Prevalence of Rotavirus Infection among under 5 years Children with Diarrhea in Wad Madani Pediatric Teaching Hospital, Gezira State, Sudan

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Abstract:  
In Sudan, rotavirus has been one of the important causative agents of diarrhea among children. The aim of this study was to detect the prevalence of rotavirus infection among children with diarrhea in Wad Madani Pediatric Teaching Hospital during the period from July 2014 to January 2015. Stool specimens from 92 children less than 5 years of age suffering from diarrhea were tested for the presence of rotavirus antigen using the solid-phase sandwich Enzyme-linked Immunosorbent Assay. The results obtained were processed and statistically analyzed using chi-square test. Rotavirus antigen was detected in 36 (39.1%) of the patients. Most of the positive cases were in children less than 1 year of age (72.2%) and the infection rate decreased with the increasing age. Children infected with rotavirus were more likely to have vomiting (72.2%) and fairly low frequency of fever (61.1%). Out of 36 rotavirus positive cases, 28 were resided Village (77.8%), 8 were from City (22.2%).
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Conclusion: Rotavirus prevalence was (39.1%) among children less than 5 years. It is mandatory to have rotavirus vaccine; routine and proper diagnosis of rotavirus infection in children with acute diarrhea helps to determine appropriate treatment, prevents the unnecessary use of antibiotics and minimizes the spread of the disease among susceptible children.

Key words: Rotavirus, Acute gastroenteritis, Watery diarrhea, Children, ELISA, Wad Madani, Sudan.

Introduction:

Rotavirus are member of the Reoviridae family can possess a genome of 11 segments of double stranded Ribonucleic acid (dsRNA) that encode six viral structural proteins and six non-structural proteins[1].

Rotaviruses are classified into at least seven serogroups: A-G on the basis of distinctive antigenic and genetic properties [2, 3]. Group A rotavirus is well known as the leading cause of diarrhea in young children worldwide and 82% of rotavirus deaths among children in the poorest countries [4].

Rotavirus is the most common cause of severe dehydrating gastroenteritis among children globally, resulting in approximately 600,000 to 850,000 deaths each year. Most deaths occur in developing countries, where access to rehydration therapy and other medical care is often limited. The disease burden is also unlikely to be significantly reduced by improvements in hygiene and sanitation [5, 6].

The symptoms of rotavirus gastroenteritis include diarrhea and vomiting, which may lead to severe dehydration and even death if rehydration therapy is not promptly initiated [7].

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The annual rate of rotavirus infection among under-five year old children in the Middle East and North Africa estimated as 42% [8]. Although gastroenteritis among children is of high clinical importance, few recent investigations were performed in human rotaviral infection in Sudan [9, 10].

The aim of this study was to investigate prevalence, associated possible risk factors for rotavirus infection among children below 5 years with diarrhea in Wad Madani Pediatric Teaching Hospital, Gezira State, Sudan.

Materials and Methods:

This was descriptive and cross-sectional study. The study group was children aged between (2 - 60) months admitted to Wad Madani Pediatric Teaching Hospital suffering from acute watery diarrhea from July 2014 to January 2015. Fecal specimens were collected in sterile stool containers. Then stools were stored at -20°C till processed. ELISA was used for rotavirus group A antigens using commercial diagnostic kits (ProSpecT Oxoid Lt, UK).

ELISA technique: A (10%) suspension of fecal sample by sample diluents (tris buffered saline solution containing antimicrobial agent and red dye). 100 μl of each diluted sample, negative control and positive control was transfer to microwells and 100 μl of conjugate (rotavirus specific rabbit polyclonal antibody conjugated to horseradish peroxidase containing antimicrobial agent and blue dye) was added.

The plate was covered and incubated at 30 C° for 60 minutes; the content was removed and washed with washing buffer 5 times. After the last wash, the contents were removed and plate tapped on absorbent paper. 100μl of substrate was added to each microwell, the plate was covered and incubated at 30 C° for 10 minutes. The substrate reaction was stopped by
adding 100μl of stop solution (sulphuric acid) and mixed well before reading the results.

The colored product was read spectrophotometrically (at 450 nm) within 30 minutes after addition of stop solution. Cut-off value was calculated by adding (0.200) absorbance units to the negative control value. The sample interpreted as positive for absorbance value more than cut-off value and negative if absorbance value less than cut-off value.

Ethical consideration: Approval was taken from faculty ethical board, Ministry of Health and Hospital from which samples were collected. Consent was also taken from children’s mothers before sampling.

Data was analyzed using SPSS.

Results:

During July 2014 to January 2015, a total number of 92 children less than 5 years of age suffering from acute diarrhea admitted to Wad Madani Pediatric Teaching Hospital were randomly selected and enrolled in this study. The fecal specimens were collected and investigated by ELISA to detect the prevalence of rotavirus infection.

Out of the total 92 fecal specimens, 36 (39.1%) were positive for rotaviral diarrhea, while 56 (60.9%) were negative (Figure1).

Regarding age distribution, the highest positive cases for rotavirus infection (72.2%) were among the age group less than 1 year and (27.8%) among the age group (1-3 years) with no significant difference (p>0.05) between the age group examined (Figure2).

Collected clinical data showed that among positive rotaviral diarrheal cases 72.2% had vomiting and 60.1% had fever; however among rotavirus negative cases 87.5% were had vomiting and 53.6% had fever (table1).
Most of positive cases observed among children who live in villages 77.8% while remain 22.2% were from city.

Figure 1: Frequency of rotavirus infection among study population. (n=92)

Figure 2: The prevalence of rotaviral diarrhea among study population according to their age.

Table 1: Clinical remarks of children under study with and without rotavirus among the diarrheal cases.

<table>
<thead>
<tr>
<th>Clinical Presentation</th>
<th>Rotavirus positive (n=36)</th>
<th>Percentage of positive*</th>
<th>Rotavirus negative (n=56)</th>
<th>Percentage of negative**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vomiting</td>
<td>26</td>
<td>72.2%</td>
<td>49</td>
<td>87.5%</td>
</tr>
<tr>
<td>Fever</td>
<td>22</td>
<td>61.1%</td>
<td>30</td>
<td>53.6%</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>39.1%</td>
<td>56</td>
<td>60.9%</td>
</tr>
</tbody>
</table>

*P-value = 0.065 (p>0.05)  
**P-value = 0.476 (p>0.05)  

Discussion:
Diarrhea remains one of the most common illnesses of children and one of the major causes of infant and childhood mortality in
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Developing countries. In this study, we focused on rotavirus as an important etiologic agent of childhood diarrhea.

In this study, rotavirus antigens were detected in 36 (39.1%) of the 92 children less than 5 years of age, suffering from diarrhea by using ELISA which was closely similar to results in Khartoum, Sudan (30%) [16] and Sudan (42%) [13].

The detected frequency of infection appears to be less than findings previously reported in the Middle East and North Africa (61%) [14] but higher than that reported in Khartoum, Sudan (8.3%) [10], Burkina Faso (14%) [18], China, India, Mexico, Myanmar and Pakistan (16%) [17], Atlanta, Georgia (22%) [15] and Khartoum, Sudan (24.6%) [9]. The low or high rates of rotavirus infections observed by different investigators in different places could be attributed to several factors including, the study population, the diagnostic techniques used, the incidence rate of the virus in different environments, the living condition and the standards of the study group, the season during which the study was conducted and different subgroups and serotypes of rotaviruses prevalent in the area of the study [11]. The highest rate of infection was found among age less than 1 year of age (72.2%). This finding is similar to the same age group in Oman [26]. Studies worldwide have reported that the most vulnerable age group to rotavirus infection is under 2 years of age with the highest prevalence between (3 to 12 months) of age [24,25,27,28]. In this study out of 36 rotavirus positive cases 28 were residence in Village (77.8%), 8 were residence in City (22.2%). Most of the mothers of the infected children were coming from rural areas where poverty and illiteracy are highly prevailing. Obviously, it is expected, that some children could have contracted the disease or become reinfected from their mothers. Rotavirus reinfections are common and it has been shown that young children can suffer up to five reinfection by 2 years of age. Asymptomic infections are more common with successive reinfection [19, 20]. Some recent studies have shown that adult contacts may be infected,
as evidenced by seroconversion; however, they rarely exhibit symptoms, and the virus is infrequently detected in their stools [20]. Although the common source of rotavirus infection is the direct contact with pediatric cases, epidemics of severe disease have occurred in adults, especially in closed population as in geriatric words [19]. In our study the clinical symptoms, fever and vomiting were found in (61.1%) and (72.2%) of infected children with rotavirus, respectively. The comparison of the clinical symptoms of the acute gastroenteritis among the rotavirus positive and rotavirus negative cases indicated that fever and vomiting were more frequently observed among diarrheal children with rotavirus infection than those without rotavirus infection, as reported in previous studies [12, 21, 22].

Obviously fever, vomiting and watery diarrhea are seen in majority of infected children lasting 2 - 7 days [10]. All of the 36 positive samples, (100%) were antibiotics treated cases. The inappropriate use of antibiotics may cause imbalance of normal microflora and consequently, this may increase the severity of gastroenteritis because the normal microflora play important role in the body defense by interference with the invasion of pathogenic bacteria. It is possible that gastroenteritis caused by pathogenic bacteria is strong predisposing factor for rotavirus included diarrhea, or vice versa [23].

**Conclusion:**

Rotavirus gastroenteritis is the most common worldwide disease associated with significant morbidity and mortality less than five years of age.

Rotavirus prevalence was (39.1%) among children (less than 5 years).

Rotavirus vaccine, routine and proper diagnosis of rotavirus infection in children with acute diarrhea help to determine appropriate treatment, prevents the unnecessary use
of antibiotics and minimizes the spread of the disease among susceptible children in Sudan.

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