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Investigation the Prevalence of Norovirus, Rotavirus, Human Bocavirus, and Adenovirus in Inpatient Children with Gastroenteritis in Tehran, Iran, During 2021-2022

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ABSTRACT

Background and Aim: Acute gastroenteritis (AGE) is one of the prevalent factors that threaten human health and is among the main causes of childhood morbidity and fatality rates globally. Viruses are considered one of the main causes of AGE. The current investigation aimed to detect the molecular prevalence of four enteric viruses in AGE.

Materials and Methods: One hundred gastrointestinal specimens were obtained from AGE patients from hospitals affiliated with the Iran University of Medical Sciences during 2021-2022. After viral nucleic acid extraction, a real-time Polymerase chain Reaction (Real-time-PCR) was performed to investigate five enteric viruses.

Results: Among 100 patients, interested viruses were diagnosed in 32 (32%) of patients, who suffered from various gastrointestinal manifestations such as diarrhea, stomach pain, and vomiting. Norovirus (n=10, 32%) was the most common enteric virus, followed by Rotavirus (n=9, 29%), Bocavirus (n=8, 25%), and Adenovirus (n=5, 14). The most virus-positive patients were males (19/32) including Norovirus (7/10), Rotavirus (5/9), Bocavirus (4/8), and Adenovirus (3/5) samples. A high proportion of viruses was detected in children under 12 months.

Conclusion: Our investigation was performed to detect the frequency of different enteric viruses in AGE patients. The finding indicated that Norovirus and Rotavirus are major viral pathogens inducing gastrointestinal infection in patients, respectively. Also, accurate diagnosis of gastrointestinal virus agents can help early treatment and prevent unnecessary prescription of antibiotics and drug resistance development.

Keywords: Gastroenteritis, Real-time PCR, Human Rotavirus, Human Norovirus, Human Adenovirus, Human Bocavirus

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1. Introduction

Acute gastroenteritis (AGE) with nearly 450,000 deaths annually is considered one of the major causes of pediatric illness and death rates across the world notably in low and middle income countries **(1, 2)**. Some young children may experience as many as 1 to 5 episodes of AGE each year. According to the Global Health Reports, AGE is considered the fifth most common cause of fatality in young children, and the eighth most common reason of mortality among every age group **(3)**. The main route of infection is oral fecal and infections are transmitted by contaminated foods and water. AGE can cause a variety of clinical

manifestations including diarrhea, stomach pain, nausea, vomiting, and some rare manifestations such as fever, rashes, muscle aches or headache **(4)**. These manifestations are usually self-limited and disappear within three to five days, but in some cases, these symptoms can be severe and last longer than one week **(5)**.

Various pathogens such as parasites (Giardia and Cryptosporidium), bacteria (Bacillus cereus, Campylobacter, Salmonella, and enterotoxigenic Escherichia coli), fungi (Candidiasis, Aspergillosis) and viruses can induce AGE **(6)**. Among the mentioned etiological agents, enteric viruses including Rotavirus(RV), Norovirus, Human astrovirus (HAstV), Human bocavirus (HBoV), Human adenovirus (HAdV) and Calicivirus are among the main causes of AGE **(7)**. The clinical manifestations and diseases caused by some of these viruses vary according to the type of virus and the immune status of the patients **(8)**.

Norovirus (NoV) is a small positive single-stranded RNA virus that often induces self-limited disease. NoV belongs to the Caliciviridae family and is among the main etiological agents of sporadic and AGE in every age group **(9)**, especially young children **(6)**. This virus accounts for 16 to 18% of all AGE cases worldwide. Small infectious doses, short-lived immune responses, and virus shedding for a prolonged duration (≥ 1-3 weeks) are the main factors of the contagiousness of this virus **(10)**. Among various genotypes, GI and GII noroviruses are the most prevalent. Currently, there are no specific antiviral drugs or treatments for this virus, and the NoV vaccines are in the development phase **(11)**.

Rotavirus is a positive double-stranded RNA virus that is a member of the Reoviridae family. RV is one of the major causes of AGE in young children, and almost every child worldwide has experienced at least one case of diarrhea caused by rotavirus **(9, 12)**. RV AGE occurs throughout the year, with a predominance in autumn and winter, and usually occurs in children 6-24 months of age. This virus has two licensed vaccines which are used in some countries **(13)**.

Human bocavirus (HBoV) is a tiny single-stranded DNA virus within the Parvoviridae family **(14)**. HBoV according to capsid proteins classified into four species (HBoV1-HBoV4). HBoV-1 is most related to respiratory illnesses but sometimes can be detected in AGE patients. HBoV2-4 species are mainly associated with AGE and detected in stool samples **(15, 16)**.

Human adenovirus (HAdV) is a double-stranded DNA nonenveloped virus with an icosahedral nucleocapsid that belongs to the Adenoviridae family **(17)**. Currently, 104 types of adenoviruses have been recognized, which are divided into seven groups based on the presence of adenine and cytosine. Among them, HAdV40 and HAdV41 are the etiological agents of AGE in young children. HAdV infections are responsible for 2-15% of AGE in Children **(18)**.

Our study aimed to analyze the frequency of four enteric viruses (Rotavirus, Norovirus, Adenovirus, Astrovirus and Bocavirus) in Children referred to hospitals affiliated with the Iran University of Medical Sciences during 2021-2022. The accurate detection of enteric viral etiology in children is critical to rapid diagnosis of these agents can help early treatment and also prevent unnecessary prescription of antibiotics and drug resistance development.

2. Materials and Methods

2.1 Sample Collection

For the present study, one hundred specimens were collected from children admitted to hospitals affiliated with the Iran University of Medical Sciences during 2021-2022. The study included children who had various gastrointestinal manifestations such as diarrhea, stomach pain, nausea or vomiting, fever, and generalized rash. Also, we exclude patients with underlying health conditions such as children with chronic gastrointestinal diseases or immunocompromised conditions. Stool samples were transferred on ice to the laboratory and were maintained at -70°C until use. Also, demographic information of patients such as gender, age, family status, clinical manifestations, and blood test results were collected from the patient's files. In the current investigation, samples were analyzed for the detection of four gastrointestinal viruses including Rotavirus, Norovirus, Adenovirus, and Bocavirus.

2.2 Nucleic Acid Extraction

The nucleic acid (RNA/DNA) of samples was extracted by a highly pure viral nucleic acid kit (Yekta Tajhiz Kit, Tehran, Iran), based on the manufacturer's instructions. We analyzed the quality and quantity of extraction by the NanoDrop™ One spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), then the elutions were kept at -70°C until use.

2.3 Real-time PCR

Extracted genomes were analyzed for specific virus detection using TaqMan Real-time PCR. We designed four sets of primers and probes to detect viruses including Rotavirus, Norovirus, Adenovirus, and Bocavirus. PCR master mixture contained TaqMan one-step PCR 2X Master Mix (10 ul), forward primer (1 ul), reverse primer (1 ul), probe (0.5 ul), and double-distilled water (2.5 ul). We mixed a 15 ul master

mixture with a 5 ul sample, in a total volume of 20 ul. The mixtures were amplified by 40 cycles of 94°C and 60°C for 10 sec and 30 sec at the steps of denaturation and annealing-extension, respectively (Table 1,2). We used an internal control to ensure the reliability of our real-time PCR results in each assay. Also, the specificity and sensitivity of the primer probes were analyzed using known positive and negative controls.

Table 1. Primers used in this study

Target virus	Type	Sequence	Amplicon (bp)
Norovirus	Forward	CGYTGGATGCGNTTYCATGA	456
	Reverse	CTTAGACGCCATCATCATTYAC	
Rotavirus	Forward	GAC GGN GCR ACT ACA TGG T	379
	Reverse	GTC CAA TTC ATN CCT GGT GG	
Adenovirus	Forward	GCTTCGGAGTACCTGAGYCC	243
	Reverse	5'-GGCCATRTCCAGCACTCKGT	
Bocavirus	Forward	GGCTCCTGCTCTAGGAAATAAAGAG	579
	Reverse	CCTGCTGTTAGGTCGTTGTTGTATGT	

Table 2. The chart for Real Time PCR

No.	Cycle	Step	Temperature	Time
1	1	Reverse Transcription	50 °C	15 min
2	1	Initial Denaturation	94 °C	3 min
3	45	Denaturation	94 °C	10 sec
		Annealing-Extension	60 °C	30 sec
4	1	Device Cooling	25 °C	1 sec

2.4 Statical Analysis

Total data were analyzed by SPSS for Windows version 22.0 (SPSS Inc., Chicago, IL, USA). All comparisons were made using the Fisher exact test. P-values < 0.05 were considered as an indication of statistical significance.

3. Results

One hundred stool specimens were obtained from AGE Children admitted to hospitals affiliated with the Iran University of Medical Sciences during 2021-2022. Patients suffered from various gastrointestinal manifestations including diarrhea, stomach pain, nausea or vomiting, fever, and generalized rash. Among 100 participants, 55 (55%) were males and 45(45%) were females. The mean (±SD) age of all patients was 19.14±16.80 months (ranging from 1 to 60 months). The viruses of interest were found in 32(32%) samples (Figure 1). According to Figure 2, Norovirus (n=10, 32%) was the most common enteric virus detected in this study, followed by Rotavirus (n=9, 29%), Bocavirus (n= 8, 25%), and Adenovirus (n=5, 14%). The most virus-positive patients were males (19/32) including Norovirus (7/10), Rotavirus (5/9), Bocavirus (4/8), and Adenovirus (3/5) samples (Table 3). A high proportion of gastrointestinal viruses was detected in children under 12 months, including Norovirus (n=5, 50%), Rotavirus (n=5, 56%), Bocavirus (n=3, 38%), and Adenovirus (n=2, 40%). The second most prevalent infected age groups belong to children between 1-2 years (n=6) including Norovirus (n=3), Bocavirus (n=3), and Adenovirus (n=1). Also, viruses were found in two age groups: children between 24-36 months and children between 48-60 months. No enteric viruses were detected in children 36-48 months in our study (Table 4).

Table 3. Demographic features of the studied participants.

Characteristic	All cases	Male	Female
Mean Age (month)	19.14±16.80	19.2±15.60	18.60±12.60
Gender (M/F)	55/45	55	45
Virus Detection (+/-)	32/100	19/32	13/32

Table 4. Distribution of viral infections in different age groups

	Detected Viruses No. (%)				
Age groups(month)	Norovirus	Rotavirus	Bocavirus	Adenovirus	Astrovirus
0-12	5	5	3	2	-
12-24	3	-	2	1	-
24-36	1	2	1	1	-
36-48	-	-	-	-	-
48-60	1	1	2	1	-

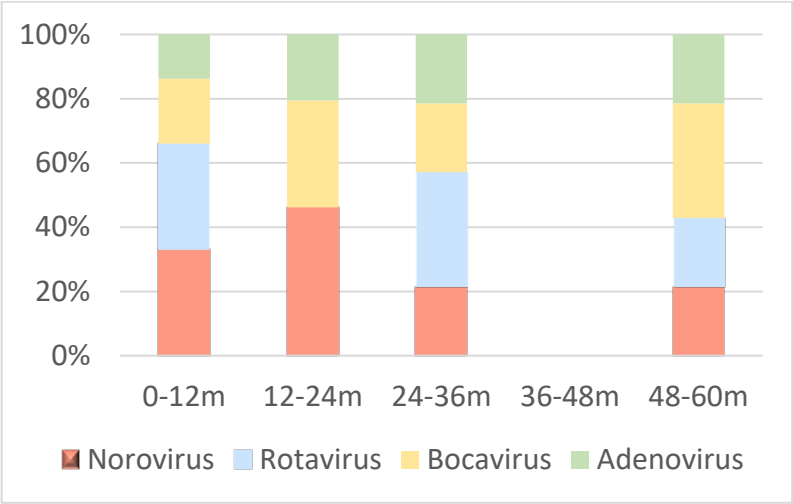

Figure 1. Distribution of viral infections in different age groups

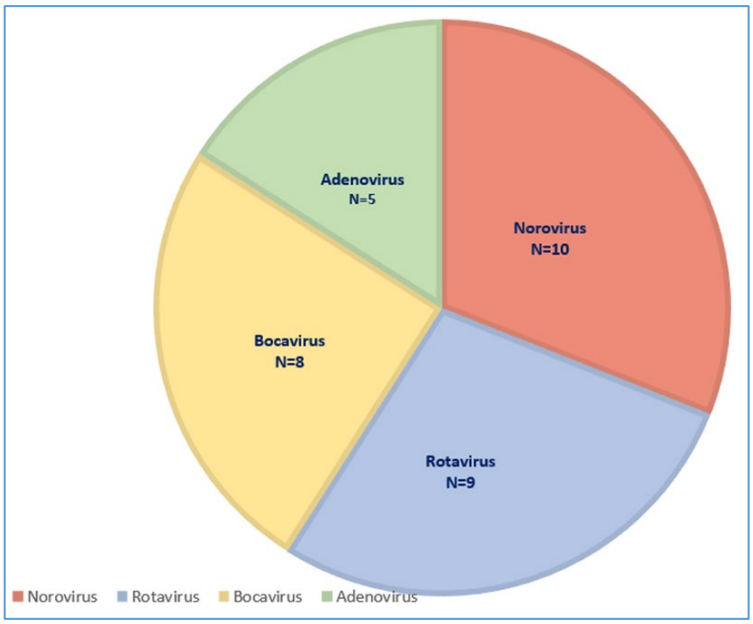

Figure2. Prevalence of enteric viruses in AGE

4. Discussion

Viruses are considered the major cause of AGE in children and adults. Enteric viruses such as Rotavirus, HAstV, HAdV, Bocavirus, Norovirus and Calicivirus are among the main causes of AGE **(7, 19, 20)**. Considering that most enteric viruses have no specific treatment and require supportive treatment, rapid diagnosis of these agents can help early treatment and also prevent unnecessary prescription of antibiotics and drug resistance development. In the current study, the prevalence of four common enteric viruses in hospitalized children who were admitted to hospitals affiliated with the Iran University of Medical Sciences during 2021-2022 was investigated. Among 100 patients, enteric viruses were detected in 32 (32%) of the samples. Norovirus (n=10, 32%) was the most common enteric virus, followed by Rotavirus (n=9, 29%), Bocavirus (n=8, 25%), and Adenovirus (n=5, 14%). Norovirus's high infectivity and resistance to disinfectants and also rotavirus vaccination can be effective on these viruses' prevalence.

Also, we found the most virus-positive patients were males (19/32) including Norovirus (7/10), Rotavirus (5/9), Bocavirus (4/8), and Adenovirus (3/5) samples. This difference can be attributed to various factors such as biological factors like hormonal variations and immune response, and behavioral factors that boys more active and have higher exposure to pathogens.

In our study, a high proportion of viruses was detected in children under 12 months. It can be due to various reasons such as the immature immune

system**,** breastfeeding and the diet of young children. Infants and young children have immature immune systems, making them particularly susceptible to infections, especially gastrointestinal ones. Additionally, when transitioning from breastfeeding to solid foods, children lose some protective maternal antibodies from breast milk, making them more vulnerable to infections.

Also, the duration of the disease varies in AGE children, with severe cases lasting longer. Factors influencing duration include the viral strain, viral load, and patient health conditions that can be effective. Various studies have been conducted to investigate the etiological role of enteric viruses in Iran and other region of the world.

Similar to the results of our study, Kaung et al analyzed genetic diversity of group A rotaviruses in 670 AGE outpatients by RT-real-time PCR method. They found that 61.7% of the samples were positive for rotavirus, and the prevalence of RNA virus was higher in children compared to adults (9.3% vs. 7.2%). Also, they indicated that G9P(B) followed by G3P(B) and G1P(B) were more prevalent genotypes in children and adults respectively **(21)**.

Eftekhari et al investigated the prevalence of various genotypes of Norovirus in 200 AGE children. Their finding indicated that among 40 positive patients, The GII.4 norovirus was found to be the most common VP1 genotype (53%) followed by GII.8 (32%), GII.7 (6%), GII.17 (6%), and GII.3 (3%). Also, the GII.P16 norovirus was found as the predominant RdRP type (53%) followed by GII.P8 (32%), GII.P7 (6%), GII.P17 (6%), and GII.P31 (3%) **(22)**.

In the study by Keita et al, the molecular prevalence of enteric viruses in 4840 cases of moderate-to-severe diarrhea (MSD), 12.6% were attributed to rotavirus 2.7% to adenovirus 40/41, 2.9% to astrovirus, and 1.9% to sapoviruses respectively **(23)**. Eifan et al. conducted surveillance on human rotavirus A (HRV) and human adenovirus (HAdV) strains associated with gastroenteritis in Riyadh, Saudi Arabia. They found a prevalence of 7% for HAdV and 2% for HRV, with HAdV infections being more prevalent in females and HRV infections in males. The study also identified a higher prevalence of HAdV in autumn, correlated with humidity, and highlighted the dominance of HAdV type 41 and the G2 lineage of HRV among circulating strains **(24)**. In a study conducted by Malek et al, the prevalence of rotavirus AGE in children in the Western Mediterranean region was reported as 40%. Their results were related to a high percentage of acute rotavirus gastroenteritis in Syria (61%) and Oman (51%). The lowest percentage of rotavirus prevalence was related to the countries of Saudi Arabia (9%), Tunisia and Egypt (16%-23%). These differences can be due to seasonal differences, geographical location, age and sex of the patients **(25)**. Monavari et al investigated the etiological role of rotavirus in inducing AGE in children in Iran, they indicated that the average prevalence of rotavirus was 39.9%, also G1 and G4 were more prevalent genotypes in children **(26)**. Also, Nazari et al., demonstrated that rotavirus (RV) infection was found among 28.5% of hospitalized children with diarrhea **(27)**. Kachooei, et al. investigated the prevalence and clinical features of human bocavirus (HBoV) in young children with AGE, HBoV was detected in 14% of patients, and HBoV3 was the predominant genotypes **(28)**. Kadhim Jwaziri, et al. analyzed the molecular prevalence and genotype distribution of human adenovirus (HAdV) in Iranian children under five with gastroenteritis. They found HAdV DNA in 15% of samples. HAdV types 2,40 and 41 are the most prevalent genotypes **(29)**. Kachooei et al. conducted molecular characterization of rotavirus infections, their finding demonstrated the emergence of uncommon G9P[4] and G9P[8] reassortants with unique genotype patterns not previously reported in Iran, and suggested the need for further analysis of rare and emerging strains **(30)**.

However, there were some limitations in the present study that should be mentioned. First, the sample size may limit the understanding of the etiological role of enteric viruses among children. Second, all specimens were collected from specific hospitals in Tehran, which may affect the accuracy of the diagnosis. Third, all specimens belonged to Tehran, without considering the potential impact of genetics and climate. Sampling from various geographical regions of Iran could be a more accurate indicator of the prevalence of enteric virus status. Future studies with larger, more geographically diverse samples are recommended.

5. Conclusion

Norovirus and Rotavirus were the predominant enteric viruses detected in our study, followed by Bocavirus and Adenovirus. The most infected group in our study was males, and a high proportion of gastrointestinal viruses was detected in children under 12 months. This result can help develop rapid diagnosis of these agents can help early treatment and also prevent unnecessary prescription of antibiotics and drug resistance development.

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Ethical Considerations

Ethical approval for the present study was provided by the Ethics Committee of Iran University of Medical Sciences: IR.IUMS.REC.1399.1385.

Authors' Contributions

SHR.M designed the study. SJ. K, Z.S and R.M performed all statistical analyses. Z.S, A.T, R.M., and MR. R wrote, reviewed, and edited the manuscript. All authors read and approved the final draft.

Conflict of Interest

The authors have no conflict of interest.

Funding

Not applicable.

Abbreviation

Acute Gastroenteritis (AGE), Realtime Polymerase Chain Reaction (Realtime-PCR), Norovirus (NoV), Rotavirus (RV), Human Bocavirus (HBoV), Human Adenovirus (HAdV).

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