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Original Article

Paediatrics Section

Prevalence of Nosocomial Diarrhea Due to Adenoviruses 40 and 41 in a Paediatric Ward in Iran

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ABSTRACT

Background: Enteric adenoviruses 40 (Ad40) and adenovirus 41 (Ad41) have been shown to be a significant cause of paediatric gastroenteritis worldwide, but no data are available for nosocomial diarrhea due to adenovirus in Iran.

Aim: The present study was performed to determine the incidence of Ad40 and Ad41 in children less than five years with nosocomial diarrhea in Shahrekord, southwest Iran.

Materials and Methods: Adenovirus was detected by polymerase chain reaction (PCR) in stool samples collected during one year (2010-2011) from children less than five years with nosocomial diarrhea admitted to a paediatric center in Shahrekord, Iran. Nosocomial diarrhea was defined as those occurring more than 72 hours after admission to hospital for non-diarrheal causes. PCR technique was used for investigation of Ad40 and Ad41.

Results: In total of 100 samples, Ad40 and Ad41 DNA was found to be positive in 14/100 (14%), and 8/100 (8%) of diarrheic patients less than five years, respectively.

Conclusion: Ad40 and Ad41 are important causes of nosocomial diarrhea in less than five-year, hospitalized Iranian children.

Keywords: Adenovirus, Children, Nosocomial diarrhea

INTRODUCTION

Diarrhea is one of the most common diseases in infants and young children in developed and developing countries. In Walker et al., study, the prevalence of diarrhea decreased in 2010 compared to 1990, with the highest incidence among infants 6-11 months [1]. In contrast to adults, in whom the most common etiologic agent is *Clostridium difficile* [2], nosocomial diarrhea in children is usually due to viruses circulating in the community such as rotavirus, enteric adenovirus, astrovirus, norovirus [3], and torovirus [4].

Adenoviruses are double-stranded, non enveloped DNA viruses. There are at least 51 distinct human adenovirus serotypes. These types classified into six species, A to F, cause human infection. Some adenovirus serotypes are associated primarily with respiratory tract diseases, and others are associated primarily with gastroenteritis (types 40, 41 and to a less extent, 31). Infection in infants and children can occur at any age. Health care-associated transmission of adenoviral respiratory tract and gastrointestinal tract infections may occur in hospital [5].

Enteric strains of adenoviruses are transmitted by the fecal-oral route. Adenoviruses do not demonstrate the marked seasonality of other respiratory tract viruses. Enteric diseases occur throughout the year and primarily affect children younger than four years [5].

Almost 50% of children under five years have been seropositive for antibody of adenovirus type 40 (Ad40) and adenovirus type 41 (Ad41) in Asia, Europe, and South America [6].

Ad40 and Ad41 have recently been recognized as important etiologic agents of gastroenteritis in children [7]. Herrmann et al., have reported that Ad40 and Ad41 are associated with a small proportion (2.0%) of gastroenteritis in Thai children attending an outpatient clinic in Bangkok, which is very similar to that reported in Rio de Janeiro, Brazil (1.9%) [8,9].

In study of Modarres et al., enteric adenovirus infection in infants and young children with acute gastroenteritis in Tehran was assessed and Ad40 and Ad41 were detected in 27 (2.6%) samples [10], but few studies have been yet conducted in Iran to investigate the prevalence of nosocomial diarrhea due to Ad40 and Ad41 in children, so this study is done to determine the prevalence of Ad40 and Ad41 nosocomial diarrhea among children less than five years at a paediatric center in Shahrekord, southwest Iran.

MATERIALS AND METHODS

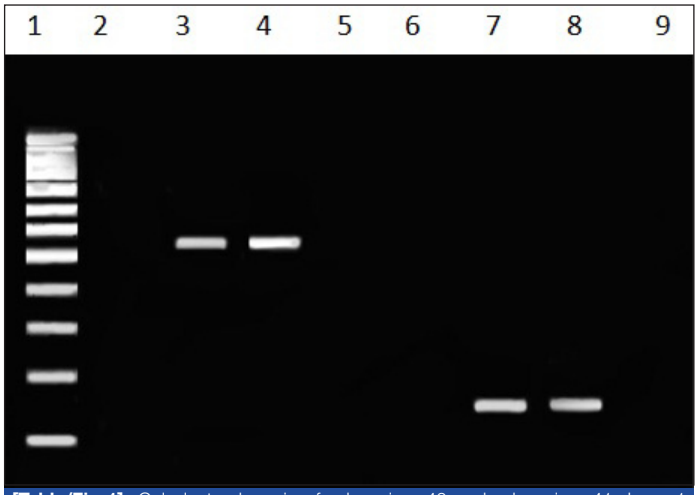
For this prevalence study conducted from December, 2010 to December, 2011, the protocol was approved by the ethic committee of the University. Informed consent was obtained from the participants' parents. The study population consisted of all patients of 6-60 months, who were admitted to the paediatric ward of the hospital because of diseases other than diarrhea. Fecal specimens were obtained from 100 children with signs of nosocomial diarrhea admitted to the hospitals and health centers in Shahrekord. The same pediatrician visited the patients every day. Fecal specimens were collected from any children and investigated. Nosocomial diarrhea was defined as occurring more than 72 hours after admission to hospital for reasons other than diarrhea. The fecal samples were transferred daily to the Cellular and Molecular Research Center, Shahrekord University of Medical Sciences in refrigerated boxes. Each specimen was stored at -70°C for later use. The methods used in this study are very similar to another study [11].

The stool specimens were obtained from 100 diarrhea patients less than five years admitted to Hajar Hospital of Shahrekord. The samples were transferred to the laboratory in sterile plastic falcon tubes (15 ml) under refrigerated conditions and stored at -20°C until analysis. The DNA virus was extracted from 200 mg stool specimens using a DNA extraction kit (DNPTM Kit Cinna Gen, Iran) according to the manufacturer's procedure. The yield of DNA was quantified after electrophoresis in 1% agarose gel containing 0.5 µg/ml of ethidium bromide.

The primers of polymerase chain reaction (PCR) for detection of ad40 were ad40F: 5'-GCCCGTGCCACCGATACCTAC -3' and ad40R: 5'-ACTTTGTAAGAGTAGGCGGTTTCC -3'.

The ad40 F primer was designed in this research and the sequence of ad40 R primer was obtained from Jiang et al., study [12]. The size of amplicon was 152 bp. The ad40R primers sequence obtained from Xu et al., study, ad41F: 5'-ACTTAATGCTGACACGGGCAC -3' and ad41R: 5'-TAATGTTTGTGTTACTCCGCTC -3' [13], were used for amplification of Ad41 and the size of amplicon was 541bp.

PCR was carried out in 25 µl total reaction volumes, each containing 2.5 µl of 10X PCR buffer, 1.5 mM $MgCl^2$, 100 ng of template DNA,



[Table/Fig-1]: Gel electrophoresis of adenovirus 40 and adenovirus 41. Lane 1 shows fermentas 100 bp molecular marker. Lanes 2 and 6 are negative controls of adenovirus 41 and adenovirus 40, respectively. Lanes 3 and 4 are positive samples for adenovirus 41 (541 bp) and lanes 7 and 8 are positive samples for adenovirus 40 (152 bp). Lanes 5 and 9 are negative samples for adenovirus 41 and adenovirus 40, respectively.

0.2 pM of each primer, 0.2 µl dNTPs, and 1 unit of Taq DNA polymerase (Fermentas, Germany).

The amplification reaction consisted of 5 min of predenaturing at 95°C, followed by 33 cycles of 1 min denaturation at 94°C, 1 min annealing at 60°C and 65°C for ad40F/R and ad41F/R respectively, and 1 min extension 72°C, and final extension at 72°C for 5 min. The samples were amplified in a Gradient Palm Cycler (Corbett Research, Australia).

The PCR amplification products (15 µl) were separated by electrophoresis in 1.5% agarose gel at 100 V for 30 min in Tris-borate-EDTA buffer, visualized by ethidium bromide staining, illuminated by UV transilluminator and images were obtained in UVIdoc gel documentation systems (UK). A 100 bp DNA ladder (Fermentas) was used as a size reference for PCR assay [12,13].

STATISTICAL ANALYSIS

Analysis of data and investigation of Ad40 and Ad41 were performed by chi-square test in the SPSS version 16 (SPSS, Chicago, IL).

RESULTS

Analysis of PCR products for the presence of Ad40 and Ad41 DNA on 1.5% agarose gel revealed 152 bp for Ad40 and 541 bp for Ad41 [Table/Fig-1].

The prevalence of Ad40 and Ad41 in stool specimens among children less than five years with nosocomial diarrhea was 22%. Ad40 and Ad41 DNA was found to be positive in 14/100 (14%), and 8/100 (8%) of diarrheic patients less than five years, respectively.

The mean age of patients was 11.8±15.3 months (range: 6-60 months), and 47% were female. The mean weight of patients was 9±2.7 kg. Chi-square and t-test showed no significant difference among positive samples in sex and age ($p>0.05$). In addition, there was no relationship between the occurrence of nosocomial diarrhea due to adenoviruses (Ad40 and Ad41) and seasons ($p>0.05$).

DISCUSSION

Next to rotavirus and norovirus, adenovirus is the most commonly identified viral agent in stools of infants and young children with gastroenteritis [14]. In some studies Ad40 and Ad41 have been detected in 5-15% of patients with diarrhea, and rate of detection was dependent on the level of economic status or geographical region of the study [15,16]; however, in the present study nosocomial adenovirus infections were detected in 22 cases (22%). This is higher than positive samples in study of Kotloff et al., In study of Kotloff et al., Ad40 and Ad41 were responsible for 6.2% of nosocomial diarrhea by a monoclonal antibody-based enzyme-linked immunosorbent assay [17]. This difference is probably related to different laboratory methods in two studies.

In study of Carraturo et al., nosocomial adenovirus infections were detected in seven patients (41.2%, ranging from 38.5% in 2005-2006 to 50.0% in 2006-2007). Overall, 71.4% of cases were children under 24 months [18], but in the present study there was no significant difference among positive samples in sex and age. Dey et al., found that among 101 samples Ad41 was more prevalent (52.5%) followed by Ad40 (24.7%), and total prevalence rate of Ad40 and Ad41 was 50.5% by ELISA and 77.2% by PCR [19]. Molecular epidemiological studies are important in the clinical adenovirus investigation [20].

Adenovirus in clinical specimens can be detected by PCR, enzyme immunoassay, direct fluorescent assay, and virus isolation from cell culture, but PCR was found as a fast, sensitive, and reliable method for the detection of adenoviruses in diarrheal disease; however, it requires special laboratory equipment [21]. In the present study, we analyzed stool samples by PCR. The actual prevalence would have been slightly higher if PCR instead of ELISA had been used as the first step in screening, so the methods of detection are not comparable in different studies. In this study, PCR-based sequence analysis of genomic DNA of adenoviruses confirmed that Ad40 is more prevalent (14%), followed by Ad41 (8%). Kotloff et al., found that Ad41 was more prevalent (68%) than Ad40 (32%) [17]. However, studies in Germany, rural Bangladesh, and the Netherlands showed that the frequencies of these two serotypes were almost similar [14,15,22].

LIMITATIONS

The present work suffers from some limitations. This study was conducted in only one hospital, but considering the fact that this hospital is the main referral hospital in the province, so the study subjects may be considered to be a good representative sample. In addition, adenoviruses other than Ad40 and Ad41 were not studied and phylogenetic analysis was not performed.

CONCLUSION

Regional epidemiological data on adenovirus infections may be important to develop strategies for intervention. Our research confirms that continuous monitoring of nosocomial gastroenteritis caused by enteric adenovirus is needed to monitor the potential adverse effects of these pathologies. We demonstrated the prevalence of Ad40 and Ad41 in a small population of Iranian children with nosocomial diarrhea for the first time. The prevalence of Ad40 and Ad41 per month of the year and at various ages suggested that they could be easily transmitted in a small community with poor hygienic conditions.

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