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Molecular Detection of Norovirus 1, 2 in Children Less than 5 Years with Gastroenteritisin Al Jazeera State, Sudan

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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 Aljazeera teaching hospital, during the period from January to May2017. For the detection of norovirus, total RNA was extracted from all samples, followed by Real time polymerase chain reaction (RT-PCR) Detected noroviruses were then genogrouped.

Results: Out of the 66 samples tested, one sample in Khartoum and 18 samples in Aljazeera were positive for norovirus infections by RT-PCR. The dected noroviruse - positive samples belonged 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 : Qiagen, RNA, NoVs, RT-PCR, Noroviruses

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, norovirus gastroenteritis has been considered to be second to rotavirus gastroenteritis as

the cause of children's hospital admissions worldwide.[3] .

In developing countries and 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) analyses 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 the cases[13]. In another study a total of 542 stool samples were collected between March 2005 and February 2006 in Leo´n, 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 are active surveillance cross sectional study, aiming to determination of viral etiology of diarrhea among children less than 5 years of age visiting children hospitals in Khartoum state and Al Jazeera State, Sudan. The study was carried out during January to May 2017. A total number of 66 stool specimens were collected from children (males, females) less than 5 years with acute diarrhea, using sterile clean containers. Then, 1mL of stool sample(female and male 34,32) respectively, was placed into sterile tube containing 5mL phosphate buffered saline, the suspensions were the centrifuged for 20 min. The supernatants were then filtered into clean tube and stored separately 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). Briefly, 140 µl of stool sample supernatant was added to 560 µl buffer AVL containing carrier RNA, and then incubated at room temperature for 10 minutes. Subsequently, 560 µl of ethanol (96- 100%) was added to the sample after which 630 µl of the resulting solution was applied to a column. A volume of 500 µl of AW1 and AW2 was added for washing and the nucleic acids were eluted with 60 µl AVE buffer and stored at -80°C until used.

Real time RT-PCR

Real-time one step RT-PCR was done to detect viral RNA by using a commercial kit following the manufacturer's instructions (One-Step Real-Time RT-PCR Master Mixes Kit, Invitrogen, genesig standard kit ,Country?).

With the primer/probe set 1, RNaseP was used as an internal control and real-time PCR was carried out following the protocol provided by manufacturer's. The real time PCR master mix for one reaction was prepared as follows: 10 µl of 2XqRT- PCR Mastre mix (consisting of a proprietary buffer system, MgSO4, dNTPs, and stabilizers),1 µl of primer/probe, 4 µl of

RNAse/DNAse free water. The final volume was 15 µl for a single reaction. The reaction was performed.

in an automated 7500 real-time PCR (AB Applied Biosystems, USA). The thermal cycling conditions were 10 minutes at 55°C for reverse transcription, 2 minutes at 95°C for enzyme activation and, 10 seconds at 95°C for denaturation and 60 seconds at 60°C for annealing and extension. A sample whose growth curve crossed the threshold line within 40 cycles (Ct < 40) was considered as positive.

Statistical Analysis

The data were analyzed using SPSS analysis software Version 21 to find any significant correlation between incidences of Genotype of noroviruses isolated from patients with Gastroenteritisin. Statistical significance was regarded at a P value < 0.05

III. RESULTS AND DISCUSSION

Out of the 66 samples taked by RT-PCR, one sample in Khartoum and 18 samples in Aljazeera proved to be positive for norovirus infections (28.8%). And (51.5%) were females and (48.5%) were males. Among the 19 noroviruses -positive samples,(18 belonged to GII and 1 to GI
(table 1,2,3)

hospital name * norovirus vaccination Crosstabulation

Table 1.1 : hospitals among NoVs

state	No positive	No samples
khartoum	1 (1.5%)	32 (48.5%)
jazeera	18 (27.3%)	13(19.7%)
total	19 (28.8%)	45(68.2%)

Table 1.2: Gender among NoVs : Age * Norovirus vaccination

Gender	No.sample tested	No. positive	No.Negetive
Male	32 (48.5%)	8(12.1%)	23(34.8%)
Female	34(51.5%)	11 (16.7%)	22(33.3%)
Total	66 (100%)	19(28.8%)	45(68.2%)

Table 1.3 : Noroviruses vaccination among various age groups

Age groupe		NoVs		
		not vaccinated	vaccinated	Total(n=66)
Age	0-2	11	18	29
	2-4	6	11	17
	4-6	3	7	10
	4	1	9	10

Table 1.4 : Symptoms * Norovirus vaccination

	clinical symptoms							
	diarrheae, fever, vomiting	diarrheae, vomiting, abdominal pain	diarrheae, vomiting, nausea	diarrheae,_vomiting	diarrheae, abdominal pain	diarrheae, fever, abdominal pain	diarrheae, fever, nausea	Total
not vaccinated	6	2	1	5	5	1	0	N=66
vaccinated	16	12	2	6	4	5	1	
	22	14	3	11	9	6	1	

The statistical analysis of my data showed no Significant differences (p>0.05) between the age group, gender and symptoms data obtained with Noroviruses genes with Gastroenteritisin.

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 RT PCR assay with specific primers.

NoVs was detected in 19(28.8%) out of the 66 samples tested For GI,GII Norovirus the higher for positive sample in al jazeera, 18 GII and one GI in al jazeera and Khartoum, respectively. This is in agreement that findings a study in tiland where 119 (23.8%) out of 941 samples were positive for NoVs. However, our finding were higher the of which 116 were GII (97.5%) and 3 were GI (2.5%). [10].

Study reported in china in 2013 carried out to investigate both rotaviruses and noroviruses in children. This study indicated that 263 (34.3%) and 80 (10.4%) were positive for rota and noro viruses respectively.

Out of the 80 case specimen positive for NoVs , there was 5 (6.3%) patients in age under 5 years [6]. Our results were also higher to that reported by Oldak et al., (2012) who detected in 105/242 single norovirus infection in 35/242 (14.5%) patients, rotavirus in 51/242 (43.4%) cases of causative agent of viral gastroenteritis of witch noroviruses infections are found in 35/242 (14.5%) patients, rotavirus in 51/242(21.1%) and adenovirus in 14/242 (5.8%) patients[3]

Similar results were also reported by Seyed D et al., (2016) in Iran who 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 showed the predominance of GII NoVs in al jazeera. This is an agreement with ying etal.,(2015) finding in shanghai, china, Who found that in patients positive for NoVs G1, GII and co infections with GI,GII 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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