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Molecular study of astrovirus, adenovirus and norovirus in community acquired diarrhea in children: One Egyptian center study

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ABSTRACT

Objective: To determine the prevalence of astrovirus, norovirus, adenovirus in children below five years old with diarrhea by multiplex reverse transcriptase polymerase chain reaction (RT-PCR) along with rotavirus antigen detection by enzyme linked immunosorbant assay.

Methods: The study was conducted on children below five years old complaining of acute diarrhea. The study included stool examination by molecular method for detection of norovirus, adenovirus and astrovirus by multiplex RT-PCR. Rotavirus antigen was detected in the stool by enzyme linked immunosorbant assay.

Results: The study included 100 children below 5 years old with acute diarrhea. Multiplex RT-PCR was positive in 34% of the children. The most frequently detected virus was rotavirus (44%), followed by norovirus (30%), adenovirus (20%) and astrovirus (14%). The clinical symptoms were more significantly associated with viral diarrhea such as fever ($P = 0.03$), bloody diarrhea ($P = 0.025$), vomiting ($P = 0.0001$) and watery diarrheas ($P = 0.05$). The frequency of diarrhea with viral pathogen was significantly presented in winter season (39.7%). There were significant frequencies of norovirus and adenovirus in age ranging 1–2 years old ($P = 0.04$, $P = 0.01$ respectively).

Conclusions: The present study spotlights on the prevalence of viral pathogens as an important etiology in diarrhea in children below five years old. Astrovirus, norovirus and adenovirus are common along with rotavirus in this group of patients. Multiplex PCR leads to improve the laboratory diagnosis of these viruses along with antigen detection method. Further longitudinal studies are required to evaluate the epidemiological data associated with these viruses and for proper management of such drastic infection.

1. Introduction

Diarrhea is a common childhood disease worldwide especially under five years old. The morbidity and mortality risks increase in developing countries in Africa and Southeast Asian countries according to the WHO report [1]. The pathogens distributions among diarrheal diseases are not well known in many countries, such as in Egypt. The knowledge of the causative pathogen is crucial for empirical treatment and preventive measures [2].

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There are various pathogens that are well known to be the etiology of diarrhea including bacterial, protozoa and viral pathogens. Viruses are recognized as a cause of diarrheal diseases in children below five years old. Among enteric viruses, four categories of viruses are associated with such condition: group A rotavirus (family Reoviridae), norovirus (family Caliciviridae), adenovirus 40/41 (subgenus F), and astrovirus [3–5].

Rotavirus is a major viral cause of diarrhea in Egypt and it was studied in previous studies [6–8]. Norovirus was also reported to be common pathogen among diarrheal diseases in children especially in an outbreak; however, its role in community acquired sporadic diarrheal diseases remains to be elucidated [7,8]. Norovirus a member of family Caliciviridae, is a single stranded RNA virus. The virus is transmitted by feco-oral route by low doses leading to vomiting and diarrhea especially in winter season [9].

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Human adenoviruses, another viral pathogen associated with diarrheal diseases, is a double strand virus belongs to the family Adenoviridae (HAdV). There are 52 serotypes classified into six species from A to G, among which species F serotypes 40 and 41 are associated with diarrheal diseases in children [10]. Human astroviruses, a single-stranded RNA virus belonging to the Astroviridae family are a leading cause of diarrhea in children [11].

The limitation of the studies concerning viral etiology of the diarrheal diseases arises from the limitation of the methodology that can be applied in routine microbiological laboratory to define the viruses associated with such infection. Viral isolation by tissue culture is the sensitive and specific method for laboratory diagnosis [12]; however, it needs specific laboratory manipulation and it is time consuming, especially in diagnosis of community acquired infections. Also, electron microscope is specific but lacks sensitivity with detection limit of 106 viral particles/gram of stool. Antigen detection methods of viral pathogen in stool are rapid, and sensitive but it is not available for all viral pathogens. Molecular detection methods have gained a special appeal in last few years for detection of various pathogens with specific and rapid results [13–17]. The choice of molecular technique as a laboratory method for diagnosis of viruses associated with diarrheal diseases appears to be an accessible method especially if multiplex method is applied for multiple viral detection in the same sample.

Therefore the aim of the present study was to determine the prevalence of astrovirus, norovirus, adenovirus in children below five years old with diarrhea by multiplex reverse transcriptase polymerase chain reaction along with rotavirus antigen detection by enzyme linked immunosorbant assay (ELISA).

2. Materials and methods

2.1. Samples collection

The study is a cross sectional observational study that was carried at Mansoura University Children Hospital from January 2015 to January 2017. The included patients were children with acute diarrhea complaint at or below five years old recruited from out patients clinics. The study included 100 children below 5 years old with acute diarrhea. The mean age ± SD was (3.4 ± 1.2) years old with male predominance gender (56%). Diarrhea was defined as presence of frequent loose stool for 3 times or more during 24 h. Children older than 5 years and those without diarrhea were excluded from the study. The study was approved by Mansoura Faculty of Medicine Ethical Committee. The study was performed according to the declaration of Helsinki and written approval was obtained from the parent of each child.

Each child was subjected to full medical history recording including age, presence of vomiting, fever and abdominal pain. Stool sample was obtained from in a clean container and transported to the laboratory.

Stool sample in the laboratory was subjected to macroscopic examination including the consistency, presence of mucus and blood. Then, each stool was divided into two aliquots: one for rotavirus antigen detection by ELISA (ELISA-Ridascreen, Germany) and the second for nucleic acid extraction for multiplex PCR detection of astrovirus, norovirus and adenovirus. The aliquots were preserved at −20 °C till time of analysis.

2.2. Rotavirus antigen detection by ELISA (Ridascreen)

The test uses monoclonal antibodies for detection of rotavirus sixth viral gene protein products in sandwich type ELISA. The method was used as described by the manufacturer.

2.3. Viral DNA and RNA extraction

For each stool sample two extraction methods were used: one for viral RNA extraction for astrovirus and norovirus by QIAamp viral RNA kit and the second for viral DNA extraction for adenovirus by QIAamp DNA stool kit (Qiagen, Germany) according to the manufacturer instructions. The extracted nucleic acid was stored at −20 °C till time of amplification.

2.4. Multiplex reverse transcriptase polymerase chain reaction

Both RT step and PCR were performed in the same tube using 5 μL of extracted sample (3 μL of extracted RNA and 2 μL of the extracted DNA). The protocol of the amplification was according to Rohayem *et al.* (2004) [18]. The primers sequences and the amplicon sizes of the used primers for norovirus, astrovirus and adenovirus were summarized in Table 1.

2.5. Statistical analysis

Data was analyzed by SPPS24 (SPSS, Inc., Chicago, IL). The qualitative data were expressed as percentage and *Chi*-square was used to compare between groups. The test was significant if $P < 0.05$.

Table 1

Viruses genes primers and base pair size amplicon of products.

Virus	Primers sequences	Size
Norovirus	Calman-29 F:5′ TATGGTGATGATGAAATAGTGTC-′3	
Norovirus I	Calman-32 R:5′ATTTCGGGCAGAAGATTG-′3	490 bp
Norovirus II	Calman-1 F:5′GCACACTGTGTTACACTTCC-′3	822 bp
	Calman-2 R:5′ACATTGGCTCTTGTCTGG-′3	
Astrovirus	Mon340 F:5′CGTCATTGTTTGTTGTCATACT-′3	347 bp
	Mon348 R:5′ACATGTGCTGCTGTTACTATG-′3	
Adenovirus	Adhex-1 F:5′GCCACCGATACGTACTTCAGCCTG-′3	261 bp
A-G	Adhex-2 R:5′GGCAGTGCCGGAGTAGGGTTTAA-′3	

Bp stands for base pair.

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