Epidemic Trends of Enterovirus Infections From Pediatric Patients in Cheonan, Korea

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Background: This study was performed to investigate the epidemiology of enterovirus (EV) infections in children at a single hospital during recent 5 years. This study was performed from December 2008 to November 2013. **Methods:** We subjected 1,067 cerebrospinal fluid (CSF) specimens from 1,053 patients and 678 stool specimens from 665 patients using real-time reverse-transcription polymerase chain reaction tests that could detect EV. The data were analyzed according to seasonality and patient age and sex. **Results:** During this period, 176 of the total 1,053 patients (16.81%) were positive for EV in CSF. The highest rate

of EV-positive samples occurred in children less than 1 year of age (114/176, 64.77%), and the most prevalent month was June. The results for CSF and stool specimens were concordant in 545 (80.38%) of 678 cases. **Conclusion:** We described the epidemiological analytic data of the EV in the population of Cheonan, South Korea. The combination of the CSF and stool specimen enhances the diagnostic yield. There is a need for continuous study of EV infections and its clinical manifestations. *J. Clin. Lab. Anal.* 30:161–164, 2016. © 2014 Wiley Periodicals, Inc.

Key words: epidemiology; distribution; viral infection; CSF; stool; children

INTRODUCTION

Enterovirus (EV) infections have been reported worldwide, with infection rate varying greatly depending on season, geographic location, age, and socioeconomic status (1). EV infection is generally found in the intestines or cerebrospinal fluid (CSF), and it occurs in temperate climates from late summer to fall (2–8) and in tropical climates year-round (1).

There are many techniques to detect EV (9). Molecular methods developed during the last decade, such as polymerase chain reaction (PCR), offer more sensitive, highly specific, and rapid test results for EV diagnosis (10, 11). Recently developed real-time reverse-transcription (RT) PCR is a quantitative method with high speed and accuracy. The development of these methods enables quick analysis of specimens collected from patients for testing viral presence.

After an initiation of EV surveillance and identification of genetic epidemiological analysis in 2006 by the Korea Centers for Disease Control and Prevention, however, few reports on EV in the region of Cheonan have been carried

out. In order to contribute to the knowledge of EV infection trends and effective diagnosis, EV infection rates according to sex, age, and season were analyzed on the basis of real-time RT-PCR results.

MATERIALS AND METHODS

From December 2008 to November 2013, 1,067 CSF specimens were obtained from 1,053 pediatric patients with symptoms consistent with EV infection who visited Dankook University Hospital in Cheonan, Korea. An additional 678 stool specimens from 665 patients were also included in this study.

Grant sponsor: Dankook University.

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Received 26 February 2014; Accepted 29 October 2014

DOI 10.1002/jcla.21830

Published online in Wiley Online Library (wileyonlinelibrary.com).

Specimens collected for DNA extraction underwent extraction on the same day or were stored at 4°C until the next day. CSF nucleic acid extraction was performed using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol by using QIAcube, which is an automated equipment for nucleic acid extraction. For stool specimens, a QIAamp DNA Stool Mini Kit was used to extract nucleic acids according to the manufacturer's protocol by using QIAcube.

Real-time RT-PCR was performed using the AdvanSure EV real-time RT-PCR kit (LG Life Science, Seoul, Korea). For the reverse-transcription step, DNA and buffer mixture was incubated at 45°C for 30 min, followed by denaturation at 95°C for 10 min. The mixture then underwent 40 cycles of PCR (15 sec at 95°C, 30 sec at 55°C) to detect fluorescence signals. Results were expressed as average and range. A chi-square test was used to analyze categorical data, with a statistically significant *P*-value less than 0.05.

RESULTS

The CSF samples were collected from 1,053 pediatric patients (less than 18 years of age) with 646 male and 407 female patients, yielding a sex ratio of 1.59:1. There were 176 of the 1,053 (16.81%) patients found positive for EV in CSF, 115 male and 61 female resulting in a sex ratio of about 1.89:1. The average age of the children analyzed was 2.1 years (range, 0–17.6 years: 2.3 for male, 1.8 for female). The average age of positive patients was 2.5 years (2.5 male, 2.6 female). The highest rate of EV-positive samples was from patients less than 1 year of age (114 of 176 positive specimens); and 86.8% (99/114) of these children were less than 3 months of age.

The highest annual number of positive specimens from December 2008 to November 2013 was 57, 51, and 47 in 2010, 2011, and 2009, respectively; however the positivity rates were 15.3%, 24.2%, and 34.3% for the same respective years. Temporal distribution of EV epidemics in Cheonan in both 2009 and 2013 showed an obvious seasonal pattern during the short period from June to August. The average EV detection rate was 19.5%, 34.3%, and 20.3% in May, June, and July, respectively.

Analysis of monthly EV incidence revealed that the highest PCR-positive rate had been recorded in summer (47.7%, *n* = 84), autumn (21.0%, *n* = 37), spring (17.1%, *n* = 30), and lastly winter (14.2%, *n* = 25). Additionally, the distribution of the annual detection of EV was mainly clustered from late spring through early fall.

EV real-time RT-PCR was conducted on 678 stool specimens from 665 patients (403 male, 262 female, sex ratio of 1.54:1). The positivity rate was 31.6% (32.8% male, 29.8% female), giving a male-to-female sex ratio of positive EV patients as 1.71:1. The average age of patients was

TABLE 1. Distribution of patients to submitted and positive

	Submitted	Positive	Positive rate (%)
CSF specimens	1,067	177	(16.6)
Patients	1,053	176	(16.8)
Male	646	115	(17.8)
Female	407	61	(15.0)
Sex ratio	1.59:1	1.89:1	
Stool specimens	678	217	(32.0)
Patients	665	210	(31.6)
Male	403	132	(32.8)
Female	262	78	(29.8)
Sex ratio	1.54:1	1.71:1	
Age (months)			
0–1	216	34	(15.7)
1–2	239	39	(16.3)
2–3	230	26	(11.3)
3–4	47	11	(23.4)
4–5	4	1	(25.0)
5–6	8	1	(12.5)
6–7	6	1	(16.7)
7–8	3	0	(0.0)
8–9	6	1	(16.7)
9–10	1	0	(0.0)
10–11	4	0	(0.0)
11–12	3	0	(0.0)
Yearly			
2009	137	47	(34.31)
2010	373	57	(15.28)
2011	211	51	(24.17)
2012	146	6	(4.11)
2013	186	15	(8.06)
Monthly			
Jan.	49	7	(14.3)
Feb	68	9	(13.2)
Mar	76	6	(7.9)
Apr	76	7	(9.2)
May	87	17	(19.5)
Jun	143	49	(34.3)
Jul	138	28	(20.3)
Aug	109	7	(6.4)
Sep	106	18	(17.0)
Oct	58	6	(10.3)
Nov	76	13	(17.1)
Dec	67	9	(13.4)
CSF	Stool	No. of results	Ratio
Negative	Negative	441	(65.04)
Negative	Positive	20	(2.95)
Positive	Negative	113	(16.67)
Positive	Positive	104	(15.34)

1.7 years, while the average age of those testing positive was 2.2 years. Patients less than 1 year of age were the largest group of positive results (36; 77.3%).

The results of the specimens that had EV real-time RT-PCR testing of both stool and CSF specimens were concordant in 545 or 678 (80.4%) of cases, and results differed between stool and CSF in 133/678 (19.6%) of specimens.

DISCUSSION

A recent study of the diagnosis of EV found RT-PCR to be faster and more sensitive for virus detection (12). Sex ratios have varied, with reports from 1.2:1 up to 2.5:1 (7, 13); generally there is a predominance of males, with approximately twice as many as females (14). In our study, the positivity rate had a male-to-female ratio of 1.89:1, suggesting that males are more prone to EV infection. Green (15) explained that sex-based immunological differences lead to males having a tendency to be infected with EV. More research is needed to confirm sex-based differences in association with EV infection in the future.

EV infections tend to affect children (2–4, 14), and among infants less than 1 month of age, mortality has been reported to be as much as 50% (16). Thus, EV is known to be dangerous in young children, and 31% of cases occurring in patients less than 1 year of age in 2011, 31.7% in 2008, and 57.0% in 2009 (12). In our study, 73.3% patients were less than 1 year of age, and 66.3% in positive patients showed less than 1 year of age. Our study analyzed only children CSF specimens, while Baek et al. (12) considered all patients, which means the age of patients included in the study played an important role, and this requires further research.

According to data from the Korea Centers for Disease Control and National Institute of Health from 1993 to 1998, 17.4% (197/1,127) of specimens tested positive and 15 species of EV were isolated; while from 1999 to 2003, the prevalence rate was 20.5% (603/2,939) and 21 species were identified as EV (17, 18). Examination of EV prevalence by year finds prevalence rates of 41%, 31%, 43%, and 56% in 1999, 2003, 2005, and 2008, respectively; it has a prevalent trend at 3-year intervals in Korea (19). In this study, the positivity rate in 2009 was 34.3%, which is similar to the rate (31.4%) reported by the Centers for Disease Control in the same year; however, considerable differences were noted in 2010 (15.3% vs. 38.3%) and 2011 (24.2% vs. 32.6%). Although the results from our study and those reported by the Centers for Disease Control cannot be directly compared, the differences may be attributable to differences in geographic region, specimen, or patient selection criteria.

EV is seldom found in winter and is more prevalent in summer and fall (3–8). Baek et al. reported an EV detection rate of 16.7%, 31.9%, and 26.4% in June, July, and August, respectively (12). In our study, there were similar prevalence rates. But, period was earlier –19.5%, 34.3%, and 20.3% in May, June, and July, respectively. This difference may be due to differences in the period and province (12). EV prevalence trends according to seasons and months should be evaluated yearly. Therefore, systematic long-term epidemiological studies need to be carried out to prevent the spread of EV.

Riding (20) reported that positivity in 1996 was 38% of stool specimens, and 25% of CSF specimens by PCR. In this study, the prevalence rate was 16.6% in CSF and 32.0% in stool specimens, which is lower than previously reported positive rates in stool but higher than positive rates in CSF. This finding could suggest that stool specimens are better than CSF specimens to EV detection, but Chung (21) showed that in 2001 EV was detected for 4–8 weeks (up to 11 weeks) in the stool, including up to 2 weeks after infection. This means we cannot rule out past infection (13, 21).

We found both stool and CSF to be useful for the diagnosis of EV infection in children using EV real-time RT-PCR. Testing stool specimens may provide a useful alternative for physicians who, for a variety of reasons, may be unable to obtain CSF from a child. However, given the disagreement in results for stool and CSF in EV positive children, we recommend testing both specimens whenever possible. Testing both specimens could increase the diagnostic yield of EV infections. This study attempted to establish a diagnosis system for EV and will provide baseline data to establish preventive measures, including prevention of outbreaks and EV spread among infants.

ACKNOWLEDGMENT

The present research was funded by Dankook University in 2012.

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