

Molecular Detection of Norovirus 1, 2 in Children Less than 5 Years with Gastroenteritis in Khartoum and Al Jazeera States, Sudan

Eman M Tatay¹, Abdel Rahim M El Hussein², Mohamed O. Mustafa², Isam M Elkhidir³ and Khalid A Enan^{2*}

¹Department of Medical microbiology, Faculty of Medical Laboratory Science, El Neelain University, Khartoum, Sudan

²Department of Virology, Central Laboratory- The Ministry of Higher Education and Scientific Research, P.O. Box 7099, Khartoum, Sudan

³Department of Microbiology and Parasitology, Faculty of Medicine, University of Khartoum, Khartoum, Sudan

*Corresponding Author: Khalid A Enan (khalid.enan@gmail.com)

ABSTRACT

Background: Noroviruses (NoVs) are human pathogens associated with acute viral gastroenteritis worldwide and an important cause of childhood morbidity and mortality in developing countries. However, there are still few epidemiological data on the occurrence of these viruses in Sudan. This study was conducted to investigate the molecular epidemiology of NoVs in children less than 5 years with acute gastroenteritis.

Methods: A total of 66 stool samples were collected from children under five years of age presenting with acute gastroenteritis, in Omdurman teaching hospital and Wad Medani teaching hospital, during the period from January to May 2017. For the detection of norovirus, total RNA was extracted from all samples, followed by Real time polymerase chain reaction (Real- time PCR) Detected noroviruses were then genogrouped.

Results: Out of the 66 samples tested, one(5.3%) sample in Khartoum and 18 (94.7%) samples in Aljazeera were positive for norovirus infections by Real-time PCR. The detected norovirus - positive samples belonged to GII and GI genogroups but the dominant genotype was GII.

Conclusions: The present study showed that norovirus are an important causative agents of gastroenteritis in children less than 5 years .There is a great need for introducing routine norovirus testing of hospitalized children with gastroenteritis.

Keywords:, NoVs, Real- time PCR, Norovirus, Khartoum, Al Jazeera, Sudan

I. INTRODUCTION AND LITERATURE REVIEW

Diarrheal diseases remain one of the leading causes of preventable death in developing countries, especially among children under 5 years of age[15].

Diarrhea is common in the developing countries, especially in areas with poor hygiene and sanitation

and with limited access to safe water. Other conditions, such as malnutrition, may further increase the risk of contracting diarrhea in developing countries. These factors may lead to a significant disease burden and negative economic effects, resulting from medical costs, loss of work, lower quality of life and high mortality [7].

Recently, noroviruses (NoVs) gastroenteritis has been considered to be second to rotavirus gastroenteritis as the cause of children's hospital admissions worldwide.[3].

In developing countries NoVs are estimated to cause 200,000 deaths each year among children aged <5 years [5, 9].

NoVs are members of the genus Norovirus within the family Caliciviridae. They can be subdivided genetically into five genogroups (G) of which GI, GII, and GIV are associated with infection in humans [4]. In 2007, 973 stool specimens were collected from children hospitalized for gastroenteritis signs or from neonates and premature cases in the north of France[13]. They were tested by rapid enzyme immunoassay (EIA) for rotavirus adenovirus, norovirus and astrovirus. The overall rates of prevalence for rotavirus, norovirus, adenovirus, and astrovirus were 21, 13, 5, and 1.8%, respectively, Mixed virus infections were detected in 32 (3.3%) of the 973 studied children and were associated with norovirus in 21 (66%) of these cases[13]. In another study a total of 542 stool samples were collected between March 2005 and February 2006 in Leon, Nicaragua and investigated for norovirus using ELISA and RT-PCR. NoVs was detected in 12% (65/542) of the children; of these, 11% (45/409) were in the community and 15% (20/133) were in the hospital [1]. According to the World Health Organization, diarrheal disease, is responsible for 1.5 million child deaths every year [11]. In Sudan, diarrhea is one of the most common reasons for children to visit healthcare clinics, but knowledge of the causative agents of these diarrhea cases is limited [8].

In Sudan infant mortality is 102 per 1000 live births and Neonatal mortality is 51 per 1000 live births [2].

II. MATERIAL AND METHOD

Study area and sample collection

This study was an active surveillance cross sectional study, aimed to determine viral aetiology of diarrhea among children less than 5 years of age visiting children hospitals in Khartoum state and AlJazeera State, Sudan. The study was carried out during January to May 2017. A total number of 66 samples (33 samples from Khartoum, and 33 samples from Aljazeera) stool specimens were collected from children (34 males, 32females) less than 5 years with acute diarrhea, using sterile clean containers. Then, 1mL of stool sample, was placed into sterile tube containing 5mL phosphate buffered saline. The suspensions were centrifuged for 20 min and the supernatants were then filtered into clean tube and stored separately at -80°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 Norovirus Genogroups 1 and 2 kit following the manufacturer's instructions (genesig® Advanced kit , UK).

Statistical Analysis

The data were analyzed using SPSS analysis software Version 21 to find any significant correlation with gender, age, and relation of the Genotypes of noroviruses detected with gastroenteritis. Statistical significance was set at a P value < 0.05.

III. RESULTS AND DISCUSSION

Results

Out of the 66 samples tested by RT-PCR, one sample in Khartoum(3%) and 18 samples in Aljazeera(54.5%) proved to be positive for norovirus infections with an

overall infection rate of 28.8%. Out of the 19 positive samples 11(32%) were females and 8 (25%) were males. Also ,among the 19 noroviruses -positive samples, Eighteen samples of the noroviruses detected (94.7%) belonged to GII and 1(15.3%) to GI (tables 1,2,3)

Table 1 : Frequency of positive and negative norovirus samples according to State

state	Total tested	No. positive	No. negative
Khartoum	33	1 (3%)	32 (97%)
Al jazeera	33	18 (54.5%)	15(45.5%)
total	66	19 (28.8%)	47(71.2%)

Table 2 : Frequency of positive and negative norovirus samples according to gender.

Gender	No. sample tested	No. positive	No. Negative
Male	32 (48.5%)	8(25%)	24(75%)
Female	34(51.5%)	11 (32.4%)	23(67.6%)
Total	66 (100%)	19(28.8%)	47 (71.2%)

Table 3. Clinical symptom observed in children with norovirus infection

Clinical Symptoms							Total
diarrheae, fever, vomiting	diarrheae, vomiting, abdominal pain	diarrheae , vomiting , nausea	diarrheae, vomiting	diarrheae, abdominal pain	diarrheae, fever, abdominal pain	diarrheae, fever, nausea	N=66
22	14	3	11	9	6	1	

The statistical analysis of the data showed no significant differences ($p > 0.05$) between the age group, gender were noted. Also no correlation between symptoms recorded in the patients and the group of Noroviruses detected was observed (data not shown).

Discussion

Diarrhea remains the second leading cause of death due to infections among children under the age of five years worldwide [12].

This study was carried out to investigate the prevalence of NoVs (GI, GII) in children with diarrhea

in Khartoum and Aljazeera States using real-time PCR assay with specific primers.

NoVs was detected in 19(28.8%) out of the 66 samples tested for GI,GII. The no. of positive sample(18, 94.7%) was much higher in Aljazeera) than in (1,5.3 %) in Khartoum State. All of the viruses detected in Aljazeera were of genogroup II while the one detected in Khartoum was of genogroup I. Our results are in agreement with the findings of a study in Thailand where 119 (23.8%) out of 941 samples were positive for NoVs of which 116 were GII (97.5%) and 3 were GI (2.5%). [10].

A similar study in china in 2013 carried out to investigate both rotaviruses and noroviruses in children indicated that 263 (34.3%) and 80 (10.4%) children were positive for rotavirus and noroviruses respectively[6]. Out of the 80 cases specimen positive for NoVs, there was only 5 (6.3%) patients in age under 5 years indicating higher incidence of NoVs infection in the above 5 years old children.

Our results also showed higher incidence of NoVs compared to that reported by Oldak *et al.*, [3]. who detected single norovirus genotype infection in 35/242 (14.5%) patients in Poland [3].

Similar results were also reported by Seyed *et al.*, [11] in Iran who by using RT-PCR analyses indicated that among 170 samples, 49(28.8%) and 15 (8.8%) were positive for rotavirus and norovirus infections, respectively[12].

The present study also showed the predominance of GII NoVs in Aljazeera. This is in agreement with Ying *et al.*, [14]. results in Shanghai, China, who found that in patients positive for NoVs ,GI, GII and their co infections represented 10.41%,85.16% and 40.41%, respectively[14].

IV. CONCLUSION

The present study showed that norovirus are an important causative agents of gastroenteritis in children less than 5 years .There is a great need for introducing routine norovirus testing of hospitalized children with gastroenteritis.

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