

# Incidence of enteric adenovirus gastroenteritis in Iranian children

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## Abstract

**Background:** Enteric adenoviruses, i.e. adenovirus 40 (Ad40) and adenovirus 41 (Ad41), have been shown to be a substantial cause of pediatric gastroenteritis in various parts of the world, but no data are available for Iran. **Objective:** The present study was performed to determine the incidence of enteric adenoviruses in children presenting to the Children's Medical Center with gastroenteritis in Iran. **Study design:** Stool specimens from 872 children less than 7 years of age attending the Children's Medical Center in Tehran, Iran, with gastroenteritis were tested for the presence of Ad40, Ad41, and adenovirus-genus by a monoclonal antibody-based enzyme immunoassay. **Results and conclusion:** 6.7% of stool specimens contained enteric adenoviruses (3.3% Ad40 and 3.4% Ad41) and 2.0% nonenteric adenoviruses. Mean ages of Ad40, Ad41 and NEAd-positive children were 21, 19 and 29 months, respectively. Among the adenovirus-positive patients, 53.9% were male and 46.1% female. Watery diarrhea was present in 86.4% of children infected by adenoviruses. In conclusion, for the first time, we demonstrated the presence of enteric and nonenteric adenoviruses in a considerable proportion of stool samples from Iranian children with gastroenteritis. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Adenovirus type 40; Adenovirus type 41; Enzyme immunoassay; Gastroenteritis

**Abbreviations:** Ad40, adenovirus type 40; Ad41, adenovirus type 41; EAd, enteric adenoviruses; NEAd, nonenteric adenoviruses; MEIA, monoclonal antibody-based enzyme immunoassay.

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## 1. Introduction

Acute gastroenteritis continues to be a major cause of childhood morbidity throughout the world and one of the leading causes of mortality among infants and young children in developing countries. Comprehensive controlled studies re-

vealed that the so-called ‘noncultivable’ or ‘enteric’ adenoviruses (EAd) are, in contrast to the conventional cultivable ‘nonenteric’ adenoviruses (NEAd), which are also commonly detected in fecal samples, a frequent primary cause of pediatric diarrhea (Brandt et al., 1979, 1985). Using special cell culture techniques, it was shown that EAd belong to two antigenically closely related serotypes, namely adenovirus 40 (Ad40) and adenovirus 41 (Ad41) which together form a new species called F (De Jong et al., 1983). Epidemiological studies performed in various industrialized countries demonstrated that EAd rank only second in the etiology of viral gastroenteritis in infants and young children (Brandt et al., 1979; Uhnnoo et al., 1986; Barnes et al., 1998; Caeiro et al., 1999). In several studies performed in various countries, EAd have been found in 1.1–12% of the stool specimens from infants and young children with acute gastroenteritis (Brandt et al., 1985; Uhnnoo et al., 1986; Shinozaki et al., 1991; De Jong et al., 1993; Jarecki-Khan et al., 1993; Barnes et al., 1998; Moore et al., 1998; Caeiro et al., 1999). Control of pediatric diarrheal disease depends on an accurate understanding of the relative prevalence of the responsible pathogens, which may differ in different communities (Barnes et al., 1998). This understanding is especially important for developing countries, in which the disease burden is heavy. Estimated incidence rates in these regions range from 3.5 to 7.0 episodes per child per year during the first 2 years of life and 2–5 episodes per child per year for the first 5 years (Brandt et al., 1983). However, only few etiological data are available for developing countries and we are currently engaged in generating such information. Recently, in agreement with earlier studies (Kidd et al., 1983; Shinozaki et al., 1987; Jarecki-Khan and Unicom, 1992), we detected the presence of antibodies to EAd in about one-half of 127 single sera from healthy Iranian children up to 7 years of age (Sadari et al., 2000). The purpose of the present study was to determine the rate of EAd and NEAd in stool samples from children with gastroenteritis attending the Children’s Medical Center in Tehran, Iran.

## 2. Materials and methods

### 2.1. Subjects and specimens

The study population consists of all children up to 7 years of age attending the Children Medical Center in Tehran, Iran, for treatment of gastroenteritis between 5 October, 1999 and 18 December, 1999. The definition of diarrhea was three or more watery or loose stool daily lasting for at least 1 day. Stool specimens were obtained from 872 patients as soon as possible after admission to the hospital. 60.1% of the children were male. The youngest patient was 1 month old. Of the children, 25.3% were less than 12 months old, 20.8% were between 1 and 2 years old, and 46.1% were between 2 and 7 years old.

### 2.2. Viruses and antibodies

Prototype strains of Ad5, Ad40 and Ad41, were obtained from the Rijksinstituut voor Volksgezondheid en Milieuhygiene (RIVM), Bilthoven, the Netherlands. Monoclonal antibodies MAAd-g1 (specific for adenovirus genus antigen), MA40-1 (Ad40-specific), MA41-1 (Ad41-specific), and horse antiserum to Ad10 were also obtained from the RIVM (De Jong et al., 1993).

### 2.3. Monoclonal antibody-based enzyme immunoassay (MEIA)

The MEIA was performed as described by De Jong et al. (1993) with some modifications. Microtitre wells (Flow Laboratories, USA) were coated overnight at 4 °C with horse antiserum to Ad10 diluted 1:500 in 0.06 M carbonate–bicarbonate buffer pH 9.6. The wells were washed with 0.01 M phosphate-buffered saline pH 7.4 (PBS) containing 0.05% (vol/vol) Tween 20 (PBS-T) three times, 100 µl of a clarified fecal homogenate (10% wt/vol) in PBS-T was added to each well, and plates were incubated at 37 °C for 2 h. The wells were washed again as described above and the plates were incubated for 1 h at 37 °C with 100 µl of the monoclonal antibodies MAAd-g1 (diluted 1:500), MA40-1 or MA41-1 (diluted 1:1000) in separate wells. Monoclonal antibodies

were diluted in PBS containing 0.5% (vol/vol) Tween 20 and 0.5% (wt/vol) bovine serum albumin (PBS-T-BSA). After washing the plates, 100  $\mu$ l of anti-mouse immunoglobulin conjugated with peroxidase (Sigma, USA) at a dilution of 1:5000 in PBS-T-BSA was added to each well and the plates were incubated for 1 h at 37 °C. The wells were washed again and orthophenylenediamine (Sigma, USA) was added as a substrate for 30 min at room temperature. The enzyme reaction was stopped with 3 M sulfuric acid and the optical density was measured at 492 nm in a 'Titertek Multiskan' spectrophotometer (Flow Laboratories, USA).

In each test, cell culture supernatants of Ad5, Ad40 and Ad41 were included as positive controls for MAD-g1, MA40-1, and MA41-1, respectively, and PBS-T was used as a negative control. A specimen was considered positive when its optical density value was 0.100 units greater than the value of a control well without specimen. This cut-off value was based on the results obtained with a series of 20 stool specimens collected from healthy children which did not test positive for adenovirus in a virus culture assay on Graham 293 cells. A specimen was considered to contain Ad40 or Ad41 if a color reaction was exhibited with the corresponding monoclonal antibody as well as MAD-g1. If a color reaction was only observed with MAD-g1, the specimen was considered to contain a NEAd.

#### 2.4. Statistical analyses

Chi-square test or Fisher's exact test was used for statistical analyses when appropriate.

### 3. Results

Of the 872 stool specimens tested by adenovirus MEIA, 76 (8.7%) were found to contain adenoviruses: 29 (3.3%) were Ad40, 30 (3.4%) Ad41, and 17 (2.0%) NEAd. None of the specimens contained both Ad40 and Ad41. The age distribution differed among infections with Ad40, Ad41, and NEAd (Fig. 1). The highest incidence of diarrhea caused by Ad40 was in children between

7 and 18 months of age (mean and median ages of 21 and 15 months, respectively), and by Ad41 in children younger than 6 months and between 2 and 7 years of age (mean and median age of 19 and 17 months, respectively). The number of NEAd infections rose to a peak at the ages of 2–7 years with a mean and median age of 29 and 22 months, respectively. Overall, 70% of the adenovirus infections occurred in children 2 years of age or younger which was statistically significant ( $\chi^2 = 18.719$ ,  $P = 0.000$ ). Moreover, there was no statistical difference in the rate of occurrence of adenovirus gastroenteritis in children under 6 months of age and others. There were 41 (53.9%) males with adenovirus gastroenteritis. Regarding the sex ratios among patients with Ad40, Ad41 and NEAd infections, 51.7, 53.3, and 58.8%, respectively, were male. These percentages did not differ statistically significantly. Watery diarrhea

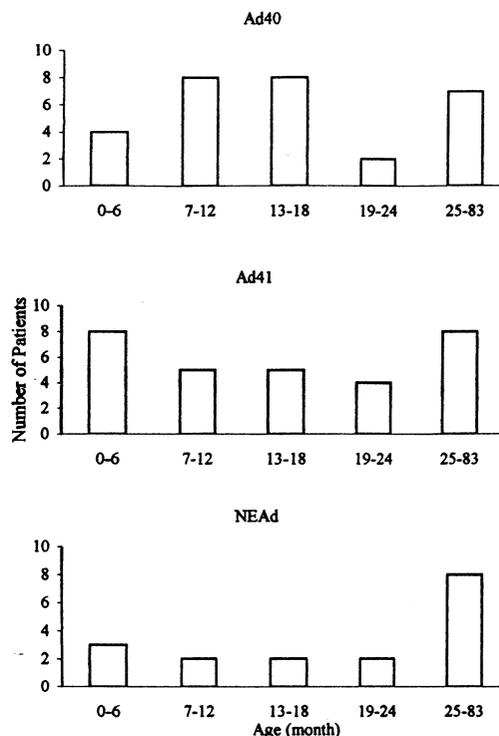


Fig. 1. Age distribution of children with adenovirus gastroenteritis.

was significantly more common in children infected with adenovirus than others (86.4 and 73.6%, respectively) ( $\chi^2 = 6.431$ ,  $P = 0.011$ ), but the rate of blood in stool was statistically similar (10.1 and 17.5%, respectively). No differences in age, sex, watery diarrhea and bloody stool distribution were observed in patients with Ad40, Ad41 and NEAd.

#### 4. Discussion

Epidemiological studies on EAd have become more frequent since the development of monoclonal antibody-based enzyme immunoassays (Herrmann et al., 1987; De Jong et al., 1993). From a clinical point of view, a major advantage of such assays over similar tests using polyclonal antibodies is their ability to differentiate between EAd and NEAd. This is important, because the presence of EAd is, and the presence of NEAd is not, statistically correlated with the occurrence of diarrhea (Brandt et al., 1979, 1985). In this study, MEIA enables us to assess the incidence of EAd and NEAd in fecal samples from Iranian children with gastroenteritis of 6.8 and 2.0%, respectively. These results and the age distribution of the infected patients are in line with those obtained in earlier studies (Brandt et al., 1985; Uhnnoo et al., 1986; Shinozaki et al., 1991; Jarecki-Khan et al., 1993; De Jong et al., 1993; Barnes et al., 1998; Caeiro et al., 1999). Despite the reports of some investigators, we did not find any difference between the two genders in the occurrence of adenovirus gastroenteritis, but confirming other reports, the majority of the patients had watery diarrhea (Uhnnoo et al., 1986; Shinozaki et al., 1991; Jarecki-Khan et al., 1993).

Several studies have reported an increase in the proportion of Ad41 infections at the expense of Ad40 infections over time (Shinozaki et al., 1991; De Jong et al., 1993). In this cross-sectional study, the incidence of Ad40 and Ad41 infections were almost the same, but long term studies are necessary to detect such shifts.

The present study confirms the importance of EAd infections in juvenile gastroenteritis in Iran and supports the implementation of rapid tests for

the laboratory diagnosis of these infections in order to avoid the unnecessary and potentially harmful use of antibiotics for this condition.

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