

ENTERIC ADENOVIRUS INFECTION IN INFANTS AND YOUNG CHILDREN WITH ACUTE GASTROENTERITIS IN TEHRAN

S. Modarres*, F. Jam-Afzon and S. Modarres

Department of Virology, Pasteur Institute of Iran, Tehran, Iran

Abstract- Adenoviruses are one of the most important etiological agents of serious gastroenteritis among infants and young children. Fecal specimens from patients with an acute gastroenteritis were evaluated for the presence of adenovirus (Ad40, 41) from April 2002 to February 2004. During the study, 1052 samples were collected from children under the age of 5 years in six educational and therapeutic pediatric centers. The specimens were tested for adenovirus (Ad40, 41) by EIA technique in the Virology Department of Pasteur Institute of Iran. Adenoviruses (Ad40, 41) were detected from 27(2.6%) samples, but were not detected in 150 samples of healthy control group. In this study the highest rate of adenovirus was found in children aged 6 to 12 months (40.7%), but the male to female ratio inpatients was approximately equal. Adenovirus (Ad40, 41) infections peaked in the winter as 48.1% was detected from December to March. There were a statistically significant difference between age and infection ($P < 0.001$), also between season with adenovirus (Ad40, 41) infection ($P = 0.005$). Breast-feeding had a protective action against adenovirus (Ad40, 41) infection. This study revealed that enteric adenovirus (Ad40, 41) is an etiological agent of acute gastroenteritis among children in Tehran.

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INTRODUCTION

Adenoviruses are one of the most important etiological agents of serious gastroenteritis among infants and young children less than five years (1-3). Subgroup F enteric adenoviruses (EAds) apparently are second only to the rotavirus a viral cause of acute gastroenteritis in infants and young children (2, 4). Antibodies to these viruses EAds (Ad40, Ad41) have been detected in approximately 50% of children less than five years of age in Asia, Africa, Europe, and South America with similar seropositive proportions in the different populations and are spread predominantly

by the fecal-oral route (3, 4). EAds have been associated with protracted diarrhea which may contribute to infant dehydration and malnutrition in developing countries (4, 5). Usually after an incubation period of 8 to 10 days, periodic diarrhea occurs, with light fever, vomiting, abdominal pains and dehydration (3, 6). They exist in all parts of the world, and are present in year-round, but are most prevalent in spring or early summer and again in midwinter in temperate climates (3-5). The purpose of this cross-sectional descriptive study was to assess the rate of EAds (Ad40, Ad41) infection among children less than five years of age with acute gastroenteritis at pediatric centers in Tehran.

In the present study, we described the development of a monoclonal antibody specifically reacting with EAds (Ad40, Ad41) in stool suspensions by EIA technique. The sensitivity of the developed test was 98% as previously described in comparison with direct electron microscopy (EM) (4, 7).

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* Corresponding Author:

S. Modarres, Department of Virology, Pasteur Institute of Iran, Tehran, Iran

Tel: +98 21 66953311-20,

Fax: +98 21 66953311

E-mail: mara@pasteur.ac.ir

MATERIALS AND METHODS

An epidemiological study on acute gastroenteritis enteric adenoviruses (Ad40, 41) was carried out in children less than 5 years of age in Tehran from April 2002 to February 2004.

Fecal specimens were collected from 1052 children under 5 years of age (including 49% male and 51% female) suffering from acute gastroenteritis, who were either inpatients and out patients at six educational and therapeutic pediatrics from Tehran University of Medical Sciences and Shahid Beheshtee of Medical Sciences in Tehran. Clinical data on each patient was collected using questionnaires including sex, age, season, clinical manifestations and clinical diagnosis. The patients had symptoms of fever, vomiting, dehydration, abdominal pain and watery diarrhoea. A total 150 nondiarrhoea healthy children of the same age group were studied as control at day-care centers in Tehran. We obtained informed consent from parents of all participants.

Stool samples were obtained within 24 hours of admission and were frozen and stored (-70°C) until processing in Virology Department of Pasteur Institute of Iran.

Specimens and enzyme immunoassay for EAdS

Enzyme immunoassay (EIA) of the stool specimen was performed in all patients. Approximately 0.1 g of solid feces or approximately 100 μl of liquid feces was suspended in 1 ml of phosphate-buffered saline (PBS) (pH 7.2) in a suitable container. This 10% fecal suspension was tested for antigens of EAdS using a commercial enzyme-immunoassay (EIA-adenovirus Ad40, Ad41) (DAKo- Denmark) was used and absorbance of test.

One hundred microliters of fecal suspension was added to the microwells, and coated with a monoclonal antibody against the group specific antigen for all human adenoviruses.

This was followed by adding 100 μl of enzyme conjugate containing antiadenovirus type 40(Ad40) and 41(Ad41) monoclonal antibodies to each microwell, and incubation at room temperature for

60 min. After a total of five washes with deionized water, 100 μl of both substrate A (urea peroxidase) and substrate B (tetramethyl benzidine) were added followed by incubation at room temperature for 10 min. Reactivity was determined by spectrophotometry at 450 nm after adding a stopping solution (1N sulfuric acid). An absorbance equal to or greater than 0.15 unit was considered positive (8, 9).

Results of this study were analyzed by Chi square test.

RESULTS

The frequency detection of adenoviruses (Ad40, Ad41) in fecal specimens among children less than five years of age with acute diarrhea is shown in Table 1. A total of 27 (2.6%) episodes of acute gastroenteritis were associated with adenoviruses (Ad40, Ad41) antigen detection. The samples of healthy control were not positive.

The highest incidence of diarrhea caused by adenovirus (Ad40, Ad41) was in children between 6 to 12 months of age (11 cases or 40.7%). There was a significant difference between the rate of infection in children under 24 months of age with older children ($P < 0.001$).

Adenoviruses (Ad40, Ad41) diarrhoea in males under five years of age to females ratio in patients of the same age was approximately equal.

Table 1. Detection by EIA of adenovirus (Ad40, Ad41) in the stools of children with acute gastro enteritis in Tehran by age group*†

Age group (months)	Total samples	Adenovirus positive samples	Positive rate (%)
6 <	176	0	0
6-12	184	11	40.7
12-24	335	9	33.4
24-36	138	3	11.1
36-48	105	2	7.4
48-60	114	2	7.4
Total	1052	27	2.6

Abbreviation: EIA, enzyme immunoassay.

* Data are given as number.

† $P < 0.001$.

From the questionnaire data, it was estimated that adenovirus (Ad40, Ad41) gastroenteritis cases in outpatients and inpatients stool at 14.2% and 23.9%, respectively, and there was a significant increase of infection in hospitalized children ($P < 0.001$).

A possible relationship between the occurrence of adenoviruses (Ad40, Ad41) infection and seasons was investigated (Table 2). The rates of adenovirus (Ad40, Ad41) infection were 33.4% in spring (April-June), 14.8% in summer, 3.7% in autumn and 48.1% in winter (December-March), with a significant difference between infection rates in the winter and other seasons ($P = 0.005$).

The adenovirus (Ad40, Ad41) related episodes of diarrhea in children according to clinical features and type of feeding are shown in Table 3. In infants up to nine months of age who were breast-fed there was an infection rate of 19.2%, significantly lower than that in the bottle-fed growth (47.8%) ($P = 0.004$). The study of clinical manifestations in adenovirus (Ad40, Ad41) gastroenteritis cases showed that most children with infection had fever (87.7%), vomiting (86.9%), abdominal pain (86.4%) and dehydration (88 %).

Table 2. Seasonal (months) distribution of adenovirus (Ad40, Ad41) isolated and isolation rates in children in Tehran from April 2002 to February 2004*

Month	Samples	No isolates (%)
Jan	118	5 (18.5)
Feb	108	4 (14.8)
Mar	94	2 (7.4)
Apr	114	4 (14.8)
May	102	3 (11.1)
Jun	87	2 (7.4)
Jul	97	2 (7.4)
Aug	109	1 (3.7)
Sep	72	1 (3.7)
Oct	64	0 (0)
Nov	35	1 (3.7)
Dec	52	2 (7.4)
Total	1052	27 (2.6)

* $P = 0.005$.

Abbreviations: Jan, January; Feb, February; Mar, March; Apr, April; Jun, June; Aug, August; Sep, September; Oct, October; Nov, November; Dec, December.

Table 3. Clinical symptoms of children with adenovirus (Ad40, Ad41) diarrhea*

Symptoms	No. of Patients	Episode of diarrhea	
		No.	Rate (%)
Fever	24	21	87.7
Vomiting	23	20	86.9
Abdominal pain	22	19	86.4
Dehydration	26	22	88
Breast-fed	26	5	19.2
Bottle-fed	23	11	47.8

* $P = 0.004$

DISCUSSION

Adenoviruses type (Ad 40, Ad41) are one of the most common etiological agents of acute gastroenteritis among infants and young children less than two years of age in both industrialized and developing countries in most parts of the world (10, 11). Adenoviruses (Ad40, Ad41) have been detected in 5 to 15% of patients with diarrhea, and rate of detection was dependent on the level of economic status or geographical region of the study area (10, 12).

In this study, laboratory diagnosis was attempted by using EIA technique for antigen detection. Adenovirus (Ad40, Ad41) infection was found in 2.6% of children with diarrhea, but was not found in the control group. There are similar studies by others from different parts of the world (13). As documented by other studies, most of the cases of adenovirus (Ad40, Ad41) diarrhea occur in children less than two years of age (14, 15). In our study the highest incidence of adenovirus (Ad 40, Ad41) occurred in children aged 6 to 12 months ($P < 0.001$), a distribution similar to that reported by others (15). In most parts of the world, the adenovirus (Ad40, Ad41) is present throughout the year, also during the period of the present study infection occurred throughout the year, but our data indicated that the outbreaks of adenovirus (Ad40, Ad41) infection were in the winter and spring (December to June), so more than 81.5% occurred in these seasons. These results are in accordance with the finding of others (16, 17). During this study,

fever, vomiting abdominal pains, watery diarrhea and dehydration were the most common specific signs and symptoms in children with adenovirus (Ad40, Ad41) infection, therefore, the clinical features of these patients are similar to those previously described (18, 19). The frequency of the adenoviruses (Ad40, Ad41) among breast-fed and bottle-fed in Tehran was 19.2% and 47.8% respectively for infants up to nine months of age. These results may indicate that breast-fed infants besides acquiring a passive immunity from the mothers, may also acquire resistance to infection with adenovirus (Ad40, Ad41) as is implied here by the low frequency of breast-fed infants among patients.

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Conflict of interests

The authors declare that they have no competing interests.

REFERENCES

1. Fields BN, Knipe DM. Virology. 4th edition. New York: Raven Press; 2001.
2. Jawetz E, Melnick JL, Adelberg EA. Medical microbiology. 22th edition. California: Appleton and Lange; 2001.
3. Behrman RE, Kliegman RM, Arvim AM. Nelson's text book of pediatrics. 16th edition. Philadelphia: Saunders; 2000.
4. Singh-Naz N, Rodriguez WJ, Kidd AH, Brandt CD. Monoclonal antibody enzyme-linked immunosorbent assay for specific identification and typing of subgroup F adenoviruses. J Clin Microbiol. 1988 Feb; 26(2):297-300.
5. Uhnnoo I, Wadell G, Svensson L, Johansson ME. Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children. J Clin Microbiol. 1984 Sep; 20(3):365-372.
6. Yolken RH, Franklin CC. Gastrointestinal adenovirus: an important cause of morbidity in patients with necrotizing enterocolitis and gastrointestinal surgery. Pediatr Infect Dis. 1985 Jan-Feb; 4(1): 42-47.
7. Schmidt NJ, Lennett DA, Lennett ET, Lennett EH, Emmons RW. Diagnostic procedures for viral, rickettsial and chlamydial infections. 7th edition. Philadelphia: Saunders; 1995.
8. Wood DJ, Bijlsma K, de Jong JC, Tonkin C. Evaluation of a commercial monoclonal antibody-based enzyme immunoassay for detection of adenovirus types 40 and 41 in stool specimens. J Clin Microbiol. 1989 Jun; 27(6):1155-1158.
9. Lin HC, Kao CL, Lu CY, Lee CN, Chiu TF, Lee PI, Tseng HY, Hsu HL, Lee CY, Huang LM. Enteric adenovirus infection in children in Taipei. J Microbiol Immunol Infect. 2000 Sep; 33(3):176-180.
10. Jarecki-Khan K, Tzipori SR, Unicomb LE. Enteric adenovirus infection among infants with diarrhea in rural Bangladesh. J Clin Microbiol. 1993 Mar; 31(3):484-489.
11. Singh-Naz N, Naz RK. Development and application of monoclonal antibodies for specific detection of human enteric adenoviruses. J Clin Microbiol. 1986 May; 23(5):840-842.
12. Blacklow NR, Greenberg HB. Viral gastroenteritis. N Engl J Med. 1991 Jul 25; 325(4): 252-264.
13. Kapikian AZ. Viral gastroenteritis. JAMA. 1993 Feb 3; 269(5):627-630.
14. Shears P, Wright A. Community-acquired infections among children in an urban environment: a 2-year prospective study in Liverpool, U.K. J Infect. 1995 Mar; 30(2):173-177.
15. Bates PR, Bailey AS, Wood DJ, Morris DJ, Couriel JM. Comparative epidemiology of rotavirus, subgenus F (types 40 and 41) adenovirus and astrovirus gastroenteritis in children. J Med Virol. 1993 Mar; 39(3):224-228.
16. Araki K, Tsai CH, Sato K, Kobayashi M, Shinozaki T, Abe T. [An outbreak of enteric adenovirus type 41 endemic in Fjieda, Japan]. Kansenshogaku Zasshi. 1994 Dec; 68(12):1459-1464. Japanese.
17. Duggan C, Nurko S. "Feeding the gut": the scientific basis for continued enteral nutrition during acute diarrhea. J Pediatr. 1997 Dec; 131(6):801-808.

18. Acra SA, Ghishan GK. Electrolyte fluxes in the gut and oral rehydration solutions. *Pediatr Clin North Am.* 1996 Apr; 43(2):433-449.
19. Branski D, Lerner A, Lebenthal E. Chronic diarrhea and malabsorption. *Pediatr Clin North Am.* 1996 Apr; 43(2):307-331.

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