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ORIGINAL ARTICLE

Clinical and epidemiologic features of Coxsackievirus A6 infection in children in northern Taiwan between 2004 and 2009

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KEYWORDS
Coxsackievirus A6;
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Background: Isolates of Coxsackievirus A6 (Cox A6) is increasing clinically in 2009 in Taiwan but detailed clinical features of Cox A6 infections in children have not been reported. This study is to define clinical manifestations and laboratory findings of Cox A6 infection in children.
Methods: From January 2004 to December 2009, a total of 4,664 children with enterovirus infections, based on throat virus culture, were treated in Chang Gung Children's hospital. Two hundred and ninety-six (6.3%) patients positive for Cox A6 infection were included in this study. One hundred and forty-one (47.6%) inpatients were further analyzed for clinical presentations, laboratory findings, and clinical diagnoses.
Results: There were two peaks of Cox A6 infection in 2007 and 2009 during the study period, especially during the warm season. The proportion of Cox A6 among total enterovirus isolates was 15.5% in 2007 and up to 22.2% in 2009. The mean age of inpatients was 2.42 ± 0.14 years. The mean hospitalization duration was 4.21 ± 0.11 days. The most common symptoms were fever (100%), oral ulcers (90.8%), and decreased oral intake (89.4%). The mean duration of fever was 2.78 ± 1 days (range, 1–7 days). Seventy-seven (54.6%) patients had fever more than 3 days. The mean leukocyte count was 14,850/mm^3, and 63 (45%) patients had leukocytosis (>15,000/mm^3). The mean serum C-reactive protein (CRP) level was 44.1 ± 3.3 mg/L (normal, <10 mg/L) and 62 (44%) had a CRP level >40 mg/L. One hundred and eight (76.6%)

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inpatients were diagnosed as herpangina and 18 (12.8%) hand–foot–mouth disease. Three patients had complications, including aseptic meningitis in one and encephalitis in two. All 141 inpatients recovered uneventfully.
Conclusions: Cox A6 is among the major serotypes of enteroviruses in Taiwan and most cases presented as herpangina and hand–foot–mouth disease. Nearly half of the cases may have leukocytosis and elevated CRP levels. Outcomes are usually good.

Introduction

Enterovirus infections are common human pathogens and traditionally classified into poliovirus, coxsackieviruses A and B, echoviruses, and the newer enteroviruses named with numbers such as enterovirus 68 to 71.[1–4] In 2000, the International Committee on Taxonomy of Viruses adopted a new classification system for human enteroviruses (HEVs). This system designates HEVs into five species, namely poliovirus and HEVs A, B, C, and D.[5] In this study, we used the traditional classification. Enteroviruses can cause a wide range of clinical diseases, including non-specific febrile illness, exanthema, respiratory infections, enteritis, aseptic meningitis, encephalitis, myocarditis, and even death.

There was a large-scale outbreak of enterovirus 71 infection in 1998 in Taiwan, which caused 78 deaths. Since then, virus surveillance for enterovirus activity has been monitored by Center for Diseases Control of Taiwan (CDC-Taiwan). According to the data from Center for Diseases Control of Taiwan (Table 1),[6] Coxsackievirus (Cox) A6 is usually among the five most common enterovirus serotypes in Taiwan from 2001 to 2008. However, the detailed clinical features of children with Cox A6 infection had not been reported. From the data of virology laboratory in Chang Gung Memorial Hospital, we found an increased number of Cox A6 isolates during the first half year of 2009, accounting for one quarter proportion of enterovirus isolates, which promoted us to review the clinical and epidemiologic features of children with Cox A6 infection.

Table 1 Five most common serotypes of clinical enterovirus (EV) isolates in Taiwan from 2001 to 2008

Year	Rank				
	First	Second	Third	Fourth	Fifth
2001	CA16	EV71	Echo30	CA6	Echo6
2002	Echo6	CA16	EV71	CA4	CB5
2003	CA16	CA2	CA6	Echo11	CA5
2004	CA4	CB4	CA6	EV71	CA5
2005	CB3	CA16	EV71	CA6	CA5
2006	CA2	CA5	CA4	Echo18	CB2
2007	CA6	CA10	CA16	CA4	Echo6
2008	CA2	EV71	CB4	CA16	CB1

Data from Center for Diseases Control of Taiwan, and modified from Ref. 14.
CA = Coxsackievirus A; CB = Coxsackievirus B; Echo = echovirus; EV = enterovirus.

Materials and methods

Patient enrollment

From January 2004 to December 2009, a total of 4,664 children with enterovirus infections, based on throat virus culture, were treated in Chang Gung Children's hospital. Two hundred and ninety-six (6.3%) patients, who were positive for Cox A6 infection, were included in this study. Of the 296 patients, 141 (47.6%) were inpatients and 155 were outpatients.

Data collection

Medical records of these patients were reviewed retrospectively. From the outpatients, only demographics were collected and analyzed, since detailed and comprehensive data cannot be collected from these patients. From the inpatients, we further collected clinical symptoms and signs, laboratory findings, clinical diagnoses, and outcomes. All data were expressed as mean ± standard deviation.

Virological studies

In our virology laboratory, virus isolation was carried out by tissue culture and each respiratory specimen was inoculated into four cell lines, including the human epidermoid carcinoma cells, canine kidney cells, human embryonic lung fibroblasts, and rhesus monkey kidney cells. Cultures were maintained in minimal essential media containing antibiotics and incubated at 33°C, rotated at 12 revolutions/hour. All cultures were observed daily for cytopathic effects. When the cell culture presented at least 25% of cytopathic effects, the cells were spotted and fixed on slides. Viral identification was done by immunofluorescent staining with virus-specific monoclonal antibody. All positive specimens were further confirmed by neutralization with type-specific pools of immune sera.

Diagnostic definition

Herpangina was defined as oral ulcers on anterior tonsillar pillars, soft palate, buccal mucosa, or the uvula. Patients with hand–foot–mouth disease had oral ulcers on the tongue or the buccal mucosa and vesicular rashes over palms, soles, knees, or the buttocks. Fever was defined as body temperature equal to or greater than 38°C. Leukocytosis was defined as white blood cell (WBC) count equal to or greater than 15,000 × 1,000/uL. For the complicated cases, meningitis was defined as pleocytosis in cerebrospinal fluid analysis. Encephalitis was characterized by the

presence of altered level of consciousness, personality changes, or hallucinations. Encephalomyelitis included both encephalitis and myelitis-like syndrome, which had the characteristics of acute paralysis, acute limb weakness with decreased muscle power.

Statistical analysis

Analyses were done with the Statistical Package for the Social Sciences (SPSS) for Windows (Version 17.0; SPSS, Chicago, IL, USA).

Results

Epidemiology

During the study period, no isolate of Cox A6 was recorded in 2004 and 2005 (Fig. 1), while there were two peaks in 2007 and 2009, respectively. In 2007, the proportion of Cox A6 among the total enterovirus isolates was 15.45% and the proportion was up to 22.27% in 2009. The prevalent season was warm season from March to September (Fig. 2). 98.6% of 229 patients with demographic data available were under 6 years old (Fig. 3), with the peak in 1 year of age. The youngest was 3 months old and the oldest was 11.6 years old. Male to female ratio was 1.27:1.

Of the 141 hospitalized children, the age ranged from 3 months to 7.3 years with a mean of 2.42 ± 0.14 years. 70.2% of the patients aged less than 3 years. The mean hospitalization duration was 4.21 ± 0.11 days (Table 2). The longest hospitalization was 11 days, because of subsequent healthcare associated acute enterocolitis. Twelve children had various underlying diseases, including asthma in two children, and G6PD deficiency, epilepsy, congenital facial palsy, febrile convulsion, allergic rhinitis, absence of right kidney, nephrotic syndrome, hyperthyrodism, and gastroesophageal reflux disease in one each.

Clinical manifestations

The most common symptoms in the 141 hospitalized children were fever (100%), oral ulcers (90.8%), and decreased oral intake (89.4%) (Table 3). The mean duration of fever was 2.78 ± 1 days (range, 1–7 days). Fifty-six (39.7%) patients had fever greater than 39°C and more than 3 days. Myoclonic jerk was noted in 30 patients (21.3%). There were seven patients with neurologic symptoms: febrile seizure in five and altered consciousness in two.

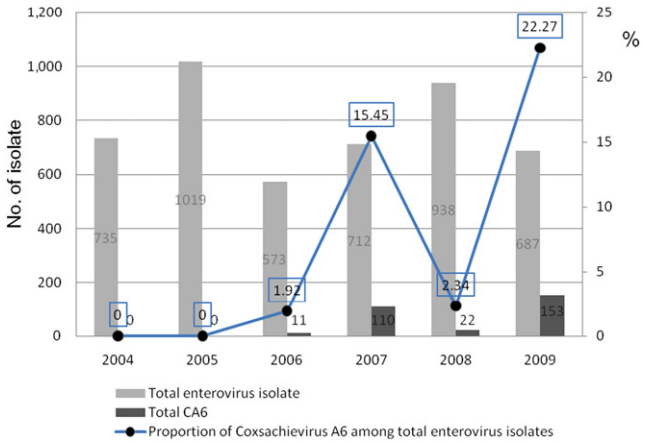

Figure 1. Number and proportion of enterovirus and Coxsackievirus A6 isolates from 2004 to 2009 June in Chang Gung Memorial Hospital.

Herpangina was the most common final diagnosis (108, 76.6%), followed by hand–foot–mouth disease (18, 12.8%). Central nervous system (CNS) involvement was noted in three patients, including meningismus in one (0.8%), and encephalitis in two (1.6%) (Table 2).

For the complicated cases, meningitis was defined as pleocytosis in cerebrospinal fluid analysis. Encephalitis was characterized by the presence of altered level of consciousness, personality changes, or hallucinations. The three cases of CNS involvement were described briefly. A 7-year-old boy had herpangina, headache, vomiting, mild lethargy, and fever up 40°C for 1 day but without respiratory tract symptom. Neither meningeal signs, nor leukocytosis, nor elevated serum C-reactive protein (CRP) level was noted. The symptoms relieved after admission. Lumbar puncture was not performed. Aseptic meningitis was highly suspected in this case. Both cases of encephalitis were 5 years of age and had

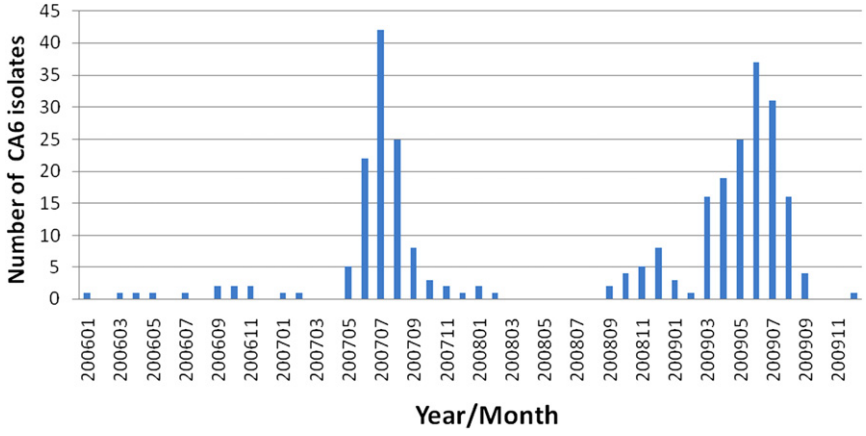

Figure 2. Monthly distribution of 296 Coxsackievirus A6 isolates from 2006 to 2009 in Chang Gung Memorial Hospital.

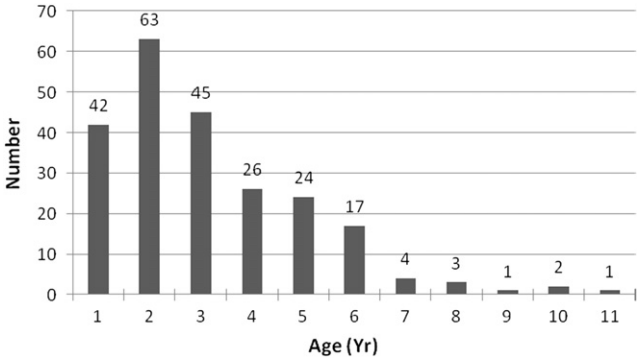

Figure 3. Age distribution of 229 patients with Coxsackievirus A6 infection from 2006 to 2009 in Chang Gung Memorial Hospital.

herpangina. One presented with fever, drooling, and visual hallucination for 3 days. No other neurologic sign was noted. No abnormal finding was recorded on electroencephalography. Hallucination resolved 2 days after hospitalization.

Table 2 Demographic data, final diagnoses and outcomes of 141 hospitalized children with Coxsackievirus A6 infection

Demographic characteristics and diagnoses	
Total number	141
Age (yr)	
Median	1.92
Mean	2.42 ± 0.14
Range	0.25–7.33
Age distribution, *n* (%)	
≤3 mo/o	1 (0.7)
≤6 mo/o	7 (5)
≤1 yr/o	33 (23.4)
≤2 yr/o	75 (53.2)
≤3 yr/o	99 (70.2)
≤4 yr/o	112 (79.4)
≤5 yr/o	126 (89.4)
≤6 yr/o	139 (98.6)
Male:female ratio	1.27:1
Children with underlying disease, *n* (%)	12 (8.5)
Duration of hospitalization (d)	
Range	1–11
Mean	4.21 ± 0.11
Median	4
Diagnosis	
Uncomplicated	No. (%)
Herpangina	108 (76.6)
Hand–foot–mouth disease	18 (12.8)
Acute exudative tonsillitis	7 (5)
Upper respiratory infection	4 (2.8)
Croup	1 (0.8)
Complicated	
Aseptic meningitis	1 (0.7)
Encephalitis	2 (1.4)
Outcome, *n* (%)	
Recovery	141 (100)
Sequelae	0

Table 3 Clinical symptoms and laboratory findings of 141 hospitalized children with Coxsackievirus A6 infection

Clinical symptoms and laboratory findings	
Symptoms	
Fever	141 (100)
Fever ≥3 d	77 (54.6)
Fever ≥ 39°C	99 (70.2)
Fever ≥3 d and ≥39°C	56 (39.7)
Oral ulcer	128 (90.8)
Skin rash	19 (13.5)
Cough	42 (29.8)
Rhinorrhea	38 (27)
Vomiting	16 (11.3)
Diarrhea	9 (6.4)
Decreased oral intake	126 (89.4)
Decreased activity	69 (48.9)
Myoclonic jerk	30 (21.3)
Febrile seizure	5 (3.5)
Alter consciousness	2 (1.4)
Laboratory findings	
WBC count (/mm^3)	
Median	14,850
Mean	$15{,}144 \pm 426$
Range	4,500–30,600
≥17,500/mm^3	42 (30%)
CRP (mg/L)	
Median	38.3
Mean	44.1 ± 3.3
Range	0.5–204
<10 (mg/L)	20 (14)
>40 (mg/L)	62 (44)
Glucose ($n = 103$)	
Mean (mg/dL)	91 ± 20
>150 (mg/dL)	0
AST ($n = 59$)	
Mean (U/L)	35 ± 7.7
>40	15/59
ALT ($n = 24$)	
Mean (U/L)	18.2 ± 6
>40	0

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CRP = C-reactive protein; WBC = white blood cell.

The other case presented with fever for 2 days and then developed bizarre behavior with eye staring, involuntary movements of both legs, and loss of attention to voice, which resolved after admission. Neither electroencephalography nor image studies were performed.

Laboratory finding

The mean value of WBC count was $15{,}144 \pm 426$/mm^3. Sixty-three cases (45%) had leukocytosis with WBC > 15,000/mm^3, while only one patient had leukocyte count < 5,000/mm^3 (4,500/mm^3) (Table 3).

The mean serum CRP level was 44.1 ± 3.3 mg/L. Only 20 patients had a normal CRP level (<10 mg/L) while 62 (44%) patients had a serum CRP level greater than 40 mg/L. Even, 12 cases (8.5%) had a CRP level > 100 mg/L. The

serum glucose was measured in 103 patients and none had hyperglycemia (>150 mg/dL). Fifteen of 59 patients with aspartate transaminase measurement were above 40 U/L.

Management

Most of the hospitalized children received supportive treatment only. Nine of the 141 (6.4%) inpatients ever received antibiotic therapy, and the indications included empiric use for suspicious bacterial infection due to elevated serum CRP level in four cases, and acute otitis media, cellulitis, positive throat swab for group A Streptococcus antigen test, and lower respiratory tract infection in one each. All of the inpatients recovered uneventfully.

Discussion

Results from this study indicated that children with Cox A6 infection were at younger age and more than 90% of the patients were less than 6 years of age. Most cases presented as herpangina, followed by hand-foot-mouth disease (HFMD); both manifestations accounted for 90% of the cases. About half of the inpatients had leukocytosis and elevated serum CRP. No fatal case was noted and all the inpatients recovered uneventfully.

Herpangina and HFMD are common contagious diseases in children, and most patients were less than 4 years of age, with the peak at 1 year of age.[7–10] A report regarding coxsackievirus infection from northern Taiwan[2] indicated that the mean age for children with Cox A10 was 40.2 ± 26 months, Cox A16 was 43.3 ± 6.4 months, Cox B1 was 36.4 ± 32.4 months, Cox B2 was 40.4 ± 29.9 months, Cox B3 51 ± 41 months, Cox B4 was 34.3 ± 22.2 months, and Cox B5 was 60.7 ± 79.8 months. Apparently, the children with Cox A6 infection in the present study were younger than those with other coxsackievirus infections.

Compared with those infected with Cox A16 or enterovirus 71,[11,12] which mostly presented as HFMD, three-fourths of the children with Cox A6 infection in the present study presented as herpangina, and only 12.8% as HFMD. However, Cox A6 was identified as a primary pathogen associated with HFMD during a nationwide outbreak in Finland in 2008.[11] Whether Cox A6 is among the major causes of epidemic HFMD needs further observation.

In the current study, though only four children had clinical/documented bacterial co-infections, nearly half of the hospitalization children had leukocytosis >15,000/mm^3 and elevated serum CRP level more than 40 mg/L, a picture which was seldom seen for infections due to other serotypes of enteroviruses and even other viruses. Most children with virus infection had a serum CRP level no more than 20 mg/L, such as respiratory syncytial virus, 17 mg/L; parainfluenza virus, 10 mg/L; influenza virus, 23 mg/L; coxsackievirus A, 20 mg/L; coxsackievirus B, 14 mg/L, except for adenovirus (49 mg/L).[13,14] In addition, none of the patients in the present study had severe enterovirus infection, reflecting that severity of Cox A6 infection can't be predicted from laboratory data despite of leukocytosis and elevated serum CRP level.

As we know, enteroviruses are the major causes of aseptic meningitis,[15,16] particularly echoviruses and enterovirus 71. In contrast, only three (2.4%) of the 141 inpatients with Cox A6 infection in the current study had probable CNS involvement and all of the inpatients recovered uneventfully. Since the case number was relatively small, the issue whether Cox A6 infection in children is relatively mild needs further observation.

There are several limitations in this study, since it is a retrospective study in nature. First, virus isolation and identification were not performed in every patient with possible EV infection, who visited our hospital during the study period. Second, not every patient with Cox A6 infection was hospitalized and subsequently not included in this study. Therefore, the clinical features shown here cannot represent the whole picture of children with Cox A6 infections but those hospitalized.

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