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Epidemiological Survey of Group A Rotaviruses Infection among Children under 5 Years with Acute Diarrhea

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Abstract

Background: Rotaviruses are one of the most important causes of acute gastroenteritis and death in children worldwide. WHO suggested hospital based surveillance all over the world in order to evaluate the prevalence of rotavirus diarrhea. This study was conducted to determine the prevalence of rotavirus gastroenteritis in hospitalized children in Shiraz. Materials and Methods: In this study 138 stool samples from children aged <5 years old with severe diarrhea (≥3 loose watery stools per 24 hours), hospitalized at Shaheed Dastgheib and Nemazee hospitals in Shiraz, were collected during 2006-2007. All the stool specimens were evaluated for Group A of rotaviruses with enzyme immunoassays (EIA). Then demographic and clinical data were analyzed by SPSS software.

Results: Out of total collected samples rotavirus infection was detected in 48(34.78%). The highest infection rate was among children less than two years old (70.83%). Diarrhea (97.92%), vomiting (77.08%) and fever (52.08%) were the most frequent reported clinical symptoms in children with rotavirus infection. The highest of isolation of virus was observed in autumn (45.83%) and the lowest in spring (8.33%) (p=0.012). Also, there was no significant difference between the frequency of the rotavirus diarrhea and the pattern of nutrition (p=0.236).

Conclusion: Regarding to high frequency of rotavirus infection, concurrently surveillance of rotavirus gastroenteritis in other hospitals of Iran is recommended.

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Introduction

cute gastroenteritis is one of the most significant public health challenges facing the world today. This disease is a leading cause of morbidity and mortality rates among infants and young children in both developed and developing countries [1]. Enteric viruses are the most significant agents of acute diarrhea. Of these, rotaviruses have been recognized as the most common cause of viral acute gastroenteritis, responsible for dehydration and death in infants and children worldwide [2]. Each year rotaviruses have been reported as being associated with approximately 111 million episodes of gastroenteritis, 25 million clinical visits, 2 million hospitalizations and 352,000-592,000 deaths among children aged less than 5 years old around the world [3]. Studies conducted in Iran have shown that 64,000 children under 5 years old die annually. Of these, 10,880 cases are due to diarrhea and 2,720 of these episodes are caused by rotaviral infection [4].

Rotaviruses are classified into seven serogroups (A to G) on the basis of their genetic and antigenic properties. Group A rotaviruses have been identified as the leading cause of more than 90% of severe gastroenteritis in infants and young children worldwide [5]. The most important clinical symptoms of rotavirus infection have been characterized as watery diarrhea, dehydration, electrolytic disorders, fever and vomiting in children.

Humans appear to be the main reservoir of rotavirus infections. The exact modes of transmission are unknown but are presumed to involve droplet or direct contact spread via the fecal-oral route [6]. In temperate climates, rotavirus diarrhea has been predominantly reported during the winter with fewer cases in other seasons. But in tropical countries rotavirus infection has been observed throughout the year and has no seasonal peak or only a minor one [7].

Most studies conducted in different regions of the world have demonstrated that rotaviruses are responsible for 14 to 61% of the cases of acute gastroenteritis [8-11]. However, there is very little data on illness caused by rotavirus in Iran.

Some studies conducted in Iran indicate that 28 to 61% of acute diarrhea is due to rotavirus infection [12-15]. According to our last investigations in Tehran [16], Jahrom [17], Marvdasht [18] and Borazjan [19] cities, the average frequency of rotavirus diarrhea have been reported to be between 24.27 to 46.02%. Epidemiological studies of rotavirus gastroenteritis are some of the most valuable research subjects around the world, highlighting the important role of rotaviruses in the community of children aged less than 5 years old.

Accurate recognition of the disease burden of rotavirus gastroenteritis is very important in the development of

vaccination programs for high risk children and in the control of rotaviral diarrhea during the epidemic period. The objective of the current study was to assess the prevalence of group A rotavirus gastroenteritis in hospitalized children less than 5 years old in Shiraz, Iran.

Materials and Methods

From December 2006 to November 2007, a cross sectional descriptive study was conducted on 138 stool samples collected from hospitalized children less than 5 years old, suffering from acute gastroenteritis, in Shaheed Dastgheib and Nemazee hospitals in Shiraz. According to WHO's protocol, acute gastroenteritis was defined as less than 3 loose watery stools every 24 hours [20]. Age and sex demographics, clinical symptoms and temperature were recorded for each case, using a standard structured questionnaire.

All the fecal specimens were transported to the virology laboratory at Shaheed Dastgheib hospital and stored at -70°C until the time of assay. In this study, all samples were collected according to the principles of ethics approved by the Shiraz University of Medical Sciences. According to the WHO's recommendation, all the children presenting gastroenteritis were classified into age groups 0-2, 3-5, 6-8, 9-11, 12-17, 18-23, 24-35, 36-47 and 48-59 months so that the distribution of samples could be identified separately in children aged less than 5 years old [20]. All the stool samples were screened for group A rotavirus antigens by enzyme immunoassay (EIA) (Rotavirus Ag ELISA, DRG, Germany).

Briefly, suspension of stool specimens was prepared by using kit's buffer. The kit is designed such that the bottom of the microplate wells are coated with polyclonal antibody against VP6 protein of group A rotavirus. Then the diluted stool samples of the patients, monoclonal antibody anti-virus and Anti-Mouse antibody (conjugated with Horse Radish Peroxidase) were added to the wells, respectively. Enzyme substrate was added after one hour. If the color changed to blue, the test was positive.

Finally, the enzyme reaction was stopped by dispensing a stopple solution into the wells, turning the solution from blue to yellow. The optical density (OD) of the solution was read at 450 nm and specimens having OD values above the cut-off value (0.1 + OD) of the negative control) were considered positive for rotavirus antigens.

Data was statistically analyzed by SPSS-17 (SPSS Inc., Chicago, IL, USA) and χ^2 . p<0.05 were considered statistically significant.

Results

In this study, 42.75% (59 cases) of the patients with acute gastroenteritis were female and the others were male with 57.25% (79 cases). In the spring, the highest frequency of the evaluated samples was related to children 9-11 and 36-47 months (3.62% each). In the summer and autumn the highest frequency were observed in children 36-47 (5.07%) and 12-17 months (5.07%), respectively. Also, the highest frequency in the winter

was related to children 3-5 and 12-17 months (7.25% each).

The lowest frequency of the collected samples in the spring, summer, autumn and winter was related to children 3-5 (5%), 0-2 (0.73%), 0-2, 12-17 (0%), and 36-47 months (0%), respectively. A statistically significant difference was observed between age group and season (p=0.001). A total of 48 (34.78%) of collected specimens were confirmed as group A rotavirus positive using the EIA assay. There was a statistically significant difference between gender and virus isolation (p=0.026). Rotaviruses were detected in 15 cases (31.25%) of the studied females and in 33 cases (68.75%) of the assessed males. The age group analysis revealed that the highest infection rate was among children under 2 years old (70.83%). Children between 9-11 months of age were the most affected (20.83%) and the lowest rates of infection were observed in children 0-2 and 48-60 months (each 4.17%) (Fig. 1).

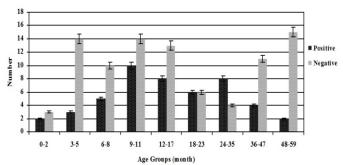


Figure 1. The frequency of rotavirus gastroenteritis in the studied age groups

However there was no significant relationship between age group and rotavirus isolation (p=0.052). The most important clinical features in children with rotavirus infection are shown in table 1.

A significant relationship was found between rotavirus infection and diarrhea (p=0.001) and convulsion (p=0.049) by using the chi square test. However, there was no statistically significant difference between virus isolation and fever (p=0.328) and vomiting (p=0.567). Although in the current study a significant relationship was observed between fever and convulsion in children with non-rotaviral infection (p=0.026), no such relationship was identified in patients with rotavirus gastroenteritis (p=0.133). In this study, a significant relationship was also found between rotavirus infection and seasonal distribution (p=0.012). The highest prevalence of infection was identified in autumn (45.83%), followed by the winter (33.33%), summer (12.50%) and spring (8.33%), respectively.

In the present study, we evaluated the relationship between the type of feeding and the frequency of rotavirus diarrhea. The highest rate of the disease burden of rotavirus infection was observed in children who used complete food. The lowest episodes of illness were related to children who were being breast-fed and bottlefed, simultaneously (Fig. 2). This finding was not statistically significant (p=0.236).

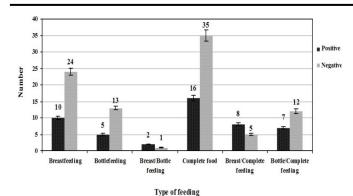


Figure 2. The frequency of rotavirus gastroenteritis based on type of feeding

Table 1. Distribution of the identified clinical symptoms in children with rotavirus gastroenteritis

Discussion

Symptoms	Rotavirus cases			
	Positive	Negative	Total	Walua
	N(%)	N(%)	N(%)	<i>p</i> -Value
Diarrhaea	47(97.92)	1(2.08)	48(100)	0.001
Vomiting	37(77.08)	11(22.92)	48(100)	0.567
Fever	25(52.08)	23(47.92)	48(100)	0.328
Convulsion	3(6.25)	45(93.75)	48(100)	0.049

In the present study, rotaviruses were detected in 48 of 138 (34.78%) of the collected samples from hospitalized children less than 5 years of age with acute gastroenteritis in Shiraz. Currently, rotaviruses are the most common leading cause of acute gastroenteritis among infants and children, worldwide. An estimated 527,000 children aged less than 5 years old die from rotavirus diarrhea each year, with 85% of all rotavirus-related deaths occurring in low-income countries of Africa and Asia [21]. Therefore, regarding the WHO's protocol, epidemiological surveillance of rotavirus gastroenteritis among children less than five years old around the world could be more important than its detection in order to gain control of this self-limited disease.

In our recent studies, we carried out hospital-based surveillance of severe gastroenteritis in the cities of Tehran [16], Jahrom [17], Marvdasht [18] and Borazjan [19]. ELIZA is one of the best and simplest methods recommended by WHO in the detection of group A rotaviruses. This is why, we also utilized this method in the current study and in our recent investigations. The prevalence of rotavirus infections were 35, 46.01, 28.37 and 24.27% in the afore-mentioned cities, respectively. Also, the disease burden of rotavirus gastroenteritis in Isfahan [12], Tabriz [14], Mashhad [15] and Kerman [22] cities has been reported as 30.81, 53.05, 28.85 and 24.60%, respectively. In addition, virus isolating rates in various geographical regions of the world is different as well. For example, the incidence of rotavirus infection in Norway [8], Brazil [9], Italy [10], Switzerland [23], France [24] and Cuba [11] countries has been 14.5%, 25.8%, 34.9%, 38.1%, 48.8% and 60.9%, respectively. The frequency of rotavirus detection in this study

(34.78%) is in keeping with results observed in Iran as well as other countries.

Studies conducted in different countries have shown that children less than 2 years old are at greatest risk for developing acute gastroenteritis from rotavirus. In the current study, the occurrence of rotavirus infection cumulatively observed in the first 24 months of life (70.83%), is more than in older age groups. Similar results were found in previous investigations in the countries of Switzerland (86%) [23], Nepal (64%) [25], Bulgaria (76.85%) [26] and the cities of Mashhad (69%) [15], Marvdasht (85.20%) [18] and Borazjan (76.92%) [19]. This demonstrates that not only the diagnosis of rotavirus in diarrhea samples is very important, but widespread immunization of children in this age group can play very significant role in prevention and control of this disease.

The clinical spectrum of rotavirus gastroenteritis ranges from mild to severe illness with abdominal cramp, fever, vomiting and watery diarrhea. That can result in dehydration, shock, electrolyte imbalance, and even death [27]. The survey of clinical manifestations in Spain revealed that vomiting (76%), fever (63%) and dehydration (35%) were the most common symptoms in patients with rotaoviral gastroenteritis [28]. Diarrhea (98.7%), vomiting (92.4%) and fever (71%) were the most prevalent clinical symptoms observed among children with rotavirus infection in Taiwan [29]. Similar studies carried out in the cities of Mazandaran [13], Tehran [16], Borazjan [19] and Kerman [22] implied that diarrhea, vomiting and fever were the most prominent spectrum presentation of rotavirus acute gastroenteritis. During this study, diarrhea (97.92%) was the symptom most commonly reported as being associated with rotavirus infection, followed by vomiting (77.08%), fever (52.08%) and convulsion (6.25%). Comparison of the identified symptoms in patients with rotavirus infection, in the current study and the other surveys, indicate that despite the difference in frequency, diarrhea and vomiting are the most common symptoms in children with rotavirus gastroenteritis. These clinical characteristics can play an important role to rapidly diagnosis of rotavirus infection.

In the present study, we evaluated the relationship between feeding status and the disease burden of rotavirus infection. The role of breastfeeding in the control of rotavirus infection has not yet been properly documented. However, it seems that passive immunity of the transferred antibodies from mother's milk may provide a protective factor against rotavirus diarrhea. Studies carried out in the cities of Isfahan [12] and Tehran [16] have shown that the disease burden of rotavirus infection in children who were breastfed and bottle-fed were 21.1, 44.8% and 15.38, 10.38%, respectively. A study conducted in Ahwaz revealed that the frequency of rotavirus diarrhea in children who were being breastfed was significantly lower among those being bottle-fed at the time of acute gastroenteritis infection [30]. Recent studies in Turkey have indicated that breast feeding more than 6 months can reduce the incidence of rotavirus

diarrhea, when compared with children who were only being bottle-fed [31]. No statistically significant differences were observed between the type of feeding and the disease burden of rotavirus diarrhea in Uganda [32]. Our results in this study imply that rotavirus-positive rates were infrequent among those being breastfed when compared with children who were being bottle-fed. This finding may be explained with more random collection of samples from hospitalized children who were being breastfed.

Epidemiological studies in temperate climates have indicated that there is a statistically significant difference between rotavirus isolation and seasonal distribution [7], but seasonal patterns in tropical climates have shown that rotavirus diarrhea occurs throughout the year [33, 34]. Investigations conducted in the countries of Japan [5] and the United States [35] and the cities of Isfahan [12], Mashhad [15], Jahrom [17] and Borazjan [19] have demonstrated that the prevalence of rotavirus infection is similar to those reported in regions with temperate climates. In these areas rotavirus diarrhea was observed predominantly during the cold season of year (autumn and winter). Our findings in the present study showed that the greatest incidence of rotavirus infection in Shiraz occurred during the cold seasons. That is in keeping with other temperate regions of the world. Regarding the seasonal variation of the prevalence of rotavirus diarrhea in different studies, it can be concluded that the frequency of rotavirus in various seasons is dependent on the geographical location of the research. Thus, according to Iran's position in the Northern Hemisphere, the highest burden of rotavirus diarrhea in the cold seasons can be demonstrated

One limitation of the current study is that the true frequency of rotavirus infection could be higher than estimated in the present study. This is because we assessed only hospitalized children with acute gastroenteritis. Meanwhile, children with home care or outpatient visits could also be infected by this virus.

Today, rotavirus diarrhea plays a very significant role in the affliction of infants and young children in the overall world community. This is why the World Health Organization (WHO), after the eradication of polio, has

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recommended a codified program in order to facilitate hospital-based surveillance of rotavirus gastroenteritis and its eradication in countries, all over the world. Current continuation of hospital-based surveillance of rotavirus infection in other regions of Iran, in order to have more an accurate recognition of the disease burden of rotavirus, can show the way for the development of a vaccination program for children at risk in the near future. Immunization against rotavirus can be effective in stopping severe types of this disease. Attempting to produce a suitable vaccine would be very helpful in saving many expenses, particularly in hospitalization costs, clinical treatment costs, etc.

The high prevalence of rotavirus infection in the studied population and the necessity for a comprehensive picture of the disease burden of rotavirus gastroenteritis provides strong support for a widespread hospital-based surveillance in other areas of Iran. On the other hand, increasing the level of public health and immunization of children at risk can also play an important role in the control and prevention of severe types of this disease while also saving in clinical treatment costs.

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Authors' Contributions

MK contributed in development of the research protocol, coordination, scientific consultation and revised the manuscript. TJ participated in sampling and carried out the practical methods. AN helped in interpretation of data, statistical analysis and draft of the manuscript. All authors studied and edited the draft and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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