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ORIGINAL ARTICLE

Nosocomial rotavirus gastroenteritis in pediatric patients: A cross sectional study using molecular analysis

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Abstract:

Objective: *Rotavirus is one of the most important etiological agents of nosocomial infections in childhood. This cross sectional study was designed to determine the incidence and the main risk factors of rotavirus nosocomial infection in children admitted to the Bahrami Children Hospital, Tehran, Iran. Analyzing the genetic diversity and phylogenetic pattern of rotavirus was also performed. Identifying the most common genotypes of rotavirus contributes in establishing a suitable vaccination program.*

Study design: *A total of 105 stool samples were obtained on the first day of admission of children admitted to different wards of Bahrami Children Hospital, Tehran, Iran during December 2009 to December 2010. An additional sample was collected from rotavirus-negative children within 48 hour of their admission. Children who were initially rotavirus-negative and became positive 2 days or more after admission were considered as certain nosocomial cases. Rotavirus infection was detected in the feces samples using RNA PAGE method and RT PCR in order to specify the rotavirus genotypes. Both VP4 and VP7 primers were utilized in order to identify the rotavirus genotypes.*

Results: *During the study period, 105 children were enrolled. The incidence of rotavirus nosocomial infection was 20% with high rates in children aged 12-24 months. Nausea, vomiting and high grade fever were the prominent symptoms in the infected patients .Existence of an underlying disease including congenital heart disease and intractable seizures predisposed the children to infection. The most commonly found genotype in nosocomial infection was G1P [8] and G1P [4].*

Conclusion*: Nosocomial rotavirus infection cause significant morbidity in hospitalized children especially young infants. According to the most common genotypes found in patients with nosocomial infection in this study, appropriate vaccination programs should be considered in developing countries*

Keywords: *diarrhea, stool sample, infancy, nosocomial infection*

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Introduction

Today, rotaviruses are recognized as the single most significant cause of severe gastroenteritis, malnutrition and diarrhea in young children in both developed and developing countries worldwide. [1] Each year about 440.000 children <5 years of age die mainly in developing countries, because of an infection with rotavirus. [2] Rotavirus is a non-enveloped virus of the family Reoviridae with an icosahedral capsid 70nm across. Its genome is made up of 11 segments of double stranded RNA held in the inner core of the three-layered virus. [3] The genome codes for 6 virus proteins (VP1, 2, 3, 4, 6, and 7) and 6 non-structural proteins (NSP1-6).

There are at least 7 groups of rotavirus (A-G) and 4 subgroups within group A. To distinguish types within group A, a dual classification system has been established

with the glycoprotein VP7 defining G types, and the protease-sensitive protein VP4 defining P types. [4].

Infection with rotavirus typically occurs in infants between ages 6 months and 2 years, although severe infection in infants younger than 6 months and infection in neonatal intensive-care units are frequently observed. [5] In all age groups, the classic presentation of rotaviral infection is fever and vomiting for 2–3 days, followed by non-bloody diarrhea. [6] The contribution of rotavirus as a cause of endemic gastrointestinal disease varies according to geographic distribution and characteristics of patients. In addition, rotavirus infection in children is seasonal, with peak incidence in winter months and in temperate climates. [7] Transmitted by the fecal-oral route, rotavirus infects cells that line the small intestine producing an enterotoxin (NSP4) that induces gastroenteritis. [8] As the disease provides short-term immunity, recontamination is not uncommon. And it is a striking fact that rotaviruses can produce a chronic symptomatic infection in immunodeficient children. [9] This virus is also considered to be the most frequent etiological agent of nosocomial infections due to diarrhea. [10].

The concept that pediatric nosocomial infections differ from those in adults has been well established. Multiple factors contribute to the differences in nosocomial infection of children and nosocomial infection of adults including host factors, source of infection, routs of transmission and distribution of pathogens. Since nosocomial infections due to rotavirus increases the duration of hospitalization and/or rehospitalization, it seems that evaluation of the risk factors of rotavirus nosocomial infections is necessary. Considering the importance of this infection and lack of exact information about rotavirus in our community, the study was conducted to evaluate the prevalence of nosocomial infections due to rotavirus to adopt efficient approaches to decrease the rate of contamination.

Material and methods

This study was designed as a cross sectional study with the aim of evaluation of the incidence and risk factors of nosocomial rotavirus infections. According to our study all of the children who were admitted to the different wards of the Bahrami children hospital, a public hospital in Tehran, Iran during December 2009-December 2010 were evaluated. The study populations were admitted in different wards of the hospital including infectious, hematology& oncology, nephrology, endocrinology, neurology, gastroenterology, immunology, and general wards. With the aim of properly controlling the present study, cases were classified as follows: A) Nosocomial: children who developed diarrhea at least two days after admission .B) Community acquired: patients who were either hospitalized with gastroenteritis or developed the symptoms within 48 hr of admission. C) Non-diarrhea: situations in which no diarrhea was recorded at least 2 days before and 2 days after collection of sample. Stool samples recruited from every patient that was admitted to the participant wards of the hospital at the first day of admission. Those patients who had negative rotavirus antigen in stool examination on the first day of admission were candidate to have another sample of stool 48 hr after admission regardless of their symptoms. And those who had community acquired gastroenteritis that was rotavirus positive did not have further specimen.

Informed consent to participation in the study was given to the children's parents. Questionnaires were designed in order to record the age, sex, the season of admission, the ward of admission, the type of feeding (breast/bottle feeding), and any underlying diseases of the patients. Additionally, compliance of the hand hygiene in mothers and the number of staff present in each ward were submitted in the participant questionnaires.

Each of the enrolled children was visited by a doctor at least once a day for the whole duration of their stay in hospital. The stool samples of the children were kept in closed containers in a refrigerator at 4 °C and were shipped in dry ice to the Virology Ward of Pasteur Institute for strain characterization. In the labratorary, the samples were preserved in -20 °C inside special refrigerators. Samples were then homogenized in PBS in a 10% (wt/vol) solution/suspension and centrifuged (2000 g for 15 min) to remove debris before being analyzed.

Poly acrylamid Gel Electrophoresis (PAGE) was performed through polyacrylamide slab gels using loading buffer. After the formation of the bands of genes, silver staining method was used in order to clarify the RNA electrophoretypes. After identifying the electrophoretypes of rotavirus and the division of rotaviruses into long, short and mixed subgroups, according to the distance between the 10^{th} and 11^{th} segment of the RNA fragment, multiplex Reverse Transcriptase Polymerase Chain Reaction (RT PCR) was performed. Nucleic acid extraction and G/P rotavirus typing is designed in mentioned method: Double-stranded viral RNA was extracted from 140 ul of the 10% fecal suspension using a commercial kit (QIAamp viral RNA minikit; QIAGEN) and manufacturer's instructions relevant to virus genotypes had been followed. Specific primers related to VP4 (P9, P8 ,P6 ,P4) and VP7 (G1, 2, 3, 4, 8, 9) were used for determination of the genotypes. In

order to identify the G type, we used the VP7-F and VP7-R consensus primers in RT-PCR. [11,12,13] Subsequently, the VP7-R primer was used in a nested multiplex PCR together with G1, G2, G3, G4, G8, G9 primers. For P types, we used Con2-Con3 consensus primers in RT-PCR [14], followed by the standard multiplex PCR including the Con3, in combination with the typing primers P4, P6, P8 and P9. [15, 16].

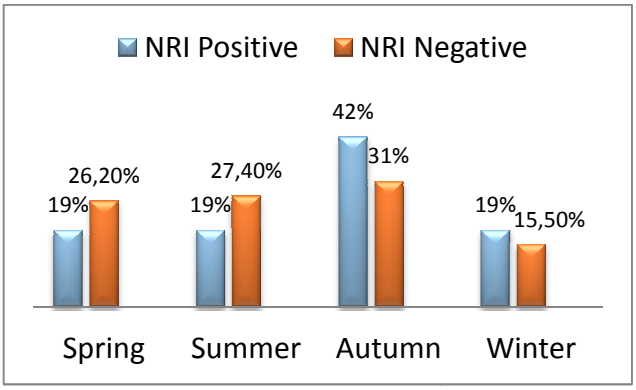
Results

During December 2009 to December 2010, 105 patients were enrolled in the study from which 21 patients became rotavirus positive within 48 hr of admission and were considered as nosocomial infection group. Ten subjects with positive rotavirus in stools at the time of admission were excluded from the study, and 25 patients developed diarrhea within 48 hours of admission (community acquired group).Consequently twenty one patients including 66.7% boys and 33.3% girls were included. Though the rate of affected girls was half of boys, the difference between them,using Chi Square method, was not statistically significant (P=0.42).

The mean age of the patients with positive stools was 28 months. With respects to age, 19% were under 6 months, 9.5% were aged from 6-11 months, 33.3% from 12 to 24 months, 23% from 3 to 5 years old and the rest were older than 5 years old. No statistically significant difference was found between the ages of children with and without nosocomial rotavirus infections.(NRI).

Though most of the affected samples were collected in autumn (43% vs. 31%), there was no statistically difference between the season of infection in children with or without nosocomial rotavirus infections. (P=0.64) (Figure 1.)

Figure 1. Comparison of the seasonal distribution between the children with and without NRVI admitted to Bahrami children Hospital, Tehran, Iran.



The clinical symptoms like nausea, vomiting and high grade fever (38-39c) was statistically higher in infected patients. (P=0.00).Approximately 80% of the infected patients had moderate and severe dehydration. (P=0.53) (Table 1.).

Table 1.The differences between clinical symptoms of children with and without NRVI admitted to Bahrami children Hospital, Tehran, Iran.

Symptoms		NRI Positive	NRI Negative
Vomiting (%)		76%	12%
Dehydration (%)	Moderate	57%	50%
	Severe	21%	50%
Fever (>38°C) (%)		76%	19%

According to this study, approximately 70% of the patients with nosocomial rotavirus infection had an underlying disorder including congenital heart disease, central nervous system problems (e.g., intractable seizures) and respiratory disorders and other conditions. Congenital heart disorder and central nervous system problems were statically higher in affected patients. (P=0.15 &P=0.04 respectively)(Table 2).

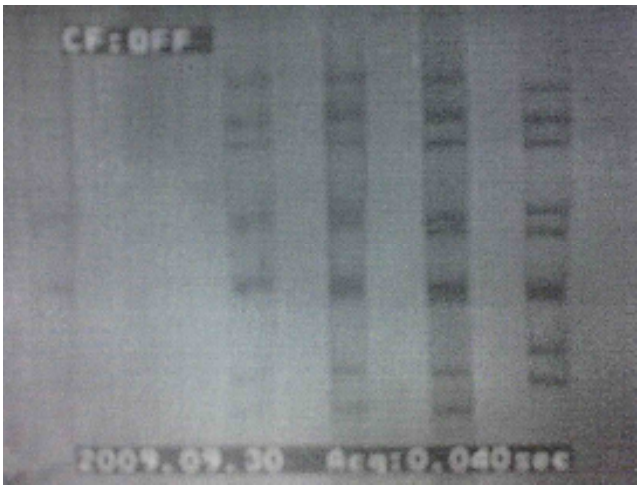
Table 2. Distribution of an underlying disease in children with and without NRVI admitted to Bahrami children Hospital, Tehran, Iran.

Underlying Disorder	NRI Positive	NRI Negative
Congenital heart disorder (%)	23%	4.8%
Central nervous system (%)	28%	7.1%
Respiratory disorder	9.5%	90%
Other problems	9.5%	70%

There wasn't any statistically difference in the number of staff and the length of hospitalization in children with and without NVRI according to our study. The most common electrophoretypes found in children with NVRI was Long (81%) and the rest were Short (20%).According to our

study, the most commonly genotype combination was G1 P [8] genotype (40%, n = 8) and G1 P [4] (19%, n = 4).The phylogenetic tree showed the similarity of the genotypes of rotavirus in our country with some countries like Russia, India and China. (Figure 2).

Figure 2. Demonestration of the electrophoretypes of rotavirus in children with NRVI admitted to Bahrami children Hospital, Tehran, Iran.



Discussion

This analysis revealed that nosocomial rotavirus infection accounts for 20% of children who were admitted to the different wards of the Bahrami children hospital, a public hospital in Tehran, Iran. The overall prevalence of the nosocomial rotavirus infection around the world is estimated approximatelly20% to 50%. [17]

In developing countries few studies have been performed on the prevalence of rotavirus-associated nosocomial infection. In one study, designed in the pediatrics wards of the main referral hospital of the Isfahan province, Iran the prevalence of the nosocomial rotavirus infection was 26.25%, of which 15% were symptomatic. [18] Currently available literature on rotavirus nosocomial burden in Central and Eastern Europe revealed that, among the pediatric population, rotaviral nosocomial infection accounts for between 22% and 55% of cases. [19]The frequency of rotavirus nosocomial infection in Brazil, Italy, France and Spain were estimated 40%, 27.7%, 11.1% and 3%.

According to our study no significant difference was found between the prevalence of rotaviral nosocomial infection in boys and girls. The same results were highlighted in most studies. In our study 63% of the infected patients were under 2 years .In the United States, rotaviruses cause about 5% to 10% of all diarrheal episodes in infants and children under 5 years old. [20] In a study performed over a 2-year period in Bangladesh, rotaviruses were the most frequently detected pathogen in children under 2 years of age, as 46% of the studied group was rotavirus positive. [21].

Rotaviral infections in most countries peaked in the winter months [19], while this study revealed the highest prevalence of the NRVI in autumn (42%). In the United Kingdom and in the USA, increasing numbers of infections begin in December or January, with peaks in March or April and incidence falling to almost zero by July [22].

High grade fever and vomiting were more prevalent in patients with NRVI based on our study while the same result was obtained in a pilot study performed by Gusmao et al in Brazil in 1995. [23]

According to this study, approximately 70% of the patients with nosocomial rotavirus infection had an underlying disorder including congenital heart disease, central nervous system problems (e.g., intractable seizures) and respiratory disorders and other conditions. There are few studies in literature investigating the effect of existence of an underlying disease on rotavirus nosocomial infection. [11].

According to our study, the most commonly genotype combination was G1 P [8] (40%, n = 8), G1 P [4] (19%, n = 4) and 31% were non-typable and partially typed. Several studies (24 studies) included information about rotaviral genotype distribution and predominance in different European countries have been performed. The most commonly isolated genotype combinations in the Central and eastern European region were G1P [8], G4P [8] and G2P [4], according to the studies from 2005/06 and 2007/08. The proportion of nontypable and partially typed genotypes reported from 2005/06 and 2007/08 ranged from 0.6% to 13.7% of the total rotavirus positive samples. [19]Data from other Asian countries showed differences in predominant strains.The predominant serotype in Hong Kong & Vietnam is G1, in China is G3 and in Korea, Taiwan & Thailand is G9 [21].

According to our study, while two or three genotype combinations currently predominate in our region, analysis of the evolution of different genotypes over time shows that the dominance of a certain genotype can change dramatically from year to year and from country to country. Vaccination programs may help to reduce the infection rates of this disease; a vaccine with broad serotype coverage would be needed to decrease the burden

of NRVI in Asian countries like Iran. Although vaccines studies are ongoing in Asia, these are still to be completed and reported. WHO in a recent position paper on rotavirus vaccines noted that "clinical efficacy of rotavirus vaccines has been demonstrated mainly in the United States, Europe and Latin America and that WHO strongly recommends the inclusion of rotavirus vaccination into the (NIPs) of regions where vaccine efficacy data suggest a significant public health impact and where appropriate infrastructure and financing mechanisms are available".

Lack of data on efficacy in Asian countries, particularly those with poor populations, has prevented WHO from making a universal recommendation. This experience suggests that up-to-date local disease burden data may be an important requirement of decision makers.

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