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Molecular Detection of Rotavirus (A and B) and Astrovirus in Children Less than 5 Years with Gastroenteritis in Khartoum and Aljazeera States, Sudan

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ABSTRACT

Background: Diarrheal diseases represent a major worldwide public health problem particularly in developing countries. Each year, at least four million children under five years of age die from diarrhoea. Although rotavirus is a leading cause, other viruses including astroviruses are also important, but have been the subject of limited studies. The objective of this study was to estimate the Rotavirus and Astrovirus among children less than 5 years with gastroenteritis.

Methods: This study was an active surveillance cross sectional study in a total of 66 diarrhoea specimens collected from children of less than 5 years old with gastroenteritis in Aljazeera Children's Hospital and Omdurman children's Hospital during January to May 2017. RNA was extracted from all specimens, followed by Real Time-PCR amplifications to detect rotavirus and astrovirus were performed.

Results: of the 66 samples tested for rota virus, one sample was positive in Aljazeera samples (1,5%) while no positives were detected in Khartoum samples. While for astrovirus there was one positive in Khartoum samples (1.5%) and one positive in samples from AlJazeera (1.5%).

Conclusion: The current study showed that infection with astroviruses may be as an important cause of gastroenteritis as rotavirus. These findings highlight the need to implement rotavirus and astrovirus detection assays in clinical diagnosis to prevent nosocomial spread of viral gastroenteritis infections in paediatric departments. It is recommended to conduct genotyping of rotavirus on larger number of samples before starting vaccination in the country.

Keywords : Rotavirus, Astrovirus, Diarrhoea, Vomiting, Sudan.

I. INTRODUCTION

Acute gastroenteritis is a common disorder in young children, and the associated dehydration is a leading cause of admission to hospitals in industrialized countries and major sources of mortality in developing countries [1]. Although global deaths from diarrheal disease have decreased from 2.6 million to 1.3 million between 1990 and

2013, diarrhea remains a major health concern, particularly in Africa. [2] Young children are known to be the most affected population, and there were an estimated 450 million diarrheal episodes among children <5 years of age in 2010 in Africa [2] Enteric viruses have been recognized as the most significant etiological agents of the disease, and four categories of viruses are considered clinically relevant: group A rotavirus , norovirus , adenovirus 40/41 (subgenus F), and astrovirus [18,19,20] .Astrovirus (AstVs) are enteric viruses that can cause gastroenteritis in children and a severe disease in immune compromised and elderly people [3, 4]. Astrovirus belong to the family Astroviridae; the non-enveloped virion is small, with a diameter of 28 nm, and contains a single stranded positive-sense RNA of 6.8 kb. The viral nucleic acid consists of open reading frames (ORFs), including ORF1a, ORF1b and ORF2 [5]. The prevalence rate of human astrovirus infection worldwide ranges from 2 % to 9 % among children with diarrhoea [6]. The morbidity varies depending on the season, with higher infection during the winter in temperate climates and the rainy season in tropical regions [7]. The main symptom of astrovirus infection is watery diarrhea, which is often associated with vomiting, fever, and abdominal pain [4].

Rotaviruses (Reoviridae family) are non-enveloped, double stranded RNA viruses with 11 gene segments. They comprise seven major groups (A-G) [8]with group A Rotaviruses being the major causes of diarrhoea among infants and young children all over the world [9].

Rotavirus is considered the most common cause of severe diarrhea in children, resulting in the hospitalization of approximately 55,000 children each year in the United States [9, 10]. In the developing world, rotavirus may account for 1 million childhood deaths as well as significant morbidity each year [11], due to poor nutrition and health care [12]. Children in the poorest countries account for 82% of rotavirus deaths [13].

The main symptoms of rotavirus gastroenteritis (RVGE) are fever, abdominal pain, lethargy; diarrhea and vomiting that may lead to hypo volemic shock and dehydration [14,15]. Severe cases can lead to death [16].

II. MATERIAL AND METHOD

Study area and patients

This study was an active surveillance cross sectional study, aiming to determine and rotavirus and astrovirus incidence among children less than 5 years of age visiting Omdurman children's Hospital in Khartoum State(33 children) and Aljazeera Children's Hospital in Aljazeera State(33 children) and The study was carried out during January to May 2017.

Thirty of the children were males while 36 were females. Their ages varied between less than 1 year(16),1year to less than 3 years(30), and 3 years to 5 years (20) (Table1).The combination of different symptoms (fever, diarrhoea, vomiting) manifested in these children were shown in table (2)

Sample collection

Stool specimens were collected from the children with acute diarrhoea, using sterile clean containers. Then 1mL of diarrhoea sample was placed into sterile tube containing 5mL phosphate buffered saline, the suspension was centrifuged for 20 min.The supernatant was then filtered into clean tube and stored at -2o°C until used.

RNA extraction:

Total RNA was extracted by using the QIAamp Viral RNA Mini spin according to the protocol of the manufacturer (Qiagen, Germany).

Real time RT-PCR

Real-time one step RT-PCR was done to detect viral RNA by using a commercial Rotaviruses A and B, and Astrovirus kits following the manufacturer's instructions (genesig® Advanced kit , UK).

III. RESULT

Out of the total of 66 sample there was one (1.5%) positive for Rotavirus A in Aljazeera samples but no positive samples were detected in Khartoum samples for Rotavirus A infection.Also among the 66 samples tested there was one(1.5%) positive for astrovirus infection in Khartoum samples and one(1.5%) positive in Aljazeera samples. One (50%) male and one (50%) female were among the 2 astrovirus positive patient(Table 3)

Table 1. Distribution of children according to age and gender

Gender / Age	Male	Female	Total
0 up to 12 months	6 (20 %)	10 (27.8 %)	16 (24.2%)
More than 12 up to 36 months	16 (53.3%)	14 (38.9%)	30 (45.5%)
More than 36 up to 60 months	8 (26.7%)	12(33.3%)	20 (30.3%)
Total	30 (45.4%)	36 (54.6%)	66 (100%)

Table 2. Distribution of clinical symptoms among infected children

Symptoms	Number	Percent %
Children with diarrhea, vomiting and fever	14	21.2 %
Children with diarrhea and vomiting	16	24.2 %
Children with diarrhea and fever	20	30.4 %
Children with diarrhea only	14	21.2 %
Children with vomiting only	2	3 %
Total	66	100 %

Table 3. Viruses detected in children with gastroenteritis in the Khartoum and AlJazeera States

Total Sample / Virus detected	Rota Virus (A)	Astro Virus
66	1	2
100%	1.5 %	3 %
Gender	Male	1 Male, 1 Female
Age (years)	3	1-3
Symptoms	• Diarrhea-vomiting - fever	• Diarrhea-vomiting-fever • Diarrhea-vomiting

IV. DISCUSSION AND CONCLUSION

Diarrhea is major causes of morbidity in developed and developing countries. In addition, diarrheal illnesses account for an estimated 12,600 deaths each day in children in Asia, Africa, and Latin America. The causes of diarrhea include a wide range of viruses, bacteria, and parasites, many of which have been recognized only in the last decade [17 In several international reports, it was evident that viral pathogens are the most common cause of gastroenteritis in developed countries Worldwide [21, 22-24]. Gastrointestinal rotavirus infections result in an estimated 440,000 deaths in children fewer than five years of age [25].

Only few studies regarding the problem of childhood diarrhea in Sudan are available, only two reports had indicated that rotaviruses and diarrheagenic Escherichia coli (DEC) were common causes of childhood diarrhea (Mustafa *et al.,* [34]; Elhag *et al.,* [33]

The present study indicates that rotavirus and astroviruses (1.5 % and 3% respectively) might be relatively important gastroenteritis viral infections in children less than 5 years with gastroenteritis in Aljazeera Children's Hospital and Omdurman children's Hospital.

Out of the 66 patients, viral pathogens were detected in only 3 cases (5%). These findings highlight the need to implement rotavirus and astrovirus detection assays in clinical diagnosis to support the prevention of nosocomial gastroenteritis viral infections in paediatric departments.

Detection of rotavirus infection in Sudanese children has previously been reported in Melut district (nowadays belongs to Republic of South Sudan) but the rate of infection was not stated [27]. In a study by Elhag *et al.,* [33] it was reported that out of the 710 patients tested, viral pathogens were detected in only 99 cases (13.9%). Of these 99 cases of viral diarrhea, 83 (83.8%) were due to rotaviruses while 16 (16.2%) were attributed to adenovirus. Of the 83 rotaviruses identified, 42 were characterized by RT-PCR, of these 40 (95.2%) were proved as type A (VP6), and 2 (4.8%) type C (VP7). Type C (VP7) rotavirus was detected in samples collected from children under 5 years only. In a more recent study . Adam *et al.,* [32] indicated 10.2% infection rate with rota virus in children less than 5 years in Khartoum state but no astrovirus was detected in their study. The infection rate of rota virus (1.5%) detected in the present study is much lower than that reported in the above two studies from Sudan. It is also much lower than that ((17%) reported from Tunisia and Kenya[28] [29] and that (13%) reported from Libya[30]. These differences in the prevalence rates recorded in our study in comparison to the other studies may be related to the smaller sample size (66 samples) investigated in our study compared to the other studies.

Our results regarding prevalence of astrovirus (3 %) were similar to those from Tunisia (4%), Italy (3.1%), but slightly lower than those reported from other countries such as in France (6 %), Spain (4.9 %) and India (5.8 %)[32 [27].

Since this study is hospital-based, the overall 5% prevalence rate may not reflect the true prevalence among Sudanese children, thus a community-based surveillance is needed.

Finally, the results of this study may be useful as an additional information that might help in the planning and implementation of efficient prevention programs. In this respect a rota vaccine with broad and consistent serotype coverage and public education would be important to help

decrease the burden of gastroenteritis in the country.

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