



Molecular Detection of Adeno and Norwalk Viruses Associated with Gastroenteritis among Juvenile in Khartoum State

Authors

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Abstract

Background: Diarrheal disease is a major public health problem for children in developing countries. Knowledge of etiology that causes diarrheal illness is essential to implement public health measures to prevent and control this disease. Adeno 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.

Methods: A total of 208 stool samples were collected from hospitalized children under five years of age presenting with acute gastroenteritis, in Khartoum State, Sudan, during the period from November 2017 – May 2021, For the detection of norovirus and adenoviruses, DNA from stool samples was extracted using Qiagen DNA kits from all samples, followed by Multiplex nested Polymerase Chain Reaction (PCR)

Results: The study revealed that 9 (4.3%) were positive for Norovirus and 4 (1.9%) with Adenovirus. The study found no significant difference in the positivity of Norovirus and Adenovirus according to the gender (p values 0.35, and 0.341 respectively). Moreover, the study found no significant difference in the positivity of Norovirus and Adenovirus according to the status of the polio-vaccination (p values 0.785, and 0.941 respectively). The study showed that there was no statistically significant association when compare age with Norovirus and Adenovirus (p values 0.081, and 0.231 respectively), The current study showed that there was no relationship between gastroenteritis diseases and Adeno and norovirus (p . value = 0.8, 0.9).

Conclusions: The present study showed that Norovirus and Adenovirus were not causative agents of gastroenteritis in children less than 5 years.

Introduction

Diarrhea has been defined as the passing of watery stool more than three times in 24 h, the etiological causes are consumption of contaminated food or drinks containing any of various pathogens (Vargas et al., 2004). Diarrhea become one of the leading causes of morbidity and mortality in children in Africa (Ngosso et al.,

2015). It can be caused by viral, bacterial, protozoan, or helminth and fungal pathogens. It can also be caused by sensitivity to certain types of food or drugs and sometimes by stress (Vargas et al., 2004; Ngosso et al., 2015). Globally , more than 1 billion diarrhea episodes and approximately 2.5 million deaths occur in children under 5 years of age in developing countries

(Vargas et al., 2004; Chen et al., 2012; Kotloff's et al., 2013; Ngosso et al., 2015). In most of these countries, diarrhea is the third most common cause for young children to visit health care centers, but little information is available in those countries regarding the causative agents (Vargas et al., 2004). Viral and bacterial infections are the most common causes of childhood diarrhea, and cannot be differentiated based on clinical presentation. Hence, in developing countries treatment of diarrhea is mainly based on symptomatic findings (Ali et al., 2005; Wang et al., 2014). Among viral causes, enteric viruses are recognized as the principal etiologic agents for childhood diarrhea. Five type of viruses are considered relevant as a cause of gastroenteritis including rotaviruses, adenoviruses, noroviruses, astrovirus, and bocavirus (Silva et al., 2008). The Norwalk virus agent (the original prototype virus is referred to as Norwalk virus in this review) was originally visualized by using immunoelectron microscopy (Kapikian et al., 1972), revealing 27-nm virus like particles. Noroviruses are single stranded, small non-enveloped RNA viruses that belong to the Caliciviridae family (Mans et al., 2016; Park et al., 2012). AdV is a relatively large nonenveloped dsDNA virus possessing a molecular weight of ~150 MDa and a diameter (dia) of ~950 Å, excluding its elongated fiber proteins. Adenovirus is the largest and complex nonenveloped viruses whose structures have been analyzed by cryoelectron microscopy (cryoEM) or X-ray diffraction. The ~36 kb AdV genome encodes more than 40 different proteins; however, only 12 of these have been shown to be constituents of the virus particle. Crystal structures of individual AdV proteins including the fiber knob (Bewley et al. 1999; Burmeister et al. 2004) and shaft (van Raaij et al. 1999) domains, penton base (Zubieta et al. 2005), hexon (Rux et al. 2003), and cysteine protease (McGrath et al. 2003) have been reported.

Material and Method

This study was a descriptive cross-sectional hospital based case study aimed to investigate viral etiology and related clinical and epidemiological factors in children with acute diarrhea in Khartoum State, Sudan, during the period from January 2018 – May 2021. A total of 207 fecal samples were collected from hospitalized children <5 years old with acute diarrhea, (107 males, 101 females) DNA from stool samples was extracted using Qiagen DNA kits for all samples, followed by investigation of Polyoma virus, norovirus and adenovirus by Multiplex nested Polymerase Chain Reaction (PCR).

DNA Extraction

Viral DNA was extracted from stool samples using Qiagen DNA kits according to the manufacturer's instruction (Qiagen, Germany).

Multiplex nested Polymerase Chain Reaction (PCR) for Adeno and norovirus

The reaction mixture was prepared by adding 17µl of master mix to 3µl of each samples nucleic acid added for negative control, we added 3 µl of diarrhea ACE negative control, the diarrhea ACE positive control was also used. The tube were placed in the preheated (94c°) thermal cycler, and the following cycles were performed one cycle at (94c°) for 15min for denaturation followed by 40 cycles for amplification (94c°) for 5 min 60 c° for 15min and 72c° for 15 min and final extension at 72c° for 10 min

Visualization of products

Agarosegel (2%) was prepared by adding 1.6 g of Agarose to 75 ml 1X Tris Acetate EDTA buffer, 5µl of the amplified product was subjected to direct analysis by gel electrophoresis. The product was visualized by staining with 0.2 µg/ml Ethidium bromide using UV gel documentation system Biometra (Germany). The expected size of Adeno and norovirus (G2, G1) were 482 bp, 330 and 387bp respectively

Table 1: Primers used for adenovirus and norovirus

Virus and primer sequence	Amplicon size
Adenovirus	
Ad1 TTCCCCATGGCICAYAACAC+ AD2 CCCTGGTAKCCRATRRTTGAT-	482
Norovirus G2	
COG2FCARGARBCNATGTTYAGRTGGATGAG+ G2SKRCCRCCNGCATRHCCRTTRTACAT-	387
Norovirus G1	
G1SKFCTGCCCGAATTYGTAAATGA + G1SKRCCAACCCARCCATTRTACA-	330

Statistical Analysis

- Data was entered, prepared, and analyzed using SPSS version 26.0
- Descriptive statistics was performed in term of frequency tables with percentages and graphs.
- Bi variable analysis will be done to assess the relation between the demographical and characteristics with results of the investigations using chi square statistical; test and t – statistical test.
- P value of 0.05 or less is considered statistically significant.

Results

A total of 208 Sudanese subjects were enrolled in this study, 101 of children were females (48.6%) and 107 (51.4%) were males ad shown in (Figure.1), the children age divided as group,(<1 years) (18.8%), (2-3 years) (23%), and (>3 years) (58.2%) as shown in (Table. 1). The majority of

the participants 169 (8.3%) were vaccinated with polio vaccine as in (Figure.2). The study revealed that 9 (4.3%) positive for Norovirus and 4 (1.9%) with Adenovirus as detailed in (Tables2,3). The study found no significant difference in the positivity of Norovirus and Adenovirus according to the gender (p values 0.35, and 0.341 respectively) as shown in (Table 4-5). Moreover, the study found no significant difference in the positivity of Norovirus and Adenovirus according to the status of the polio-vaccination (p values 0.785, and 0.941 respectively) as shown in (Table 6-7). The study showed that the majority of ag less than 4y in Norovirus and Adenovirus but the association was not statistically significant (p values 0.081, and 0.231 respectively) as detailed in (Tables 8-9).The current study showed that there was no association between gastroenteritis diseases with adenovirus and noroviruses as shown in (Table 10,11)

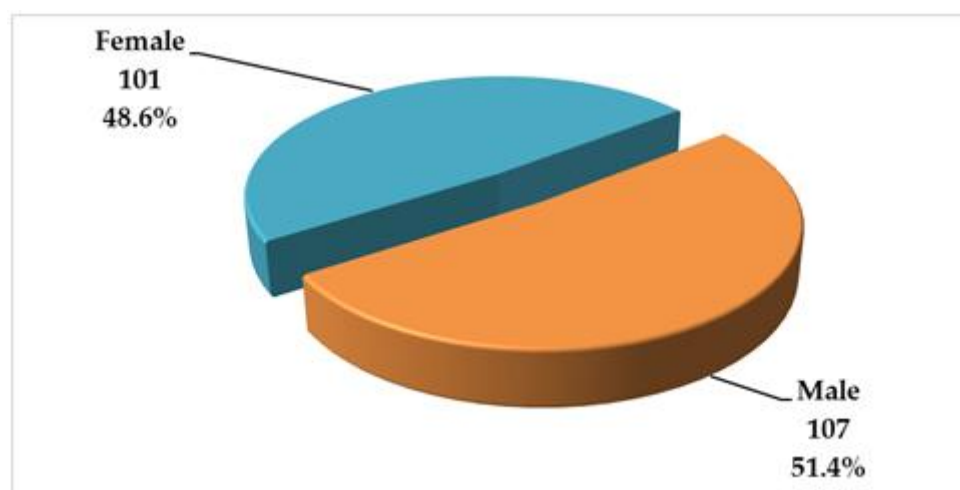


Figure (1) the distribution of the participants according to their gender (n = 208 children under 5 years age who admitted with acute diarrhea at three teaching hospitals in Khartoum state between November 2017 – May 2021)

Table (1) The distribution of the participants according to their age (n = 208 children under 5 years age who admitted with acute diarrhea at three teaching hospitals in Khartoum state between November 2017 – May 2021)

Age	Frequency	Percent
<1 years	39	18.8
2 - 3 years	48	23.0
>3 years	121	58.2
Total	208	100.0

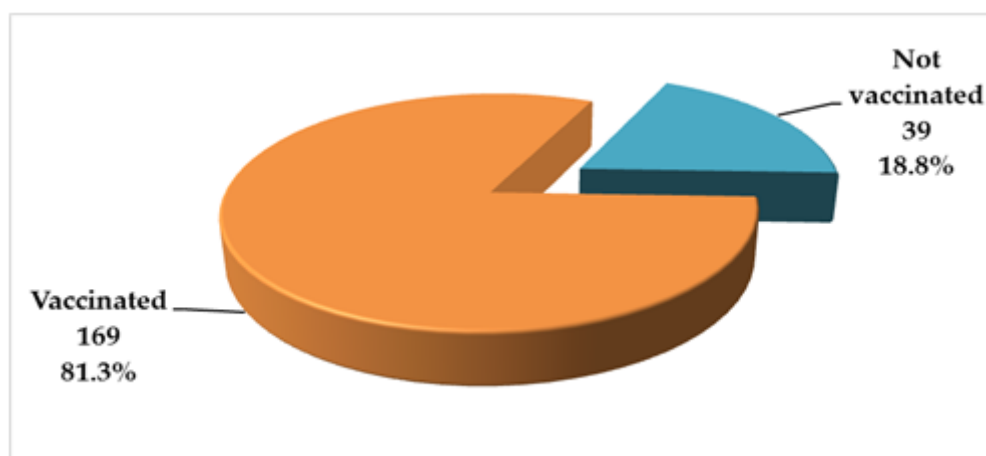


Figure (2) The distribution of the participants according to their polio vaccination status (n = 208 children under 5 years age who admitted with acute diarrhea at three teaching hospitals in Khartoum state between November 2017 – May 2021)

Table (2) The distribution of the participants according to the molecular

Table (2) The distribution of the participants according to the molecular detection of Norovirus (n = 208 children under 5 years age who admitted with acute diarrhea at three teaching hospitals in Khartoum state between November 2017 – May 2021)

Norovirus results	Frequency	Percent
Positive	9	4.3
Negative	199	95.7
Total	208	100.0

Table (3) The distribution of the participants according to the molecular detection of Adenovirus (n = 208 children under 5 years age who admitted with acute diarrhea at three teaching hospitals in Khartoum state between November 2017 – May 2021)

Adenovirus results	Frequency	Percent
Positive	4	1.9
Negative	204	98.1
Total	208	100.0

Table (4) The relation between the Norovirus molecular detection results with the participants gender (n = 208 children under 5 years age who admitted with acute diarrhea at three teaching hospitals in Khartoum state between November 2017 – May 2021)

Gender	Norovirus molecular detection results					
	Positive		Negative		Total	
	Freq.	%	Freq.	%	Freq.	%
Male	6	66.7	101	50.8	107	51.4
Female	3	33.3	98	49.2	101	48.6
Total	9	100.0	199	100.0	208	100.0
Chi square	0.8729					
P value	0.350					

Table (5) The relation between the Adenovirus molecular detection results with the participants gender (n = 208 children under 5 years age who admitted with acute diarrhea at three teaching hospitals in Khartoum state between November 2017 – May 2021)

Gender	Adenovirus molecular detection results					
	Positive		Negative		Total	
	Freq.	%	Freq.	%	Freq.	%
Male	3	75.0	104	51.0	107	51.4
Female	1	25.0	100	49.0	101	48.6
Total	4	100.0	204	100.0	208	100.0
Chi square	0.9061					
P value	0.341					

Table (6) The relation between the Norovirus molecular detection results with the participants Poliovirus vaccination (n = 208 children under 5 years age who admitted with acute diarrhea at three teaching hospitals in Khartoum state between November 2017 – May 2021)

Poliovirus vaccination	Norovirus molecular detection results					
	Positive		Negative		Total	
	Freq.	%	Freq.	%	Freq.	%
Vaccinated	7	77.8	162	81.4	169	81.3
Not vaccinated	2	22.2	37	18.6	39	18.8
Total	9	100.0	199	100.0	208	100.0
Chi square	0.0744					
P value	0.785					

Table (7) The relation between the Adenovirus molecular detection results with the participants Poliovirus vaccination (n = 208 children under 5 years age who admitted with acute diarrhea at three teaching hospitals in Khartoum state between November 2017 – May 2021)

Poliovirus vaccination	Adenovirus molecular detection results					
	Positive		Negative		Total	
	Freq.	%	Freq.	%	Freq.	%
Vaccinated	4	100.0	165	80.9	169	81.3
Not vaccinated	0	0.0	39	19.1	39	18.8
Total	4	100.0	204	100.0	208	100.0
Chi square	0.332					
P value	0.9412					

Table (8) The relation between the Norovirus molecular detection results with the participants age (n = 208 children under 5 years age who admitted with acute diarrhea at three teaching hospitals in Khartoum state between November 2017 – May 2021)

Age – years	Norovirus molecular detection results					
	Positive		Negative		Total	
	Freq.	%	Freq.	%	Freq.	%
< 1 years	3	33.3	36	18.1	39	18.8
2 - 3 years	4	44.4	44	22.1	48	23.1
>3 years	2	22.2	119	59.8	121	58.2
Total	9	100.0	199	100.0	208	100.0
Chi square	5.0182					
P value	0.081					

Table (9) The relation between the Adenovirusmolecular detection results with the participants age (n = 208 children under 5 years age who admitted with acute diarrhea at three teaching hospitals in Khartoum state between November 2017 – May 2021)

Age – years	Adenovirusmolecular detection results					
	Positive		Negative		Total	
	Freq.	%	Freq.	%	Freq.	%
<1 years	4	100	35	19.1	39	18.8
2 - 3 years	0	0	48	23.5	48	23.1
>3 years	0	0	121	57.4	121	58.2
Total	4	100.0	204	100.0	208	100.0
Chi square	2.93					
P value	0.231					

Table (10) The relation between the norovirus molecular detection results with Gastroenteritis

Results	Frequency	Percent	Chi square	P. value
Positive	9	4.4	117.0	0.8
Negative	199	95.6		
Total	208	100.0		

Table (11) The relation between the norovirus molecular detection results with Gastroenteritis

Results	Frequency	Percent	P. value
Positive	4	1.9	0.9
Negative	204	98.1	
Total	208	100.0	

Discussion

Acute gastroenteritis (AGE) is a common illness affecting all age groups worldwide. Even in developed countries, most of the population will average one episode of AGE per year. Among young children and the elderly, the rate is higher and the disease more severe. Globally, approximately 1.5 billion episodes and 1.5 to 2.5 million deaths annually in children under age five are estimated to be associated with AGE, the majority occurring in developing countries.

This a cross sectional study was conducted to demonstrate the association between acute

gastroenteritis and Adeno and Norwalk viruses in Sudanese children patients less than five years . This study was carried on 208 children less than 5 years age, the result of present study was reported that male gender dominance 107 (51.4%) with male: female ratio of 1.1:1

The current study found that 39 (18.8%) were less than one year in age, 48 (23%) 2 – 3 years and 121 (58.2%) were above three years in age

The majority of the participants 169 (8.3%) were vaccinated with polio vaccine

The present study revealed that 9 (4.3%) positive for, with Norovirus and 4 (1.9%) with

Adenovirus, this frequency similar to Elhag et al., 2013 which found that Adenovirus frequency (16.2%) was lowest infection virus out of 710 stool sample also the result agreed with meta analysis study performed on Arab population by Fadi in 2022 (Fadi et al; 2022) which showed that the lowest frequency of Adeno virus was observed in sudan in compersion to other gastroenteritis virus, in addition Javad et al., 2015 in Zabol, Southeastern Iran, the frequency of Adenoviruses and Noroviruses among infants with diarrhea were 20.3% and 9.5%, respectively be the lowest infection and this agreed with current study (Javad et al., 2015) also in Afghanistan Diao et al., 2014, the lowest frequency of adenovirus (1.6%; 7/432) and norovirus (0.7%; 3/432) (Diao et al., 2014) and this was consistent with the present study. also Adam reported that norovirus and adenovirus had lower frequency and this was agree with current study (Adam *et al*; 2018).incidence of norovirus and adenovirus was lower than previous studies in Sudan (Elhag et al., 2013; Mustafa et al., 2013). This difference may reflect differences in sample size, age tested and timing of these studies. All discussed pervious study illustrated that there was no statistically significant association between Adenoviruses and Noroviruses Polyomavirus virus this was consistent with current study. In study performed in Khartoum and Aljazeera Tatay et al showed that norovirus are an important causative agents of gastroenteritis in children less than 5 years and this was disagree with present study and this may due to low sample size of the previous study (Tatay *et al*; 2018).

The present study was showed that there was no statistically significant association with Adenoviruses and Noroviruses, this was similar to Paula and his colleagues observed shedding of Polyomavirus in most of samples (Paula *et al*; 2008), also eman M Tatay, the present study showed that norovirus are an important causative agents of gastroenteritis in children less than 5 years and this was disagree with present study.

The present study found no significant difference in the positivity of Norovirus and Adenovirus according to the gender (p values 0.66, 0.35, and 0.341 respectively) this was agree with Tatay which showed in related to Adeno virus there was no significant differences ($p > 0.05$) between the age group, gender were noted (Tatay *et al*; 2018). Moreover, the study found no significant difference in the positivity of Norovirus and Adenovirus according to the status of the polio-vaccination (p values 0.946, 0.785, and 0.941 respectively).

The study showed that the majority of positive cases of Adeno and Norovirus were younger than 4 years, with no significant association this was agree (p values 0.081, and 0.231 respectively) with Siebrasse et al in 2012 positive patients were from children aged between 1 and 2 years.

Conclusion

The present study showed that no association between norovirus and adenovirus with gastroenteritis infection in children less than 5 years.

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