RESEARCH ARTICLE

Molecular study of sapovirus in acute gastroenteritis in children: a cross-sectional study [version 1; peer review: 1 approved with reservations]

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Abstract

Background: Sapovirus has emerged as a viral cause of acute gastroenteritis. However, there are insufficient data about the presence of this virus among children with acute gastroenteritis. The present study aimed to evaluate the presence of sapovirus in children with acute gastroenteritis by reverse transcriptase-polymerase chain reaction (RT-PCR).

Methods: A cross-sectional study enrolled 100 children patients with acute gastroenteritis from outpatient clinics with excluded bacterial pathogens and parasitic infestation. A stool sample was collected from each child for laboratory examination. Each stool sample was subjected to study by direct microscopic examination, study for rotavirus by enzyme-linked immunoassay (ELISA) and the remaining sample was subjected to RNA extraction and RT-PCR for sapovirus.

Results: The most frequently detected virus was rotavirus by ELISA (25%). RT-PCR detected sapovirus in 7% of the stool samples. The children with sapovirus were all from rural regions and presented mainly during the winter season in Egypt (42.9%). The main presenting symptoms were fever (71.4%) and vomiting (57.1%). None of the children with sapovirus had dehydration. Rotavirus was significantly associated with sapovirus infections in 5 patients (71.4%, $P=0.01$). There was an insignificant difference between symptoms of gastroenteritis in children with sapovirus and children with gastroenteritis without sapovirus as regards vomiting ($P=0.7$), fever ($P=0.46$), and abdominal pain ($P=0.69$).

Conclusion: The present study highlights the emergence of sapovirus as a frequent pathogen associated with acute gastroenteritis in

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1. **Marta Diez Valcarce** , Centers for Disease Control and Prevention, Atlanta, USA

2. **Nicola A. Page** , Centers for Disease Control and Prevention, Atlanta, USA

Any reports and responses or comments on the article can be found at the end of the article.

children. There is a need for a national survey program for the study of sapovirus among other pathogens associated with acute gastroenteritis for better management of such infection.

Keywords

sapovirus

This article is included in the **Cell & Molecular Biology** gateway.

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Introduction

Sapovirus is a single strand non enveloped RNA virus that belongs to the Caliciviridae family¹⁻⁴. The length of its genome is 7.1 to 7.7 kb, and its polyadenylated 3' terminal is responsible for viral replication, while its 5' terminal is associated with viral translation through production of VPg^{5,6}. The viral genome consists of two to three open reading frames⁵. There are 15 genotypes of the virus with only four genotypes that can infect humans, namely GI, GII, GIV and GV^{2,5,7,8}.

The laboratory methods for detection of sapovirus include enzyme-linked immunosorbent assay, electron microscopy, reverse transcription-polymerase chain reaction (RT-PCR) and next-generation sequencing³. The sensitivity and specificity of RT-PCR are good and can be used for routine identification of the virus³. The primers used for the amplification of the virus depend upon the use of a segment from VP1 encoding gene compared to the RdRp region^{3,9}. The classification of sapovirus depends upon complete sequence analysis of VP1^{6,10,11}.

Sapovirus infection is associated with viral gastroenteritis, especially in children below five with, around two million deaths around the world, and sapovirus causing viral gastroenteritis represents the second common cause of death¹². The common symptoms are vomiting and diarrhoea⁵. The transmission of sapovirus occurs via ingestion of contaminated food and water and also by direct contact with affected individuals^{13,14}. The infection occurs both sporadically and as an outbreak^{2,9}. Treatment is symptomatic to prevent the aggravation of the disease^{3,9}, and prevention of the infection depends mainly upon access to clean drinking water and food¹⁵.

There are data available about sapovirus infection in different countries such as Peru¹⁶ Iran¹⁷ and Ethiopia¹⁸. However, to our best of knowledge, there are no available reports about this in Egypt. Therefore, the present study aimed to evaluate the presence of sapovirus in children with acute gastroenteritis by RT-PCR.

Methods

Study design and participants

The present study was a cross-sectional study that enrolled 100 children patients (sample size calculated by a statistician) with acute gastroenteritis from outpatient clinics from Mansoura Children's Hospital, Mansoura, Egypt within the previous 48 hours with excluded bacterial pathogens and parasitic infestation by microbiological culture and microscopic examination to exclude parasites. The study was carried out from January 2019 till February 2020.

The procedures followed were approved by the Mansoura Faculty of Medicine, Egypt ethical committee on human experimentation (R.20.11.1053) and were carried out in accordance with the Helsinki Declaration of 1975, as revised in 1983. Written informed consent was obtained from the parents of the included children.

Each child was subjected to full medical history by asking the parents about the residence area and age of the child, which was then followed by clinical examination. A stool sample was collected from each child for laboratory examination.

Stool samples

Each stool sample was subjected to study by direct microscopic examination, study for rotavirus by ELISA Ridascreen® (R-Biopharm AG- An der Neuen Bergstraße 1764297 Darmstadt, Germany), and the remaining samples was subjected to RNA extraction and RT-PVR for sapovirus.

ELISA for rotavirus. The kit is ELISA for qualitative detection for rotavirus in the stool sample. The kit used monoclonal antibodies in a sandwich-type method. The monoclonal antibody is directed to the protein of the six viral genes (VP6), and it is used for the coating of the wells of the microplate. The VP6 is a group-specific antigen present in all rotaviruses genotypes. In brief, the stool suspension was prepared using ready to use stool diluent (protein-buffered NaCl solution). A total of 1 ml of the diluent was added to 100 microliter of the stool and homogenized the suspension by aspiration into and ejection from a disposable pipette or, alternatively, blending in a Vortex mixer. The suspension was allowed to stand for a short period of time (10 minutes) for the coarse stool particles to settle, and the clarified supernatant of the stool suspension was used directly in the test.

The control supplied with the kit were added to the wells of a microplate with biotinylated monoclonal anti-rotavirus antibodies and incubated at room temperature for one hour. The wells were washed with the supplied wash buffer after incubation and streptavidin poly-peroxidase conjugate was added before a second incubation was performed for a half-hour at room temperature. After a second wash, the substrate of the enzyme was added leading to a colour change of colourless to blue if the test is positive. The samples were then incubated at room temperature for 15 minutes. A stop reagent was added, changing the colour from blue to yellow. The colour intensity was proportional to the concentration of rotavirus present in the stool samples in comparison with a control, and was measured at 450 nm using a microplate ELISA reader (Statfax Chromate 4300).

Sapovirus PCR

Extraction of RNA and complementary DNA preparation. The stool samples were subjected to the extraction of RNA of sapovirus using QIAamp Viral RNA kit (Qiagen). The extraction was performed according to the instructions supplied by the manufacturer.

Complementary DNA (cDNA) was generated by adding 100 nanograms of the extracted RNA to 32.5 µl prepared mixture composed of 1 µl hexamers primers (Fermentas, Latvia), 4 µl of 5 x buffer, 0.5 µl of Ribolock RNase inhibitor, 2 µl of dNTP mixture composed of 10 mM each of dNTP, 200 units

of reverse transcriptase enzyme and 19 µl of RNase free water (Fermentas, Latvia). The RT assay was performed at 42°C for one hour.

PCR for sapovirus. The amplification process was carried out using previously reported primers with nucleotide sequences of primers as follows: SLV5317 forward 5'-CGGRCYT-CAA AVSTACCBCCCCA-3'; SLV5317 reverse 5'- CTCGCCAC-CTACRAWGCBTGGTT-3'¹⁹.

The amplification mixture used was ready to use Qiagen *Taq* PCR Master Mix Kit mixture (Cat No./ID: 201443) with 5 µl of cDNA added to a 20 µl PCR mix. The amplification procedures were performed using the following conditions: denaturation at 94°C for 5 minutes, then 35 cycles composed of 94°C for 45 seconds- 55°C for 45 seconds and 72°C for 1 minute, then final extension of 7 minutes at 72°C (MiniAmp Thermal Cycler, Applied Biosystem).

. PCR products were visualised under UV illumination after electrophoresis on a 1% agarose gel stained with ethidium bromide. The estimated amplified fragment size for sapovirus was 434 bp¹⁹.

Statistical analysis

Data were analysed with SPSS 22 (SPSS Inc, Chicago, Illinois, USA). Qualitative values were calculated as numbers and percentages. The use of the chi-square test performed comparisons, and the P-value was considered significant if it was <0.05.

Results

The study included 100 children with acute gastroenteritis manifested by diarrhoea associated predominately with fever (56%), vomiting (47%), abdominal pain (42%). A minority had dehydration (11%). Their mean age± SD was 53.33± 11.71 months. Most cases presented in the spring season (34%) followed by winter (24%). Demographics of the children are shown in [Table 1](#).

The most frequently detected virus was rotavirus by ELISA (25%). RT-PCR detected sapovirus in 7% of the stool samples.

The children with sapovirus were all from rural regions (Belkas, Dekrnes, Aga) and presented mainly during the winter season in Egypt (42.9%). The main presenting symptoms were fever (71.4%) and vomiting (57.1%). None of the children with sapovirus had dehydration. Rotavirus was significantly associated with sapovirus infections in 5 patients (71.4%, P=0.01). There was an insignificant difference between symptoms of gastroenteritis in children with sapovirus and children with gastroenteritis without sapovirus as regards vomiting (P=0.7), fever (P=0.46), abdominal pain (P=0.69) ([Table 2](#)).

Discussion

Viral pathogens represent a significant aetiology for acute gastroenteritis. These infections are usually self-limited in developed countries while it may lead to mortality in underdeveloped countries, especially in children¹³.

Table 1. Demographic and clinical data of the studied children (n=100).

Parameter	
Age, months (mean± SD)	53.33± 11.71
Gender, n (%)	
Male	66 (66)
Female	34 (34)
Season, n (%)	
Summer (21 June-22 September)	20 (20)
Autumn (22 September-21 December)	22 (22)
Winter (22 September -20 March)	24 (24)
Spring (20 March-21 June)	34 (34)
Residence, n (%)	
Rural	68 (68)
Urban	32 (32)
Abdominal pain, n (%)	42 (42)
Fever, n (%)	56 (56)
Vomiting, n (%)	47 (47)
Dehydration, n (%)	11 (11)

The present study included 100 children with acute gastroenteritis manifested by diarrhoea with the majority experiencing fever (56%), vomiting (47%), and abdominal pain (42%). A minority had dehydration (11%). These are common symptoms of viral gastroenteritis. These patients usually present to outpatient clinics and do not require hospital admission except for dehydration²⁰.

The proper management of children with acute gastroenteritis relies upon appropriate and robust diagnosis of the aetiology. In the present study, the most frequently detected virus was rotavirus by ELISA (25%). Previous meta-analysis study revealed that rotavirus is associated with acute gastroenteritis in 31.5% of children and 25.7% in the general population¹⁴. Previous studies in Africa reported that the prevalence of rotavirus infections ranged from 22.73% up to 30%^{21,22}. A previous study from Egypt reported that the prevalence of rotavirus was 31% among children with acute gastroenteritis²³.

The study of sapovirus as an emerging pathogen associated with acute gastroenteritis has gained importance in recent years. Research has been facilitated by the emergence of the molecular techniques in laboratory diagnosis^{16,24,25}. In the present study, sapovirus was detected among 7% of children with acute gastroenteritis by RT-PCR. A previous meta-analysis study reported that the prevalence of sapovirus was 6.5% with a remarkable difference in the presence of sapovirus between low income and high-income countries²⁶. Another study reported a lower prevalence of sapovirus 4.6% (10/219)²⁷. Previous studies reported the prevalence of 3% up to 17% of sapovirus in

Table 2. Comparison between sapovirus positive children and sapovirus negative children.

	Sapovirus positive (n=7)	Sapovirus negative (n=93)	Odds ratio	95%CI	P-value
	n (%)				
Gender					
Male	4 (57.1)	62 (66.7)	0.667	0.14-3.155	0.61
Female	3 (42.9)	31 (33.3)			
Age, months (mean± SD)	48.86± 9.40	51.52± 11.88			0.6 F=0.33
Rural residence	7 (100)	61 (65.6)	0.897	0.828-0.972	0.09
Season					
Summer (21 June-22 Sept)	0 (0)	20 (21.5)	1.55	0.32-7.315	0.41
Autumn (22 Sept-21 December)	2 (28.6)	20 (21.5)			
Winter (22 September -20 March)	3 (42.9)	21 (22.6)			
Spring (20 March-21 June)	2 (28.6)	32 (34.4)			
Vomiting	4 (57.1)	43 (46.2)	1.6	0.32-7.31	0.7
Abdominal pain	2 (28.6)	40 (43.01)	0.53	0.1-2.87	0.69
Fever	5 (71.4)	51 (54.8)	2.059	0.38-11.157	0.46
Dehydration	0 (0)	11 (11.8)	1.085	1.02-1.15	0.43
Rotavirus	5 (71.4)	20 (21.5)	9.12	1.646-50.594	0.01

acute gastroenteritis in children with gastroenteritis in high and low-income countries, respectively^{16,24,25}. The variation of the prevalence rates reported may be due to the variation of the climate, environment, socio-economic factors, and cultural practices beside the difference of the used method of diagnoses.

The management of sapovirus depends mainly upon oral rehydration solution and zinc supplementation²⁸. The risk factors for sapovirus infection are not fully understood. The prevention of sapovirus infection depends mainly upon efficient hand hygiene practice, environmental disinfection, proper sewage disposal, and limited contact with ill individuals. There is an argument about the role of improvement of water sanitation in the prevention of sapovirus as it is a common pathogen in both high and low-income countries. However, as it is transmitted by contaminated water and food²⁹, increasing food and water sanitation will reduce the burden of the infection.

The use of new molecular technologies for sapovirus detection in different samples from patients, food and environment, is mandatory to recognize the mode of sapovirus transmission. Infection at a young age may predispose to durable immunity. Therefore, the development of a vaccine toward this virus may reduce the burden of this infection³⁰.

In the present study, there was an insignificant difference between the clinical presentation of sapovirus positive and sapovirus negative children. The clinical symptoms associated with acute gastroenteritis usually include diarrhoea, vomiting, and fever, making laboratory diagnosis essential for appropriate management. Therefore, there is a need for a national survey program to improve the monitoring of the circulation of the new virus such as sapovirus alongside other pathogens associated with gastroenteritis to improve the control measures³⁰.

Conclusions

The present study highlights the emergence of sapovirus as a frequent pathogen associated with acute gastroenteritis in children. There is a need for a national survey program for the study of sapovirus among other pathogens association with acute gastroenteritis for better management of such infection.

Data availability

Underlying data

Figshare: Molecular study of sapovirus in acute gastroenteritis in children: a cross-sectional study, <https://doi.org/10.6084/m9.figshare.13574933.v1>³¹

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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Marta Diez Valcarce

Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

The article titled 'Molecular study of sapovirus in acute gastroenteritis in children: a cross-sectional study' by El Sayed Zaki *et al.* includes some very interesting data on the prevalence of sapovirus in Egypt.

Overall, the article is well presented but there are a few minor comments that I would like to make, and also some edits that I consider necessary for clarity and accuracy.

- In the abstract section, results paragraph, I think it could be informative which months are considered winter season in Egypt, as this varies in different parts of the world.
- In the introduction I would suggest to write the family *Caliciviridae* in italics. Also, and that is a major edit that need to be done, the sapovirus genus contains or can be divided into 15 genogroups, not genotypes.
- I am not sure what the authors mean when they say that the primers used depend upon the use of a segment from VP1 encoding gene compared to the RdRp region. I am not aware of any comparison made between the capsid and the polymerase for classification of sapovirus. In some cases, both regions are sequenced to give a more accurate classification, but not compared. Maybe the authors could rewrite this sentences so they are clearer.
- The third paragraph of the introduction mentioned a reference but I think the authors misinterpreted the findings of that work. What the article said is that globally, diarrhea is the second cause of mortality and the most common cause of illness in children younger than 5, and that it causes approximately 2 million deaths per year. But that is diarrhea, from any cause, not only sapovirus. Please correct.
- In the methods section, under the stool samples paragraph the acronym for the molecular detection method used is incorrect, it should read RT-PCR and not RT-PVR.
- Also in the methods section, when the mentioned the Ridascreen method to detect

rotavirus, I think they should refer to the method as a semiquantitative one, since as they mentioned, the color intensity is proportional to the concentration of rotavirus present compared with the control.

- I also suggest to rewrite the part in which they explain how the kit uses monoclonal antibodies that specifically react with the VP6 protein (not the protein of the six viral genes, please correct).
- In the statistical analysis paragraph, if results were expressed as numbers and percentages, those are quantitative values, not qualitative. Please correct.
- In the discussion section, the fifth paragraph, I suggest to replace the word 'management' for 'treatment'. Also, I would rewrite the sentence and instead of using the word 'argument', I would say something like: There are conflicting/contradictory opinions about the role of water sanitation improvement in the prevention of sapovirus ...
- I suggest to replace the expression 'an insignificant difference' for 'no significant difference', throughout the manuscript. Similarly, I also suggest to just mention acute gastroenteritis with all the letters once, with the acronym AGE between brackets, and from then on just use AGE throughout the article.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular epidemiology of caliciviruses

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 15 May 2021

Maysaa El Zaki

Dear Dr. Diez Valcarce,

Thanks for your valuable comments. I have made all the required corrections.

Best Regards.

Competing Interests: No competing interests were disclosed.

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