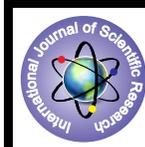


Molecular Detection of Rotavirus Antigens in Stools of Children with Diarrhea in Khartoum State, Sudan



Microbiology

KEYWORDS : Latex agglutination technique, real time-PCR, rotavirus, Sudan

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ABSTRACT

Aims: To identify rotavirus antigens in stools of children with diarrhea using serological and molecular techniques in Khartoum State, Sudan. Materials and methods: Two hundred stool specimens were collected from 200 children less than 5 years of age suffering from diarrhea, all samples were tested for rotavirus antigens using latex agglutination technique and then 30 of them were diagnosed for rotavirus group A real time - PCR molecular technique. Results: Rotavirus antigens were detected in 42 subjects (21%) while 158 were found negative (79%). The study showed that, the disease incidence among males was (18%) and females were (26%). Most of the positive cases were among children less than one year of age (57.1%) followed by (38.1%) in children between 1-3 years of age, and lowest (4.8%) in the children less than 5 years. Using real time-PCR molecular technique rotavirus A were detected in 8 out of 20 positive latex agglutination samples (40%), while 12 samples (60%) showed no viral load. Ten cases of latex agglutination negative samples were confirmed by real time PCR, 5 of them were shown positive (50%) while the rest samples were confirmed negative. Conclusion: the study concluded that these differences in results were confirmed the importance of the application of rapid molecular methods for clinical services in hospital laboratories to provide definitive diagnoses.

Introduction

Rotavirus is a major cause of acute gastroenteritis in infants and young children worldwide (Desselberger et al, 2006). It has been estimated that about 39% of childhood diarrhea hospitalizations are caused by rotaviruses and nearly half a million children die from rotavirus infections each year (Parashar et al, 2003). Furthermore, rotavirus mortality is concentrated in the developing countries on the Asian subcontinent, Africa and Latin America where access to health care facilities is limited (Phua et al, 2006). This may result in a significant disease burden and economic effect of direct medical costs, loss of work, quality of life and mortality.

Rotavirus A, which accounts for more than 90% of rotavirus gastroenteritis in humans, is endemic worldwide (Leung et al, 2005). Each year rotavirus causes millions of cases of diarrhea in developing countries, almost 2 million resulting in hospitalization (Simpson et al, 2007) and an estimated 453,000 resulting in the death of a child younger than five years (Tate et al, 2012), 85% of whom live in developing countries (WHO, 2008).

Various molecular techniques have been exploited for the development of highly sensitive and rapid assays for the detection of causative agents of viral gastroenteritis (Pring-Akerblom et al 1997; Sen et al, 2000; O'Neill et al, 2002). Reverse transcription-PCR (RT-PCR) has reportedly increased the detection rate of rotavirus A by up to 48% compared to EIA or electron microscopy (Pang et al, 1999; Gunson et al, 2003).

Rotaviruses play a major causative agent especially in developing countries including Sudan; however the clinical significance of rotavirus species is not fully understood. The majority of the research done in Sudan on rotavirus strains was on animals and there are a limited number of studies in human rotaviruses (Hemidan, 2011). The World Health Organization studied rotavirus in Sudan during 2009 and reported that rotavirus causes approximately 42% of childhood diarrhea in Sudan hospitals (WHO, 2010). For these reasons and because of the clinical importance of the disease we found it highly important to study the frequencies of rotaviruses in children with diarrhea presenting to many teaching hospitals in Khartoum State, Sudan.

This study aims to identify Rotavirus antigens in stools of children with diarrhea using serological and molecular techniques and to detect the possible risk factors predisposing to the disease among children in Khartoum State, Sudan.

Material and Methods

1. Collection of specimens

Two hundred stool samples were carefully collected in sterile labeled containers directly from children below five years of age and transported immediately to the laboratory. Ethical consent and written questionnaire were obtained from each patient.

2. Latex agglutination technique (LAT):

Fecal samples were processed as per instructions given in the kit protocol provided with Rota-Virus Latex Test Kit manufactured by Plasmatec Laboratory Products, UK.

3. Molecular diagnosis

Real time PCR master mix was prepared according to manufacture (PrimerDesign™ Ltd), which included: 2 x Precision OneStep™ qRT-PCR MasterMix, Rotavirus A Primer/Probe mix, Internal extraction control primer/probe mix, RNase/DNase free water and RNA sample. Fifteen µl from PCR master mix and 5 µl of RNA template were added separately to Eppendorf tube, each sample in duplicate form. Multiple positive, negative and internal controls were included in each real time-PCR assay. For negative control tube use 5 µl of RNase/DNase free water. The final volume in each tube was 20 µl. Then the Eppendorf tubes inserted into real time PCR machine (Swift™ Spectrum 48 Real Time Thermal Cyclers- Esco Micro Pte. Ltd.), and Real time-PCR was performed using universal thermal cycling conditions: 10 mins at 55°C (Reverse Transcription step), 8 mins at 95°C (Enzyme activation), and 50 cycles of 10 secs at 95°C (Denaturation), and 1 min at 60°C. Fluorogenic data were collected during this step through the FAM and VIC channels, by Sequence detection software version 20 was used for all data analysis. The data obtained was analyzed using the SPSS computer program (software version 20).

RESULTS

A total of 200 fecal specimens was collected from boys and girls less than five years of age suffering from non-specific diarrhea was screened for rotavirus infection using latex agglutination technique. Then 30 specimens showing positive and negative results by Latex test were selected for further analysis using molecular technique by real time-PCR with a set of primers and TaqMan probes. The patients included in this study were 122 males (61%) and 78 females (39%). They were distributed into 3 age groups as follows; 97 patients (48.5%) less than 1year, 87 patients between 1-3 years (43.5%) and 16 patients above 3 and less than 5 years of age (8%).

Figure.1 demonstrates that out of the 200 stool specimens examined by LAT, 42 were shown positive (21%) for rotavirus antigens, while 158 subjects were found negative (79%).

Figure.2 illustrates that out of 122 male subjects 22 were found positive for rotavirus antigens (18%), while 20 out of the 78 female subjects were shown rotavirus antigens positive (26%), showing no significant difference (p = 0.216) of gender on rotavirus infection among children examined by LAT.

The results in table. 1 revealed that the highest positive cases of rotavirus infection were 57.1% among the children less than 1 year of age. However, the children above 3 years, but less than 5 years of age had the lowest (4.8%) rotaviral infection. These findings showed no significant difference in rotavirus infection using the different age groups of children examined by LAT.

The result of real-time PCR revealed that 13 out of these 30 samples were positive for rotavirus A (43.3%). Rotavirus A was detected in 8 out of 20 positive latex agglutination samples (40%), while 12 samples (60%) showed no viral load. However, when the 10 negative subjects obtained by LAT, re-examined by real-time PCR, 5 of them were found to be rotavirus A positive (50%) (table.2).

Discussion

The frequency of rotavirus antigens detected in this study among children less than 5 years of age (21%) were slightly lower than those previously reported in different parts of Sudan by WHO (2010) in Sudan (42%). Our results were also lower than those recently reported by Bonkoungou et al (2010) in Burkina Faso (33.8%), Soltani et al (2012) in Tunisia (30%), Florence et al (2012) in Cameroon (28.7%), Anupam et al (2010) in India (50%), and Mehmet et al (2011) in Turkey (52.7%).

However, the results obtained in this study were similar to those reported by Esona et al (2003) in Cameroon (21.9%), but higher than those observed by Moyo et al (2011) who found that 18.1% of diarrhea in hospitalized children less than five years of age in Dar es Salaam, Tanzania was caused by rotaviruses, and by Garcia-Puebla and Garcia-Fragoso (2012) in Puerto Rico (15%).

The low or high rates of rotavirus infection observed in this study and by other investigators could be attributed to several factors including, the study population, the season during which study conducted, living conditions and the diagnostic techniques used in investigations.

During this study; although males admission to hospitals was observed to be higher than females, but they showed no differences in their response to the disease.

The finding that most of rotavirus-infected children with diarrhea were less than one year of age (57.1%) was in agreement with many recent studies reported by Bonkoungou et al (2010) in Burkina Faso, Moyo et al (2011) in Tanzania, Soltani et al (2012) in Tunisia and Florence et al (2012) in Cameroon. It is of great interest to note that all of these recent studies were done in different African countries, from east (Tanzania), to central (Cameroon), to west (Burkina Faso) to north Africa (Tunisia), all in conformance that children less than one year of age were the main target of rotavirus infection.

The results reported in this study highlight the inadequacies of latex agglutination method currently used for the routine detection of gastrointestinal viruses and emphasize the importance of the application of rapid molecular methods for clinical services in hospital laboratories to provide definitive diagnoses.

Table.1. The effect of age of children on rotavirus infection using latex agglutination test

Age of children examined (years)	Subjects examined	Rotavirus Antigens			
		Positive		Negative	
		Number	Percentage	Number	Percentage
(less than one)	97	24	57.1 %	73	46.2 %

1-3	87	16	38.1 %	71	44.9 %
>3-5	16	2	4.8 %	14	8.9 %
Total	200	42	100 %	158	100 %

Table.2. Detection of rotavirus type A by real time – PCR in 20 RV – positive fecal samples and 10 RV negative fecal samples pre – examined by latex agglutination test.

Latex agglutination result	Subjects examined	Results of real time – PCR			
		Positive		Negative	
		Number	Percentage	Number	Percentage
Latex positive	20	8	40 %	12	60 %
Latex negative	10	5	50 %	5	50 %

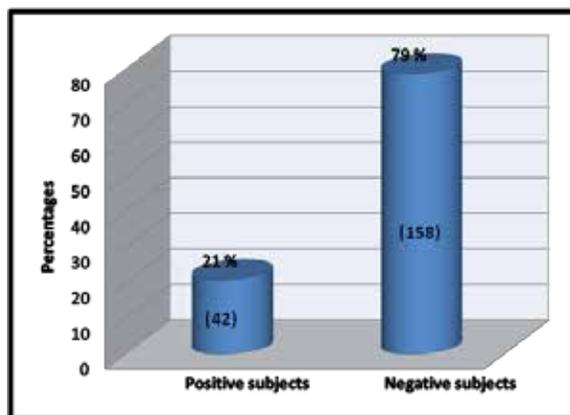


Figure.1. Detection of rotavirus antigens from fecal specimens of children pre-examined by Latex agglutination technique.

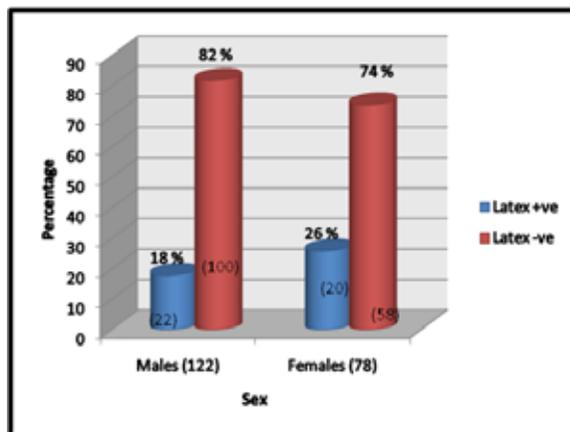


Figure.2. The effect of sex of children on rotavirus infection examined by latex agglutination technique.

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