**Original article**

# Prevalence of adenoviruses 40 and 41 in children less than five years suffering from acute gastroenteritis hospitalized in Ahvaz Abuzar Hospital

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## Abstract

**Introduction and objective:** Gastroenteritis, the inflammation of stomach and intestine, is caused by a variety of microorganisms including viruses. Especially adenoviruses type 40 and 41 of group F adenoviruses are two etiologies of gastroenteritis in newborns and infants of less than five years old. The aim of this study was to determine the prevalence of ad40 and ad41 inducing gastroenteritis in children hospitalized in Ahvaz Abuzar Hospital, Iran, during October 2007-2008.

**Materials and methods:** Fecal samples collected from the patients were tested first by an ELISA kit, specific for adenovirus detection. All samples including positive specimens by ELISA method were subjected to two rounds of PCR test. A specific pair of primers for adenoviruses 40 and 41 was applied PCR method.

**Results:** Out of 280 fecal specimens collected from diarrheic children, 7(2.5%) were positive by ELISA test and 12 (4.3%) by PCR method. All of positive samples belonged to ad41.

**Conclusion:** From group F of adenoviruses, adenovirus 41 is the major etiology of gastroenteritis in Ahvaz area.

**Keywords:** Group F adenovirus, Gastroenteritis, PCR, Adenovirus, Diarrhea

## Introduction

Viral gastroenteritis is a common infection in all age groups worldwide. Usually four types of viruses are the main causes of this infection in all over the world: Rotaviruses, caliciviruses, astroviruses and adenoviruses types 40 and 41 [1]. After rotavirus infection, adenoviruses are the second cause

of severe and acute gastroenteritis in children under five years [2]. Adenoviruses are non-enveloped, icosahedral with linear dsDNA genome. Based on hemagglutination (HI) and neutralization tests, six groups (A-F) and 51 serotypes of adenoviruses have been determined. Group F of adenovirus including types 40 and 41 are the main etiologies of 1-20% of acute gastroenteritis [3] and 50% of all adenoviruses found in stool specimens are types 40 and 41 [4]. Since prevalence of adenovirus type 40 and 41 was not determined in Ahvaz, this project was undertaken to clarify the significance of these viral agents in diarrheic patients living in our area.

## Materials and methods

Two hundred eighty stool specimens were collected during one year (October 2007-2008) from diarrheic patients admitted to Ahvaz Abuzar Hospital, Iran for this cross-sectional study. The fecal samples were kept at -70°C till experiment. The fecal samples have been tested for bacterial agents and the negative specimens for routine bacterial pathogens were included in this study. ELISA test was performed for all stool samples (Microgen Bioproduct, Adenoscreen, UK), specifically for to detect adenoviruses. Viral DNA was extracted by Fermentas DNA extraction kit (Ukraine) and the DNA was used as template for PCR reaction. Roche PCR buffer (Mg+), dNTPs (10mM) and five units *Taq* DNA polymerase were used in PCR test.

The primers of PCR reaction for detection of ad40 were as follow, ad40F: 5'-ACCCACGATGTAACCACAGACA-3', ad40R: 5'- ACTTTGTAAGAGTAGGCG-GTTTCC-3; the size of its amplicon is 88bp [5]. And primers ad41F: 5'-TGGCCAC-CCCCTCGATGA-3' and ad41R: 5'-TTT-AGGAGCCAGGGAGTT-ATA-3' [6] were used for amplification of adenovirus type 41. PCR products for ad41 were 381bp. Both PCR reactions were performed in 50µl volume: 5µl 10x PCR buffer (1.5mM $MgCl_2$/reaction), 50pmol of each primer (1µl), 1µl of dNTPs, 5µl DNA template, 0.3µl *Taq* DNA polymerase and 36.7µl deionized $H_2O$.

Thermocycler (Techne, TC-512, UK) program for ad40 was as follows: one cycle 95°C, four minutes, 30 cycles including denaturation temperature 94°C, 30 seconds, annealing temperature 55°C, 30 seconds and extension temperature 72°C 30 seconds and a final extension temperature (72°C) for six minutes. Annealing temperature for amplification of adenovirus 41 was 60°C. PCR products were loaded onto 2% agarose gel, horizontally electrophoresed at 100 volts for about 45 minutes. Following staining with ethidium bromide, the gel was visualized under UV transilluminator (Vibrant, France). Figure 1 shows the result of PCR of two clinical specimens.

## Results

Out of 280 fecal samples tested with ELISA method, 7 (2.5%) were positive for adenovirus. Positivity of these seven samples was confirmed by PCR. The remaining negative specimens in ELISA test were subjected to PCR method experiment. Another five samples turned positive with PCR test. Altogether, prevalence of gastroenteritis due to group F adenovirus was 4.3% (12 out of 280 samples) based on PCR method, and all of the 12 samples belonged to type ad41. Of 12 positive samples, seven specimens belonged to male and five to female patients. Table 1 shows distribution of adenovirus infection according to age of the patients. According to table 1, the age group 7-12 months shows the highest prevalence of adenovirus infection.

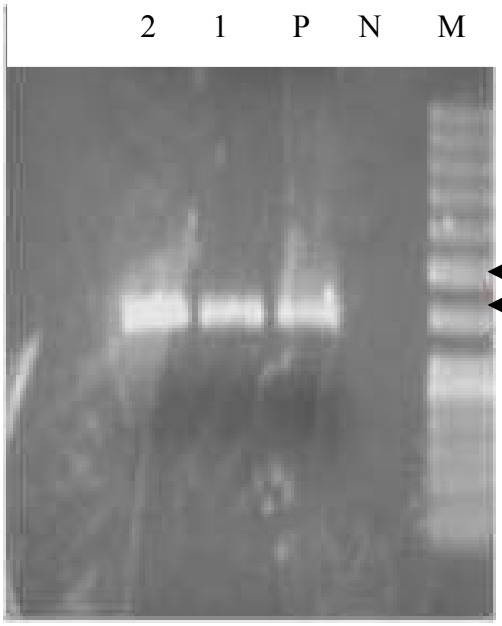


**Fig. 1:** Gel electrophoresis of ad 41 PCR products. Track M shows fermentas 50bp DNA molecular marker. N and P stands for negative and positive controls respectively. Tracks one and two are the PCR products of two clinical samples. The arrows show the sizes of fragments

**Table 1:** Age distribution of adenovirus infection

| Age group (Months) | Frequency | % |
|---|---|---|
| 0-6 | 2 | 16.7 |
| 7-12 | 4 | 33.3 |
| 13-24 | 2 | 16.7 |
| 25-36 | 3 | 25 |
| 36-60 | 1 | 8.3 |
| Total positive samples | 12 | 100 |

## Discussion

Gastroenteritis is one the commonest disease in children especially in developing countries [7]. More than 20 types of viruses cause this illness [8]. The medically most important enteric viruses are, group A rotaviruses, caliciviruses, adenoviruses and astroviruses [9]. Adenovirus enteritis occurs in children younger than two years old through the year with no seasonality restriction [10]. In this study, ELISA and PCR could detect 2.5% and 4.3% of positive gastroenteritis cases respectively. The result of this study is in consensus with the results of some of the reports from other parts of the world as the prevalence of group F adenovirus infection has been reported 2.6%-14% [11]. In 1991, Shinozaki *et al*. [12] determined the prevalence of adenovirus diarrhea in Japanese children 3.7%. They cultured fecal specimens on Graham 293 culture cells and DNA restriction enzyme analysis. Prevalence of group F adenovirus gastroenteritis was determined by ELISA test in some countries including in Thailand 9% [13], Korea 2.8% [14], Bangladesh 14% [15], Australia 3.1% [11], Italy 2.6% and USA 4.8% [16].

In 1998, Wood *et al.* [17] examined fecal samples of gastroenteritis patients by electron microscope. Prevalence of adenovirus in their study was reported 4%. The reports from other countries show that adenovirus gastroenteritis is very common in children less than two years and 7-12 month children are the most susceptible ones [13,18-20]. In our study, prevalence of adenovirus infection in age group 7-12 was 33.3% that was higher than other age groups and in concordant with the studies reported from other countries. PCR test demonstrated that only type ad41 was involved in the gastroenteritis of patients of this study.

This result (absence of type ad40 of adenovirus) was not unexpected since Fukuda *et al.* [21] in Japan carried out an

ELISA test for 892 diarrheic patients. They have reported 30 positive results for adenovirus and 29 (96.7%) of them were type ad41 and only one samples (3.3%) was ad40. Banyai *et al.* [22] conducted an investigation for adenovirus infection in diarrheic patients of Hungry by PCR from 2003 to 2006. They found that while type ad41 has been circulating in all four year duration, type ad40 has been found only in the years 2003 and 2004.

Primary reports indicated that both ad40 ad41 has been equally responsible for adenovirus infection in gastroenteritis cases [15,23], but recent investigations imply a decline in type ad40 infection and elevation of infection with type ad41 [11,18,24,25]. In justification of predominant ad41 in clinical specimens, research demonstrated that in comparison with prototype ad41-TAK of adenovirus, nucleotides of newly isolated ad41 have altered so that there is 94% similarity between nucleotides of the prototype and isolated ad41 of adenoviruses. The nucleotide alteration may have been translated to antigenic changes of ad41 and escape of this type from immune system [26].

## Conclusion

We believe if we increase the number of diarrheic samples to about 1000, prevalence of adenovirus type ad40 could be determined as well. Prevalence of group A rotaviruses in our city has already been determined 29.5% by the authors and reported [27]. Relative frequency of astrovirus, sapovirus and norovirus in our area is currently under investigation by the authors.

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