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Molecular Detection of Polyomavirus Associated with Gastroenteritis among Juvenile in Khartoum State

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Abstract

Background: *Polyomaviruses are human virus associated with acute viral gastroenteritis globally and an essential cause of childhood morbidity and mortality in developing countries. However, there are still few published data on the happens of these viruses in Sudan. This study was conducted to investigate the molecular epidemiology of polyomaviruses in children less than 5 years with acute gastroenteritis.*

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 Polyomavirus 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 26 (12.5%) were positive for Polyomavirus. The study found no significant difference in the positivity of Polyomavirus according to the gender (p values= 0.66). Moreover, the study found no significant difference in the positivity of Polyomavirus according to the status of the polio-vaccination (p values= 0.946). The study showed that the majority of positive cases of Polyomavirus were younger than 4 years, with significant association (p < 0.001), The current study showed that there was strong association between Polyoma virus and gastroenteritis diseases (p. value = 0.00)*

Conclusions: *The present study showed that Polyoma virus are an important causative agents of gastroenteritis in children less than 5 years. There is a great need for introducing routine Polyoma virus testing of hospitalized children with gastroenteritis.*

Introduction

Diarrheal diseases are important cause of morbidity and mortality during young children. Annually, more than one billion diarrhea episodes and approximately 2.5 million deaths occur among children less than five years (Bern *et al*., 2009). Insipid of the decline in mortality, it become one of the basic causes of morbidity in developing countries (Parshar *et al*., 2010). In tropical countries diarrhea designed as the second most common infection in children after acute respiratory infection (Kosek and Guerrant, 2003). About 80% of deaths related to diarrhea occur in the first 2 years of life due to dehydration, which results from loss of blood fluid and failure to absorb nutrients (Vargas *et al*., 2014). Diarrheal disease is most common in non-breast –fed infant than exclusively breast-fed infants for the first four to six months, and this emphasized by low diarrheal infection such as shigellosis and cholera among prolonged breast-fed. Other risk factors that related in the transmission of diarrhea include inappropriate stored food, contamination of water, poor hygiene and poor sanitary conditions. Host

factors include, malnutrition, recurrent infection and human immunedeffiency virus (HIV) (Vargas et al., 2014).Infant may have Leiner disease, which characterized by recurrent diarrhea, wasting, and generalized seborrheic dermatitis. The disturbance in persons with Leiner disease is usually attributed to a defect of the fifth component of complement (C5). However, a child was described by Sonea and associates who had Leiner disease associated with diminished C3 (Sonea et al., 2005). In addition, age is also considered as another factor with most diarrheal episodes occurring at the first 2 years of life, specifically in the 6-11 month age group when supplementary food starts. This age specific pattern reflects the combined decline of maternal-acquired antibodies and intake of food that is contaminated with enteric pathogens (Vargas et al., 2014). Most enteric pathogens stimulate a partial immunity. A few years ago pathogenic organisms could be diagnosed in the stool of only about 25% of patient with diarrhea. Diarrhea can be caused by awide range of bacteria, viruses, and parasites (Guerrant et al., 2005).Viruses are a cause of gastrointestinal disease worldwide and commonly infect children less than five years of age in developing countries (Cavallo and Garrabé, 2007) Polyoma viruses are DNA-based (double-stranded DNA, ~5000 base pairs, circular genome) viruses. They are small (40–50 nanometers in diameter), and icosahedral in shape, and do not have a lipoprotein envelope. Moreover, the genome possesses early and late genes, contributing to its complex transcription program. They are potentially oncogenic (tumor-causing); and often persist as latent infections in a host without causing disease, but may produce tumors in a host of a different species, or a host with an ineffective immune system. The name Polyoma refers to the viruses'that can produce multiple (poly-) tumors (-oma). (Perez et al., 2006).

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, 101females)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 manufacture's instruction (Qiagen, Germany).

Multiplex nested Polymerase Chain Reaction (PCR) for Polyomavirus

The test was carried out with first-round PCR amplification using the outer primer pairs that are specific for large Tantigen gene to amplify a conserved DNA region of the large T antigen gene of JCV, BKV, and SV40 (table1).

The reaction was performed in 25µl volume using Maxime PCR PreMix Kit master mix (Intron. South Korea). The volume included: 5µl master mix, 1 µl forward primer (10mM), 1 µl reverse primer (10mM), 5 µl extracted DNA and 13µl distilled water. The DNA was amplified in thermo- cycler (Techno ,Japan) using the following condition: initial denaturation at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 1 min and extension at 72°C for 1 min, with a final extension 72°C for 5 min.The product of the first round was used as template for the second round using another pair of primers to obtain products of different sizes for each related gene, inner primer pairs used consisted of BKV Sense 5′-GAATGCTTTCTTCTATAGTATGGTATG -3′ and JCV Sense 5′-ATATTATGACCMCCAAAACCATG -3′ and SV40 Sense 5′-ATAATTTTTTTGTATAGTATAGTAGTGCA -3′ with reverse Polyomavirus Antisense 3′-CCTTTCAGRAAYCCCATAAGATGG-5′ The

reaction was performed in 25µl volume using Maxime PCR PreMix Kit master mix (Intron. South Korea). The volume included: 5µl master mix, 2 µl of primers mix included the inner primer pairs that mentioned above (10mM), 15µl distilled water and 3 µl of first round PCR product, second round was performed under the following conditions: 94°C for 30 s, 56°C for 1min, 72°C for 30 s for 30 cycles with a final extension 72°C for 5 min.

Visualization of products of Polyomavirus

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 SV40, BKV and JCV amplicons were 135 bp, 353 and 189 respectively.

Figure 1: Multiplex PCR shown on 2% agarose electrophoresis. The expected size 135bp, on lane 1 L: ladder, 100 bp ladder (Fermentas, Germany).

Table 1: Primers used for Polyomavirus

Primer name	Sequence	Expected Size of the product
forward primer	5′-TCYTCTGGNNTAAARTCATGCTCC-3′	
reverse primer	3′-CAAGGTATCCAACCKTRGATWAA-5′.	
SV40_FW	5′- ATAATTTTTTTGTATAGTATAGTAGTGCA -3	135bp
SV40_RV	3′- CCTTTCAGRAAYCCCATAAGATGG-5′	
BKV_FW	5′-GAATGCTTTCTTCTATAGTATGGTATG -3′	353bp
BKV_RV	3′- CCTTTCAGRAAYCCCATAAGATGG-5′	
JCV_FW	5′- ATATTATGACCMCCAAAACCATG -3	189bp
JCV_RV	3′- CCTTTCAGRAAYCCCATAAGATGG-5′	

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 26 (12.5%) were positive for Polyomavirus, 9 (4.3%) as detailed in **(Tables2)**. The study found no significant difference in the positivity of Polyomavirus, according to the gender (*p values*

=0.66) as shown in **(Table3)**. Moreover, the study found no significant difference in the positivity of Polyomavirus according to the status of the polio-vaccination (*p values= 0.946*) as shown in **(Table 4)**. The study showed that the majority of positive cases of Polyomavirus were younger than 4 years, with significant association (*p < 0.001*) as detailed in **(Tables 5)**.The current study showed that there was strong association between Polyoma virus and gastroenteritis diseases (*p. value = 0.00*) as shown in **(Table 6)**.

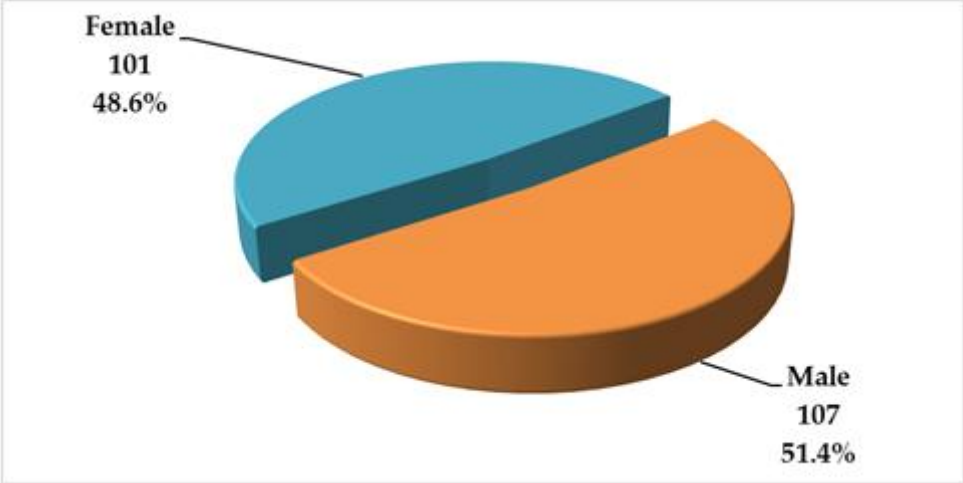

Figure (1) The distribution of the participants according to their gender (n = 208 children under 5 years age who admitted with acute diarrhea at three teaching hospitals in Khartoum state between November 2017 – May 2021)

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**

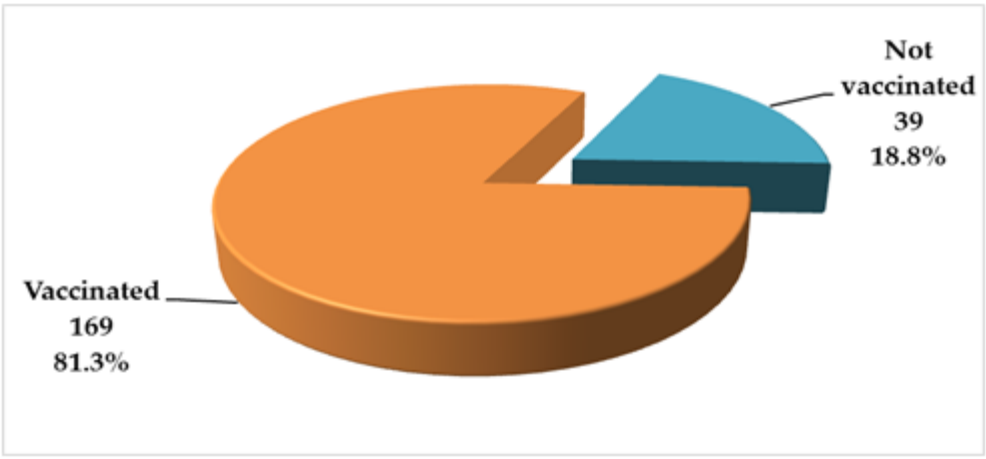

Figure (2) The distribution of the participants according to their polio vaccination status (n = 208 children under 5 years age who admitted with acute diarrhea at three teaching hospitals in Khartoum state between November 2017 – May 2021)

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

Polyomavirus results	Frequency	Percent
Positive	26	12.5
Negative	182	87.5
Total	**208**	**100.0**

Table (3) The relation between the Polyomavirusmolecular 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	Polyomavirus molecular detection results					
	Positive		Negative		Total	
	Freq.	%	Freq.	%	Freq.	%
Male	9	34.6	98	53.8	107	51.4
Female	17	65.4	84	46.2	101	48.6
Total	**26**	**100.0**	**182**	**100.0**	**208**	**100.0**
Chi square	**3.368**					
P value	**0.66**					

Table (4) The relation between the Polyomavirus 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	Polyomavirus molecular detection results					
	Positive		Negative		Total	
	Freq.	%	Freq.	%	Freq.	%
Vaccinated	21	80.8	148	81.3	169	81.3
Not vaccinated	5	19.2	34	18.7	39	18.8
Total	**26**	**100.0**	**182**	**100.0**	**208**	**100.0**
Chi square	**0.0045**					
P value	**0.946**					

Table (5) The relation between the Polyomavirus molecular detection results with the participants age. Maximum prevalence was revealed in 1 year age group less than 3 year (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	Polyomavirus molecular detection results					
	Positive		Negative		Total	
	Freq.	%	Freq.	%	Freq.	%
<1 years	17	65.4	22	12.1	39	18.8
2 - 3 years	6	23.1	42	23.1	48	23.1
>3 years	3	11.5	118	64.8	121	58.2
Total	**26**	**100.0**	**182**	**100.0**	**208**	**100.0**
Chi square	**45.5738**					
P value	**< 0.001**					

Table (6) The relation between the Polyomavirus molecular detection results with Gastroenteritis (strong statistically association with disease)

Results	Frequency	Percent	Chi square	P. value
Positive	26	12.5	117.0	0.000*
Negative	182	87.5		
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 Polyoma, 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 26 (12.5%) were positive for Polyomavirus, 9 (4.3%) 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 Diaa et al., 2014, the lowest frequency of adenovirus (1.6%; 7/432) and norovirus (0.7%; 3/432) (Diaa et al., 2014) and this was consistent with the present study in addition study worked by Cherati showed that 2 out of 237 samples (0.8%) were positive for polyma virus, (Cherati et al; and other study performed in 2013 by Alejandro which reported that Polyomavirus was detected in 5 of 327 clinical stool specimens. In china 10 human Polyoma viruses were detected in fecal specimen were detected from hospitalized children with diarrhea (Alejandro et al; 2013) also in 2013 Ke et al that the frequency of polyma virus was detect in 4 stool sample also Siebrasse et al in 2012 the frequency of polyma virus was detected in 11(4.9%) symptomatic fecal specimens (Ke et al; 2013)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 statistically significant association between gastroenteritis and Polyomavirus and 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 Polyomavirus, 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 Polyomavirus, 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 Polyomavirus were younger than 4 years, with significant association (p < 0.001) this was agree with Siebrasse et al in 2012 positive patients were from children aged between 1 and 2 years. similar age distribution was reported in Norovirus and Adenovirus but the association was not statistically significant (p values 0.081, and 0.231 respectively)

Conclusion

The present study showed that Polyomavirus are important causative agents of gastroenteritis in children less than 5 years and there is no association between norovirus and adenovirus with infection.

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