Characterization of rotavirus among Sudanese children under 5 years of age, Khartoum state, Sudan

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Abstract

Background: Diarrheal diseases are a big public health problem worldwide, particularly among developing countries. The current study was conducted at ElBuluk Pediatric hospital to detect and genotyping of genogroup A Rota virus (GARVs) from children, admitted with gastroenteritis to one of the major pediatric hospitals in Khartoum state, Sudan.

Methods: A total of 755 stool samples were collected from children less than 5 years of age presenting with acute gastroenteritis to one of the major pediatric hospitals in Khartoum. Rota virus was investigated via enzyme-linked immunosorbent assay (ELISA). Positive stool samples were used in Ribonucleic acid (RNA) extraction and thereafter RNA product was loaded on formaldehyde agarose gel, and then visualized under ultraviolet light. VP4 (P) and VP7 (G) genotyping for Rota virus was done using Reverse transcription-Polymerase chain reaction (RT PCR) and nested PCR followed by sequences.

Results: Out of the 755 stool samples from children with acute gastroenteritis, 121 (16%) were positive for rotavirus A. There was enough RNA for sequencing in only 24 samples where the VP7 predominant G type was G1 (83.3%), followed by G9 (16.7%). Out of these samples, only one VP4 genotype was detected and showed high similarity (more than 98%) to P8 Genbank strain.

Conclusion: As a conclusion the VP7 predominant G type was G1, followed by G9 whereas only one VP4 genotype was detected and showed high similarity to P8 Genbank strain. It appears that the recently approved rotavirus vaccines in Sudan are well matched to the rotavirus genotypes identified at this hospital, though larger studies are needed.

Keywords: Rotavirus, VP4, VP7, children, Sudan
Introduction

Rotavirus A is the most frequent cause of diarrhea in under-five year old children worldwide where it is estimated to cause 114 million cases of gastroenteritis resulting in 527,000 deaths annually [1-3]. Five strains are the most common among human. Geographical changes in strain prevalence patterns as well as periodic emergence of unusual G genotypes have been observed [4-6]. Assessment of the potency of rotavirus vaccine against constantly changing strains will significantly depend on continuous global strain surveillance [7]. The diversity of VP4 and VP7 rotavirus strains is a major challenge to the efficacy of the currently used vaccines [8]. Characterization of the common strains in Sudan will have an impact on the implementation of rotavirus vaccines in the country and the neighboring countries since the region is known for its mass population displacement. To the best of our knowledge there is no published data about the genotypes of rotavirus in Sudan despite its potential impact in the implementation of vaccines. However the Rotarix® vaccine was introduced to the most gathering state (Khartoum state) in Sudan on July 2011. Therefore, in this study the VP4 and VP7 were selected for sequencing because of their capacity in protection against rotavirus and their impact upon vaccine implementation. This study was conducted to determine the sequence of VP4 and VP7 rotavirus strains among Sudanese children with acute gastroenteritis.

Methods

Nature of the study

The study was a cross sectional hospital based study, conducted in ElBuluk pediatric hospital in Khartoum state, Sudan, from April to October 2010.
Samples

A total of 755 stool specimens were collected from children (430 males and 325 females) under 5 years of age. A single stool sample was taken from each patient on the first day of hospitalization before administration of any treatment. After taking a written informed consent from of the parents or guardians stool samples were collected along with demographic Data and medical history which were collected using pretested questionnaires. Ethical approval was obtained from the ethical and scientific committee of Faculty of Medical Laboratory Sciences, Khartoum University. Each sample was collected in a dry clean container and immediately placed in an ice chest and then sent directly to the virology department, National Public Health Laboratory, Khartoum, Sudan for preservation in -80°C.

Enzyme Linked Immunosorbent Assay (ELISA)

The stool specimens were brought to room temperature. A sum of 100 g from each specimen was diluted in 1 ml of specimen diluents in a clean dry tube and delay was avoided during pipetting. VP6 monoclonal antibody (ELISA) was utilized to detect rotavirus antigen as per the manufacturer’s instructions (GA Generic Assay GmbH ®, Germany) [9]. The Cut-off point was calculated depending on the optical density of the mean negative control plus 0.2 as a constant. Samples reflecting optical densities of more than the predetermined cut-off point were considered positive while those reflecting optical densities equal or less than the set cut-off point were taken as negative.

Extraction of viral RNA

The stool specimens which showed a positive result by ELISA technique were preserved as raw specimens in ice (- 80oC). These specimens were used later for RNA extraction. RNA was
extracted utilizing the QIAamp® Viral RNA Mini Kit according to the manufacturer’s instructions [10].

**RNA Formaldehyde Agarose (FA) Gel Electrophoresis**

The integrity and size of bands distribution of the extracted RNA was examined by denaturing formaldehyde agarose gel electrophoresis and ethidium bromide stain in order to confirm the ELISA results.

**Polymerase chain reaction**

The remaining of the extracted viral RNA was stored directly at -70°C and later was used for the Reverse Transcription Polymerase Chain Reaction (RT-PCR). The RNA was reverse transcribed using the Ominscript Reverse Transcriptase kit with the use of Oligo-dt primer, and then the resulting DNA was amplified by Hot Star Taq plus Master Mix (GIAGEN). For VP4 the primers Fcon3 (TGG CTT CGC TCA TTT ATA GAC A), Fcon2 (ATT TCG GAC CAT TTA TAA CC) were used while for VP7 the primers F VP7 (TAGTGGATGTCGTTGATGG) and R end 9 (GGTCACATCATACAATTCTAATCTAAG). Nested PCR was carried for VP7 and VP4 by the same primers [11, 12].

**Sequence**

The obtained PCR product (300-400bp) for VP7 and (800-900bp) for VP4 were shipped to the First Base laboratories, Malaysia for sequencing. The sequence reaction of the product was conducted using ABI Prism BigDye® kits (applied biosystem).

The nucleotide sequence data were listed by the Sequence Scanner Software. (http://www.appliedbiosystems.com/sequence scanner). The nucleotide list data were aligned using the
Basic Local Alignment Search Tool (BLAST) with Genbank strains. Phylogenetic and molecular evolutionary analysis was carried out using MEGA 5[13]. The nucleotide sequences obtained in this study were deposited in GenBank under accession numbers [KC741477- KC741500]. The data obtained was coded and entered into the SPSS software (version 16). Chi-square test was used to test for significant differences between the variables. A p value of less than 0.05 was considered as significant.

**Results**

Of the 755 fecal samples analyzed, 121 (16%) tested positive for rotavirus by ELISA. A total of 79 males (65.3%) and females 42(34.7%) were rotavirus positive; this makes a ratio of 1.9:1. Of these the 121 positive samples were amplifiable by RT-PCR and only 24 samples showed sufficient RNA for sequencing. The age of children ranged 1-60 month with a mean of 15.6±13.3 months, a median and a mode of 11months. A total of 24 rotavirus-positive samples were G and P genotyped. All of the fecal specimens contained only one rotavirus strain. Overall, during the study, 83.3% of strains identified were genotype G1 and 16.7% were G9. The only rotavirus strain detected was characterized as P 8. See Figure-1.

**Discussion**

This study described the rotavirus infections among patients presenting to one of the major Sudanese pediatric hospitals in the period April through October 2010. As referred to in another publication by Magzoub et al [14], a total of 755 fecal specimens were tested for rotavirus and 16% were positive a finding that has been found by Elhag et al[15] Parashar et al estimated 18.8% percent of diarrhea at clinic due to rotavirus in Sudan , a result which is not far from what has found in the current study[2]. The 16% prevalence results are consistent with previous
findings on rotavirus prevalence in Tunisia and Kenya [16,17] but higher than the 13% prevalence reported from Libya [18] and lower than the 21% and 32% prevalence reported from United Arab Emirates [19]. In comparison to the rotavirus infection rate between males and females, the study results showed a prevalence rate of rotavirus infections in males 79(65.3%) and females 42(34.7%) [14], the ratio of male to female infection was 1.9:1. This is similar to results reported from Vietnam and Nigeria respectively [20, 21]. In addition other worldwide studies have indicated that males are more susceptible to rotavirus infection and actually exhibited a higher rate of rotavirus in their faeces than females [22, 23]. The differences in detection rates might be explained by different conditions of the studies, such as the season of sampling and the sampling methods.

One of the main aims of this study was to characterize the VP7 (G genotype) and VP4 (P genotype) gene segments of the Sudanese rotavirus strains. For VP4 Only P8 was detected. This finding is similar to a report from Saudi Arabia [24] and to another one from Austria [25]. Moreover in agreement with our result, recent literature worldwide showed that P8 is the most prevalent genotype [26, 27]. In this study, the detection rate of 83% VP7 genotype G1 in Sudan is relatively similar to the 82.4% and 84% reported from Egypt [28] and Austria respectively [25]. Furthermore, results from 8 countries in the Middle East and North Africa showed the prevalence of VP7 genotype G1 to be more than 56% [27]. The VP7 G9 genotype has been documented since the early 1980s [29, 30]. Throughout the 1980s and 1990s, G9 was considered to be a very rare strain; however, recent reports have described its increasing importance [30, 31]. VP7 G9 strains firstly came forth into view in 1993 as one of the five globally prevalent genotypes and in 2001 accounted for a rate of at least 5.8% of rotavirus cases [32]. In the United States, the G9 genotype was detected through an outbreak in 1995–1996 [33] and maintained its
presence in the subsequent 2 years with an average detection rate of 7% across 10 United State cities [34]. Nevertheless, this may not reflect the current prevalence of the G9 genotype, since recent regional epidemiological reports have shown prevalence as high as 50–90% in some settings [35, 36]. In the present study the 17% rate of G9 (G9P8) is similar to an Iranian 17% rate [37] as well as results from Brazil [38]. Moreover this rate is within the range of 1-28% which was reported from 17 countries throughout the African continent [39]. On the other hand our G9 prevalence rate is a little bit higher than the Kuwaiti 10.2% rate [40] and the 11% reported from Saudi Arabia [24] and Iraq [41]. In this study the presence of G1P8 as predominant strains in our results is in agreement of introducing of the monovalent Rotarix™ vaccine in Sudan. Moreover the G9 detected strain in the present study had a P8 antigen which is included in the monovalent Rotarix™ vaccine, so severe gastroenteritis due to G1P8 and G9P8 strain in Sudan is otherwise expected to wane by implementing the current vaccine.

It has to be mentioned that this study dealt with strains isolated from ElBuluk pediatric hospital in Khartoum state, Sudan but the surveillance was limited to hospitalized patients only, furthermore the period of surveillance was only 6 month. Conducting a surveillance which is community based and carrying it for a longer period can produce more accurate results in terms of characterization of rotavirus strains and in order to reflect true prevalence.

**Conclusions**

As a conclusion the VP7 predominant G type was G1, followed by G9 whereas only one VP4 genotype was detected and showed high similarity to P8 Genbank strain. It appears that the recently approved rotavirus vaccines in Sudan are well matched to the rotavirus genotypes identified at these hospitals, though larger studies are needed.
Competing interests

Authors declare no competing interests.

Authors’ contribution

MAM, NEB and NI designed the study and coordinated and helped to draft the manuscript. MAM, BKE carried out the data collection and molecular genetic studies, participated in the sequence alignment and drafted the manuscript. MAA participated in the laboratory work and sequence alignment. JAB, GIG participated in the statistical analysis and drafted the manuscript. All authors read and approved the final manuscript.

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Figure 1. A flowchart illustrating the process of sampling ending up with the results:

1. 755 patients presenting with diarrhea
2. 121 patients were positive for rotavirus using ELISA
3. 24 samples underwent sequencing
4. 4 patients were G9 P8
5. 20 patients were G1 P8