus shedding by neonates and possible modes of transmission. J Trop Pediatr 1987;33:246-8.
25. Huq MI, Rahman AS, Al-Sadiq A, Al-Shahri A, Alim AR. Rotavirus as an important cause of diarrhoea in a hospital for children in Dammam, Saudi Arabia. Ann Trop Paediatr 1987;7:173-6.

Author Help: Online submission of the manuscripts

Articles can be submitted online from http://www.journalonweb.com. For online submission, the articles should be prepared in two files (first page file and article file). Images should be submitted separately.

1) **First Page File:**
   Prepare the title page, covering letter, acknowledgement etc. using a word processor program. All information related to your identity should be included here. Use text/rtf/doc/pdf files. Do not zip the files.

2) **Article File:**
   The main text of the article, beginning with the Abstract to References (including tables) should be in this file. Do not include any information (such as acknowledgement, your names in page headers etc.) in this file. Use text/rtf/doc/pdf files. Do not zip the files. Limit the file size to 1024 kb. Do not incorporate images in the file. If file size is large, graphs can be submitted separately as images, without their being incorporated in the article file. This will reduce the size of the file.

3) **Images:**
   Submit good quality color images. Each image should be less than 4096 kb (4 MB) in size. The size of the image can be reduced by decreasing the actual height and width of the images (keep up to about 6 inches and up to about 1800 x 1200 pixels). JPEG is the most suitable file format. The image quality should be good enough to judge the scientific value of the image. For the purpose of printing, always retain a good quality, high resolution image. This high resolution image should be sent to the editorial office at the time of sending a revised article.

4) **Legends:**
   Legends for the figures/images should be included at the end of the article file.