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Detection and Genotyping of Viral Gastroenteritis in Hospitalized Children Below Five Years Old in Cairo, Egypt

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Abstract

This work aimed to study the detection and genotyping of rotavirus, enteric adenovirus, and astrovirus in Egyptian hospitalized children below five years old, associated with non-bacterial diarrhea. In total, 119 fecal samples were obtained from the target population, admitted to Abu El-reesh hospital in Cairo, Egypt. Based on the findings, the detection rate of at least one viral infection was 36.7% in children below five years old, whereas, the overall detection rate of rotavirus, adenovirus, and coinfection was 31%, 6.7%, and 0.8%, respectively. No astrovirus infection was observed. Spring was the peak season for rotavirus and enteric adenovirus. The findings showed that higher rates of rotavirus (78%) and enteric adenovirus (100%) were identified in children less than two years of age. The dominant genotypes were G1P [8] (29.7%), G3P [8] (27%), and G1P [4] (18.9%) for rotaviruses and genotype 41 for enteric adenoviruses. Uncommon genotypes G1P [6] and G9P [8] were also detected in five (13.5%) and four (10.8%) samples, respectively. The present findings indicated that the high incidence of rotavirus and adenovirus in children below two years old. Thus, highlighting the necessity of vaccine development to reduce the incidence of acute viral gastroenteritis in Egypt.

Keywords: Rotavirus, Adenovirus, Children, Egypt

1. Background

According to recent statistics by the world health organization (WHO), the annual mortality rate associated with diarrhea is 30 deaths per 100,000 among Egyptian children under five years old. Nearly 3.9% of reported deaths are due to rotavirus infection, whereas enteric adenovirus is the second leading cause of acute diarrhea after rotavirus (1, 2). Both viruses, as extremely etiological agents for diarrhea, constitute a major health problem in developing countries (3).

Rotavirus and enteric adenovirus exhibit comparable manifestations. However, the symptoms of gastroenteritis due to enteric adenovirus are associated with less vomiting and dryness. Globally, 35% - 50%, 1% - 2%, and 2% - 31% of hospitalized patients with acute diarrhea are infected by rotavirus, astrovirus, and enteric adenovirus, respectively (4-9).

In Egypt, several studies have been performed on rotavirus epidemiology, whereas a limited studies have been carried out on rotavirus, astrovirus, and enteric adenovirus at the same time (10-12). Therefore, monitoring of rotavirus, astrovirus, and enteric adenovirus is necessary for the assessment of public health risk factors in Egypt. In this study, we examined the detection and genotyping of rotavirus, astrovirus, and adenovirus among hospitalized children below five years old in Cairo, Egypt.

2. Methods

2.1. Specimens Setting

This study was performed at the Affiliated Abou El-reesh Children's hospital of pediatrics in Cairo. It included 320 inpatient beds and 32 incubators at two Neonatal and Pediatric Intensive Care Units (NICU, PICU). Samples were collected from children under five years of age, who were presented to the hospital with acute watery diarrhea and required hospitalization. Information was collected on the frequency of diarrhea (more than three times per day), gender and age. Fecal specimens documented to be free of common bacterial and protozoan pathogens were collected from children and categorized to five groups and two groups according to age and gender, respectively as in Table 1.

Table 1. Distribution of Virus-Positive Cases According to Gender, Age and Season

Variables	Rotavirus	Adenovirus	Coinfection	P Value
Percentage (%) of cases according to gender				0.67
Male, %	20/69 (29)	5/69 (7.2)	-ve	
Female, %	17/50 (34)	3/50 (6.0)	1/50 (2.0)	
Total	37/119 (31)	8/119 (6.7)	1/119 (0.8)	
Percentage (%) of cases according to age group, mo				0.98
< 12	24/73 (33)	7/73 (9.5)	1/73 (1.3)	
13 - 24	5/15 (33.3)	1/15 (6.6)	-ve	
25 - 36	3/12 (25)	-ve	-ve	
37 - 48	2/9 (22)	-ve	-ve	
> 60	3/10 (30)	-ve	-ve	
Percentage (%) of cases according to season				0.068
Winter (Dec., Jan, and Feb.)	11/27 (40.7)	-ve	-ve	
Spring (March, April and May)	14/26 (53.8)	4/26 (15.3)	1/26 (3.8)	
Summer (June, July and August)	6/43 (13.9)	1/43 (2.3)	-ve	
Fall (Sep., Oct., and Nov.)	6/23 (26.0)	3/23 (13.0)	-ve	
Total	37/119 (31)	8/119 (6.7)	1/26 (3.8)	

2.2. Fecal Sample Collection

The ethics committee of the national research centre, Egypt, approved this study. A total of 119 fecal diarrhea samples were obtained weekly over 12 months from children below five years old, admitted to Abu El-reesh hospital, Cairo during June 2015 to May 2016. The fecal samples were directly transferred in a sterile plastic cup to the virology lab of National Research Centre, Egypt, and were placed in a cooler.

2.3. Fecal Sample Processing

Nearly 0.1 g of fecal diarrhea samples was weighed and diluted in phosphate buffer saline (1:10). The samples were vortexed for 30 seconds before clarification via centrifugation at 7,000 rpm for 10 minutes at room temperature. The samples were kept at -80°C until further use.

2.4. Viral Detection Using the Enzyme Linked Immunosorbent Assay

Using enzyme immunoassay kits (RIDASCREEN® viral antigen, R-Biopharm AG, Germany), the antigens of rotavirus, astrovirus, and enteric adenovirus were identified, according to the manufacturer's instructions. Briefly, 100 μL of diluted stool sample (1:10 diluent) was added with positive and negative controls to microwell plates. Then, 100 μL of conjugate I was added to each well. Incubation was performed for one hour at room temperature. After incubation, the plate was rinsed with 300 μL of washing buffer, five times. Next, 100 μL of conjugate II was poured in each well and then incubated at room temperature for half an hour. The plate was washed five times again, and then, the substrate solution was added. Following incubation at room temperature for 15 minutes, a stop reagent was added, and the plate was read at 450 nm using an ELISA reader.

2.5. DNA and RNA Extraction

Extraction of viral DNA and RNA was performed on positive ELISA samples for molecular detection and genotyping, using QIAamp DNA Stool Minikit (QIAGEN, Germany) and BioZOL reagent (BIOFLUX, Japan), respectively, based on the guidelines by the manufacturer. The DNA and RNA samples were stored at -80°C for further use.

2.6. Rotavirus Genotyping with Real Time-Polymerase Chain Reaction Amplification

For rotavirus genotyping, VP4 (P-type) and VP7 (G-type) genes were detected, using RT-PCR amplification of dsRNA with specific primer pairs, Con2-Con3 and Beg9-End9, to amplify the full-length VP4 (876 bp) and VP7 (1062 bp) genes, respectively. The second round of RT-PCR amplification of cDNA was performed with specific primer sets: 1T-1 (P[8]), 2T-1 (P[4]), 3T-1 (P[6]), 4T-1 (P[9]), and 5T-1 (P[10]) for P-typing; aBT1 (G1), aCT2 (G2), aET3 (G3), aDT4 (G4), FT5 (G5), DT6 (G6), HT8 (G8), aFT9 (G9), ET10 (G10), and BT11 (G11) for G-typing (13-15).

2.7. Enteric Adenovirus Genotyping with Nested Polymerase Chain Reaction Amplification

According to Puig et al. (16), two rounds of PCR amplification were applied for genotyping of enteric adenovirus. A specific primer set, consisting of hex AA 1913 and hex AA 1885, was applied for the first round, while for the second round, nehex AA 1905 and nehex AA 1893 were used. The products were examined using electrophoresis on 2% agarose gel. In accordance with the kit guidelines (QIAquick Gel Extraction Kit; Qiagen, Germany), purification and sequencing of positive amplicons were carried out with the same PCR primers. Consensus sequences were compared with the existing adenovirus sequences in the nucleotide collection database, using the BLASTN program, http://blast.ncbi.nlm.nih.gov/Blast.cgi

2.8. Statistical Analysis

Chi square was applied for data analysis, and the significance level was set at 0.05.

3. Results

3.1. Circulation of Viral Acute Gastroenteritis

In this study, 119 fecal samples were obtained from Egyptian children below five years old with acute diarrhea to investigate the detection of rotavirus, astrovirus, and enteric adenovirus genotypes in Cairo. Among 119 specimens, 31%, 6.7%, and 0.8% were positive for rotavirus, enteric adenovirus and coinfection, respectively, as in Table 1. No astrovirus infection was detected. In terms of gender, 29% of male and 34% of female subjects were positive for rotavirus, while 7.2% of male and 6% of female subjects were positive for enteric adenovirus. The children's age ranged from less than 12 months to 60 months. Nearly 78% and 100% of rotavirus- and enteric adenovirus-positive samples were reported in children less than 24 months, respectively. No positive samples were observed for enteric adenovirus in children above 24 months old.

Both rotavirus and enteric adenovirus were detected throughout the year, except the winter season, for enteric adenovirus. In total, rotavirus and enteric adenovirus (53.8% and 15.3%, respectively) had the highest frequencies, in spring followed by winter (40.7% and 0%), fall (26% and 13%), and summer (13.9% and 2.3%), respectively. Both rotavirus and enteric adenovirus were peaked in spring, as shown in Table 1. However, no statistically significant differences were observed.

3.2. Rotavirus and Enteric Adenovirus Genotyping

Using nested RT-PCR, rotaviruses G1P [8], G3P [8], and G1P [4] were introduced as the dominant genotypes in Egyptian children (29.7%, 27.0% and 18.9%, respectively). In addition, G1P [6] and G9P [8] were detected in five (13.5%) and four (10.8%) cases, respectively, as shown in Table 2. On the other hand, positive adenovirus samples, amplified using nested PCR, were confirmed by sequencing for genotyping, which showed similar sequences with 94% homology to human adenovirus 41 strain.

4. Discussion

Currently, many studies have indicated the importance of viral diarrhea diseases (particularly rotavirus and adenovirus infections) as one of the main causes of morbidity and mortality in developed and developing countries. There are a few studies on the combined prevalence and genotyping of rotavirus, astrovirus, or enteric adenovirus in children below five years old in Egypt.

The present one-year study showed that 36% of the specimens were infected by at least one viral infection. The overall detection rate of rotavirus and enteric adenovirus was 31% and 6.7%, respectively in all fecal samples. However, no astrovirus infection was observed. These findings are relatively similar to the detection rates reported in Egypt and other countries for the epidemiology of rotavirus and adenovirus (associated with acute diarrhea) in children below five years old (4, 5, 10, 11, 17).

There is no significant difference in the detection rate between male and female subjects. However, the higher prevalence was reported among females (34%; 17/50), in contrast to males (29%; 20/69). Most positive rotavirus (29 cases) samples and all positive enteric adenovirus (eight cases) were found in children below two years old. These findings are consistent with previous research, in which the majority of rotavirus and enteric adenovirus infections were found among children below two years old (17-21).

In developed countries, winter is the peak season for rotavirus, which can be due to temperate climate, whereas in developing countries with subtropical or tropical climates, rotavirus circulates in all months of the year (21, 22). The present findings regarding the seasonal distribution are partially in agreement with previous reports. The current study found that rotaviruses circulated throughout the year, although the spring was the peak season; on the other hand, enteric adenovirus circulated only during spring (eight cases). This finding is in line with previous studies, which suggested no seasonal variations (8, 21).

According to previous reports, rotavirus genotypes have changed over the years. In Egypt, most reports

Table 2. Seasonal Distribution of Rotavirus Genotypes in Cairo, Egypt

Season/G-Type	P-Type			Total
	P4	P6	P8	
Winter (Dec., Jan and Feb.)				
G1	2	ND	6	8
G3	ND	ND	2	2
G9	ND	ND	1	1
Spring (March, April and May)				
G1	3	2	3	8
G3	ND	ND	4	4
G9	ND	ND	2	2
Summer (June, July and August)				
G1	1	2	1	4
G3	ND	ND	2	2
G9	ND	ND	0	0
Fall (Sep., Oct. and Nov.)				
G1	1	1	1	3
G3	ND	ND	2	2
G9	ND	ND	1	1
Total	7	5	25	37

have identified G1P [8] as the predominant genotype of rotavirus, whereas some others have identified G2P [4] as the dominant genotype. In this study, G1P [8], G3P [8], and G1P [4] were the dominant genotypes among Egyptian children (29.7%, 27.0%, and 18.9%, respectively). These findings are in partial agreement with previous studies conducted in Egypt (10-12, 17), and in complete agreement with global studies, which reported P[8], P[6], and P[4] as the most common P genotypes with frequent combinations of G1P[8], G4P[8], G3P[8], G9P[8], G2P[4], and G9P [6] as the dominant genotypes globally (23). On the other hand, enteric adenovirus type 41 was the most common genotype in Cairo. This result is in complete agreement with previous study conducted in Egypt (17).

In conclusion, the present study confirmed that human rotavirus, followed by enteric adenovirus (with a more limited role), is the dominant etiological agent of acute diarrhea in hospitalized children below five years old in Cairo, Egypt. This study revealed the high incidence of rotavirus and adenovirus in children less than two years old, thus, highlighting the necessity of vaccine development to reduce the incidence of viral gastroenteritis in Egypt. Finally, this work was subject to some limitations. The authors collected fecal specimens from small number of children and this was due to excluding the specimens infected with bacterial and protozoan pathogens. Further work is necessary to monitor the prevalence of viral gastroenteritis throughout Cairo.

Footnote

Conflict of Interests: No conflict of interest.

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