Frequency of Rotavirusamong under 5 Children admitted to Wad Madani PediatricTeaching Hospital with diarrhea, Gezira State, Sudan (2021 -2022)

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 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 suffer 2 to 3 attacks of diarrhea every year ,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. Stools samples were collected from 384 children suffering from diarrhea and were tested for rotaviruses by immune chromatography test antigen (ICT - Ag), ELISA and RTPCR. The data were analyzed using statistical package for the Social Sciences (SPSS). The results showed That prevalence of rotavirus was 51/384 (13.3%) by ICT AgandELISA and confirmed by RT-PCR. 337/384 (87.8%) of the children were vaccinated by Rota vaccine, 70% of their mothers not used soap for cleaning the children and thought diarrhea not infectious disease and believed itwas caused by teething. In conclusion there wasa decrease in prevalence of diarrhea by Rotavirus due to insertion of Rota vaccine in routine vaccination, rotavirus infection mostly occur in poor family that not able to provide safety water and due to the poor sanitation and low education of motherbeside 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, laboratorydiagnosis. Sudan

Introduction:

Diarrhea disease remain one of the leading cause 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 (WHO, 2008).Diarrhea remain a leading cause of childhood morbidity and mortality in many parts of the world, especially in Africa, Asia and South America, it increase health care costs by admission to hospital and increasing the need of investigation, treatment and nursing care (Sherchand et al 2009). Rotavirus is the leading cause of acute gastroenteritis and responsible for 20% of death in children under 5 years of age (Natro J.P *etal* ,1999).

Rotaviruses are the member of family Reovirdae and characterized by their segmented double stranded RNA genome, 70 nm , non enveloped icosahedra structure (Knipe et al , 2007). 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.(desselberger and Gray, 2009). Rotaviruses are shed in high concentrations in stool of infected children and are transmitted primarily by fecal -oral rout(parshar,et al,2006).Also can be transmitted by water and food and respiratory droplets (Gray et al,2008).Incubation period1-3 days, symptoms include watery diarrhea, abdominal pain, fever and vomiting (sever 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 a symptomatic (Brooks et al , 2010). 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 (Brokoks , et al ,2010).

Ling and Cheng et al (1993) , studied the role of entericpathogen in children in Hong Kong Gastro enteric salmonella were the most common pathogens (45%) followed by Rota virus (34%) and E coli (1%) (Ling .J M, 1993).In study curried 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 (AbuElamrrEan, et al, 2006) . A recent study carried out in Sudan by WHO during Jan-Des,2009 indicate that Rotavirus causes 42% of childhood diarrhea hospitalization (WHO, 2009). Recant study carried out in Khartoum state indicated that (30%) of childhood diarrhea due to rotavirus infection (Nussiba Mustafa, et al, 2013).

Methodology:

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.

Study population and subject selection

384 diarrhea sample were taken by simple random sampling fromchildren under 5 years old attending Wad Medani Pediatric teaching hospital, (Gezira State)with diarrhea during study period (2021 - 2022) (50%) male minimum age 5 month, maximum age 5 years , mean age (1.26) , (46%) had fever , (80.7%) had vomiting (Child above 5 years were excluded) also ethical permission was taken from the ministry of health and from the faculty of medical laboratory science, permission from the head director of the hospital and consent from patient's family.

Comment [M1]: Rewrite

Comment [M2]: Mention the months

Data Collection and Analysis

A well designed questionnaire includes personal information, clinical data and laboratory tests results

SAMPLE PREPARATION:

Stool samples were collected in clean containers 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 -20ºC. 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.

Diagnosis of Rotavirus:

Rotavirus Combo Rapid Test Cassette (Feces) (ICT Ag), Package Insert(REF IMVD-645 | English) were used. After preparation of samples, 80ML 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:

Comment [M3]: What is type of ELISA and what is the manufacture

Washing bufferwas 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 dilutedbuffer;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 to the well, add 100ML of biotin Conjugated antibody (conjugate 1) Incubate for 60 min thin wash using wash buffer 5 time (each time use 300 ML wash buffer).

Second incubation:-

Add 100ML streptavidin poly- peroxidase conjugate (conjugate2) into the well then incubated for 30 minutes and then washed (similar to first wash).

Third incubation:-

Comment [M4]: Delite

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

Comment [M5]: What is the OD value and calculation
Or how read the results

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)

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RTPCR:

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 reversetranscription 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 µl
GPSTM-mix-RT (BLUE CAP)	5 µl
TargetSpeciesdtec-RT-qPCR-mix (AMBER TUBE)	1µl
Reaction pre-mix volume.	15 µl

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

Machine Protocol (BioRad)

	Step	**Time**	**Temperature**
	Retrotranscription	10 min	50 °C
	Activation	2 min	95 °C
40 Cycles	Denaturation	5 sec	95 °C
	Hybridization / Extensionand data collection	20 sec	**60 °C**

Flurogenic 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

Comment [M6]: Pleas rewrite the paragraph also why mention the all steps of ELISA and PCR

Not

Comment [M7]: Add the statical analysis

Result

The Study was conducted during the period from January 2021 to March 2022 to identify Rotavirus A that cause diarrhea among under 5 children in Wad Medani pediatric teaching Hospital.From the total 196 stool samples(in which causative agents identified) found prevalence of Rotavirus (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 (Figure1)(46%)of patienthad fever and (80%) had vomiting , (87.8%) of children were take Rota vaccine , the study found there is Nodifference 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 (2)most of patient residesin rural(80.7%).(Table2)

This study found ICT sensitivity (89.3%) and specificity (95.8%) while ELISA sensitivity (96.4%) and specificity (100%)(Figure 3, Figure 4, Figure 5)

Comment [M8]: Where are the results of ELSA and PCR
You most add
In addition to can make table for the clinical signs

Table (1) : Demographic and characteristic of study subject

Sex	13Male (50%) 13Female (50%)
Fever	12 have fever (46%)
Vomiting	21 have vomiting (80,7%)
Rural	21 Reside in rural(80,7%)
Rota vaccine	(87,8%) take Rota vaccine

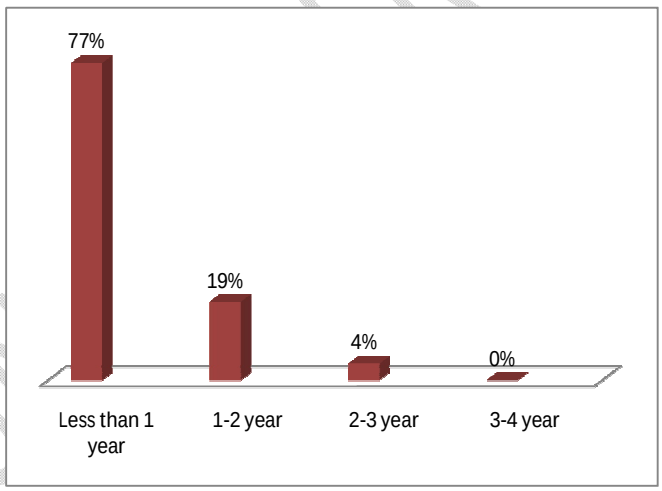


Figure (1) : Distribution of Children infected by Rotavirus according to age.

Table (2): Distribution of study population according to Residence

Rotavirus	Rural	Urban
Positive	21	5
Negative	270	88

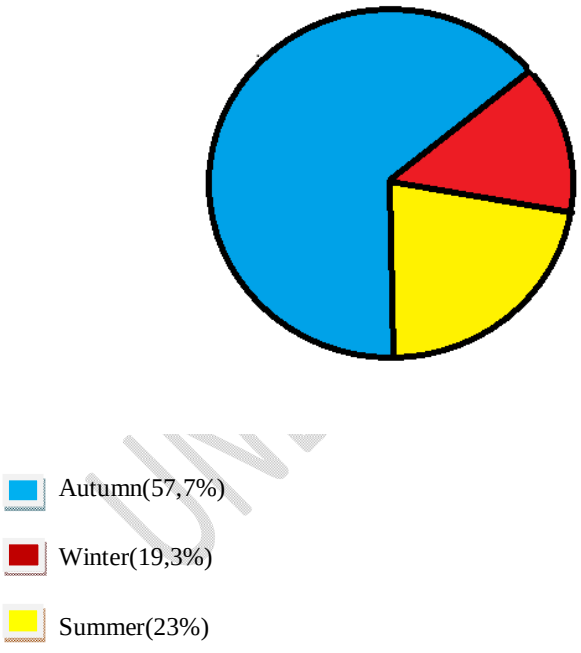
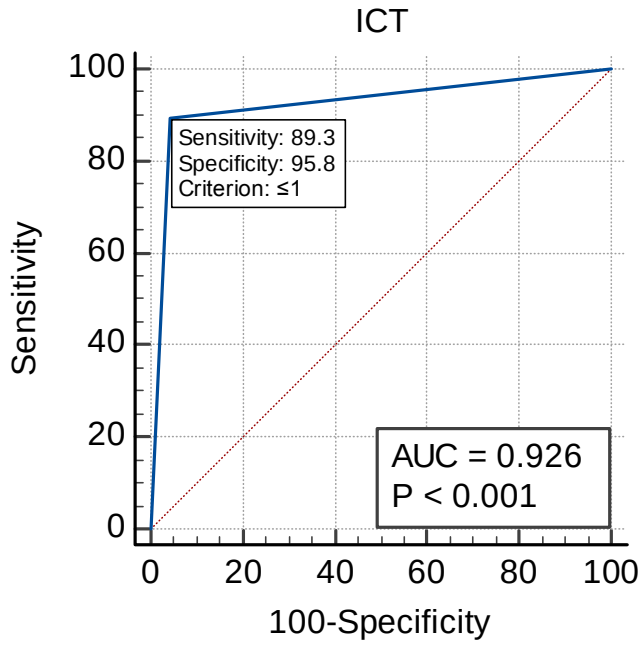


Figure (2) :Distribution of study population according to season



Figure(3): Sensitivity and Specificity of ICT

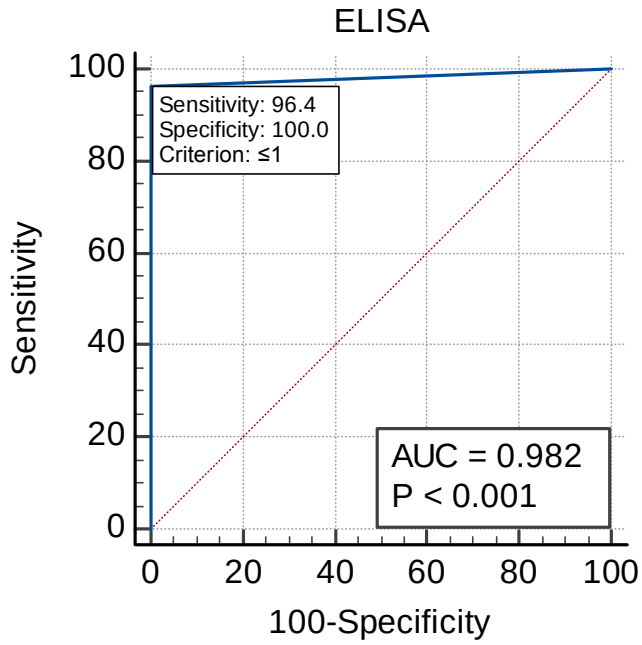


Figure (4):Sensitivity and Specificity of ELISA

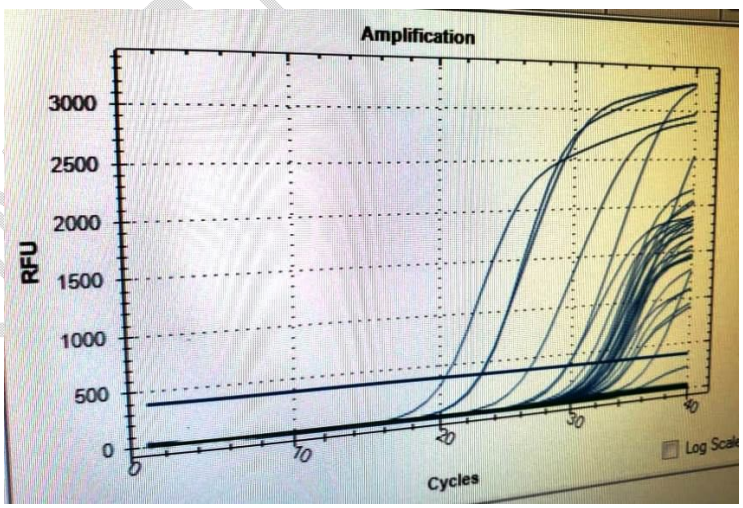


Figure (5):RT PCR

Discussion:

diarrhea 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 poorest countries account 82% of rotaviruses death .(Ramig , 2004). 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 (ling and cheng et al 1993) which found Rotavirus (34%) because this study is very old and in that time. Rotavirus vaccine is not apply in Routine vaccinations (ling and cheng et al 1993) Also Result of our study less that study conducted byWHO in Sudan during Jan-Des 2009 which found.Rotavirus causes (42%) of childhood diarrhea hospitalization (WHO 2010). And also less thanstudy conducted by(AbELAMeeren 2006) in Gaza Palestin in wichRotavirus (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 ofRotavirus also children more attract to play in the ground in autumn and may contaminate hands with Rotavirus. This result agree with result conducted by Mohamed Babekir in Wad Madani Pediatric Teaching Hospitalin 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 oneyearmay be due to the immunity is not well developed and inter food meals and drink to the child after 4 months whichmaybe contaminated by virus this disagree with study conducted by AbELAMeeren 2006 in Gaza Palestine in which found 90% of patient (1-24)month.Most of child infected by Rotavirus (80%) suffer from vomiting and thereforemore 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).

In the present study ICT and ELISA and PCR were used for identification of Rotavirus gastroenteritis in under five children.This study found the best standard method used for retain diagnosis of Rotavirus is ELISA because sensitivity (96.4%) and specificity (100%)which more better than ICT which sensitivity (89.3%) and specificity (95.8%) also more useful than PCRwhich is very expensive and Time consuming and not available reagents and need expertise Person and expensive PCR machine so although its gold standard, method.not suitable for routinediagnosis.

Conclusion:

This study show prevalence of Rotavirus is (13.3%). The incidences of disease increase 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 need expertise Person and expensive PCR machine, So ELISA more suitable for clinical diagnosis of Rotavirus in Sudanese population (which 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 .

References:

Comment [M9]: References are little and add more recent references

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