



RESEARCH ARTICLE

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MOLECULAR DETECTION OF ADENOVIRUS AND ROTAVIRUS CO-INFECTION IN CHILDREN WITH GASTROENTERITIS

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Abstract

Background: Acute gastroenteritis is one of the leading causes of illness and hospitalization among children under five years of age. Rotavirus and enteric adenovirus are among the most common viral pathogens, while co-infection with both viruses may contribute to a more severe clinical course.

Objective: To determine the frequency of rotavirus, adenovirus, and rotavirus–adenovirus co-infection among children with acute gastroenteritis and to evaluate their association with demographic characteristics, clinical manifestations, and disease severity.

Methods: A prospective cross-sectional study was conducted from January to June 2025 at Ibn Saif Al-Janabi Hospital for Pediatrics, Babylon, Iraq. One hundred children aged 6 months to 5 years presenting with acute gastroenteritis were enrolled. Stool specimens were screened for rotavirus and adenovirus antigens using enzyme-linked immunosorbent assay (ELISA), and positive samples were confirmed by real-time polymerase chain reaction (RT-PCR). Demographic and clinical data, were analyzed using SPSS version 17.

Results: Viral infection was detected in 45 (45%) children. Rotavirus mono-infection was identified in 24 (24%) cases, adenovirus mono-infection in 12 (12%), and rotavirus–adenovirus co-infection in 9 (9%). Both rotavirus and adenovirus mono-infections were significantly associated with fever, severe dehydration, and longer hospital stay ($p < 0.001$). Compared with children with mono-infection, those with co-infection had a significantly longer mean hospital stay (2.89 ± 0.78 vs. 1.61 ± 0.64 days, $p < 0.001$) and a higher frequency of severe dehydration (77.8% vs. 22.2%, $p = 0.006$). No significant differences were observed regarding age, sex, vomiting, or Ct values between mono-infected and co-infected patients

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Conclusion: Rotavirus was the most frequently detected viral pathogen, followed by adenovirus. Rotavirus–adenovirus co-infection was associated with greater clinical severity, particularly severe dehydration and prolonged hospitalization.

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Introduction:-

Gastroenteritis remains a common infectious disease with a substantial global health burden, affecting individuals across all age groups. Young children are particularly susceptible to acute gastroenteritis and its associated complications. In developing countries, viral pathogens are recognized as one of the principal causes of gastroenteritis and contribute considerably to the overall incidence of the disease(1). Diarrhea is a frequent symptom of gastroenteritis and continues to be a major cause of morbidity and mortality in children. In the past, bacteria were regarded as the main cause of gastroenteritis; however, advances in viral diagnostic techniques during the last 20 years have identified viruses as the leading cause of the disease(2). Among children under five years of age, diarrhea remains a major public health concern and an infectious disease associated with high morbidity and mortality. Rotavirus gastroenteritis is estimated to cause approximately 197,000 deaths each year in children under five, and its high incidence places a considerable economic burden on both low- and high-income countries(3). Globally, rotavirus is recognized as a major cause of acute diarrhea in infants and is responsible for nearly 9% of deaths occurring in children younger than five year(4). Structurally, rotavirus is characterized by the absence of an envelope and possesses a double-stranded RNA genome surrounded by a triple-layered icosahedral capsid.

The viral genome produces six structural and six non-structural proteins that contribute to the virus's replication and virulence (5). After rotavirus, adenovirus is another common viral agent associated with acute gastroenteritis in infants. Studies have shown that enteric adenoviruses are responsible for about 2–6% of acute diarrhea and gastroenteritis cases among young children worldwide (6). Human adenoviruses are non-enveloped viruses containing a linear double-stranded DNA genome of about 34–36 kb enclosed within an icosahedral capsid. Based on their biological and genetic characteristics, 88 serotypes have been classified into seven species (HAdV-A to HAdV-G).(7). These viruses also create coinfections in different combinations. It has been reported that viral mixed-infection rates range from 4.4% to 29% in classical or molecular-based studies(8). In areas with poor sanitation and limited healthcare resources, infection with more than one enteric virus is relatively common. Such co-infections have been linked to increased disease severity and reduced vaccine efficacy. They may also facilitate viral evolution because the concurrent presence of different viral strains provides opportunities for genetic events such as recombination and reassortment(9).

Materials and Methods:-**Study Design:-**

A prospective cross-sectional study was conducted between January and June 2025 at Ibn Saif Al-Janabi Hospital for Pediatrics, Babylon, Iraq. The study included 100 children aged 6 months to 5 years who presented with signs and symptoms of acute gastroenteritis. Demographic and clinical information, including age, sex, fever, vomiting, dehydration status, and duration of hospital stay, were collected using a structured data collection form.

Sample Collection:-

Fresh stool specimens were collected from all enrolled children in sterile screw-capped containers under aseptic conditions. Samples were transported immediately to the laboratory and processed on the day of collection whenever possible. If immediate analysis was not feasible, specimens were stored at 2–8°C for short-term storage and at –20°C until further laboratory investigations.

Detection of Rotavirus and Adenovirus Antigens by ELISA:-

All stool specimens were screened for Rotavirus and Adenovirus antigens using the EDI™ Fecal Rotavirus Antigen ELISA Kit (KT-841) and the EDI™ Fecal Adenovirus Antigen ELISA Kit (KT-842) (Epitope Diagnostics Inc., San Diego, CA, USA), according to the manufacturer's recommendations. Stool samples were diluted in the supplied sample diluent and transferred into microplate wells pre-coated with virus-specific antibodies. After incubation, horseradish peroxidase (HRP)-conjugated antibodies were added to form antigen-antibody complexes. Following repeated washing steps to remove unbound materials, tetramethylbenzidine (TMB) substrate was added and incubated for color development. The reaction was terminated using stop solution, and the optical density (OD) was measured at 450 nm using a BioTek microplate ELISA reader (BioTek Instruments Inc., USA). Test results were interpreted according to the manufacturer's recommended cut-off values. Only ELISA-positive samples were subjected to molecular analysis.

Viral Nucleic Acid Extraction:-

Viral nucleic acids were extracted from ELISA-positive stool specimens using the Ribo-Sorb RNA/DNA Extraction Kit (Sacace Biotechnologies, Como, Italy) according to the manufacturer's instructions. Briefly, 100 µL of clarified

stool suspension was mixed with lysis buffer containing an internal control and incubated at room temperature to ensure complete viral lysis. Viral nucleic acids were adsorbed onto silica sorbent particles and purified through sequential washing steps using the supplied washing solution, 70% ethanol, and acetone. The purified nucleic acids were eluted in 50 µL of elution buffer after drying the sorbent at 60°C for 10 minutes. Extracted nucleic acids were either analyzed immediately or stored at -80°C until amplification.

Detection of Human Rotavirus by Real-Time PCR:-

Rotavirus RNA was detected using the Rotavirus/Norovirus/Astrovirus Real-TM Kit (Sacace Biotechnologies, Italy). PCR reactions were prepared according to the manufacturer's instructions using 15 µL of the prepared reaction mixture and 10 µL of extracted RNA, giving a final reaction volume of 25 µL. Amplification was performed using the Rotor-Gene Q Real-Time PCR System (QIAGEN, Germany). The thermal profile consisted of reverse transcription at 50°C for 30 min, initial denaturation at 95°C for 15 min, followed by 45 cycles of 95°C for 10 sec, 60°C for 35 sec, and 72°C for 10 sec, with fluorescence acquisition during the amplification stage. Positive and negative controls supplied with the kit were included in each PCR run to verify assay performance. Cycle threshold (Ct) values were generated automatically by the Rotor-Gene software, and samples showing amplification in the FAM channel with Ct values <40 were considered positive according to the manufacturer's recommendations.

Detection of Human Adenovirus by Real-Time PCR:-

Samples positive for Adenovirus antigen by ELISA were further analyzed using the RealStar® Adenovirus PCR Kit 1.0 (Altona Diagnostics GmbH, Germany). The assay detects and quantifies human adenovirus DNA using fluorogenic hydrolysis probes specific for adenovirus DNA (FAM channel) together with an internal control detected in the JOE channel. Extracted DNA served as the template for amplification. PCR reactions were prepared according to the manufacturer's instructions using 5 µL Master A and 15 µL Master B to prepare the master mix. Since the internal control had been added during nucleic acid extraction, 20 µL of master mix was dispensed into each reaction tube followed by 10 µL of extracted DNA, resulting in a final reaction volume of 30 µL. Each PCR run included positive and negative controls. Amplification was carried out on the Rotor-Gene Q Real-Time PCR System (QIAGEN, Germany) under the following thermal conditions: initial denaturation at 95°C for 10 minutes, followed by 45 amplification cycles consisting of 95°C for 15 seconds and 58°C for 60 seconds, with fluorescence acquisition performed during the annealing/extension step.

Statistical Analysis:-

Statistical analysis was performed using were entered and analyzed using SPSS (statistical package of the social science) package version 17. Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as mean $\pm$ standard deviation (SD). Associations between categorical variables were assessed using Pearson's Chi-square test or Fisher's exact test where appropriate. Continuous variables, including Ct values, were compared using the independent-samples t-test. A p-value of less than 0.05 was considered statistically significant.

Ethical Considerations:-

The study protocol was approved by the Scientific and Ethical Committee of Al-Furat Al-Awsat Technical University. Written informed consent was obtained from the parents or legal guardians of all participating children before enrollment. All patient information was kept confidential and used exclusively for research purposes.

Results:-

A total of 100 stool samples were analyzed. Rotavirus mono-infection was detected in 24 (24.0%) children, adenovirus mono-infection in 12 (12.0%), and co-infection with both viruses in 9 (9.0%). Overall, viral infection was confirmed in 45.0% of the studied children, whereas 55.0% tested negative for both viruses (table 1).

Table (1): Distribution of of Rotavirus, Adenovirus and Co-infection among the studied children

Infection Status	no	Percentage%
Rotavirus mono-infection	24	24%
Adenovirus mono-infection	12	12%
Rota and adenovirus co-infection	9	9%
Negative	55	55%
Total	100	100%

Table 2 demonstrates the association between rotavirus mono-infection and the studied demographic and clinical characteristics. No significant differences were observed regarding age ($p=0.230$) or sex ($p=0.675$). In contrast, fever was significantly more common among rotavirus-positive children than in the negative group (90.9% vs. 47.8%, $p<0.001$). Severe dehydration was also significantly associated with rotavirus infection (33.3% vs. 6.0%, $p<0.001$). Furthermore, children with rotavirus infection experienced significantly longer hospital stays compared with non-infected children ($p<0.001$). Although vomiting was more frequent among infected patients (90.9%), the association was not statistically significant ($p=0.388$).

Table (2): Association between Rotavirus mono-infection and study variables

Variables	Rotavirus(-ve) N(%)	Rotavirus(+ve) N(%)	P value
Age(months) Mean $\pm$ SD	25.86 $\pm$ 16.3	21.82 $\pm$ 14.5	0.230
Sex			0.675
Male	39(58.2)	17(51.5%)	
Female	28(41.8%)	16(48.5%)	
Fever			<0.001
No	35(52.2%)	3(9.1%)	
Yes	32(47.8%)	30(90.9%)	
Vomiting			0.388
No	12(17.9%)	3(9.1%)	
Yes	55(82.1%)	30(90.9%)	
Dehydration			<0.001
None	47(70.1%)	7(21.2%)	
Mild	16(23.9%)	15(45.5%)	
Severe	4(6.0%)	11(33.3)	
Hospital Stay			<0.001
0 day	37 (55.2%)	2(6.1%)	
1 day	17(25.4%)	8(24.2%)	
2 day	10(14.9%)	14(42.4%)	
3 day	3(4.5%)	7(21.2%)	
4 day	0(0.0%)	2(6.1%)	

As presented in Table 3, adenovirus mono-infection was not significantly associated with age ($p=0.505$) or sex ($p=0.898$). However, all adenovirus-positive patients presented with fever, resulting in a significant association ($p<0.001$). Severe dehydration occurred more frequently among adenovirus-positive children (52.4%) than among adenovirus-negative children (8.8%) ($p<0.001$). Hospital stay was also significantly prolonged in the adenovirus-positive group ($p<0.001$). No statistically significant association was observed between adenovirus infection and vomiting ($p=1.000$).

Table (3): Association between Adenovirus mono-infection and study variables

Variables	Adenovirus(-ve) N(%)	Adenovirus(+ve)N(%)	P value
Age(months)	25.06±16.5	22.52±12.9	0.505
Sex	0.898		
Male	51(56.0%)	11(52.4%)	
Female	40(44.0%)	10(47.6%)	
Fever	<0.001		
No	38(41.8%)	0(0.0%)	
Yes	53(58.2%)	21(100.0%)	
Vomiting	1.000		
No	14(15.4%)	3(14.3%)	
Yes	77(84.6%)	18(85.7%)	
Dehydration	<0.001		
None	54(59.3%)	2(9.5%)	
Mild	29(31.9%)	8(38.1%)	
Severe	8(8.8%)	11(52.4)	
Hospital stay			
0 day	39 (42.9%)	2(9.5%)	<0.001
1 day	25(27.5%)	4(19.0%)	
2 day	22(24.2%)	5(23.8%)	
3 day	4(4.4%)	9(42.9%)	
4 day	1(1.1%)	1(4.8%)	

Table (4) shows the relationship between adenovirus–rotavirus co-infection and the studied variables. No statistically significant differences were found for age ($p=0.394$) or sex ($p=1.000$). Fever was present in all co-infected patients (100%) and showed a significant association with co-infection ($p<0.001$). Severe dehydration was markedly more frequent among co-infected children (77.8%) compared with non-co-infected children (5.1%), representing a highly significant difference ($p<0.001$). In addition, co-infected patients had significantly longer hospital stays ($p<0.001$). Vomiting was common in both groups without a statistically significant difference ($p=1.000$).

Table (4): Association between Co infection and study variables

Variables	Co-infection (-ve) N(%)	Co-infection (+ve) N(%)	P value
Age(months)	25.27±16.3	20.53±10.9	0.394
Sex			1000
Male	45(57.0%)	5(55.6%)	<0.001
Female	34(43.0%)	4(44.4%)	
Fever			
No	38(48.1%)	0(0.0%)	<0.001
Yes	41(51.9%)	9(100.0%)	
Vomiting			1.000
No	12(15.2%)	1(11.1%)	<0.001
Yes	67(84.8%)	8(88.9%)	
Dehydration			
None	52(65.8%)	0(0.0%)	
Mild	23(29.1%)	2(22.2%)	<0.001
Severe	4(5.1%)	7(77.8)	
Hospital stay			
0 day	37 (46.8%)	0(0.0%)	<0.001

1 day	21(26.6%)	0(0.0%)
2 day	19(24.1%)	2(22.2%)
3 day	1(1.3%)	6(66.7%)
4 day	1(1.30%)	1(11.1%)

Table 5 compares the demographic and clinical characteristics between children with mono-infection and those with adenovirus–rotavirus co-infection. No significant differences were detected regarding age, sex, fever, or vomiting ($p>0.05$). Nevertheless, co-infected children had a significantly longer mean hospital stay than those with mono-infection (2.89 ± 0.78 vs. 1.61 ± 0.64 days, $p<0.001$). In addition, severe dehydration was significantly more common among co-infected patients (77.8%) compared with children with mono-infection (22.2%) ($p=0.006$).

Table (5): Comparison of demographic and clinical characteristics between children with mono-infection (Rotavirus or Adenovirus) and co-infection

Variables	Mono-infection(n=36)	Co-infection (n=9)	P-value
Age(Months) Mean± SD	24.4± 13.2	22.4± 11.8	0.638
Male,n(%)	18(50.0%)	5(55.6%)	1.000
Female,n(%)	18(50.0%)	4(44.4%)	1.000
Fever ,n(%)	33(91.7%)	9(100.0)	1.000
Vomiting, n(%)	32(88.9%)	8(88.9%)	1.000
Hospital stay(days),Mean±SD	1.61± 0.64	2.89± 0.78	<0.001
No dehydration ,n(%)	9(25.0%)	0(0.0%)	
Mild dehydration,n(%)	19(52.8%)	2(22.2%)	
Severe dehydration,n(%)	8(22.2%)	7(77.8%)	0.006

Table 6 compares the real-time PCR cycle threshold (Ct) values between children with mono-infection and those with adenovirus–rotavirus co-infection. The mean rotavirus Ct value was slightly higher in the co-infection group than in the mono-infection group (28.11 ± 6.79 vs. 27.42 ± 5.11), although the difference was not statistically significant ($p=0.785$). Similarly, the mean adenovirus Ct value was higher among co-infected children (31.44 ± 4.61) compared with mono-infected patients (27.83 ± 5.36), but this difference also failed to reach statistical significance ($p=0.115$). Overall, these findings indicate that the viral Ct values were comparable between mono-infected and co-infected patients.

Table (6) Comparison of Ct value between mono-infection and Co-infection

Variable	Mono-infection(n=36)	Co-infection(n=9)	Pvalue
Rotavirus Ct(Mean±SD)	27.42±5.11	28.11±6.79	0.785
Adenovirus Ct(Mean±SD)	27.83±5.36	31.44±4.61	0.115

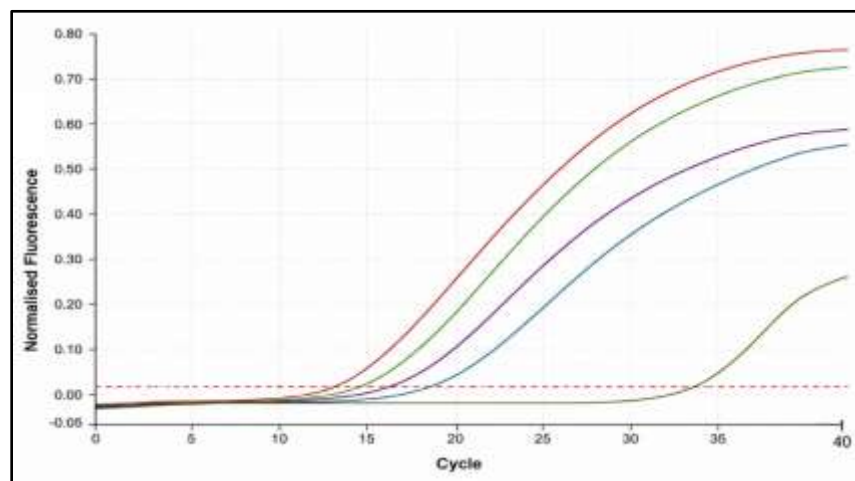


Figure (1): amplification curve of rotavirus by real time

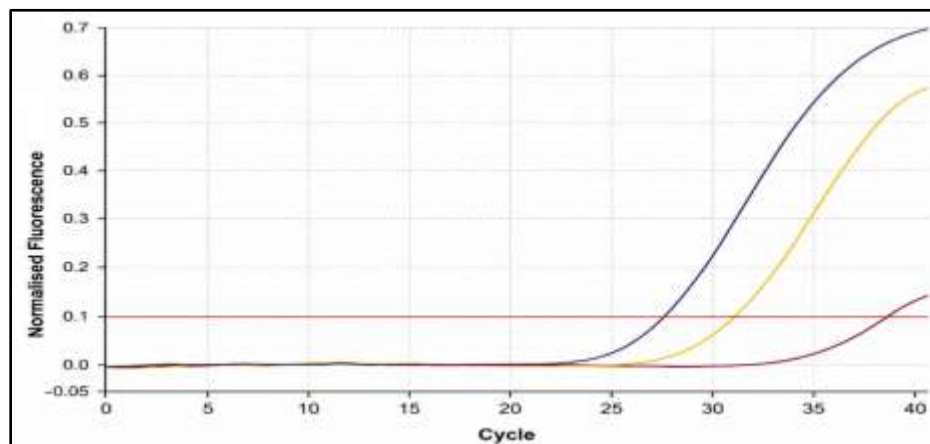


Figure (2) amplification curve of adenovirus by Real time PCR

Discussion:-

Acute gastroenteritis (AGE) is still a major health problem among children younger than five years. It remains one of the main causes of illness and hospital admission worldwide, especially in low- and middle-income countries. Although improvements in sanitation and the introduction of rotavirus vaccination have reduced the burden of diarrheal diseases, viral pathogens continue to account for a substantial proportion of pediatric AGE cases. Among these viruses, rotavirus and enteric adenovirus are recognized as two of the most important etiological agents, frequently causing severe diarrhea, dehydration, and hospitalization(10).

The present study showed that viral pathogens were detected in 45% of stool samples collected from children with acute gastroenteritis. Rotavirus mono-infection was the most frequently detected virus (24%), followed by adenovirus mono-infection (12%), while rotavirus–adenovirus co-infection accounted for 9% of cases. The predominance of rotavirus observed in the present study is expected because rotavirus remains the leading viral cause of severe acute gastroenteritis requiring hospitalization among children under five years of age. The lower prevalence of adenovirus may be attributed to its relatively lower circulation compared with rotavirus, while the occurrence of co-infection may reflect simultaneous exposure to multiple enteric viruses, particularly in hospitalized children. Furthermore, differences in viral prevalence may be influenced by seasonal variation, and geographical location. Our findings are consistent with a recent Iraqi study conducted in Baghdad, which reported that rotavirus was the predominant viral pathogen (47%), followed by adenovirus (12%) among hospitalized children with acute watery diarrhea, confirming that rotavirus remains the major cause of viral gastroenteritis in Iraqi children (11). However, our prevalence of rotavirus was lower than that reported in another recent Iraqi molecular study from Baghdad, where rotavirus was detected in 45% of cases and adenovirus in 6%(12), possibly because of differences in study population, diagnostic approach, season of sample collection.

In the current study, the absence of a significant association between both rotavirus and Adenovirus infection and age or sex suggests that all young children are susceptible to infection regardless of demographic characteristics. In contrast, both viral infection were strongly associated with fever, severe dehydration, and longer hospitalization reflects the well-recognized pathogenicity of rotavirus and adenovirus, which cause extensive damage to the intestinal epithelium, resulting in excessive fluid loss, electrolyte imbalance, and the need for prolonged inpatient management. Furthermore, several studies have demonstrated that vomiting is a common symptom in viral gastroenteritis regardless of the causative virus, explaining why no significant difference was observed between infected and non-infected groups in the present study (13).

The findings of the current study are consistent with a recent study by Onur et al. (2025) (14) and Shams et al (2022) (15), which demonstrated that children positive for rotavirus or adenovirus had significantly higher rates of fever, dehydration, and hospitalization than virus-negative children, while no significant differences were observed regarding age or sex. In contrast, an Iranian study found that rotavirus infection was significantly associated with younger age and the degree of dehydration but showed no significant association with fever or vomiting (16). These discrepancies may be attributed to differences in the study population, geographic region, sample size, and diagnostic methods used.

The present study demonstrated that children with rotavirus–adenovirus co-infection had a significantly longer hospital stay and more severe dehydration than those with mono-infection, whereas no significant differences were observed regarding age, sex, fever, or vomiting. These findings suggest that simultaneous infection with more than one enteric virus may increase disease severity, leading to greater fluid loss, prolonged recovery, and consequently a longer duration of hospitalization. The enhanced clinical severity may result from the combined pathogenic effects of both viruses on the intestinal mucosa, causing greater disruption of absorptive function and increased gastrointestinal fluid loss.

The observed association between viral co-infection and increased disease severity is consistent with several previous studies that was reported that children with mixed viral infections experienced more severe clinical manifestations and required longer hospital care than those with single viral infections (11,17,18). In contrast, other investigators have reported that viral co-infection does not necessarily increase disease severity. For example, Mikounou et al and Abdel-Rahman et al (19,20) found that the presence of co infection of Rotavirus and Adenovirus enteric viruses was not associated with prolonged hospitalization or worse clinical outcomes compared with mono-infection. These discrepancies may be explained by differences in study populations, circulating viral strains, sample size, age distribution, geographic location, and the diagnostic methods used to detect viral pathogens.

There is no statistically significant differences found in the present study in the Ct values of either rotavirus or adenovirus between mono-infected and co-infected children. These findings suggest that concurrent infection with both viruses did not significantly influence the viral replication of either pathogen. One possible explanation is that enteric viruses replicate independently within the intestinal epithelium, and viral load is primarily determined by factors such as the host immune response, the timing of specimen collection in relation to disease onset, and the natural kinetics of viral shedding rather than the presence of another virus (21,22). Furthermore, the relatively small number of co-infected cases in the present study may have reduced the ability to detect subtle differences in Ct values between the two groups.

In contrast, De Grazia et al (23). reported significantly lower Ct values (indicating higher viral loads) for rotavirus in mixed viral infections than in mono-infections, suggesting that viral interactions may enhance replication under certain conditions. The discrepancy between the two studies may be explained by differences in study population, sample size, circulating viral genotypes, the spectrum of co-infecting enteric viruses, and the timing of stool sample collection, all of which are known to influence Ct values and viral load measurements.

Conclusions:-

Rotavirus was the most frequently detected viral pathogen among children with acute gastroenteritis, followed by adenovirus, while rotavirus–adenovirus co-infection was identified in a smaller proportion of cases. Both rotavirus and adenovirus mono-infections were significantly associated with fever, severe dehydration, and prolonged hospitalization. Furthermore, children with co-infection experienced significantly longer hospital stays and a higher frequency of severe dehydration than those with mono-infection, indicating a more severe clinical course. No significant differences were observed in age, sex, vomiting, or viral Ct values between mono-infected and co-infected patients.

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