







Diagnosis of Causative Acute Childhood Viral Diarrhea by Multiplex Real-Time PCR Among Children in a Referral Hospital, Tehran, Iran

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Abstract

Background: Viral gastroenteritis has significant public health implications and can greatly affect individuals' overall well-being. It spreads rapidly within communities, particularly among pediatric populations in settings like schools.

Objectives: This study aimed to identify the most common viral causes of gastroenteritis in children aged one month to 18 years over a year in a referral tertiary children's hospital, highlighting the burden of the illness in this population.

Methods: In this cross-sectional study, stool samples from children with viral gastroenteritis admitted to Mofid Children's Hospital were collected from January 2023 to October 2024. Total RNA extraction was performed following stool preparation. Detection of SARS-Cov-2, norovirus, sapovirus, rotavirus, adenovirus, and astrovirus was carried out using multiplex Real-Time PCR.

Results: A total of 100 participants were included in this study, of which 62% were male and 38% female, with a mean age of 2.7 ± 3.44 years. Among all patients with diarrhea, 55.5% tested positive via PCR. Rotavirus was the most frequently detected virus, accounting for 43.6% of cases, followed by Norovirus and Adenovirus at 23.6%, Sapovirus and Coronavirus at 14.5%, and Astrovirus at 7.3%. Co-infection with two viruses was observed in 23.6% of patients, and 1.8% were co-infected with three viruses. Conclusions:

Conclusions: This study highlights that gastroenteritis in young children has decreased in many countries following the introduction of the rotavirus vaccine. An important finding of this research is the prevalence of co-infection in pediatric patients, which should be taken into consideration by physicians.

Keywords: Viral Gastroenteritis, Children, Multiplex Real-time PCR

1. Background

Viral gastroenteritis, commonly known as the stomach flu, is an illness caused by various viruses that affect the gastrointestinal tract (1). Understanding the significance of viral gastroenteritis is crucial due to its impact on public health and individual well-being (2).

Viral gastroenteritis is highly contagious and can spread rapidly within communities, particularly in crowded places such as schools, hospitals, and workplaces (3-5). Awareness of its significance is essential for implementing preventive measures to limit virus transmission. These measures include practicing good hand hygiene, maintaining proper sanitation, and avoiding close contact with infected individuals during their illness (6).

Additionally, viral gastroenteritis can cause significant morbidity and discomfort due to symptoms such as watery diarrhea, vomiting, abdominal pain, and sometimes fever (7, 8). While most cases resolve within a few days, severe cases can lead to dehydration, electrolyte imbalances, and other complications, particularly in vulnerable populations such as young children, older adults, and immunocompromised individuals (9). Recognizing the importance of viral gastroenteritis promotes early detection and proper management to prevent complications and ensure timely medical intervention when necessary.

Monitoring the incidence and prevalence of viral gastroenteritis cases allows healthcare authorities to identify patterns, implement control measures, and provide appropriate guidance to healthcare professionals and the public (10). This helps minimize

the impact of outbreaks and ensures the efficient allocation of resources.

The identification and management of the causative agents of viral gastroenteritis are crucial for public health, individual well-being, and effective outbreak control (10).

2. Objectives

This study aimed to identify the most common viral pathogens responsible for gastroenteritis, enabling proactive measures to prevent transmission, manage symptoms, and mitigate the impact on affected individuals and communities.

3. Methods

3.1. Data Collection and Study Setting

In this cross-sectional study, stool samples were collected from children with viral gastroenteritis admitted to Mofid Children's Hospital, Tehran, Iran, from January 2023 to October 2024. Fresh stool samples from hospitalized children, aged between one month and 18 years, in the emergency and infectious wards, were included in the study after obtaining informed consent from the children's parents.

3.2. Inclusion Criteria

- Children with watery diarrhea (no WBCs or RBCs in stool examination).
- Children with no history of antibiotic use.
- Children without other gastrointestinal diseases.

3.3. Sample Preparation

The stool samples were homogenized by vortexing with sterile DNase and RNase-free water at a 1: 5 (weight/volume) ratio. The samples were then centrifuged at 5000 rpm for 5 minutes, and 300 μ l of the supernatant was reserved for total RNA extraction.

3.4. RNA Extraction

The samples were processed and prepared for RNA extraction using a specialized RNA extraction kit (SIMBIOLAB, Lot No. 010502-2326-202N100).

3.5. Multiplex Real-time PCR

The most common causes of viral gastroenteritis include Norovirus, Sapovirus, Astrovirus, Rotavirus,

Adenovirus, and SARS-CoV-2. In this study, commercial multiplex real-time PCR (Genova, Cat No. GA-5GAS.25) was used. SARS-CoV-2 was detected using COVITEC (Lot No. COVIT-21008-1, Iran).

3.6. Statistical Analysis

Statistical analysis was performed using SPSS software version 23. Logistic regression analysis was used to evaluate the effect of factors such as gender and age on the detected viruses. A P-value of ≤ 0.05 was considered statistically significant. For descriptive data analysis, percentages with 95% confidence intervals (95% CI) were reported.

4. Results

A total of 100 participants were included in this study, with 62% being male and 38% female. The mean age of participants was 2.7 ± 3.44 years (ranging from 1 month to 8 years). Among all patients with diarrhea, 55.5% showed positive PCR results. Rotavirus was the most frequently detected virus at 43.6%, followed by Norovirus and Adenovirus at 23.6%, Sapovirus and Coronavirus at 14.5%, and Astrovirus at 7.3%. Additionally, 23.6% of patients had co-infections with two viruses, and 1.8% had co-infections with three viruses. Among the co-infections, Rotavirus plus Norovirus was the most frequent (Table 1).

Fever and vomiting were the most common symptoms in PCR-positive patients. All the main symptoms are presented in Table 2.

Among PCR-positive children, 36.4% had leukocytosis (WBC $> 11,000$), and 30.9% had leucopenia (WBC $< 4,000$). This variable was assessed in both PCR-positive and PCR-negative patients (Table 3).

None of the considered variables showed a significant difference between the positive and negative PCR groups ($P \geq 0.05$). There was also no significant difference (P -value > 0.05) in the clinical symptoms (vomiting, fever, cough, rhinorrhea, severity of dehydration, duration of diarrhea, duration of admission) of hospitalized patients based on the type of virus identified. However, the presence of leukocytosis in patients with adenovirus and norovirus (P -value: 0.04), and the presence of leukopenia in Covid-19 patients (P -value: 0.05), were significant. The frequency of detected viruses across different months is shown in Table 4.

There was no significant difference (P -value > 0.05) in the frequency of the viruses across different months.

5. Discussion

Table 1. Frequency of Different Co-infections

Variables	Frequency (%)
SARS-Cov-2 + norovirus+ rotavirus	7.1
Norovirus + rotavirus	28.5
SARS-Cov-2 + rotavirus	14.3
Sapovirus + adenovirus	14.3
SARS-Cov-2 + adenovirus	7.1
Norovirus + adenovirus	21.4

Table 2. The Main Symptoms in PCR Positive Patients

Symptoms	Frequency (%)
Fever	74.5
Vomiting	69
Abdominal cramp	49
Malaise	43.6
Cough	36.4
Sore through	20
Rhinorrhoea	16.3
Seizure	12.7
Headache	3.6
Bloody diarrhea	3.6

Table 3. Survey of Variables in Positive and Negative PCR Groups^a

Variables	Stool. PCR Positive	Stool. PCR Negative
Age (y)		
<1	19 (52.7)	17 (47.3)
1-5	27 (56.2)	21 (43.8)
>5	9 (56.3)	7 (43.7)
Severity of dehydration		
Mild	25 (48)	27 (52)
Mod	25 (65.8)	13 (34.2)
Severe	5 (50)	5 (50)
Duration of admission; mean (range) (day)	4.87 (2-16)	4.69 (1-18)
Duration of diarrhea; mean (range) (day)	4.9 (1-13)	5.1 (1-12)
Leukocytosis	20 (34.6)	16 (36.6)
Leucopenia	8 (14.5)	7 (15.6)
ESR (mean)	32	30
CRP (mean)	15	18

^a Values are expressed as No. (%) unless otherwise indicated.

This study reports the prevalence of six gastrointestinal viruses and their associated clinical signs and laboratory findings in children admitted with diarrhea over the past year (January-November 2023) in a pediatric teaching hospital in Iran. The prevalence of these viruses aligns with most studies conducted in the

Middle East (11-13), with the exception that in regions where the rotavirus vaccine is administered, the percentage of rotavirus cases is lower.

Borkakoty et al. (2020) from India reported the prevalence of norovirus, rotavirus, adenovirus, and sapovirus as 18.4%, 67.1%, 45.9%, and 5.9%, respectively (11).

Table 4. Frequency of the Viruses in Different Months (%)

Months	SARS-Cov-2	Rotavirus	Adenovirus	Norovirus	Astrovirus	Sapovirus
January	1	2	1	2	0	1
February	0	2	0	1	1	1
March	3	2	1	0	0	0
April	1	7	5	4	0	1
May	0	2	3	1	0	3
June	0	4	0	1	1	2
July	0	1	0	0	0	0
August	1	2	1	2	2	0
September	2	2	2	2	0	0

Kara et al. (2018 - 2021) from Turkey observed a 65% positivity rate for viruses and bacteria in symptomatic patients, with multiple pathogens detected in 35% of cases (12). In our study, co-infections were observed in 25.4% of patients. Another study from Saudi Arabia (2021 - 2022) revealed rotavirus as the most frequently detected virus, with a frequency of 54% (13). Similarly, our study found that rotavirus was the leading cause of viral gastroenteritis in children, consistent with these other studies (11-13).

In Iran, while some studies have focused on rotavirus and adenovirus, research on other viral agents has been limited. In this study, leukocytosis was significantly more frequent in children infected with adenovirus and norovirus compared to the PCR-negative group (P-value: 0.04). Additionally, leukopenia was more prevalent in Covid-19 patients (P-value: 0.05).

The results of this study highlight the utility of multiplex molecular testing as a reliable diagnostic method due to its speed and high sensitivity, enabling the simultaneous detection of multiple pathogens. However, it can be challenging to differentiate between asymptomatic viral shedding and disease-causing pathogens. As expected, rotavirus had the highest prevalence among the detected viruses in this study, and rotavirus-norovirus co-infections were the most frequent.

Given that Mofid Hospital is a referral center and all children who visited the emergency department and met the inclusion criteria were enrolled in this study, it is reasonable to conclude that these findings may be generalizable to the pediatric population in Tehran.

5.1. Conclusions

It is important to note that gastroenteritis in young children, particularly in infancy, has decreased in many

countries following the introduction of the rotavirus vaccine. As expected, rotavirus was the most frequently detected virus in our study, and among co-infections, the combination of rotavirus and norovirus was the most common. Additionally, multiplex PCR offers a reliable, time- and cost-efficient method for detecting multiple viruses in stool samples. However, distinguishing between active infection and viral shedding remains a challenge.

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Footnotes

Authors' Contribution: M. R. designed study, improve the manuscript; L. A. methodologist, designed the lab part of study, improve the manuscript; H. Kh. lab data gathering, prepare the primary draft of the manuscript; A. K. designed the study, pediatric infectious disease consultant.

Conflict of Interests Statement: This study does not have any conflict of interest.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences (IR.SBMU.RICH.REC.1402.003).

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Informed Consent: Inform consent was obtained from parents.

References

- Mojahed N, Mohammadkhani MA, Pourasgari M, Gol-Jah Rad G, Mohamadkhani A. Viral Gastroenteritis Prevalence in Iranian Pediatric Population: A Systematic Review. *Avicenna J Clin Microbiol Infect.* 2022;**9**(3):124-9. <https://doi.org/10.34172/ajcmi.2022.3389>.
- Khales P, Motlaghzadeh S, Ghorbani S. Acute Viral Gastroenteritis. *Int J Enteric Pathog.* 2023;**11**(2):81-2.
- Krenzer ME. Viral gastroenteritis in the adult population: the GI peril. *Crit Care Nurs Clin North Am.* 2012;**24**(4):541-53. [PubMed ID: 23089659]. [PubMed Central ID: PMC7172911]. <https://doi.org/10.1016/j.ccell.2012.07.003>.
- Reynolds KA, Beamer PI, Plotkin KR, Sifuentes LY, Koenig DW, Gerba CP. The healthy workplace project: Reduced viral exposure in an office setting. *Arch Environ Occup Health.* 2016;**71**(3):157-62. [PubMed ID: 26066784]. [PubMed Central ID: PMC4676746]. <https://doi.org/10.1080/19338244.2015.1058234>.
- Li J, Gao X, Ye YL, Wan T, Zang H, Mo PH, et al. An acute gastroenteritis outbreak associated with person-to-person transmission in a primary school in Shanghai: first report of a GL5 norovirus outbreak in China. *BMC Infect Dis.* 2018;**18**(1):316. [PubMed ID: 29986649]. [PubMed Central ID: PMC6038313]. <https://doi.org/10.1186/s12879-018-3224-4>.
- Lee MB, Greig JD. A review of gastrointestinal outbreaks in schools: effective infection control interventions. *J Sch Health.* 2010;**80**(12):588-98. [PubMed ID: 21087255]. <https://doi.org/10.1111/j.1746-1561.2010.00546.x>.
- Jagirdhar GSK, Pulakurthi YS, Chigurupati HD, Surani S. Gastrointestinal tract and viral pathogens. *World J Virol.* 2023;**12**(3):136-50. [PubMed ID: 37396706]. [PubMed Central ID: PMC10311582]. <https://doi.org/10.5501/wjv.v12.i3.136>.
- Hellysaz A, Neijd M, Vesikari T, Svensson L, Hagbom M. Viral Gastroenteritis: Sickness Symptoms and Behavioral Responses. *mbio.* 2023;**14**(2). e0356722. [PubMed ID: 36976000]. [PubMed Central ID: PMC10128049]. <https://doi.org/10.1128/mbio.03567-22>.
- Wilson M, Wilson P. Gastroenteritis Due to Salmonella. *Close Encounters of the Microbial Kind: Everything You Need to Know About Common Infections.* Berlin: Springer; 2021. p. 451-61. https://doi.org/10.1007/978-3-030-56978-5_33.
- Shaheen MNE. The concept of one health applied to the problem of zoonotic diseases. *Rev Med Virol.* 2022;**32**(4). e2326. [PubMed ID: 35060214]. <https://doi.org/10.1002/rmv.2326>.
- Borkakoty B, Bali NK, Jakaria A, Hazarika R, Temsu T, Gohain M, et al. Norovirus gastroenteritis in children under-five years hospitalized for diarrhea in two cities of northeast India: A retrospective study. *Indian J Med Microbiol.* 2023;**45**:100397. [PubMed ID: 37573056]. <https://doi.org/10.1016/j.ijmmb.2023.100397>.
- Kara Y, Kızıl MC, Kılıç Ö, Us T, Dinleyici EÇ. Investigation of the use of multiplex PCR in childhood diarrhea with clinical and epidemiological features. *J Trop Pediatr.* 2022;**68**(6). [PubMed ID: 36272732]. <https://doi.org/10.1093/tropej/fmac090>.
- M. Alsubaiei R, Gharbi J, Almalki M, Alahmed K, Ali Brahim A, Alshakes F, et al. Viral Etiology and Epidemiology of Children Acute Gastroenteritis (AGE) in Al-Ahsa Province - Saudi Arabia during the period 2021-2022. *Iranian J Med Microbiol.* 2023;**17**(5):541-9. <https://doi.org/10.30699/ijmm.17.5.541>.