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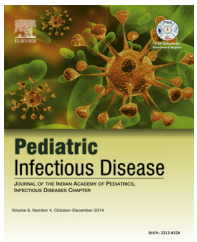

Original Article

Rotavirus infection markers in children with rotaviral gastroenteritis and their relation to disease severity

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ABSTRACT

Introduction: Although natural infection with rotavirus causes damage to the enteric tract resulting in diarrheal disease in humans and animals, recent studies evidenced the presence of serum rotavirus antigen/RNA in children with rotavirus diarrhea.

Methods: In this study, we investigated the markers of acute rotavirus infection (antigenemia, viremia and anti-rotavirus IgM antibody) in a group of 50 rotavirus infected children, using enzyme-linked immunosorbant assay and conventional polymerase chain reaction in stool and serum specimens.

Results: Rotavirus antigenemia and viremia were identified in 50% and 54% of acute-phase serum samples respectively. The mean level of rotavirus antigen in stools was greater than in serum. The rate of viremia was significantly higher in the serum of children with antigenemia than in those without ($P < 0.001$). Children with viremia showed significantly greater level of serum antigen and lower level of IgM titers ($P < 0.001$, 0.004 respectively). Among the manifestations tested, the frequency of diarrhea was significantly higher among antigenemia group ($P = 0.031$), and it was correlated with serum and stool antigen levels, and the level of rotavirus antigen in the sera of children with fever was significantly higher than those without fever ($P = 0.002$).

Conclusion: Accordingly, we can conclude that rotaviral antigenemia and viremia were common in children with rotaviral diarrhea, however, the impact of rotavirus antigenemia/viremia on clinical manifestations of infection is unknown.

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1. Introduction

Rotaviruses are non-enveloped, double-stranded segmented RNA viruses (dsRNA) comprising a genus within the family *Reoviridae*.[1] It is the most important cause of early childhood nonbacterial gastroenteritis in both developed and developing countries.[2] The clinical spectrum of rotavirus disease varies from asymptomatic infection to acute, severe, dehydrating diarrhea with vomiting that can be fatal, especially in developing countries.[3] Meanwhile, rotavirus induced gastroenteritis causes a large economic burden in developed countries; therefore, treatment has been directed exclusively at correcting dehydration and the loss of fluids and electrolytes.[4]

Rotavirus infection induces innate and adaptive immune responses, including the production of cytokines and virus-specific antibodies.[5,6]

However, it is unclear, whether dehydration is the only reason that rotaviral infections are more often fatal among children in the developing world than in the developed world. Recently, another possible explanation for this difference in disease severity and outcome has been suggested by findings from both animal and human studies demonstrating that rotavirus can also infect extraintestinal sites,[7] as rotavirus gastroenteritis is sometimes complicated by high fever, elevated transaminase levels, seizures, and encephalitis, which may be caused by systemic viral infection.[4] So, it has been reported that rotavirus infection is commonly associated with antigenemia (the presence of antigen in blood) in children with diarrhea.[8]

In the acute phase of infection, this antigenemia ranges from 43% to 90%. Its levels usually peak 1–3 days after symptom onset and are undetectable beyond 1 week. Antigenemia level is reported to be directly associated with antigen levels in stools, and inversely related to the titer of specific anti-rotavirus antibodies in the serum.[9]

Early work suggested that rotavirus antigenemia was the result of host immunological defects,[10] but recent reports suggest that rotavirus antigenemia is commonly observed in immunocompetent children with rotavirus diarrhea. Therefore, antigenemia could be at the center of the pathogenesis of various extraintestinal infections with rotavirus.[11]

Also, rotavirus RNAemia (the presence of viral RNA in blood) has been reported but appears to be less common. Detection rates of extraintestinal RNA in blood have varied widely, ranging from 0% to 64% in rotavirus-infected children and are not always concordant with presence of antigenemia.[12]

If extraintestinal rotaviral infection is an additional cause of more-severe and more-lethal disease, alternative strategies for treatment might be required to improve outcome.[7]

Two licensed live oral rotavirus vaccines, RotaTeq® and Rotarix™, have shown high efficacy among infants in developed and middle-income countries, but are much less immunogenic and efficacious in protecting children in many low-income countries. These findings indicate a need to better understand early response events to oral vaccines and design new approaches to rotavirus vaccination.[8]

In this study, we quantified rotaviral load in stool from rotavirus infected immunocompetent, non-malnourished children with acute rotaviral gastroenteritis; then we investigated the prevalence of rotavirus antigenemia, and assessed rotavirus viremia and anti-rotavirus IgM antibody as markers of acute rotavirus infection and their relation to disease severity in the same group of children.

2. Materials and methods

2.1. Ethical considerations

The protocol of the study was approved by the Faculty of Medicine, Alexandria University Ethics Committee prior to its start. An informed consent was taken from the parents of each child involved in the research prior to his/her participation.

2.2. Patient selection and specimen collection

One hundred and fifty paired stool–serum specimens were collected from children, aged 6 months to 4 years, who attended the outpatient clinic and those, who were hospitalized for acute diarrhea in Alexandria University Children Hospital at El-Shatby, during the period from November 2012 through February 2013. The specimens were collected within 48 h of hospitalization.

2.3. Clinical features, demographic and laboratory data

All the patients were examined for fever, number of episodes and duration of vomiting and diarrhea, extent of dehydration and treatment. The standard definition of malnutrition and dehydration were strictly followed.[2] Severity of the disease was assessed by using 0–20 point score and based on the sum of points, the disease was described as mild, moderate, severe and very severe.[13] Extra-intestinal manifestations were also noted if present. Laboratory investigations including serum electrolytes (Na, K), kidney function tests (BUN, Cr) and liver function tests (ALT, AST) were performed.

2.4. Clinical specimens

The fecal samples were subjected to naked eye examination for consistency, color and atypical components (mucus, blood). Stool samples showing the presence of blood and mucus (dysentery) were excluded from the study. A 10% (w/v) suspension of each of the stool specimens was made in 0.01 M phosphate buffered saline (PBS) pH 7.2 containing 0.01 M $CaCl_2$. The suspensions were centrifuged at 805 × *g* for 15 min to remove the debris. The supernatants were stored at −70 °C until tested.

Clotted blood specimens were centrifuged at 805 × *g* for 10 min. The sera were separated and stored at −70 °C until tested.[14]

2.5. ELISAs, RNA extraction, RT-PCR

ELISA for the detection of rotavirus antigen was performed on all stool specimens using (Ridascreen® Rotavirus, R-Biopharm,