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Original Article

Incidence of Coinfection between Rotavirus and Some Enteropathogenic Agents in Children Referred to Children Medical Center Hospital, Tehran, 2009

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Abstract

Background and Aims: One of the major causes of acute diarrhea in children during the cold season in Iran is infection by rotavirus. Children infected with rotavirus often require hospitalization. Several studies on human and animals have shown that enterotoxigenic Escherichia.coli and rotavirus are the most common coinfection causing diarrhea. There are other reports indicating occurrence of rotavirus coinfection with some other enteric agents such as Salmonella, Giardia, and Shigella flexeneri. In this study the rate of rotavirus infection and its coinfection with some other enteropathogenic agents in children which could influence the severity of the disease was investigated.

Methods: Approximately 100 stool samples were collected from children with acute gastroenteritis. The specimens were cultured for bacteria isolation. They were also clarified and tested for rotavirus using the techniques of latex agglutination and negative staining electron microscopy.

Results: Using negative staining, rotavirus particles were observed in 43 of the 100 stool specimen. The highest prevalence was observed in 6-12 months old children consisting 39.5% of the total specimens. Patients with mixed infection particularly rotavirus and E.coli had the highest incidence of severe vomiting and dehydration.

Conclusion: Coinfection of children with rotavirus and other enteric agents can occur frequently. This coinfection has a synergistic effect which increases the severity of the clinical manifestation.

Keywords: Rotavirus; Escherichia.coli; enteropathogenic agents; coinfection

Introduction

Rotavirus is a major cause of acute severe diarrhea in children worldwide.Prevalence of rotavirus infection in children requiring admission to hospitals has been reported from 17.7% to 69% in different countries (1-3). Numerous human and veterinary surveys have determined that enterotoxigenic Escherichia coli and rotaviruses are two of the most frequently implicated etiological agents in infectious diarrhea (4, 5).

Mixed infections are common, and it has been demonstrated that mixed infection with E. coli and rotavirus caused a greater mortality than either disease agent alone in mice (5). Also, in humans, mixed Escherichia coli and rotavirus infections have been noted (6, 7). The results of clinical observations demonstrated

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coinfection between rotavirus and Escherichia coli (11.1-45.5%), Salmonella (0.5-4.8%), Giardia (1.7-8.6%) and Shigella flexeneri (3.2-8.0%) (7, 8).

The clinical outcomes of coinfection with rotavirus and other pathogens are based on in vitro experiments and animal model studies (7, 9). In the clinical studies, children with a mixed (rotavirus and bacterial) infection had the highest incidence of severe vomiting and dehydration (10, 11). In a study carried out in Iran on 197 children under the age of 3 years with diarrhea and enterotoxigenic E.coli infection duration of diarrhea was longer than in patients with E.coli or rotovirus infection alone (12).

There is a lack of data concerning potential interactions between rotavirus and protozoan parasitic infections. In a study, it was shown that diarrheal episodes were more severe in children infected with rotavirus alone than in cases where there was a mixed infection with rotavirus and Giardia lamblia (13). However, the number of relevant cases was small.

The purpose of this study was to assess the rate of rotavirus infection and the coinfection of this virus with some enteropathogenic agents among children less than six years old with acute gastroenteritis referred to Children Medical Center Hospital, Tehran.

Methods

Stool specimens

Stool samples were collected from 100 infants and children up to 6 years old with acute gastroenteritis during the winter 2009. For latex agglutination test and electron microscopy, specimens were diluted 1:2 in phosphate buffer saline (PBS) and clarified by centrifugation at 2000 rpm for 10 min. Supernatants were collected and stored at -70^{0}c until used.

Latex Agglutination Test

Fifty µl of clarified stool suspension were mixed with 50µl of latex suspension which had been developed before by the authors (14).

The test was carried out on a slide and reaction was read in 3-5 min.

Electron Microscopy

Clarified stool specimens were negatively stained using 1% phosphotungstic acid (PTA) on 400 mesh formvar coated copper grids. The grids were examined in a Zeiss EM 10 electron microscope.

Stool Examination and Stool Culture

Laboratory analysis including light microscopic examination and stool culture on differential bacterial media were done to find out if bacteria or protozoa may be causing the infection.

Results

One hundred children with acute diarrhea were obtained from the referred patients. The specimens were divided into two parts. One part was cultured on differential bacterial culture media and also examined directly with light microscope and the other part was processed for electron microscopy as described .Negative staining examination of the specimen revealed presence of rotavirus particles in 43 from the total of one hundred. Virus particles were observed both in single shell and complete double shell particles (Fig. 1).

The distribution of positive cases by age is demonstrated in Table 1. The highest prevalence was observed in children 6-12 months of age consisting 39.5% of the all positive cases.

Table2 shows the clinical characteristics of patients with acute diarrhea which were infected with rotavirus alone or coinfected with some enteropathogenic agents.

Children with a mixed infection, specially rotavirus and Escherichia coli, had the highest incidence of severe vomiting and dehydration among this cohort of 100 children.

Discussion

Several methods have been used for the diagnosis of viral gastroenteritis (15). We used latex agglutination test which had been developed before and the sensitivity and specificity of the test were 90% and 98.1%, respectively (14) which was based on electron microscopy as gold standard for detection of

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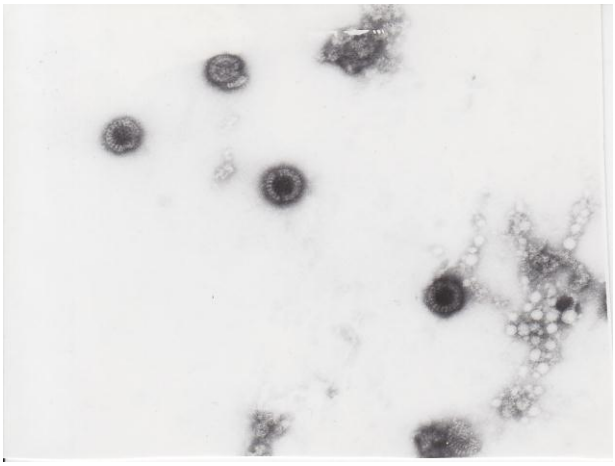

Fig. 1. EM of a positive stool sample by negative staining technique showing the presence of rotavirus particles.

rotavirus (16,17). Our findings showed that rotavirus is an important etiological agent of acute diarrhea throughout the winter in Tehran, accounting for nearly 43% of all cases with acute gastroenteritis.

There are only limited studies showing the incidence of rotavirus infection in Tehran. Zarnani *et al* (18) reported the incidence of rotavirus infection in children with acute diarrhea to be 15.3%. Most of infected children in our study were under 2 years old, with highest prevalence between 6 and 12 months. This age distribution is comparable to previous reports (1,18,19).Also because of the lack of data about incidence of coinfection between rotavirus and some enteropathogenic agents in Iran, we tried to study the incidence of coinfection with other enteric agents which could be problematical in industrialized countries (7). The results of our clinical observations showed coinfection between rotavirus and some bacteria and Giardia lamblia as coinfecting microorganisms. Those were: Escherichia coli (27.9%), Shigella flexeneri (2.3%), Salmonella (2.3%) and Giardia lamblia (6.9%). These results are comparable to previous studies by Souza and Hori who carried out similar investigation in Brazil and Ghana.

Also children with a mixed infection had highest incidence of sever vomiting and fever. We observed the highest incidence of severe dehydration among children with coinfection between rotavirus and Escherichia coli.

The evidence for potentiation of synergism between infections with rotavirus and bacterial enteropathogens -particularly E.coli- is strong. This study showed that coinfection between rotavirus and an enteropatogenic agent often produces more severe disease the infection by any of the agents alone.

On the other hand, rotavirus gastroenteritis is a vaccine – preventable disease (20), which could reduce the morbidity and mortality associated with coinfection in vaccinated individuals.

However, furture studies are needed to define the role of rotavirus vaccination in the context of coinfections.

Table 1. Distribution of rotavirus positive cases by age.

Age	Patients	Rota positive (EM)	Rota positive (LA)
0-6 months	32	8	8
6-12 months	29	17	16
1-2 years	17	11	11
2-5 years	22	7	6
Total	100	43	41

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Table 2. The incidence of vomiting, fever and dehydration in children (n= 100) with acute diarrhea according to the detection of rotavirus and/or enteropathogenic agents in feces.

pathogens / cases	Rota	E.coli	Shigella	Salmonella	Giardia lamblia	Rota +E.coli	Rota+ Shigella	Rota+ Salmonella	Rota + Giardia lamblia
number (positive cases)	43	20	4	3	6	12 (27.9%)	1(2.3%)	1(2.3%)	3(6.9%)
Vomiting (%)*	79	30	100	66	0	91.6	100	100	33.3
Acute** Dehydration (%)	65	58	0	0	0	72	0	0	0
Fever***	69.7	80	100	100	0	86	100	100	100

*Vomiting: % of patients with vomiting at least once a day

**Acute dehydration: % of patients reported by the physician to be dehydrated.

***Fever: % of patients with fever 38

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