Frequency of Norovirus among Children with Gastroenteritis in Khartoum State

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قال تعالى:

(الَّذِينَ قَالَ لَهُمُ النّاسُ إِنّ النّاسُ قُدّ جَمَعُوا لَكُمُ فَاحْشَؤُهُمْ قَرَادُهُمْ إِيمَانًا)

وقالوا: حسبنا الله ونعم الوكيل.

سورة آل عمران، الآية 173
DEDICATION

To

My mother, father, great brothers and husband
Acknowledgment

First of all great thank to AL-Mighty ALLAH for offering the power to complete this work.

I am greatly indebted to my Supervisor Prof. Humodi Ahmed Saeed, for his constructive guidance, support and encouragement all throughout this study.

Thanks go to the staff members of Ibrahim Malik Teaching Hospital for help and cooperation.

My appreciation are extended to the staff members of microbiology Department and all colleagues in Sudan University of science and technology.

I also owe may thanks to the patients and their parents for their understanding and cooperation during the study period.
ABSTRACT

*Norovirus* is the most common cause of viral gastroenteritis in human.

This study was carried out in Khartoum State during the periods from January to September 2016. The study aimed to detect *Norovirus* antibody in children with gastroenteritis.

A total of eighty four (n= 84) children with gastroenteritis were enrolled in this study. Three ml blood was collected from each patient. Serum was obtained by centrifugation at 3000 rpm for 20 minutes. The sera was examined for the presence of *Norovirus* antibody using Enzyme Linked Immune Sorbent Assay (ELISA).

The results revealed that 40(47.6%) were positive of *Norovirus* antibodies. The rest 44(52.4%) were negative. Of the positive blood samples 18(45%) were obtained from males and 22(55%) from females, and 25(62.5%) were less than 2.5 years and 15(37.5%) more than 2 years.

The study concluded that there is considerable percentage of *Norovirus* among children with gastroenteritis under five years in Khartoum state.
المستخلص

نورو فيروس هو سبب شائع لالتهاب المعدة والأمعاء في الإنسان. قد اجريت هذه الدراسة في مدينة الخرطوم خلال الفترة من يناير وحتى مارس 2016. وذلك للكشف عن الجسم المضاد للنورو فيروس عند الأطفال المصابين بالتهاب المعدة والأمعاء.

تم جمع 84 عينة دم من الأطفال المصابين بالتهاب المعدة والأمعاء، ثم جمع 3 مل من الدم من كل مريض ثم فصل منها المصل من العينات وخضعت للفحص عن الجسم المضاد للنورو فيروس باستخدام الاليزا. ظهرت النتائج أنه من مجموع 84 عينة فحصت 40 عينة اظهرت نتائج ايجابية، بينما 44 عينة اظهرت نتائج سلبية. وجمع عينات الإيجابية كانت (45%) 18 عينة من الذكور بينما (55%) 22 عينات انتان، (62.5%) 25 اعمارهم اقل من سنتين ونص، (37.5%) 15 اعمارهم أكثر من سنتين ونص.

وخلصت هذه الدراسة إلى ان هناك نسبة كبيرة من نورو فيروس بين الأطفال السودانيين المصابين بالتهاب المعدة والأمعاء دون سن الخامسة.
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CHAPTER ONE
INTRODUCTION AND OBJECTIVES
CHAPTER ONE
INTRODUCTION AND OBJECTIVES

1.1. Introduction

*Norovirus* was first discovered from an outbreak of gastroenteritis in an elementary school in Norwalk, Ohio U.S.A in 1968 (Hansman *et al.*, 2004). It was the first virus identified as cause of gastroenteritis in humans, but recognition of its importance as a pathogen has been limited because of the lack of available, sensitive, and routine diagnostic methods (Roger *et al.*, 2009). The virus causes the majority of acute viral gastroenteritis worldwide. The increased recognition of *Noroviruses* as the cause of outbreaks and sporadic disease is due to the recent availability of improved *Norovirus*-specific diagnosis. Transmission of these viruses is facilitated by their high prevalence in the community, shedding of infectious virus particles from a symptomatic individuals and the high stability of the virus in the environment (Anne *et al.*, 2004).

*Gastroenteritis* is a major public health problem worldwide. With the application of new and sensitive diagnostic techniques *Noroviruses* are now recognized as the
leading cause of outbreaks and cases of non-bacterial gastroenteritis (Du ping zheng et al., 2006).

The two types of caliciviruses that cause acute gastroenteritis in humans have been identified, those in genus *Norovirus* and those in genus *Sapovirus*. *Norovirus* is a leading cause of gastroenteritis worldwide and cause outbreaks in various epidemiological setting including hospitals, cruiseships, schools and restaurants (Hansman et al., 2004).

*Noroviruses* are one of the leading causes of gastroenteritis in young children; however, the duration of *Norovirus* shedding in young children is not well known (Murata et al., 2007).

1.2. Rationale

*Norovirus* is one of the leading causes of gastroenteritis in young children (Murata et al., 2007). Epidemiological data from developing countries about the importance of *Norovirus* in pediatric diarrhea are limited. Recently, in Nicaragua, it has been observed that *Norovirus* is responsible for 11% of the diarrhea cases occurring in
children less than five years of age at community level and 15% of the moderate to severe cases requiring intravenous (Bucardo et al., 2011).

The *virus* affect people of all ages (Morillo and Timenetsky, 2011). Recent findings indicated that *Norovirus* is the most common cause of gastroenteritis (Kroneman et al., 2006).

Revising the literature, there is no previous published data in Sudan about *Norovirus*. This study was to detected *Norovirus* among Sudanese children with gastroenteritis.
1.3. Objectives

1.3.1. General objective

To determine the frequency of *Norovirus* among Sudanese children with gastroenteritis in Khartoum state.

1.3.2. Specific objectives

1. To detect the *Norovirus* antibody in children with gastroenteritis and to detect the frequency of virus among children.

2. To determine the association between *Norovirus* infection and child age and sex.
CHAPTER TWO

LITERATURE REVIEW
CHAPTER TWO

LITERATURE REVIEW

2.1. Norovirus

The *Norovirus* was originally named the “Norwalk agent” after Norwalk Ohio, in the United states, where an outbreak of a cute gastroenteritis occurred among children in Bronson Elementary school in November 1968 (Hansman *et al.*, 2004).

In 1972, electron microscopy was conducted on stored human stool samples identified a virus, which was given the name “Norwalk virus”. Numerous outbreaks with similar symptoms have been reported since that time. The cloning and sequencing of the Norwalk virus genome showed that this virus has a genomic organization consistent with viruses belong to the family Caliciviridae (Kapikian, 1996). The name was shortened to “*Norovirus*” after being identified in a number of outbreaks on cruise ships and receiving attention throughout the United States. The name “*Norovirus*” was approved by the international committee on Taxonomy of virus (ICTV) in 2002; however, a press release and a newsletter were published by ICTV, which strongly encouraged the media, national health authorities and the scientific community to use the virus name Norwalk virus, rather than the genus name *Norovirus*, when referring to outbreaks of the disease (Kapikian, 1996).
Norovirus is RNA, non-enveloped virus which belongs to the Caliciviridae family. According to ICTV Noroviruses are genetically diverse group of single-strand viruses, the genus Norovirus has one species, which is called Norwalk virus (Eric et al., 2011). Noroviruses are the most common cause of viral gastroenteritis in human, and affect people of all ages (Morillo and Timenetsky, 2011). Norovirus infection is characterized by nausea, forceful vomiting, watery diarrhea, and abdominal pain, and in some cases, lost of taste. General lethargy, weakness, muscle aches, headache, coughs, and low-grade fever may occur. The disease is usually self-limiting, and severe illness is rare. The virus affects around 267 million people and cause over 200,000 deaths each year; these deaths are usually in less developed countries and in the very young, elderly and immune compromised patient (Debbink et al., 2012). The species causes approximately 90% of epidemic non-bacterial outbreaks of gastroenteritis in the United States (Widdowaon et al., 2005).

“Winter vomiting bug” is a common term for Noroviruses in the UK, because the virus tends to cause vomiting and to spread more easily in winter, when people tend to spend more time indoors and close to each other. Outbreak of Norovirus infection often occurs in close or semi closed communities, such as long-term care facilities, overnight camps, hospital, prisons, dormitories, schools and cruise ships. Where the
infection spread very rapidly either by person to person transmission or through contaminated foods (Noda et al., 2007).

2.2. Transmission

Norovirus are transmitted directly from person and indirectly via contamination water and food. They are extremely contagious, and fewer than twenty virus can case an infection (Morillo and Timenetsky, 2011). Some researches suggests as few as five (Leon, 2008). Transmission occur through ingestion contamination food and water by person to person spread, transmission can be aerosolized when those stricken with the illness vomit, and can be aerosolized by a toilet flush when vomit or diarrhea is present; infection can follow eating food or breathing air near an episode of vomiting, even if cleaned up. The viruses continue to be shed after symptoms have subsided and shedding can still be detected many weeks after infection (Atmar et al., 2008). In one outbreak at an international scout jamboree in the Netherland, each person with gastroenteritis infected an average of 14 people before increased hygiene measures were put in place. Even after these new measure were enacted, an ill person still infected an average 2.1 other people (Heijne et al., 2009).
2.3. Pathophysiology

When a person becomes infected with *Norovirus* the virus begins to replicate within the small intestine. After approximately one to two days symptoms can appear. The principal symptom is acute gastroenteritis that develops between 24 and 48 hours after exposure, and lasts for 24-60 hours. The disease is usually self limiting, and characterized by nausea, forceful vomiting, watery diarrhea, abdominal pain, and in some cause, loss of taste. General lethargy, weakness, muscle aches, headache, coughs, and low-grade fever may occur. Severe illness is rare; although people are frequently treated at the emergency ward, they are rarely admitted to the hospital. The number of deaths from *Norovirus* in the United State is estimated to be around 300 each year, with most of these occurring in the very young, the elderly, and persons with weakened immune systems (Goodgame, 2006). *Norovirus* are extremely infectious and cause epidemic gastroenteritis. The infectious dose is believed to be as low as 10-100 virus particles (Caul, 1996). Recent dose-response studies show that both the infective dose and host susceptibility may vary according to the infecting *Norovirus* strain (Lindesmith *et al*., 2005; Moe *et al*., 2003). The mechanism of pathogenicity of *Noroviruses* is still not clearly understood because of the inability to propagate these viruses, but information is being obtained from the in vitro culture of a mouse *Norovirus* (Wobus *et al*., 2004). It is known that the mature enterocyte cells
in the small intestine become infected and the malabsorption of fats, d-xylose, and lactose occurs for up to 2 weeks. Unusually, gastric emptying is also delayed, and this may explain the nausea and characteristic projectile vomiting associated with *Norovirus* infection. Large numbers of *Norovirus* are excreted in feces from the onset of symptoms and continue to be shed in decreasing numbers for up to weeks after infection. Animals infected with the Newbury agent, bovnic calicviruses assigned to *Norovirus* genogroup 111, show similar symptoms, pathological changes and processes as seen in human (Appleton *et al*., 2001). In the past in absence of reliable laboratory tests for *Norovirus* developed epidemiological and clinical criteria were stools negative for bacterial gastroenteritis outbreak (Kaplan *et al*., 1982). These criteria were stool negative for bacterial pathogens, a mean or incubation period of 24-48hrs. These criteria are still widely used. The symptoms of acute-onset projectile vomiting, watery non-bloody diarrhea with abdominal cramps, and nausea may develop within 12 hrs of exposure, and low-grade fever also occurs occasionally. Dehydration is a common complication that can particularly affect the young and elderly, necessitating rehydration therapy. There is no evidence of any long-term sequels after *Norovirus* infection (Kaplan *et al*., 1982). The mechanism of immunity to *Norovirus* infection is not clear. Infection normally stimulates production of both gut and serum antibody, and although immunity to the infecting *Norovirus* strain may develop, it is generally short lived, strain-specific, and does not confer protection
against future infection. Reinfection with a different strain can occur soon after the initial infection. Thus, given the genetic variability of *Norovirus*, people are likely to be re-infection many times during their lifetime. Recent research has suggested that there may be a genetic determinant involved in susceptibility to *Norovirus* infection, with people belonging to histo-blood group O being at greater risk for severe infection (Hutson *et al.*, 2004., Hutson *et al.*, 2002).

Evidence that *Norovirus* transmission occurs through aerosolization of vomit was clearly demonstrated at a UK hotel. During a meal, a guest vomited at the table, and *Norovirus* infection spread in a radial pattern through the restaurant, progressively decreasing form 91% attack rate among those seated at the same table to an attack rate of 25% in those patrons who were seated the farthest distance away from the guest who vomited (Marks *et al.*, 2003).

### 2.4. Gastroenteritis

*Noroviruses* are now recognized as playing a major role in sporadic gastrointestinal illnesses, as well. Early serosurveys documented a high prevalence of *Norovirus* antibodies in children, but because the virus was rarely detected in fecal specimens, its role in causing the infection seemed questionable. With the use of RT-PCR, *Noroviruses* are routinely detected in fecal specimens of children and adults with
gastroenteritis. A recent review of studies documented *Norovirus* in 5 to 31% of patients hospitalized for gastroenteritis and in 5 to 36% of those visiting a clinic, making it the most common cause of diarrhea in adults and the second most common cause in children (Patel *et al.*, 2008).

### 2.5. Previous studies

In study conducted for the determination of *Rotavirus*, *Norovirus* and *Adenovirus* in stool samples of children with gastroenteritis by qualitative polymerase chain reaction, and the determination of co-infections due to acute viral gastroenteritis were tested for *Rotavirus* group A and Adenovirus with commercial immune-chromatographic test and for *Norovirus* with EIA assay. Single *Norovirus* infection was found in 35/242 (14.5%) patients and in a further 5 (2.1%) children as co-infection with *Rotavirus*. Overall, *Norovirus* was detected in 16.5% of stool specimens. *Norovirus* infection tended to peak from October to November and again from February to March, In autumn months and in February, the proportion of *Norovirus* gastroenteritis cases was equal or even surpassed those of *Rotavirus* origin. Both *Norovirus* and *Rotavirus* infection most commonly affected children between 12 and 13 month of age. The low-garde or no fever was significantly more common in children infected with *Norovirus* (94.3%) compared to *Rotavirus* cases (52.9%). Overall *Norovirus* gastroenteritis was less severe than *Rotavirus* disease
with regard to 20-point severity scale (p<0.05). Norovirus have emerged as a relevant cause of acute gastroenteritis in polish children. There is a great need for introducing routine Norovirus testing of hospitalized children with gastroenteritis (Oldak et al., 2012).

Epidemiological data from developing countries about the importance of Norovirus in pediatric diarrhea are limited. Recently, in Nicaragua, it has been observed that Norovirus is responsible for 11% of the diarrhea cases occurring in children less than five years of age at community level and 15% of the moderate to severe cases requiring intravenous (Bucardo et al., 2011).

Norovirus has captured increasing attention as an agent of childhood diarrhoea. However, it is not known whether Norovirus causes severe diarrhoea like Rotavirus, particularly among children in developing countries. In a 1-year study conducted between May 2004 and April 2005 in Recife, Brazil, Norovirus was detected by ELISA in 34/233 (15%) diarrhea, children less than 5 years of age. The severity of clinical illness, as indicated by the presence of dehydration, the requirement for hospitalization, and the duration of hospital stay, was similar between children with Norovirus and Rotavirus infection. These data underscore the importance of Norovirus as a cause of severe diarrhoea in children (Nakagomi et al., 2008).
During July 2003–June 2004 from infants and children with acute gastroenteritis, encompassing five localities (Maizuru, Tokyo, Sapporo, Saga, and Osaka) of Japan, were tested for the presence of *Norovirus* by RT-PCR. It was found that 58 (14.4%) fecal specimens were positive for *Norovirus*. The virus was detected throughout the year with the highest prevalence in December (Tung *et al.*, 2006).

In Shanghai, China, between 2001 and 2005 stool specimens collected (n=5411) from children under 5 years of age who were hospitalized with acute gastroenteritis 3,975 were rotavirus-negative, indicating the presence of another causative agent. From these specimens, 484 were selected at random for genotyping, and 45 were *Norovirus*-positive. *Norovirus* infection was detected in all age groups, but infants less than 6 months old showed the lowest prevalence (5.4%). *Norovirus* infections peaked from August to November. This study demonstrated the impact of *Norovirus* infection causing acute gastroenteritis in hospitalized children (Jin Xu *et al.*, 2009).

*Norovirus* was detected in 21% of young children (278 of 1295) seeking medical attention for acute gastroenteritis in 2009 and 2010, with *Norovirus* detected in 22% (165 of 742) in 2009 and 20% (113 of 553) in 2010. The virus was also detected in 4% of healthy controls (19 of 493) in 2009. *Rotavirus* was identified in 12% of children with acute gastroenteritis (152 of 1295) in 2009 and 2010 (Daniels *et al.*, 2013). *Rotavirus* and *Norovirus* are leading viral causes of diarrhoea in children. A
A cross-sectional study was undertaken among children aged <5 years with acute gastroenteritis at Al-Jala Children's Hospital, Tripoli, Libya, from October 2007 to September 2008. Of 1,090 fecal samples collected, 260 from inpatients and 830 from outpatients, all inpatients and approximately a third of outpatients, selected systematically, were investigated for Rotavirus and Norovirus infection by ELISA and real-time RT-PCR, respectively. Of 520 fecal samples examined (inpatients=260, outpatients=260), 164 (31.5%) had Rotavirus and 91 (17.5%) had Norovirus. Rotaviruses and Noroviruses are both important causes of gastrointestinal infection among young children in Libya (Abugalia et al., 2011).

Three-hundred eighteen fecal samples were collected from January to December 2004, from children with acute gastroenteritis in 3 public hospitals in Rio de Janeiro, Brazil. Norovirus infections were detected in 65 (20%) of the samples, of which 11 (4%) were mixed infections with Rotavirus. Infants up to 1-year-old were the most affected and a peak of virus detection was observed in autumn and spring seasons. Dehydration and diarrhea were the inclusion criterion; coughing (51%), vomiting (33%), and fever (22%) were the main clinical manifestations (Victoria et al., 2004). In a study in Ghana 13 (15.9%) of the 82 diarrheic stool samples tested for caliciviruses were positive for Noroviruses. Noroviruses were present in all age
groups and were detected only during the diarrhoea peak that coincided with the peak *Rotavirus* season (George *et al.*, 2006).
CHAPTER THREE

MATERIALS AND METHODS
CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This was a cross-sectional study.

3.1.2. Study area

Specimens for this study were collected from Ibrahim Malik Teaching Hospital, Khartoum state. The experimental work was carried out in the Research lab., Sudan University of Science and Technology.

3.1.3. Study duration

This study was conducted during the period from January to March 2016.

3.1.4. Study population

Boys and girls less than five years with gastroenteritis were enrolled.

3.1.5. Sample size

A total of 84 blood specimens were obtained from patients.
3.1.6. Ethical consideration

This study was approved by the college of Medical Laboratory Science Ethical committee, SUST, to collect blood specimen sign was obtained from children parent before sample collection.

3.1.7. Specimens collection and storage

Three ml of venous blood was collected using venous puncture technique. The collected blood was drawn into plain containers, allowed to clot and then centrifuged at the speed of 2000-3000 r.p.m for 20 minutes, the supernatant was removed, then if precipitation appeared, centrifugation repeated, then collected into epindorff tube and stored at -20°C until the serological analysis was done.
3.2. Materials supplied in the test kit

<table>
<thead>
<tr>
<th>No.</th>
<th>Item Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard (800ng/ml)</td>
<td>0.5ml</td>
</tr>
<tr>
<td>2</td>
<td>Standard diluent</td>
<td>3ml</td>
</tr>
<tr>
<td>3</td>
<td>Microelisa strip plate</td>
<td>12well X 8strips</td>
</tr>
<tr>
<td>4</td>
<td>Str- HRP – Conjugate Reagent</td>
<td>6ml</td>
</tr>
<tr>
<td>5</td>
<td>30 X wash solution</td>
<td>20ml</td>
</tr>
<tr>
<td>6</td>
<td>Biotin – (Noro virus) Ag</td>
<td>1ml</td>
</tr>
<tr>
<td>7</td>
<td>Chromigen Solution A</td>
<td>6ml</td>
</tr>
<tr>
<td>8</td>
<td>Chromigen Solution B</td>
<td>6ml</td>
</tr>
<tr>
<td>9</td>
<td>Stop solution</td>
<td>6ml</td>
</tr>
<tr>
<td>10</td>
<td>Instruction</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>Closure plate membrane</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>Sealed bags</td>
<td>1</td>
</tr>
</tbody>
</table>

3.2.1. Principle of the assay

The kit uses a double – antigen sandwich – linked immunosorbent assay (ELISA) to assay the level of human *Norovirus* antibody (*Norovirus* - Ab) in samples. Norovirus antibody (*Norovirus* - Ab) was added to Enzyme well which is pre-coated with Human *Norovirus* antibody (*Norovirus* - Ab) antigen, incubation: then, *Norovirus* antibody antigen labeled with biotin, and combined with streptavidin –HRP to form immune complex: then incubated and washed again to remove the uncombined enzyme. Then chromogen solution A.B was added the color of the liquid changes into blue. the effect of acid, the color finally becomes yellow. The chroma of color
and the concentration of the Human substance Norovirus antibody (Norovirus - Ab) of sample were positively correlated.

3.2.2. Assay procedure

A) Standard dilution

The test kit original standard reagent was diluted according to the manufacture instructions as follow.,

<table>
<thead>
<tr>
<th>Conc</th>
<th>STD No.</th>
<th>Required concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>400ng/ml</td>
<td>Standard No. 5</td>
<td>120u1 original standard + 120u1 standard diluents</td>
</tr>
<tr>
<td>200ng/ml</td>
<td>Standard No. 4</td>
<td>120u1 original standard No5 + 120u1 standard diluents</td>
</tr>
<tr>
<td>100ng/ml</td>
<td>Standard No. 3</td>
<td>120u1 original standard No4 + 120u1 standard diluents</td>
</tr>
<tr>
<td>50ng/ml</td>
<td>Standard No. 2</td>
<td>120u1 original standard No3 + 120u1 standard diluents</td>
</tr>
<tr>
<td>25ng/ml</td>
<td>Standard No. 1</td>
<td>120u1 original standard No2 + 120u1 standard diluents</td>
</tr>
</tbody>
</table>

B) The quantity of the plates depends on the quantities of to-be tested samples and the standards. Every sample shall be made according to required quantity, and duplicated well possible was used.
C) Inject samples

All reagent and specimens were settled to reach room temperature, 40ul of specimens added then 10ul of *Norovirus*-antigen to each sample except blank then 50ul stander, 50ul of streptavidin-HRP was added to each well except blank, the plate was covered and incubated for 60 minutes at 37°C, by the end incubation period each well was washed 3 times with distilled water. Finally 50ul of chromogen A and chromogen B solutions were added to each well including blank, then the plate was incubated at 37°C for 10 minutes and stop solution was added.

### 3.2.3. Interpretation of the results

Samples with a concentration lower than 400ng/ml are considered negative for *Norovirus* antibody.

Samples with a concentration higher than 400ng/ml are considered positive for *Norovirus* antibody.

### 3.3. Data analysis

The data that collected from questionnaire and laboratory results were analyzed by SPSS version 15 computerized program.
CHAPTER FOUR

RESULTS
CHAPTER FOUR

RESULTS

This study was carried out during period from January to March 2016 to detect *Norovirus* antibody in children with gastroenteritis in Khartoum State.

A total of 84 blood specimens were collected from children of < 5 years with gastroenteritis hospitalized in Ibrahim Malik Teaching Hospital.

The results showed that out of 84 blood samples investigated, 40(47.6%) were positive for *Norovirus* antibody. The rest were negative 44(52.4%).(Table1). From the positive samples18(45%) was males, and 22(55%) were females (Table2). 25(62.5%) of the patients under 2.5 years showed positive results, and 15(37.5%) 2.5 - 5 years showed positive result (Table 3).

Table 1. Frequency of *Norovirus* among gastroenteritis children

<table>
<thead>
<tr>
<th>Result</th>
<th>no.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>44</td>
<td>52.4</td>
</tr>
<tr>
<td>Positive</td>
<td>40</td>
<td>47.6</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 2. Frequency of *Norovirus* according to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>no.</th>
<th>%</th>
<th>Result</th>
<th>Result</th>
<th>Result</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>negative</td>
<td>negative</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>no.</td>
<td>%</td>
<td>no.</td>
<td>%</td>
</tr>
<tr>
<td>male</td>
<td>48</td>
<td>57.1%</td>
<td>30</td>
<td>62.5</td>
<td>18</td>
<td>45</td>
</tr>
<tr>
<td>female</td>
<td>36</td>
<td>42.9%</td>
<td>14</td>
<td>38.9</td>
<td>22</td>
<td>55</td>
</tr>
<tr>
<td>total</td>
<td>84</td>
<td>100</td>
<td>44</td>
<td>100</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3. Frequency of *Norovirus* according to age

<table>
<thead>
<tr>
<th>Age</th>
<th>no.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 2.5 years</td>
<td>25</td>
<td>62.5</td>
</tr>
<tr>
<td>2.5 _ 5 years</td>
<td>15</td>
<td>37.5</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>
CHAPTER FIVE

DISCUSSION
5.1. Discussion

Norovirus constitutes the second most common viral pathogen causing gastroenteritis and pediatric diarrhea after Rotavirus. In Africa, diarrhea is a major health problem for children, and yet few studies have been performed regarding Norovirus (Nordgren et al., 2013).

The present study aimed to detection Norovirus antibody among children with gastroenteritis in Khartoum State. Out of 84 blood samples investigated, only 40 (47.6%) were positive. This result was near to that obtained in Mexican by Garcia et al., (2006) who reported 30% of Norovirus among children under five years with gastroenteritis.

In Egypt Zaghloul et al., (2013) showed 310/500 (62%) virus positive case by qualitative PCR among children with gastroenteritis; Rotavirus, Norovirus, and Adenovirus were detected in 39%, 16.2% and 6.8% of the children with gastroenteritis respectively.

Tamura et al., (2010) reported that in Vietnam, Rotavirus and Norovirus were detected in 87 (47.5%) and 12 (6.6%) of the 183 fecal specimens form children
hospitalized with acute gastroenteritis, respectively. The majority of patients with
Rotavirus and Norovirus were children under younger than 2 years of age.

Liu et al., (2010) reported that Norovirus was identified in 26.4% of the children
with acute nonbacterial gastroenteritis in Beijine.

In the present study the high percentage (47.6%) may be due to endemic area with
Norovirus infection.

5.2. Conclusions

This study concluded that there is considerable percentage of Norovirus among
children with gastroenteritis under five years of age in Khartoum state sudan.

5.3. Recommendation

1. Norovirus detection in every case of gastroenteritis was recommended.

2. Attention to hygienic measures such as hand washing, safe disposal of feces and
disinfection of contaminated surfaces is essential in reducing the risk of transmission.

3. Use of large sample size for different hospitals and employing the confirmatory
techniques such as real- time PCR to reflect real picture of the disease in Sudan is
highly recommended.

4. Screening for Norovirus should be included in routain investigation.
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