Medical Journal of the
Islamic Republic of Iran

Volume 19
Number 3
Fall 1384
November 2005

PREVALENCE OF ROTAVIRAL DIARRHEA IN AHVAZ ABUZAR HOSPITAL

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ABSTRACT

Introduction: In developing countries, rotaviral diarrhea is the cause of death of many children, and is the main etiology of gastroenteritis in children under 2 years old. Determination of the prevalence of rotaviral infection can reveal the impact of this infection in this group of the population and provides clues as to the strategies for prevention and treatment protocols for children suffering from diarrhea.

The aim of this study was to determine the prevalence of group A rotavirus in diarrheic children in Ahvaz, Iran.

Methods: For this purpose, 200 stool samples were collected from diarrheic children under 2 years old who were referred to Ahvaz Abuzar Hospital. The samples were examined by electron microscope and the electropherotype of extracted RNA of the virus were also studied.

Results: The results showed that 59 samples (29.5%) were rotavirus positive.

Conclusion: These results suggest that the prevalence of this virus in diarrheic children is moderately high in comparison with some reports from other developing countries.

MJIRI, Vol. 19, No. 3, 267-270, 2005.

Keywords: Rotavirus, EM, Electropherotype.

INTRODUCTION

The Reoviridae are non-enveloped icosahedral viruses and their genomes consist of 10-12 dsRNA segments. The genome of rotavirus, the most important genus of this family, is comprised of 11 dsRNA segments located in the core of the virion encoding 6 structural and 5 non-structural proteins.[1] These agents are the etiology of severe diarrhea in children worldwide especially in developing countries.[2,3] Annually more than 125 million cases of rotavirus diarrhea are reported in children under 5 years old. Eighteen million of these cases are moderately severe and these agents are responsible for one-fourth of all deaths from diarrhea.[4] Since the immune response to the infection reduces the occurrence and severity of subsequent infections, the risk of mortality due to this agent may be reduced through vaccination.[5] Rotaviruses are divided into seven groups (A-G) according to their dsRNA mobility on SDS-PAGE gel. Most diarrheic cases are caused by group A rotaviruses. Group A rotaviruses are divided further based on their VP6 (subgroup), VP4 (P-serotypes) and VP7 (G-serotypes) proteins. In addition to VP6 criterion, subgroup assignment in group A rotaviruses is determined accord-

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ing to short (S = subgroup I) and long (L = subgroup II) electropherotypes of their dsRNA on SDS-PAGE gel. Long and short patterns are because of relative migration of segments 10 and 11 of viral RNA.[1]

Rotaviruses are transmitted mainly by the fecal-oral route,[6] although respiratory route transmission of these agents is also speculated.[7] Laboratory detection of rotaviruses is performed by electron microscopy (EM), electropherotyping of their RNA, RT-PCR, ELISA, latex agglutination and HI.[1]

After an incubation period of 48 hours, clinical manifestations which range from a mild diarrhea to severe and sometimes fatal gastroenteritis appear. Fever and vomiting is also frequent (413 fields).

The main treatment of this infection is replacement of water and electrolytes containing either glucose or sucrose.[8]

Efforts are underway to develop attenuated vaccines from animal (bovine) or human strains of rotavirus.[9]

In this paper, the prevalence of rotaviral diarrhea in children under 2 years old has been reported in Ahvaz, capital of Khuzestan province, southwest of Iran. Detection of rotavirus in this study was performed by direct examination of stool samples of the patients by EM and electropherotypes of viral RNA extracted from the stool specimens.

MATERIAL AND METHODS

Sample collection

A total of 200 stool samples from children under 2 years old with acute diarrhea were collected between November 2002 and March 2003. All the specimens were collected from Ahvaz Abuzar hospital (the pediatric referral hospital of Ahvaz). The specimens were kept at -70°C till the experiment. For each patient, a data sheet including name, age, sex, residential address, the date of onset of diarrhea, average number of defecations, symptoms and degree of dehydration was obtained.

RNA extraction

All solutions were purchased from Merck Company (Germany) unless otherwise stated. For extraction of RNA from stool, briefly 0.5 gram solid or 0.5 mL liquid sample was dissolved and homogenized in 100µL phosphate buffered saline (PBS) in an Eppendorf tube. One microliter of RNase T_1 (100units) was added to the suspension and incubated for 15 minutes at 56°C. One-hundred microliter Tripure isolation reagent (Roche Biochemicals, Germany) was added to the suspension and mixed well. The mixture was incubated at RT for 5 minutes. Ten microliters chloroform was added to the tube and incubated at RT for 5 minutes. The tube was centrifuged for 10 minutes at 12000rpm. After centrifugation, the clear upper layer containing the RNA of the virus was transferred to a fresh tube carefully. The RNA was isolated by RNA isolation kit (Roche Biochemicals, Germany) as per manufacturer instruction. The RNA was eluded in 10-15µL sterile deionized water and saved at -70°C or immediately resolved on a 10% SDS-PAGE gel. After completion of electrophoresis, the gel was stained using a silver nitrate-based staining[10] and visualized. χ^2 and SPSS software were applied for statistical analysis of the data and the level of statistical significance was calculated with 95% confidence.

Electron microscopy

Of positive samples, 10 specimens were selected randomly for further examination by electron microscopy and shipped on ice to Shiraz University for this purpose. Negative staining for electron microscopy was performed at pH 3-4 and visualized with ×278000 magnification.

RESULTS

Of the 200 stool samples collected from the pediatric hospital in Ahvaz, 59 cases (29.5%) were positive for rotavirus based on electropherotype of extracted dsRNA on SDS-PAGE gel Fig1. All positive samples showed the typical 4-2-3-2 pattern of group A rotavirus. Besides, the 10 selected samples for EM demonstrated the typical morphology of rotavirus Fig 2. Of the positive samples, 30 cases (50.84%) had short electropherotype pattern (subgroup I) and 28 (47.45%) had the long pattern (subgroup II). One strain had a mixed long and short pattern. Comparison of the patterns of electropherotype of isolated dsRNA with representative human rotavirus electropherotypes, demonstrated that the predominant strains obtained in this study were Wa strain (46%). Twenty patterns (34.48%) were similar to DS-1 strain, 7 (12%) were similar to P strain and 5 (8.6%) had a pattern of W161 strain of group A human rotavirus.

The highest incidence of diarrhea was in age group 12-17 months (40.24%) and the lowest incidence belonged to age group 1-6 months (19.4%) (Table I).

Out of 200 patients, 109 cases were male and 91 cases were female. Of 109 male cases, 34 (31.1%) were positive and of 91 female specimens, 25 (27.4%) were positive for rotavirus.

Forty-four positive samples belonged to December and 7 (17.9%) positive samples were collected in March.

DISCUSSION

This research was conducted from November 2002 to February 2003 to determine the prevalence of acute

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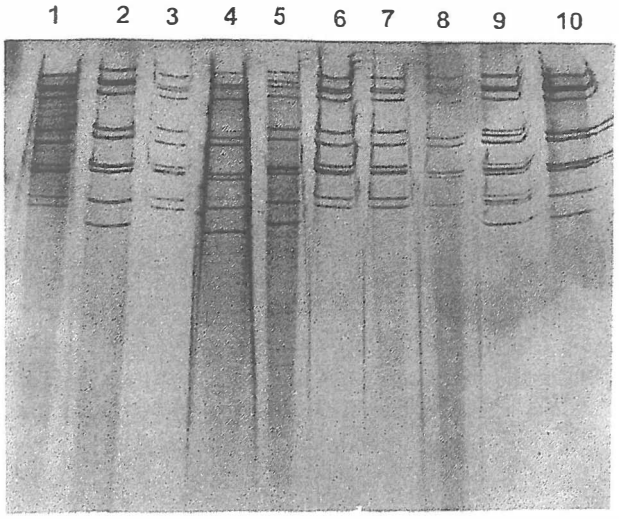


Fig. 1. Electropherotype profile of genomic group A rotavirus dsRNA isolated from the stool of patients. Number 9 shows a mixed pattern.

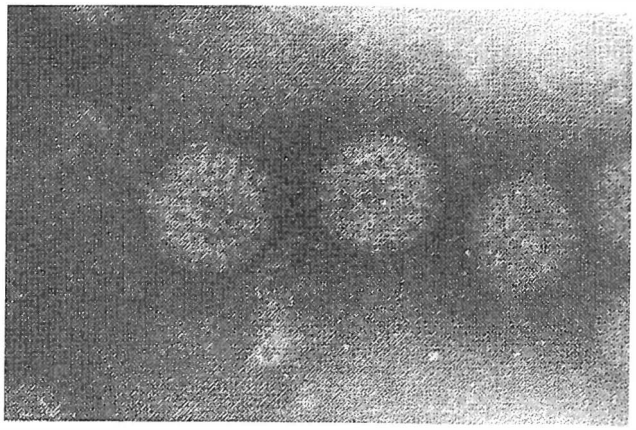


Fig. 2. EM image of human group A rotavirus particles isolated from a stool specimen (×27800).

Table I: Distribution of frequency and relative frequency of rotaviral diarrhea in different age groups of Ahvaz children.

Age group (Month)	Total cases	Frequency of positive cases	Relative frequency
1-6	63	12	19.04
7-12	82	33	40.24
13-18	21	7	33.3
19-24	34	8	23.52

rotaviral diarrhea among children under 2 years old in Ahvaz, southwest of Iran.

Of 200 stool samples collected from diarrheic children referred to Ahvaz Abuzar Hospital, 29.5% were positive for group A rotavirus. Similar studies on epidemiology of rotaviruses have been conducted by other researchers in our country mainly by ELISA and latex agglutination test. Prevalence of group A rotaviruses has been determined in Tehran and Tabriz to be 15.3% and 29% respectively by ELISA. In Zahedan, another provincial capital, this prevalence was 41% by latex agglutination test. Recently a group of researchers in Shahrekord, a neighbor provincial city in central Iran, studied the epidemiology of rotaviruses by RT-PCR and electropherotyping.[12] According to their results, rotavirus was responsible for 78% of diarrhea in children under 5 years old in Shahrekord.[12]

Routine hygienic measures such as hand-washing, especially in nursery homes satisfactorily prevents the spread of the virus.

The results of this research are similar to data collected in some other countries such as China. In a report from China, Tong and colleagues have shown that rotavirus was responsible for 40% of diarrhea in age group 7-12 months admitted as out-patients or in-patients in Beijing hospitals.[13]

In our study the most prevalent age group for rotavirus was 7-12 months with 40% infection and the least incidence of rotaviral diarrhea was in age group 1-6 months with 19% of positive cases. The prevalence of rotavirus infection in these two groups was statistically significant. The age group distribution was in concordance with the results found in Tehran and Tabriz[11] and some other countries such as Australia.[14] The rate of infection of rotavirus among sex groups statistically was not significant. This result has been confirmed in Tehran and Tabriz as well.[11]

The impact of breast-feeding in prevention of

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rotavirus diarrhea is contradictive. According to various researches conducted in different areas, breast-feeding does not decrease the rate of rotaviral infection in children under 2 years old. In our study 20% of diarrheic infants were breast-fed and 37.2% were bottle-fed. These two figures were not statistically significant. These results have been confirmed in other projects performed in Tehran and Tabriz.[11] The role of neutralizing antibodies of milk in preventing rotaviral diarrhea in cattle has been reported[1], but in human only the severity of diarrhea was lowered in breast-fed children in comparison with their bottle-fed counterparts. But in a report from Tehran, breast-feeding could significantly protect children against rotaviral diarrhea.[15]

Fever and vomiting were clinical manifestations in most patients.

In 59 cases of this study, 94% had fever and vomiting. These figures were 75% for vomiting and 69% fever in a study performed in Tehran.[11] In USA 96% of the patients were suffering from vomiting and 80% had fever.[1]

Historically rotavirus infection had been called winter vomiting disease.[16] The peak of infection in this study was November (44 %) and the lowest incidence of infection was in March (18%). The difference of these two figures was statistically significant. Therefore, the seasonality of distribution of rotaviral infection in our area is in full agreement with the usual distribution of this agent reported from other places such as Shahrekord.[12]

Knowledge about the epidemiology of rotaviruses is necessary to estimate the impact of these agents on public health. The data will in turn be useful for producing a vaccine against these agents, especially following molecular epidemiological surveillance of rotavirus.

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