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Original article

A prospective study of enterovirus infection in Thai infants presenting as sepsis

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Objective: To study prospectively the prevalence, clinical presentations and laboratory findings of enterovirus (EV) infection in infants under 3 months of age who present as a sepsis-like syndrome.
Method: All infants less than 3 month of age admitted as a sepsis-like syndrome to King Chulalongkorn Memorial Hospital between April 2003 and February 2004 were included. Patients who were immunocompromised or who had been admitted for longer than 14 days before developing symptoms were excluded. A detailed history, physical and laboratory findings were recorded and analyzed. Specimens of blood and cerebrospinal fluid were tested for enteroviruses using Nucleic Acid Sequence-Based Amplification (NASBA). Patients were followed to determine the clinical outcome and duration of hospitalization.
Results: Of 56 infants, thirty-six were admitted to the pediatric wards and 20 had been hospitalized since birth in the neonatal intensive care unit (NICU) or nursery wards. Enterovirus infection was diagnosed in 13 (36.1 %) of the patients admitted to the pediatric wards and none in the group of NICU/nursery patients. The most common clinical presentations were high grade fever (92 %), rashes (77 %) and lethargy (54 %) as compared to fever (78.3 %), poor feeding (60.9 %) and lethargy (56.5 %) in the EV negative group. Ten (76.9 %) of the enterovirus positive infants had evidence of central nervous system (CNS) involvement as evidenced by the presence of EV RNA in cerebrospinal fluid (CSF) or CSF pleocytosis plus EV RNA in blood and/or CSF. Nevertheless, CSF pleocytosis was found in only 7 infants (53.8 %). Average duration of illness was 3.2 days as compared to 3.5 days in the nonenteroviral group with similar clinical features. All enterovirus positive patients had an uncomplicated recovery. Ten (76.9 %) received parenteral antibiotics for a mean of 5 days (versus 4.8 days in enterovirus negative group). The average length of stay was 8.1 days as compared to 15 days in enterovirus negative group.
Conclusion: Enterovirus infections are important causes of a sepsis-like syndromes in infants under 3 months of age. Most enterovirus infected patients presented with fever without localizing signs and rashes. Detection of enterovirus RNA by NASBA in serum and/or CSF represents a rapid method for the diagnosis of enterovirus infection in infants presenting with a sepsis-like syndrome.

Keywords: Enterovirus, infant, NASBA, sepsis.

Acute severe illness in infants under 3 months of age is usually considered a bacterial sepsis. The infant is hospitalized and treated with parenterally administered antibiotics pending the results of blood cultures [1]. However, a study showed that 90 % of such cases in this age group are the result of viral infections [2]. Review of the literature showed that enteroviruses are the most common etiology (25-45 %) [3-6]. The most common syndromes associated with enteroviral infection are meningitis and encephalitis (62-75 %). Enteroviruses are usually transmitted horizontally through fecal-oral and possibly oral-oral (respiratory) routes but perinatal infection may also occur. Clinical presentations can vary from barely symptomatic to severe disease affecting many organ systems, such as meningitis/meningoencephalitis, severe hepatitis and myocarditis [7]. Neonatal enteroviral hepatitis and coagulopathy can be life

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threatening. They can become apparent within the first 7 days of life [8] and the case fatality rate may be as high as 25-40 % [9]. Presenting signs and symptoms may be indistinguishable from those seen in bacterial sepsis.

Cell cultured-based tests for enteroviruses are of limited use because of slow turn around time (2-3 weeks) and limited sensitivity [10]. Molecular techniques such as polymerase chain reaction (PCR) or nucleic acid sequence-based amplification (NASBA) are more sensitive and rapid [10-14] in detecting enterovirus ribonucleic acid (RNA). We studied the potential of this diagnostic method in an effort to improve our understanding of the epidemiology and clinical spectrum of enterovirus infection in infants under 3 months of age who presented with a sepsis-like syndrome.

Patients and methods

Patients

Between April 2003 and February 2004, 56 infants were admitted with a diagnosis of sepsis at King Chulalongkorn Memorial Hospital. They were included in this study. Twenty infants had been hospitalized since birth in the neonatal intensive care unit (NICU). Inclusion criteria were individuals with age of less than 90 days and those who had more than 2 of the following symptoms: temperature instability, abnormal behavior (lethargy or irritability), respiratory symptoms (apnea or tachypnea), gastrointestinal symptoms (feeding intolerance, vomiting, diarrhea, abdominal distension), neurological symptoms (seizure, signs of menigeal irritation), rash, hypotension, metabolic dearangement, abnormal complete blood count (CBC) (leukocytosis or leukopenia and/or thrombocytopenia). Exclusion criteria were patients in an immunocompromised state and those who had been admitted longer than 14 days before developing such symptoms. Demographic and clinical information was recorded on a prepared data collection form including date of birth; gestational age; gender; birth weight; date of discharge; maternal history; immunization history; symptoms and timing of onset of the current illness; temperature; results of complete physical examination; ill contacts; duration of illness; cerebrospinal fluid (CSF) profile; CBC and other laboratory tests; results of bacterial cultures; results of viral diagnostic testing; discharge diagnosis and clinical outcome. Daily follow ups were conducted by the attending staff until the patient was discharged from the hospital. CSF pleocytosis was defined as greater than 22 white blood cell (WBC) in infants less than 4 weeks, greater than 15 WBC in infants 4 to 8 weeks, and greater than 7 WBC in infants older than 8 weeks [15]. Informed consent was obtained from the parents of the patients entering the study which was approved by the ethics committee of King Chulalongkorn Memorial Hospital.

Specimen Collection

We used specimens of blood and CSF that remained after completion of routine tests for the enterovirus NASBA assays This avoided additional sample collections. If there was not a sufficient volume of blood for both bacterial cultures and NASBA assays, only the former was performed. The specimens consisted of whole blood in edetic acid- and CSF in polypropylene-collection tubes. All specimens were refrigerated at 4 °C. Specimens were sent to the laboratory within 48 hours and frozen at -70 °C until the NASBA assay was performed. It was performed on all specimens within 30 days of collection. Viral cultures were not performed routinely.

NASBA assay

Enterovirus was detected using a formatted NASBA assay (NucliSens Basic Kit). The primers permit genetic detection of probably all human pathogenic members of the genus enterovirus by amplification of a segment of the highly conserved 5' non-coding region. The assay is able to detect d"10 enterovirus RNA copies and d"1.5 $TCID_{50}$ per amplification reaction. Enterovirus NASBA achieves a similar sensitivity as RT-PCR [16, 17].

Statistical analysis

The differences between clinical and laboratory data for infants with and without enterovirus infection were analyzed by Student's *t* test for continuous variables and chi-square test or Fisher's exact test for dichotomous variables. A P-value <05 was set as the level of significance.

Results

A total of 56 infants were enrolled and divided into 2 groups. Group 1 included patients admitted to the pediatric wards. Group 2 included NICU patients. The main difference between the two groups was that all infants from the NICU had been hospitalized

since birth, whereas all infants from the pediatric wards had been at home prior to admission. Their characteristics are shown in **Table 1**.

Group 1

Thirty six patients were included in group 1. Their demographic and clinical characteristics are presented in **Table 2**. An enterovirus infection was detected in 13 patients (36.1 %). Males predominated (69 %) and ranged in age from 14 to 80 days. Our patients were previously healthy term infants with high Apgar scores. Thirteen were infected with enterovirus, Ten (76.9 %) presented with fever without localizing signs. Only 3 (23.1 %) patients had signs of CNS infection (seizure, meningeal irritation). The most common clinical presentations were high grade fever (92 %), rashes (77 %) and lethargy (54 %). This was compared to fever (78.3 %), poor feeding (60.9 %) and lethargy (56.5 %) in the EV negative group. (**Table 3**) Fever persisted for an average of 3 days. The longest duration of fever was 7 days. Hypothermia was not noted. Rashes tended to appear between the second and third day of symptoms. 10 of 13 (76.9 %) enterovirus positive infants had a wide variety of rashes as compared to only 3 of 23 (13 %) in the enterovirus negative group. The most common form was a maculopapular rash. It was found in 5 of 13 enterovirus positive infants. Three of them had petichial rashes.

Mottling and pustulo-vesicular rashes were found in one patient. None of the 13 enterovirus-infected infants died. The average duration of acute illness was 3.2 days as compared to 3.5 days in nonenteroviral group with similar clinical features. All enterovirus-infected infants had an uncomplicated recovery. 10 of 13 (76.9 %) enterovirus-infected infants received parenteral antibiotics for a mean of 5 days. (versus 4.8 days in the enterovirus negative group).The average length of stay was 8.1 days as compared to 15 days in the enterovirus negative group.

Laboratory data

High white blood cell counts were common in enterovirus infection. They ranged from 7,600 to 15,900 cells/mm^3 (mean =10,433 cells/mm^3). The absolute neutrophil counts were usually within the normal range. None of the children had leukopenia or thrombocytopenia. Five enterovirus negative infants had abnormal complete blood counts. Two of 23 enterovirus negative infants had leucopenia and 2 had leukocytosis. Only one patient had thrombocytopenia. Each infant had a lumbar puncture performed. In two infants, CSF specimens were not available for NASBA test. The CSF findings of the enterovirus-infected infants are shown in **Table 4**. Seven infants had a CSF pleocytosis (53.8 %). Enterovirus RNA was recovered from 9 of 11 CSF specimens that were tested by NASBA. Three infants that were CSF enterovirus positive had no CSF pleocytosis. Of the 13 enterovirus-positive infants, eleven blood specimens were available for NASBA tests. Ten of 11 blood specimens were positive for enterovirus. All blood enterovirus positive infants were febrile. Only one patient (Number 7 in **Table 4**) who had a negative enterovirus blood test had no fever when admitted to the hospital. This patient presented with clinical encephalitis and was CSF enterovirus positive. 76.9 % of enterovirus infected infants had evidence of CNS infection (EV positive in CSF or CSF pleocytosis and EV positive in either blood or CSF). However, only 53.8 % had CSF pleocytosis. The final diagnosis for all infants in group1 is shown in **Table 5.**

Table 1. Characteristics of 56 infants under 3 months of age with clinical sepsis.

Characteristics	Group 1 (N=36)	Group 2 (N=20)	P- value
Birth weight, mean gram (range)	2744 (1350-3660)	2604 (1290-4020)	0.445[a]
Gestational age, mean weeks(range)	36.7 (30-40)	36.5 (30-40)	0.766[a]
Age at onset, mean days (range)	38 (7-87)	2.8 (1-11)	<0.001[a]
Sex, male/female (ratio)	20/16 (1.25)	12/8 (1.5)	0.968[b]
Positive results of NASBA for EV	13 (36.1 %)	0	0.002[c]
Positive results of bacterial culture	7 (19.4 %)	5 (25 %)	0.737[c]

[a]Two tailed *t*-test; [b]Two sided Chi-square; [c]Two sided Fisher's exact; Group 1: admission from community; Group 2: newborn ICU patients.

Table 2. Characteristics of 36 infants who were admitted from the community to the pediatric wards with a clinical diagnosis.

Characteristic	Enterovirus positive n=13	Enterovirus negative n=23	P- value
Birth weight, mean gram (range)	2983 (2500-3500)	2619 (1350-3660)	0.035[a]
Age at onset, mean days (range)	35.7 (14-80)	39.3 (7-87)	0.672[a]
Sex, male/female (ratio)	9/4(2.3)	11/12 (0.9)	0.372[b]
Syndrome classification			
- Clinical sepsis, without localizing signs	10 (76.9 %)	10 (43.5 %)	
- meningitis/meningoencephalitis	3 (23.1 %)	4 (17.4 %)	
- clinical sepsis with focal signs/symptoms	0	9 (39.1 %)	
Peak temperature, mean celcius (range)	38.9 (37.5-40)	38.4 (36.8-40.5)	0.064 [a]
Antibiotics, no. of patients	10 (76.9 %)	16 (69.6 %)	1.000[c]
Acyclovir, no. of patients	2 (15.4 %)	2 (8.7 %)	0.609[c]
Duration of antibiotic therapy, mean days (range)	5 (0-13)	4.8 (0-14)	0.858[a]
Duration of acute illness, mean days (range)	3.2 (2-7)	3.5 (1-14)	0.787[a]
Length of stay, mean days (range)	8.1 (3-17)	15 (2-84)	0.143[a]
ill contacts, no. of patients	9 (69.2 %)	4 (17.4 %)	0.003[c]
Bacterial culture positive, no. of patients	0	7 (30.4 %)	0.034[c]

[a]Two-tailed *t* test; [b]Two-sided Chi-square (continuity correction); [c]Two-sided Fisher's exact.

Table 3. Signs and symptoms for 36 infants with clinical sepsis in group 1.

Signs and symptoms	Enterovirus positive n=13	Enterovirus negative n=23	P-value
Fever	12 (92.3 %)	18 (78.3 %)	0.385
Rash	10 (76.9 %)	3 (13.0 %)	<0.001
Lethargy	7(53.8 %)	13 (56.5 %)	1.000
Poor feeding	5 (38.5 %)	14 (60.9 %)	0.344
Hypereflexia	5 (38.5 %)	4 (17.4 %)	0.235
Irritable	3 (23.1 %)	4 (17.4 %)	0.686
Meningeal irritation	2 (15.4 %)	0	0.124
URI	1 (7.7 %)	5 (21.7 %)	0.385
Vomiting	2 (15.4 %)	7 (30.4 %)	0.438
Diarrhea	1 (7.7 %)	5 (21.7 %)	0.385
Seizure	1 (7.7 %)	4 (17.4 %)	0.634
Hypertonia	1 (7.7 %)	2 (8.7 %)	1.000
Apnea	0	4 (17.4 %)	0.274
Respiratory distress	0	3 (13 %)	0.288
Poor tissue perfusion	0	2 (8.7 %)	0.525

Table 4. The CSF findings of the enterovirus-infected infants.

Patient	CSF WBC ($1/mm^3$)	CSF EV	Serum EV	CSF ANC ($1/mm^3$)	CSF Glucose (mg/dL)	CSF Glucose/ BS ratio	CSF protein (mg/dL)
1	8	Neg.	Pos.	0	43	0.56	65
2	330	Pos.	Pos.	293	46	0.56	120
3	10	Neg.	Pos.	0	42	0.57	59
4	5	Pos.	ND	0	52	0.48	41
5	1	ND	Pos.	0	50	0.84	71
6	161	ND	Pos.	0	49	0.65	62
7	30	Pos.	Neg.	0	29	0.4	146
8	115	Pos.	Pos.	23	39	0.39	69
9	205	Pos.	Pos.	148	54	0.67	112
10	12	Pos.	Pos.	0	47	0.49	32
11	18	Pos.	ND	0	46	0.51	104
12	0	Pos.	Pos.	0	51	0.65	53
13	560	Pos.	Pos.	73	44	0.44	55

ND=not done; ANC=Absolute neutrophils count.

Table 5. Final diagnosis for infants in group 1.

Final diagnosis	Number
Enterovirus infection	13
Viral infection, unspecified	3
Dengue infection	1
RSV pneumonia	3
Rotavirus gastroenteritis	2
Other viral gastroenteritis	1
Herpes simplex encephalitis	2
Chlamydia pneumonia	1
Bacterial meningitis	1
Urinary tract infection	2
Bacterial skin infection (pustulosis)	1
Orbital cellulitis	1
Necrotizing enterocolitis	1
Sepsis, unspecified	1
Noninfectious diagnosis	3
Total	36

Group 2

The 20 patients (12 term and 8 preterm infants) in group 2 had been hospitalized since birth in the NICU (**Table 1**). Enterovirus was detected in none of these patients. Five patients (25 %) had positive bacterial blood cultures.

Discussion

Sepsis in neonates and infants is an important clinical entity that leads to hospitalization and empirical antibiotics treatment. Several previous studies showed that the majority of septic appearing infants under 3 months of age were infected with enteroviruses (25-45 %) [3-6]. During the summer and fall, the incidence was as high as 50 % [3, 18].

We use NASBA to identify enterovirus infection. Thirty six percent of the infants admitted to our pediatric wards from home with an admission diagnosis of sepsis were found to have an enterovirus infection. During the past, enterovirus infection was diagnosed by viral cultures or serologic studies. NASBA or PCR improved diagnostic capability significantly [10-14]. NASBA assay of blood and CSF is very sensitive for diagnosing enterovirus infection [10, 11]. In our study, NASBA detection for enterovirus was positive in 10 of 11 blood specimens. All blood NASBA enterovirus positive patients had fever. The blood negative ones had no fever at admission. The utility of whole blood in diagnosing enterovirus infection was encouraging especially when infants were febrile. NASBA results can become available within 6 hours and may avoid prolonged antibiotic therapy and will expand the current understanding of the epidemiology and consequences of enterovirus infection in this age group. Enterovirus infections were detected throughout the year and a male predominance was shown. Our patients were usually otherwise healthy, term infants without history of perinatal complications. A non-specific febrile illness was the most common presentation of enterovirus infection (76.9 %) and the high grade fever was striking. Fever was found in most of our patients (92 %). Different forms of rashes were

also observed. The most common types were maculopapular (38.5 %), and petichial (23.1 %). The presence of a rash, together with other clinical features suggesting sepsis, suggests enterovirus infection as one possible diagnosis.

Seventy-six percent of enterovirus positive infants had evidence of CNS involvement (EV positive in CSF or CSF pleocytosis plus EV positive in either blood or CSF). Most of them had no obvious signs of meningeal inflammation such as bulging anterior fontanelles or nuchal rigidity. Of the 10 patients with enterovirus CNS involvement, only 7 patients had CSF pleocytosis. The diagnosis of meningitis was established by the presence of CSF pleocytosis or positive CSF NASBA, or both. The CSF cellular reaction to infection is a dynamic process, and it is possible that in some of the infants who were diagnosed as having meningitis, pleocytosis developed later. Moreover, it is also possible that with single lumbar puncture, cases of meningitis in enterovirus infected infants might be missed. The use of CSF NASBA, may help diagnosing enterovirus CNS infection before the presence of CSF pleocytosis. This information is important since language delays have been demonstrated in infants who had enterovirus meningitis before 3 months of age [19, 20]. In another long term follow-up study of 19 children who had documented enterovirus infection, three children (16 %) had definite neurologic impairment, five (26 %) had possible impairment in later ages during follow up period of 2-5 years [21]. This underscores the importance of careful monitoring of children who have meningitis of any etiology during early infancy for language development and long term neurological impairments. An enterovirus infection was detected in none of the NICU patients who had a sepsis or a sepsis-like syndrome. All these infants had been hospitalized since birth. Although previous reports show that intrauterine infection by enteroviruses, can be another mode of transmission, this was not found in our study. Usually, intrauterine infection was more severe and almost always occurred within the first week of life [7-9]. It is likely that our infants were infected horizontally. Enterovirus detection by NASBA can be used as a diagnostic adjunct in infants who have a clinical sepsis-like syndrome. Rapid diagnosis of enterovirus infection is important to prevent unnecessary exposure to broad spectrum antibiotics. It may also decrease the length of hospitalization and reduce anxiety in parents about the cause of the disease and prognosis [22-26].

Conclusions

Enterovirus infections are an important cause of a sepsis-like presentation in infants under 3 months of age. Febrile infants admitted to the hospital from the community are likely to be infected with enteroviruses. Most enterovirus infected patients presented with fever without localizing signs and rashes. NASBA assay in blood and/or CSF was a rapid method for diagnosis of enterovirus infection in infants presenting with a sepsis-like syndrome and if carried out early, should prevent unwarranted antibiotic therapy.

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