



## Enteric adenovirus infection in children in Taipei

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Enteric adenoviruses (EAd), including type 40 (Ad40) and 41 (Ad41), can cause acute and severe diarrhea in young children. To delineate the epidemiological features of pediatric EAd infection in Taiwan, we conducted a retrospective study of all cases of EAd gastroenteritis in children treated at National Taiwan University Hospital for the period from July 1993 to December 1997. Stool samples were tested for the presence of Ad40 or Ad41 by enzyme immunoassay (EIA). A total of 64 cases of EAd infection in 63 children aged from 8 days to 81 months old with a median age of 9.5 months treated during the study period were identified. The male-to-female ratio was 1.63 (39/24). No obvious seasonal clustering of EAd cases was noted. Most patients (76.6%) were infected before the age of 2 years. Clinical features included diarrhea (96.9%), fever (54.7%), vomiting (45.3%), mild dehydration (43.8%), symptoms of upper respiratory tract infection (21.9%), and abdominal pain (12.5%). Analysis of fecal samples in patients with diarrhea showed watery diarrhea in 72.2%, diarrhea with mucus in 20%, diarrhea with blood in 3.1% and diarrhea with mucus and blood in 1.6% of all patients. Nearly one-half (43.5%) of the patients had diarrhea for more than 7 days. Thirty-seven patients (57.8%) were hospitalized due to gastroenteritis or other unrelated diseases, and 11 patients (17.2%) acquired enteric adenovirus infection during hospitalization for other underlying disease. Twelve patients (18.8%) had mixed infections, which included rotavirus, respiratory syncytial virus (RSV) and *Salmonella* species. There were no deaths in this series. The findings of this study suggest that EAd are important etiologic microbes of pediatric gastroenteritis.

**Key words:** Adenovirus types 40 and 41, diarrhea, enteric adenovirus, gastroenteritis, Taipei

At least 49 immunologically distinct types of adenovirus have been recovered from humans [1]. Respiratory symptoms with or without concurrent gastroenteritis are the most common manifestations of adenovirus infection. Some types of adenoviruses induce gastroenteritis only with few respiratory symptoms and are thus referred to as enteric adenoviruses (EAd). Flewett *et al* first identified EAd in pediatric fecal specimens which failed to grow in conventional cell culture by using electron microscopy [2]. They named these organisms fastidious or uncultivable adenovirus [2]. The successful use of Chang conjunctival, Graham 293 and PLC/PRF5 cells allowed EAd to be propagated and well recognized [3-5]. EAd are viral particles measuring from 70 nm to 80 nm in diameter with double-stranded DNA. Restriction enzyme analysis demonstrated that EAd have a unique pattern and led to their classification as a new subgenus, F, including two serotypes, 40 and 41 [6].

Studies in Europe, North America, South Africa, Australia, and Asia have shown that EAd are one of the most common causes of pediatric viral gastroenteritis, secondary to rotavirus, and account for 2.6% to 14% of cases [7,20]. EAd have also been implicated in nosocomial infections, day care center outbreaks, and infection of immunocompromised patients [12,21-24]. EAd mainly infect children younger than 2 years of age. No seasonal variation in the incidence of EAd infection has been reported [25]. The clinical features include watery diarrhea persisting for a mean of 4.5 days, vomiting, with or without fever [26].

So far, there has been no report describing the characteristics of pediatric EAd infection in the Taiwan population. The purpose of this study was to provide a baseline set of data about the clinical features of EAd gastroenteritis in Taiwanese children.

### Materials and Methods

#### Subjects

A total of 64 stool sample tests which were positive for EAd antigen were identified from virology laboratory

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records of pediatric patients treated from July 1993 to December 1997. The charts of these 63 patients were reviewed. These specimens had been collected from patients in the out-patient department, emergency department, and pediatric wards. Acute diarrhea was defined as more than three daily loose or watery stools with diarrhea lasting for a total duration of at least 24 h. Symptoms of upper respiratory infection (URI) were defined as the concurrence of sneezing, coughing or rhinorrhea.

### 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  $\mu$ L 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 an EIA kit (Adenoclone-Type 40/41 EIA, Cambridge Biotech., Worcester, USA). 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  $\mu$ L of enzyme conjugate containing antiadenovirus type 40 (Ad40) and 41 (Ad 41) monoclonal antibodies to each microwell, and incubation at room temperature for 60 min. After a total of five washes with deionized water, 100  $\mu$ L of both substrate A (urea peroxidase) and substrate B (tetramethylbenzidine) were added followed by incubation at room temperature for 10 min. Reactivity was determined by spectrophotometry at 450 nm after adding a stopping solution (1 N sulfuric acid). An absorbance equal to or greater than 0.15 unit was considered positive.

### Examination of other enteropathogens

Testing for the presence of other potentially pathogenic enteric microorganisms was performed depending on the physician's request. For the detection of *Salmonella* spp. and *Shigella* spp., fecal specimens were inoculated in *Salmonella-Shigella* agar. Rotavirus antigen was detected by EIA (Premier Rotaclone, Meridian Diagnostics, Inc., Cincinnati, OH, USA).

### Results

Sixty-four specimens from 63 patients were found to be positive for EAd40/41. The age of these children ranged from 8 days to 81 months, with a median age of 9.5 months (Table 1). The male-to-female ratio was 1.63 (39/24). More than half of the symptomatic EAdS infections (62.6%) were in children younger than 1 year

of age, and only a few of the infections (7.8%) were in children older than 3 years of age. A total of 62 (96.9%) out of 64 episodes of infection were accompanied by symptoms of diarrhea. Fecal samples were watery in 72.2% of patients; contained mucus in 20% of patients, blood in 3.1%, and mucus and blood in 1.6%. Half of the patients in this series experienced fever (54.7%), and vomiting (45.3%), mild dehydration (43.8%) and URI (21.9%) were also common symptoms (Table 2). Twenty-eight patients (43.5%) had diarrhea for more than 7 days.

Eighteen patients (28.6%) had been hospitalized for acute gastroenteritis at the time of diagnosis of EAdS, and 19 cases were admitted for other unrelated underlying diseases. The underlying diseases included congenital heart disease in eight patients, biliary atresia post-Kasai operation in two, and one each with imperforate anus, prematurity, acute myeloid leukemia, acute lymphoblastic leukemia, floppy infant syndrome, congenital nephrotic syndrome, congenital hypothyroidism, and nesidioblastosis. Among the 18 patients with community-acquired EAdS infection (including dual infection), the mean duration of hospitalization was 9.2 days (ranging from 2 to 31 days). The mean hospital stay in the nine children with pure EAdS infection was 5.4 days (ranging from 2 to 10 days). Nosocomial infection was common and 11 patients (17%) acquired

**Table 1.** Age distribution of children with enteric adenovirus infection (n = 64)

| Age (month) | No. of cases (%) |
|-------------|------------------|
| 0-6         | 28 (43.8)        |
| 7-12        | 12 (18.8)        |
| 13-24       | 9 (14.0)         |
| 25-36       | 10 (15.6)        |
| > 36        | 5 (7.8)          |

**Table 2.** Clinical manifestations of enteric adenovirus infection in children

| Symptom/sign                 | No. of cases (%) |
|------------------------------|------------------|
| Diarrhea                     | 62 (96.9)        |
| Watery stool                 | 48 (72.2)        |
| Stool with mucus             | 12 (20.0)        |
| Stool with blood             | 2 (3.1)          |
| Stool with mucus & blood     | 1 (1.6)          |
| Fever                        | 35 (54.7)        |
| Vomiting                     | 29 (45.3)        |
| Dehydration (mild)           | 28 (43.8)        |
| Symptoms of URI <sup>a</sup> | 14 (21.9)        |
| Abdominal pain               | 8 (12.5)         |
| Metabolic acidosis           | 2 (3.2)          |

<sup>a</sup>URI = upper respiratory tract infection

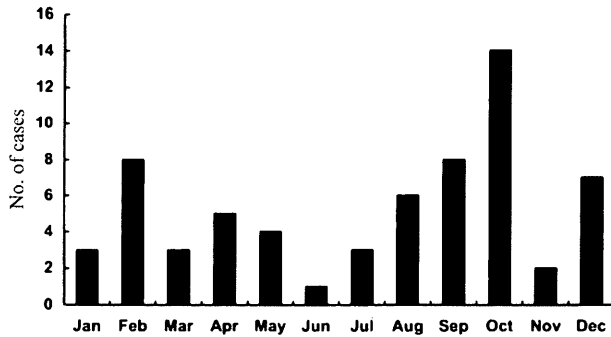


Fig. 1. Monthly distribution of children with enteric adenovirus infection, during the period from 1994 to 1997.

the EAds infection during hospitalization. EAds infection was not a cause of death in this series. The analysis of seasonal distribution of incidence was done using the subjects treated from the second half of 1993 to 1997, including nine patients treated in the second half of 1993, 14 in 1994, 20 in 1995, 14 in 1996, and seven in 1997. The number of cases declined after 1995, although the study population was not large enough to evaluate the significance of this finding. Two clusters of cases of EAds infection were noted in February and October, respectively (Fig. 1).

Twelve patients (18.8%) showed mixed infections in addition to EAds, with respiratory syncytial virus (RSV) and *Salmonella* sp. in one child (1.6%), *Salmonella* sp. in one (1.6%), rotavirus and *Salmonella* sp. in one (1.6%), and rotavirus in nine (14.1%). One patient had a second episode of EAds infection 18 months after the first episode.

## Discussion

This is the first study to delineate the clinical features of EAds gastroenteritis in Taiwanese children. Enteric adenovirus-associated gastroenteritis in this study was usually mild and self-limited, and was characterized by watery diarrhea, vomiting, and fever. Concomitant symptoms of upper respiratory tract infection were found in one-fifth of EAds patients. Mild dehydration was found in 43.8% of patients. The majority of the patients in this series were younger than 2 years of age (76.6%) with a slight male predominance. Nosocomial infection was common in this series of patients (17.2%). Mixed infection was not uncommon and repeated infection was found in one patient 18 months apart. There was no fatalities due to EAds infection in this series. No major seasonal variation in the incidence of EAds infection was found despite some seasonal clusters.

The true incidence of EAds infection in the study population was unclear from the data of this retrospective study because we could not know the total number of fecal specimens for enteric pathogens examination. Reports throughout the world have shown variable results depending on the methods of detection used and the socioeconomic status of the study populations. Uhnouo *et al* [8] showed that EAds were the sole recognizable etiologies of diarrhea in 7.2% of children. They also demonstrated that infection with most adenoviruses had a different clinical picture from EAds infection, characterized by milder diarrhea, higher fever, and more common respiratory symptoms [8]. With the aid of electron microscopy and immune electron microscopy, the prevalence of EAds gastroenteritis was estimated to be 4% in children younger than 2 years of age in England [10]. Shinozaki *et al* found that EAds in 3.7% of children with diarrhea in Japan by using Graham 293 cell culture and DNA restriction enzyme analysis [17]. Survey studies of diarrhea in children using EIA showed a 4.4% detection rate of EAds in Thailand [14], 9% in Korea [15], 2.8% in rural Bangladesh [16], 14% in Guatemala [19], 3.1% in Australia [20], 4.8% in the US, and 2.6% in Italy [29]. Most of these studies demonstrated that EAds usually infected children younger than the age of two [8,14-18,20,27], with the highest incidence, ranging from 20% to 45.4%, found in infants aged from 7 to 12 months old [14,16,17,25,27]. A compatible result of 76.6% was found in the present study [8,14-18,20,27]; however, it is noteworthy that approximately one-half of the patients in this series (43.8%) were younger than 6 months of age. A controlled study conducted by Cruz *et al* in Guatemala [19] showed that infants aged below 6 months had a lower incidence of EAds diarrhea than children in other age groups. Factors attributed to this difference included breast-feeding, socioeconomic status, hygiene, culture, and climate.

The most common clinical manifestations of EAds infection were watery diarrhea and URI in this series, and the nature and prevalence of other clinical presentations, such as mucus or blood tinged diarrhea, were similar to previous reports, except for metabolic acidosis in two patients. A previous study in Australia found that most children with EAds infection had diarrhea for longer than 5 days, and were less febrile or dehydrated than children with rotavirus infection [20]. Symptoms of URI were present in 21% of patients, which was compatible with data from other regions (21%-46%) [8,15,20,21,23]. The frequency of respiratory symptoms was much lower than those caused by nonenteric adenovirus [8]. In the present

study, 12 patients (18.7%) had diarrhea caused by dual infections, a finding noted in 6% to 24.8% of patients in previous reports [15,16,20]. Dual infections with other potential enteropathogens, including rotavirus, astrovirus, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Cryptosporidium* spp., *Vibrio cholerae*, *Giardia lamblia*, and *Escherichia coli*, have also been reported [8,15,16,19,20]. One patient in the present series suffered from two episodes of EAd infection. However, we could not differentiate whether this patient had Ad 40 or Ad 41 type infection. Infection with Ad40 (Ad41) and followed by Ad41 (Ad 40) was the most likely clinical scenario in this patient, although reinfection by the same viral type is possible. Reinfection by the same type of EAds has been demonstrated by Unicomb *et al* [30], who showed that neutralizing antibodies alone could not protect infants against symptomatic EAds reinfection.

The mean duration of hospital stay (5.4 days) in patients with community-acquired EAds infection in this series was longer than that in previous reports, including 4.5 days reported by Kim *et al* [15], and 4 days by Grimwood *et al* [20]. Nosocomial infection was not rare in our study population (17.2%). The contributing factor might have been that 30% of our patients had severe underlying diseases, such as congenital heart disease and acute lymphoblastic leukemia, and, hence, required longer hospital stays, which increased their likelihood of acquiring nosocomial infections. The results of this study were similar to previous reports from Canada, London, Australia, Sweden, Korea, Guatemala, USA, and Thailand [8,14,15,18-21,24], which showed no significant seasonal distribution of EAds infection. However, in Japan, EAds infections were found throughout the year, but peaked between September and November [17].

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