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Original Article

Detection of Rotavirus and Enteric Adenovirus among children with Acute Gastroenteritis admitted to Benghazi Pediatric Hospital

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ABSTRACT

Rotavirus A and enteric adenovirus are common causes of acute gastroenteritis (AGE), especially in children under five years of age worldwide. This study aimed to detect the incidence of rotavirus and enteric adenovirus antigens in stool specimens of children with AGE admitted to a Benghazi pediatric hospital between February and April 2019. The presence of rotavirus and adenovirus antigens in the samples was tested using an immunochromatographic method (ICT). Between the 273 stool samples, 119 (43.58%) were positive for viruses causing AGE. Rotavirus 74 (27.1%) was the most frequently detected, followed by adenovirus 45 (16.5%). However, 22 (8.1%) of the samples were detected to be positive for both virus antigens (co-infection). The highest incidence of infection with both viruses was seen in the 1-6 month age group. There was a significant difference (P = 0.000 and P = 0.005) between the results of the rotavirus and adenovirus samples of infected patients within age groups as well as inpatients and outpatients (P = 0.000 and P = 0.001). Whereas with rotavirus and enteric adenovirus antigens, there was no significant difference between genders (P = 0.06 and P = 0.2, respectively). A rapid, simple ICT method is recommended for routine diagnosis to effectively decrease unnecessary antibiotic usage and reduce overall medical costs; nevertheless, for epidemiological studies, PCR-based assays are needed.

Keywords: rotaviruses, enteric adenoviruses, acute gastroenteritis, immunochromatographic test (ICT).

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Introduction

Acute gastroenteritis (AGE) is a significant cause of mortality and morbidity globally. The microbes that cause AGE spread through the fecal-oral route and infect people from person to person, as well as through contaminated food, drinking water, or human handling (1). Many pathogens cause aging. Rotavirus and enteric adenovirus serotypes 40 and 41 are major causes of AGE in children. Little is known about the epidemiology of adenovirus and rotavirus infections in Libya (2,4). Rotavirus and enteric adenovirus are fundamental causes of diarrhea and death globally in children, and these viruses continue to greatly impact childhood morbidity and mortality (5). For proper treatment and patient management, rapid pathogen identification is essential. The number of articles using multiplex detection assays to diagnose AGE is rising, which allows the detection and identification of many targets in the same or parallel analysis of one sample (6, 7). However, these methods are more sensitive than immune analytical tests. However, their application for the routine clinical diagnosis of AGE is still limited because commercial multiplex tests are often expensive per sample (8). Currently, the detection of viral antigens in stool samples performed by enzyme-linked immunosorbent assay (ELISA) or immunochromatographic (ICT) techniques is recommended because of their simplicity and low cost (9). ICT has the advantages of being quicker, more cost-effective, and valuable for single testing specimens; being convenient; not requiring additional equipment; being readily available; being simple to perform; and having easy-to-read results (10, 11). While the ICT test may be suitable for rotavirus detection, a *polymerase chain reaction* (PCR)-based assay is better adapted for enteric adenovirus detection in stools (12).

Material and Methods

Patient samples

Two hundred and seventy-three (273) stool samples were collected from infants and young children diagnosed with AGE and diarrheal disease. Samples were stored at -20 oC until processing and investigation. The study was carried out between February and April 2019.

Immunochromatographic Test (ICT)

Immunochromatographic testing was performed using the Rotavirus and Adenovirus Combo Rapid Test Kit (Biopanda Reagents Ltd., Catalogue Number: RAPG-RAV-001).The test was performed and interpreted according to the manufacturer's instructions. The kit was highly specific (97.8% to detect rotavirus and 99.5% to detect adenovirus) and also highly sensitive (99.9% to detect rotavirus and 99.9% to detect adenovirus).

Statistical analysis

Data analysis was performed using the SPSS (Statistical Package for Social Sciences) software package (version 23) (IBM Corp., Armonk, N.Y., USA). A significance test such as the Chi-square test was used to examine the variable differences. The level of significance was set at 5% (P 0.05).Correlation analysis was used to find a high degree of correlation between age groups and the positive co-infections with rotavirus and adenovirus.

Results and discussion

The present study detected rotavirus and enteric adenovirus among children with AGE admitted to Benghazi Pediatric Hospital. The demographic characteristics and details of the patients are presented in Table 1.

Table 1. Demographic characteristics of patients.

Characteristics	Number of patients (N=273)	Percentage %
Age group in months		
Neonate	9	3.3
1-6 M	90	33.0
6-12 M	68	24.9
13-18 M	3	1.1
19-24 M	28	10.3
25-60 M	37	13.6
> 60 M	38	13.9
Gender		
Male	147	53.8
Female	126	46.2
Cases		
Inpatients	215	78.8
Outpatients	58	21.2
Month		
February	32	11.7
March	77	28.2
April	164	60.1

The number and percentage of rotavirus and adenovirus cases

In a study conducted in Zliten, Libya, from April 2000 to March 2001 in children aged a few days to 12 years, 74 of 273 patients (27.1%) tested positive for rotavirus antigen, and cases of rotavirus antigen positivity were very close to positive infection (26.6%) in 169 stool samples tested by monoclonal latex agglutination. On the other hand, the rotavirus antigen's positivity was lower than that observed in other studies. For instance, a study carried out in three public hospitals in northwest Libya reported that 58% of a total of 410 diarrhea patients under 5 years of age were positive from August 2012 to April 2013 using ELISA (13), and 57% of positive rotavirus cases were reported in a study conducted in Almarj, Libya, from January 2011 to March 2012 in children under 5 years of age using ELISA (14). The rotavirus antigen's positivity was found to be higher than that reported by other studies (15–17).

45 of 273 (16.5%) patients were detected to be positive for enteric adenovirus antigen. Cases of adenovirus antigen positivity were slightly closer to the results of a study

conducted in Turkey (14%), which used 320 stool samples among children under 5 years of age from March 2004 to March 2005, using the ICT test (18). At the same time, the infection rate of adenovirus in this study was found to be lower than the rate observed in a study conducted in Al-Najaf province, Iraq, where the infection rate was 23.33 percent among children under the age of five between November 2016 and February 2017 (19). On the other hand, the infection rate in our study was higher than the rate reported by a study conducted in Sana, Yemen, where the infection rate was 11% in children under 5 years of age with 326 stool samples and a study period from January 2008 to May 2009 using ELISA (20).

The results of rotavirus detection according to characteristics of patients

The distribution of rotavirus positive antigen and rotavirus negative antigen according to the characteristics of patients is presented in Table 2. A significant association was noted between the rotavirus results (positive or negative) and age groups in months (P = 0.000). The majority of patients infected with rotavirus were between 1-6 months old (32.2% of the age group); 0% of patients who didn't have an infection of rotavirus were between 13 and 18 months, children over 60 months, and neonates. There was no statistically significant difference in positive rotavirus infection between males and females (P = 0.06). Rotavirus was found in 22.4% of males and 32.5% of females. Furthermore, a significant difference was observed between inpatients and outpatients (P = 0.000). 32.1% of the inpatients had a positive rotavirus infection; only 8.6% of the outpatients had a positive rotavirus infection.

Table 2. Rotavirus detection rates according to characteristics of patients

Characteristics	Rotavirus		Total of row No. (%)
	Positive No. (% of row)	Negative No. (%of row)	
Age group in months			
Neonate	0 (0%)	9 (100%)	9 (100%)
1-6 M	29 (32.2%)	61 (67.8%)	90 (100%)
6-12 M	23 (33.8%)	45 (66.2%)	68 (100%)
13-18 M	0 (0%)	3 (100%)	3 (100%)
19-24 M	10 (35.7%)	18 (64.3%)	28 (100%)
25-60 M	12 (32.4%)	25 (67.6%)	37 (100%)
> 60 M	0 (0%)	38 (100%)	38 (100%)
χ^2 =35.7 P = 0.000*			
Gender			
Male	33 (22.4%)	114 (77.6%)	147 (100%)
Female	41 (32.5%)	85 (67.5%)	126 (100%)
χ^2 = 3.49 P = 0.06			
Cases			
Inpatient	69 (32.1%)	146 (67.9%)	215 (100%)
Outpatient	5 (8.6%)	53 (91.4%)	58 (100%)
χ^2 = 15.1 P = 0.000*			

* P<0.05 is statistically significant

The results of adenovirus detection according to characteristic of patients

The distribution of adenovirus positive and negative antigens according to the characteristics of patients is presented in Table 3. A significant association was noted between adenovirus results (positive or negative) and age groups in months (P = 0.005). Most patients infected with adenovirus were between 1-6 months (24.4% of the age group) and 6-12 months (20.4% of the age group). In contrast, 0% of patients who did not have an adenovirus infection were between the ages of 13 and 18 months, as well as neonates. There is no significant difference between males and females (P = 0.2); 19% of males and 13.5% of females had a positive infection with adenovirus. Furthermore, a significant difference was observed between inpatients and outpatients (P = 0.001), where 20.0% of the inpatients and only 3.4% of the outpatients had a positive infection with adenovirus.

Table 3. Enteric adenovirus detection rates according to characteristics of patients

Characteristics	Adenovirus		Total of row No. (%)
	Positive No. (% of row)	Negative No. (%of row)	
Age group in months			
Neonate	0 (0%)	9 (100%)	9 (100%)
1-6 M	22 (24.4%)	68 (75.6%)	90 (100%)
6-12 M	14 (20.6%)	54 (79.4%)	68 (100%)
13-18 M	0 (0%)	3 (100%)	3 (100%)
19-24 M	5 (17.9%)	23 (82.1%)	28 (100%)
25-60 M	3 (8.1%)	34 (91.9%)	37 (100%)
>60 M	1 (2.6%)	37 (97.4%)	38 (100%)
χ^2 =18.8 P = 0.005*			
Gender			
Male	28 (19%)	119 (81%)	147 (100%)
Female	17 (13.5%)	109 (86.5%)	126 (100%)
χ^2 = 1.54 P = 0.2			
Cases			
Inpatient	43 (20%)	172 (80%)	215 (100%)
Outpatient	2 (3.4%)	56 (96.6%)	58 (100%)
χ^2 = 11.8 P = 0.001*			

* P<0.05 is statistically significant

Relation between positive cases of co-infectionand age groups

About 22 of 273 (8.1%) patients were detected to be positive for both rotavirus and adenovirus antigens. Co-infection with both viruses was most common in children

aged 1-6 months (54.5% of 22 patients), while no co-infection with rotavirus and adenovirus was found in children aged 13-18 months, more than 60 months, or neonates, as shown in table 4. A detection rate of 8.1% in rotavirus and adenovirus co-infection positive cases recorded in this study is similar to the finding of another study that reported a detection rate of 8% (21), whereas another study recorded a detection rate of 6.09% out of 450 stool samples from infected children under four years of age in Baghdad using the same detection method as in this study (22). Moreover, the finding of co-infection observed here is higher than the 2.3% reported by Balkan and Celebi. (23); Furthermore, other studies did not agree with this study, as they showed lower detection of co-infection in several regions of the world (15, 17). Moreover, the correlation coefficient (r = - 0.134) was a weak negative correlation, indicating that positive rotavirus and adenovirus co-infection was more common in younger patients (less than one year).

Table 4. Relation between positive cases of co-infection and age groups

Age group in months	Rotavirus &Adenovirus No. (% of positive)
Neonate	0 (0%)
1-6 M	12 (54.5%)
6-12 M	6 (27.3%)
13-18 M	0 (0%)
19-24 M	2 (9.1%)
25-60 M	2 (9.1%)
> 60 M	0 (0%)
Total	22 (100%)
Correlation	r = - 0.134

Distribution rate of infected children between hospital admitted cases and outpatient department cases

Out of 215 (78.8%) children admitted to the hospital, the percentage of overall positive cases of viral infection was about 79%. In comparison, out of 58 (21.2%) children in the outpatient department, about 21% were detected to be positive cases of viral infection. The significant difference in the relationship between outpatients and inpatients may be because the children with underlying severe symptoms who were admitted to the hospital for follow-ups were rationally more likely to have a high rate of infection and also have a higher chance for hospital-acquired infections, which increase the possibility of the viral infection, than outpatients.

Monthly distribution of rotavirus and adenovirus in infected patients

In February, the majority of patients were detected to be positive for the rotavirus antigen (86%), while 14% were detected to be positive for the adenovirus antigen. In March, the majority of patients were detected to be positive for the rotavirus

antigen (57%), while 43% were detected to be positive for the adenovirus antigen. In April, the majority of patients were detected to be positive for the rotavirus antigen (65%), while 35% were positive for the adenovirus antigen (Figure1).

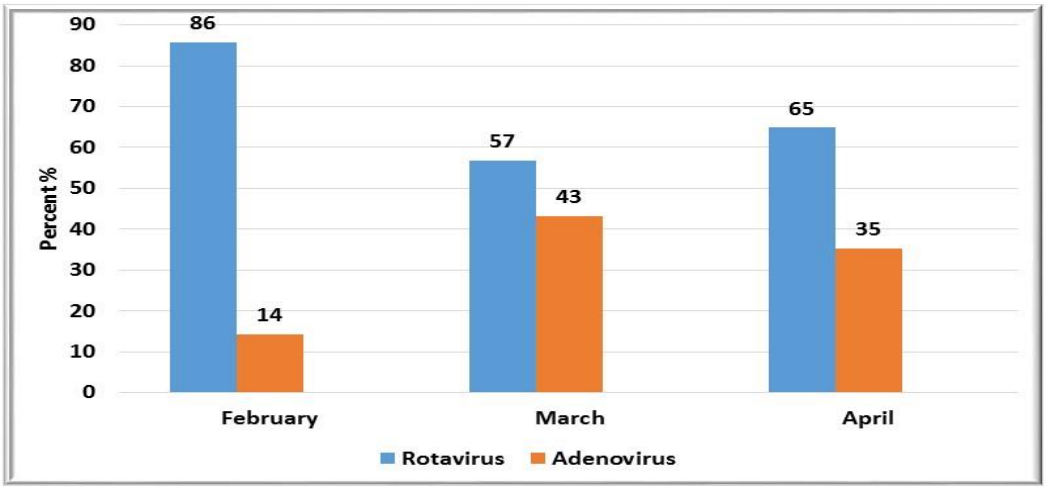

Figure 1. Monthly distribution of rotavirus and enteric adenovirus infections

The difference in the identification rates of positive rotavirus and enteric adenovirus detection reported in this study is due to the various factors that might influence the rate of diagnosis, such as the duration of the study, socio-economic status, geographic region, seasonality, sample size, and diagnostic method. Delayed hospital discharge might also increase the likelihood of detecting positive cases of rotavirus and enteric adenovirus. The months of the year sometimes play an essential role in the distribution of multiple infectious diseases caused by these enteric viral pathogens, as demonstrated in the study that identified the highest detection rate and proportion of dual infection in February, March, and April, which agrees with our study (23). The previous study reported that adenovirus plays a significant role in co-infection with rotavirus and the severity of diarrhea among children less than two years of age (22).

Conclusion

Rotavirus and enteric adenovirus infections, as well as co-infection with both viruses, occur in AGE children under the age of five, with a significant frequency of the highest positive cases occurring between the ages of 1-6 months. Studies with larger sample sizes are required. ICT for rapid testing provides scientific evidence of the causative agent in AGE patients from other agents that cause the same symptoms, which helps in disease monitoring, management, and choosing a proper treatment to reach rapid recovery.

Author Contribution

AE conceived the paper and wrote most of it. SA participated in the writing and performed laboratory testing. GA and As participated in the writing and final revising of the work. All authors approved the final version of the manuscript.

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Conflict of Interests
The authors declare that there is no conflict of interest.

Financial Disclosure
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Ethics
This study was performed according to Libyan National Committee for Biosafety and Bioethics, and the approval Ref. No: NBC:000.H.23.5

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