

Frequency of Rotavirus among under 5 Children Admitted to Wad Madani Pediatric Teaching Hospital with Diarrhea, Gezira State, Sudan (2021-2022)

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How to cite this paper: Babekir, R.I.A., Babiker, A.M. and Nour, B.Y.M. (2024) Frequency of Rotavirus among under 5 Children Admitted to Wad Madani Pediatric Teaching Hospital with Diarrhea, Gezira State, Sudan (2021-2022). *Open Journal of Medical Microbiology*, **14**, 206-214.

<https://doi.org/10.4236/ojmm.2024.143015>

Received: February 16, 2023

Accepted: September 23, 2024

Published: September 26, 2024

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Abstract

Rotavirus is the leading cause of acute gastroenteritis and responsible for 20% of death in children under 5 years of age approximately 111 million episodes of gastroenteritis due to *Rotavirus* in under 5 children, which result in 25 million visits to clinic, 2 million hospitalization and 352,000 - 592,000 deaths. Children in the poorest countries account 82% of rotaviruses death. Diarrhea disease are major cause of death and disease among children under five years, a child on average suffers 2 to 3 attacks of diarrhea every year. Stools samples were collected from 196 children suffering from diarrhea and were tested for rotaviruses by immune chromatography test antigen (ICT-Ag), ELISA and RT PCR The data were analyzed using statistical package for the Social Sciences (SPSS). The results showed that prevalence of rotavirus was 26/196 (13.3%) by ICT Ag and ELISA and confirmed by RT-PCR. 87.8% of the children were vaccinated by Rota vaccine, 70% of their mothers not using soap for cleaning the children and thought diarrhea not infectious disease and believed it was caused by teething. In conclusion there was a decrease in prevalence of diarrhea by Rotavirus due to insertion of Rota vaccine in routine vaccination, rotavirus infection mostly occurs in poor family that not able to provide safe water and due to poor sanitation and low education of mother beside that some children suffered from malnutrition, so it recommended to incorporate ELISA as a diagnostic tool in routine diagnosis of rotavirus among children suffered from gastroenteritis.

Keywords

Diarrhea, *Rotavirus*, Under 5, Laboratory Diagnosis, Sudan

1. Introduction

Diarrhea disease remains one of the leading causes of preventable death in developing countries especially among children under five years of age, diarrhea is common in the developing countries especially in areas with poor hygiene and sanitation and with limited access to save water [1]. Diarrhea remains a leading cause of childhood morbidity and mortality in many parts of the world, especially in Africa, Asia and South America, it increases health care costs by admission to hospital and increasing the need of investigation, treatment and nursing care [2]. Rotavirus is the leading cause of acute gastroenteritis and responsible for 20% of death in children under 5 years of age [3].

Rotaviruses are the member of family Reoviridae and characterized by their segmented double stranded RNA genome, 70 nm, non-enveloped icosahedra structure [4]. There are six structural viral proteins (VPs) that form virus particle called VP1, VP2, VP3, VP4, VP6 and VP7. In addition to the VPs there are six nonstructural proteins (NSPs), that are only produced in cells infected by Rota virus [5]. Rotaviruses are shed in high concentrations in stool of infected children and are transmitted primarily by fecal-oral route [6]. Also can be transmitted by water and food and respiratory droplets [7]. Incubation period 1-3 days, symptoms include watery diarrhea, abdominal pain, fever and vomiting (severe loss of electrolytes and fluids leading to dehydration which is fatal unless treated), symptoms of dehydration include (decreased urination, dry mouth and throat—feeling dizzy when standing up, crying with few or no tears and unusual sleepiness) also infection may be asymptomatic [8]. Estimated range from 3 - 5 billion for annual diarrhea episodes in children under 5 years of age in Africa, Asia and Latin America, resulting in 1 million death [8].

Ling and Cheng *et al.* (1993), studied the role of enteric pathogen in children in Hong Kong Gastro enteric salmonella were the most common pathogens (45%) followed by Rota virus (34%) and E coli (1%) [9]. In study carried in Gaza, Palestine, rotavirus was detected in 28% of the fecal specimens examined and 90% of patients who were positive for the virus were aged 1 - 24 month and the infections rate decrease with increasing age [10]. A recent study carried out in Sudan by WHO during Jan-Dec, 2009 indicate that Rotavirus causes 42% of childhood diarrhea hospitalization [11]. Recent study carried out in Khartoum state by Nussiba Mustafa 2013 indicated that (30%) of childhood diarrhea due to rotavirus infection. The aim of this study was to determine the frequency of Rotaviruses among children under five years of age admitted Wad Medani Pediatric Teaching Hospital in Central Sudan, during January 2021 to March 2022. Using different serological and molecular techniques.

2. Methodology

2.1. Study Design and Study Area

This is a prospective descriptive study to determine the frequency of Rotavirus among Children under 5 years admitted Wad Medani Pediatric Teaching Hospital with diarrhea Gezira State. The Study conducted during the period (Jan 2021-march 2022). This hospital in the center of Wad Medani City and has important

rule in treating the children in Wad Medani City and all villages around Wad Medani also its teaching hospital.

2.2. Study Population and Subjects Selection

From the attendee at outpatient's clinic in Wad Medani Pediatric Teaching Hospital, Gezira State, Sudan, between January 2021-March 2022, Stools samples were collected from 384 children less than 5 years old suffered from diarrhea. The children age was from 5 month to 5 years with a mean age of 1.26 years.

2.3. Data Collection and Analysis

Structured questionnaire was designed including demographic and clinical signs and the results of the different laboratory techniques used in this study. (all children under five in the word of diarrhea in the Wad Medani pediatric Teaching Hospital during study period were included in this study all tripe and both sex included (children above five years were excluded)

2.4. Sample Preparation

196 Stool samples were taken in clean containers by simple random sampling and processed as soon as possible to guarantee the quality of the test as the fresh samples are recommended for the laboratory tests.

For longer storage, the samples were stored at -20°C . In this case, the sample will be totally thawed and brought to room temperature before testing. Homogenize stool sample as thoroughly as possible prior to preparation. Freezing and thawing cycles are not recommended.

2.5. Diagnosis of Rotavirus

Rotavirus Combo Rapid Test Cassette (Feces) (ICT Ag), Package Insert (REF IMVD-645|English) were used. After preparation of samples, 80 ML was added to ICT strip, after migration of sample the reaction were observed, the result considered as positive when 2 lines appeared in while 1 line reported as negative.

ELISA: (RIDASCREEN Rotavirus) Co 901:

Washing buffer was prepared and I part of it was concentrated with a part distilled water.

Preparing the Specimens:

100 ML of stool sample were added to the diluted buffer; then the mixture was homogenized in vortex mixer, then Incubated for 10 min.

First Incubation:

Then 100 ML of positive control, the Negative control or stool sample suspension added to the well, and 100ML of biotin Conjugated antibody (conjugate 1) added and incubated for 60 minutes then washed using wash buffer 5 times (each time use 300 ML wash buffer).

Second Incubation:

100 ML streptavidin poly-peroxidase conjugate (conjugate2) were added to the

well then incubated for 30 minutes and then washed (similar to the first wash).

Third Incubation:

All wells were filled with 100ML substrate (substrate) then incubated for 15 minutes in darkness at room temperature, all wells were filled with 50 ML stop reagent and measured the extinction at 450 nm.

2.6. RNA Extraction

Automatic, commercially method was used to extract RNA of rotavirus from the stools according to the manufacturer's instructions in the kit (ABT Beijing Applied Biological Technologies Co. Ltd (Z C H S-C-YF-TQ03-06).

2.7. RT PCR

Detection of rotavirus RNA by RT PCR Kit (genetic PCR Solutions RNA detection RT qPCR (spin) was designed for the diagnosis of gastroenteritis in human stool samples. The detection was done in one step real time RT format where the reverse transcription and the subsequent amplification of specific target sequence occur in the same reaction well. The isolated RNA targeted was transcribed generating complementary DNA by reverse transcriptase which was followed by the amplification of a conserved region gene using specific primers and a fluorescent-labeled probe.

Rotavirus RT-PCR Detection Kit is based on 5' exonuclease activity of DNA polymerase. During DNA amplification, this enzyme cleaves the probe bound to the complementary DNA sequence, separating the quencher dye from the reporter. This reaction generates an increase in the fluorescent signal which is proportional to the quantity of the target template. This fluorescence could be measured on RT-PCR platforms (Bio Rad).

The following were the Preparation of master mix.

DNase/RNase free water (GREEN CAP)	9 μ l
GPSTM-mix-RT (BLUE CAP)	5 μ l
Target Species dtec-RT-qPCR-mix (AMBER TUBE)	1 μ l
Reaction pre-mix volume	15 μ l

5 microliter of samples or diluted standard template were added to each PCR tube to reach final PCR volume 20 microliter.

Machine Protocol (Bio Rad)

	Step	Time	Temperature
40 Cycles	Retro transcription	10 min	50°C
	Activation	2 min	95°C
	Denaturation	5 sec	95°C
	Hybridization/Extension and data collection	20 sec	60°C

Fluorogenic signal was collected during this step by using the FAM channel for the target and by using the HEX channel for the internal control. Then after at the end the result was reported.

2.8. Data Analysis

Data were analyzed by Statistical Package for the Social Sciences SPSS (version 20).

2.9. Ethical Consideration

For this study, ethical approval was obtained from the ethical committee of the Gezira State Ministry of Health and signed informed consents were obtained from the guardians of the participants.

3. Results

The Study was conducted during the period from January 2021 to March 2022 to identify Rotavirus A that cause diarrhea among under5 children in Wad Medani pediatric teaching hospital. From the total 196 stool samples we found that the prevalence of Rotavirus was (13.3%). Most of child infected by Rotavirus within age groups (less than one year) and incidence of *Rotavirus* infection decrease with increases of age and demographic and clinical characteristics of patients, and the residence of the enrolled subjects were 80.7% in rural and the urban were 19,3% as indicated in (Table 1), (46%) of the patient had fever and (80%) had vomiting, (87.8%) of children were take Rota vaccine, the study found there is No difference between prevalence of diarrhea between male (50%) and female (Table1). The study show that prevalence of Rotavirus infection is higher in the autumn (54%) than other season Figure 1.

Table 1. Demographic and clinical characteristics of patients.

Sex	13 Male (50%)
	13 Female (50%)
Age	Less than 1 year = 20
	1- 2 years = 5
	2 - 3 years = 1
Fever	12 have fever (46%)
Vomiting	21 have vomiting (80.7%)
Residence	Rural = 21 (80.7%)
	Urban = 5 (19.3%)
Rota vaccine	(87.8%) take Rota vaccine

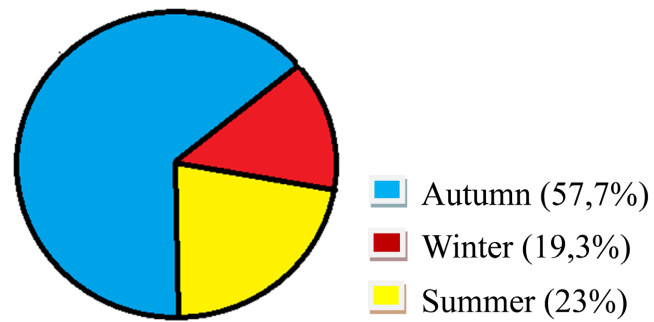


Figure 1. Distribution of study population according to season.

This study found ICT sensitivity (89.3%) and specificity (95.8%) while ELISA sensitivity (96.4%) and specificity (100%) (**Figure 2, Figure 3, Figure 4**).

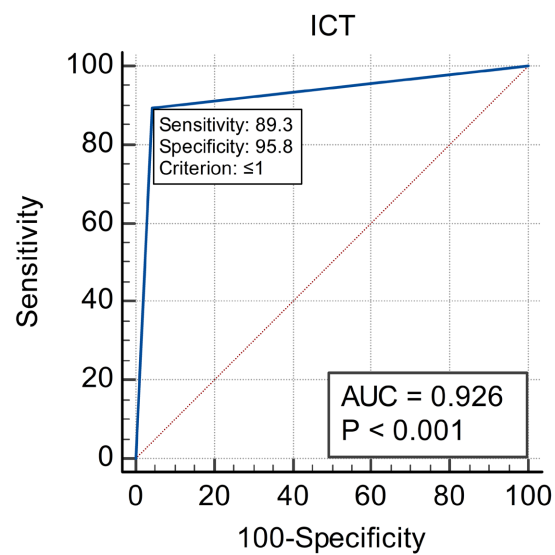


Figure 2. Sensitivity and specificity of ICT.

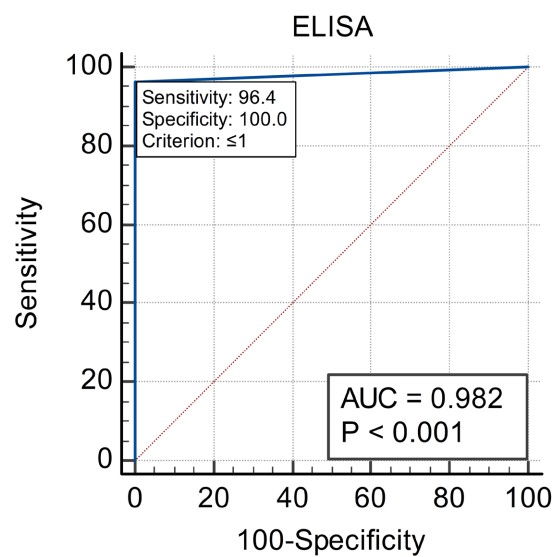


Figure 3. Sensitivity and specificity of ELISA.

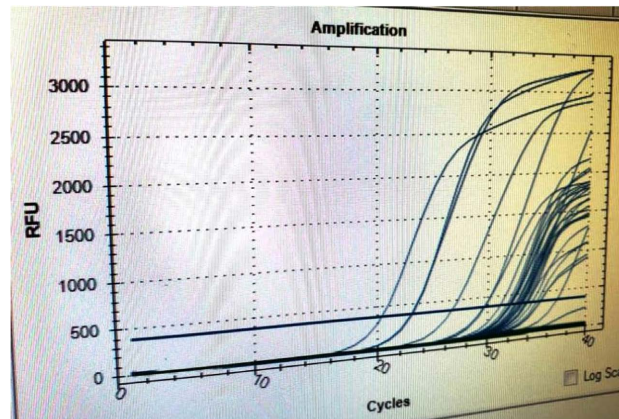


Figure 4. RT PCR.

4. Discussion

Diarrheal diseases remain a serious public health and causes morbidity and mortality worldwide. Although diarrhea in children cannot be completely eliminated, a reduction of infection rate to a minimal level could have significant benefits by reducing diarrhea morbidity and mortality.

Each year rotaviruses cause approximately 111 million episodes of gastroenteritis in children, which result in 25 million visits to clinic, 2 million hospitalization and 352,000 - 592,000 deaths children in the poorest countries account 82% of rotaviruses death [12]. Therefore, periodic molecular detection of organism's level and elimination is crucial to prevention of infection. In the present study found Prevalence of Rotavirus in (13.3%) of population and (86.7%) not found Rotavirus because gastroenteritis in those children cause by other virus, or parasite or bacteria. In this Present study found Prevalence of Rotavirus (13.3%) which considered less in comparison with study conducted by [9] which found Rotavirus (34%) because this study is very old and in that time. Rotavirus vaccine is not apply in Routine vaccinations [9] Also Result of our study less that study conducted by WHO in Sudan during Jan-Des 2009 which found. Rotavirus causes (42%) of childhood diarrhea hospitalization [13]. And also less than study conducted by [10] in Gaza Palestin in which Rotavirus (28%)

Most Rotavirus infection occur in autumn. This may be due to increase mainly multiplication of insect like house fly during autumn which increase the transmission of Rotavirus also children more attract to play in the ground in autumn and may contaminate hands with Rotavirus. This result agrees with result conducted by Mohamed Babekir in Wad Madani Pediatric Teaching Hospital in 2015 which found Rotavirus infection increase in autumn The Rate of diarrhea in Rural more than rate of diarrhea in urban due to different environment and education and economic condition which agree with Mohamed Babekir study in 2015. The Prevalence of Rota virus infection is higher in children less than one year may be due to the immunity is not well developed and inter food meals and drink to the child after 4 months which may be contaminated by virus this disagree with study conducted by Abelameeren 2006 in Gaza Palestine in which found 90% of patient (1 - 24) month

and prevalence of Rotavirus 28%. Our study result less than result of WHO which found prevalence of Rotavirus 42%. Most of child infected by Rotavirus (80%) suffer from vomiting and therefore more susceptible to dehydration due to acute diarrhea and vomiting. This study showed decrease in the prevalence of Rota virus from 28% in 2015 to 13.3% in (2021-2022). Our study found there is no difference between female and male and this result disagree with result of study conducted by Roy et al 2012 in which prevalence of Rotavirus infection in male more than in female due to different geographic and environmental condition (14).

In the present study ICT and ELISA and RT-PCR were used for identification of Rotavirus gastroenteritis in under five children. This study found the best standard method used for routine diagnosis of Rotavirus is ELISA because sensitivity (96.4%) and specificity (100%) which more better than ICT which its sensitivity was 89.3% and its specificity was 95.8,) also more useful than RT-PCR although it is the gold standard and this is attributed to its high cost: machine, reagents and high qualified experts, so renders it not suitable for the routine diagnostic tool.

5. Conclusion

This study shows prevalence of Rotavirus is (13.3%). The incidences of disease increased during the autumn with high rate among infant (less than one year) Eighty percent (80.7%) of the study population was rural, (19.3%) was urban, (46%) developed fever, (80%) developed vomiting. Although PCR is gold stander method and more sensitive and specific but very expensive and time-consuming and not available reagents and needs expertise Person and expensive PCR machine, so ELISA more suitable for clinical diagnosis of Rotavirus in Sudanese population (better than ICT) (ICT sensitivity (89.3%) and specificity (95.8%)). This study showed definite impact of Rotavirus vaccine in reducing the prevalence of Rotavirus from (42%) before the implementation of vaccine to (13.3%) at the present time.

Acknowledgements

The authors thank all patients who participated in this study and acknowledgements extended to the laboratory staff of molecular labs in the Faculty of Medical laboratory Sciences in University of Gezira and Tropical Institute for Medical Research for their technical assistance.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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