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Association of viral isolates from stool samples with intussusception in children

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SUMMARY

Background: Intussusception is the most common cause of intestinal obstruction in young children. The pathogenesis of intussusception is still not well understood. In this study the pathogens from stool specimens were investigated in children with intussusception.
Methods: Patients diagnosed with primary idiopathic intussusception were enrolled. Pathogenic bacteria and viruses were detected in the stool samples by routine culture, cell culture, polymerase chain reaction, reverse transcriptase-polymerase chain reaction, enzyme immunoassay, and electron microscopy examinations.
Results: A total of 71 samples were analyzed during the 2-year study period. The patients ranged in age from 4 to 47 months. Viruses were detected in 56 of the 71 stool samples (78.9%). Adenovirus was found in 19 of 35 cases aged <2 years, whereas it was found in 17 of 21 cases aged ≥2 years. The majority of adenovirus isolates were non-enteric organisms generally associated with respiratory tract symptoms.
Conclusions: These results suggest a casual association of viral infections in children with intussusception. Adenovirus infection, especially with the primary non-enteric types, is a significant risk factor for developing intussusception in children, particularly those aged over 2 years.

1. Introduction

Intussusception is the most common cause of intestinal obstruction in young children. The majority of cases occur in children under the age of 24 months.[1,2] Several studies have demonstrated that the incidence of intussusception varies among countries and also over time. A recent report prospectively compared the incidence of intussusception between Vietnam and Australia, and showed that the incidence in Vietnam was more than four-fold higher than that in Australia.[3] The incidence of intussusception observed in Vietnam is also higher than that in any other country for which incidence data are available, including recent reports from the USA and Latin America.[4–7] Studies from China also suggest a high incidence,[8] however, those reported in the USA, Denmark, and Australia have significantly declined over the past decade.[2,4,9] These results may reflect the presence of an environmental risk factor and/or infectious etiology in developing intussusception.

The underlying cause of intussusception in children remains unknown, but it has been associated with several pathogens, including adenovirus. Previous studies have demonstrated adenoviruses in from 30% to 50% of stool specimens, and in intestinal or lymphoid tissue specimens from children with intussusception.[3,10–16] Murphy et al. reported an increased risk of intussusception after the administration of tetravalent rotavirus vaccine composed of rhesus rotavirus (RRV) and three human RRV reassortant strains, RRV-TV.[17] These results suggest an association between viral infection and the development of intussusception in young children.

The present study analyzed the association of infectious pathogens with the development of intussusception in children by a detailed examination of stool specimens during hospitalization. Results showed a high prevalence of viral isolates and a strong association with adenoviruses in patients with intussusception, particularly those over 2 years of age.

2. Materials and methods

2.1. Patients

The study was conducted at Hiroshima City Funairi Hospital, Hiroshima, Japan, over a 2-year period (August 2006 through July 2008). The study complied with the Declaration of Helsinki. The research protocol was approved by the ethics committee, and

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informed consent was obtained from the patients' guardians. Children with signs and symptoms suggestive of intussusception and seeking care for this problem were considered for enrollment into the study. The diagnosis of primary idiopathic intussusception was made according to the clinical history, physical findings, abdominal radiograph, ultrasounds, and finally an air and/or contrast enema, using the case definition of the Brighton Collaboration Intussusception Working Group.[18] All patients with intussusception were admitted to the hospital. During the same 2 years, all in-patients with uncomplicated gastroenteritis aged <4 years who were examined for the presence of viral isolates in stool specimens in order to identify the cause, were enrolled in the study.

2.2. Assessment of infectious pathogens

Stool samples were collected from patients with intussusception during hospitalization. All samples were collected within 24 h of hospitalization and stored at 4 °C. Routine culture media were used to assay for common bacterial pathogens. Screening for enterovirus, poliovirus, echovirus, and adenovirus was performed using routine cell culture, HE, Hep-2, RD-18S and Vero cells, to second-generation of serial passage, and immunofluorescence detection.[19] Calicivirus detection was conducted using reverse transcriptase-polymerase chain reaction (RT-PCR) assays.[20] Rotavirus was tested for using an enzyme-linked immunosorbent assay (ELISA) and RT-PCR assays.[21] All patients positive by culture were examined by neutralization tests for enterovirus, poliovirus, echovirus, and adenovirus. In those patients negative by cell culture and by the other tests described above, additional examinations were performed for the detection of viral inclusion by electron microscope and using PCR assays for adenovirus, parechovirus, sapovirus, astrovirus, and aichivirus.[22,23]

2.3. Statistical analysis

Statistical significance was determined using the Chi-square test, Fisher's exact test, or Ryan's test according to the StatView software program (version 5.0l; SAS Institute, Inc., Cary, NC, USA). These tests were used to assess differences in stool findings between age groups and differences in the clinical symptoms between patients with the detection of adenovirus and those with virus other than adenovirus and negative isolation. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Study participants

During the 2-year study period, 83 children with primary idiopathic intussusception were diagnosed and admitted to the hospital. Twelve patients were excluded from the analysis because the stool specimens from these patients were not obtained during their hospitalization. Therefore, 71 patients were enrolled in the study. There was no discernible seasonal variation in the incidence of intussusception, nor was there any significant variation in the annual presentation rate over the 10-year period 1998–2008 (data not shown).

3.2. Infectious pathogens

Figure 1 shows the results of viral detection assays and age distribution in the patients. The 71 patients ranged in age from 4 to 47 months; 23 were aged <12 months (32.4%), 25 were aged 12–24 months (35.2%), and 23 were aged >24 months (32.4%). The male to female ratio of patients was approximately 2.6:1 throughout the age groups (data not shown). Fifty-six of the 71 patients (78.9%) had stool specimens positive for viruses. The rate of viral detection gradually increased with age: 15 of 23 (65.2%) aged <12 months, 20 of 25 (80%) aged 12–24 months, and 21 of 23 (91.3%) aged >24 months. Similarly, the prevalence of adenovirus isolation increased significantly with older age. Of those patients with virus detected, adenovirus was found in seven of 15 (46.7%) aged under 12 months, 12 of 20 (60%) aged 12–24 months, and 17 of 21 (81.0%) aged over 24 months. The detection of adenovirus from stool specimens of intussusception patients gradually increased with older age. Table 1 shows the number of patients with adenovirus, as well as other viral pathogens, namely rotavirus, enterovirus, parechovirus, norovirus, and poliovirus, detected in the stool specimens. Multiple viral infections occurred in one patient. Viruses causing enteritis were more frequently detected in patients under 2 years of age. A bacterial pathogen, *Campylobacter jejuni*, was simultaneously cultured in one patient (22 months of age) whose stool was positive for adenovirus type 1 (data not shown). No other bacterial pathogens were cultured from the patient stool specimens.

During the same 2 years, stool specimens from 82 patients with uncomplicated gastroenteritis aged under 48 months were screened for viral isolates (Table 2). The rate of patients with

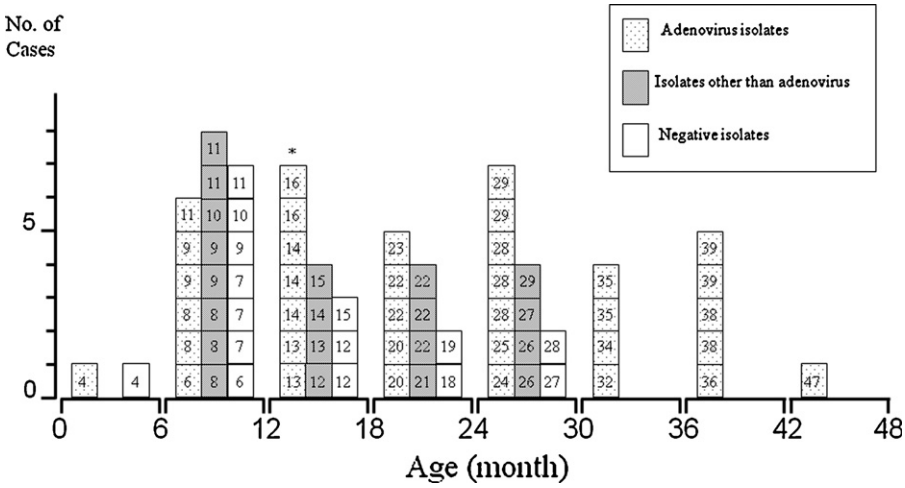

Figure 1. The number of cases with intussusception by age and viral isolate. Data represent the number of cases with adenovirus isolates (n = 36), isolates other than adenovirus (n = 20), and no isolates (n = 15) according to age. The numeral in the square represents the accurate age (in months) for each individual. The figure includes one case of multiple viral infections (*): a 14-month-old girl in whom both adenovirus type 5 and norovirus were detected.