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Analysis of enterovirus types in patients with symptoms of aseptic meningitis in 2014 in Shandong, China

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ABSTRACT

We reviewed the epidemiological and clinical characteristics of 927 aseptic meningitis patients in Shandong in 2014, and the phylogeny of predominant enterovirus (EV) types causing this disease was analyzed. A total of 209 patients that were positive for EV were identified by both cell culture and a reverse transcription-seminested PCR in cerebrospinal fluid samples. The positive patients were most likely to be children within 15 years of age, had symptoms such as fever, vomiting and nausea ($P < .05$). The 209 EV sequences belonged to 11 types, and coxsackievirus B5, echovirus types 6 and 30 were predominant types. VP1 analysis exhibited multiple lineages were co-circulating. The significance of the study could come from the fact that surveillance is important to monitor the prevalence of EV types in population, which shows enterovirus meningitis maintains an important public health problem in China.

1. Introduction

Enteroviruses (EVs) are small, non-enveloped RNA viruses in the *Picornaviridae* family. They are known to cause a spectrum of clinical manifestations in humans, from mild presentations including minor febrile illness, hand, foot, and mouth disease (HFMD), and herpangina to the more severe syndromes such as meningitis, encephalitis, myocarditis, acute flaccid paralysis, and sepsis (Pallansch et al., 2013). According to the latest data of EV taxonomy from Pirbright Institute available at http://www.picornaviridae.com, a total of 116 EV types belonging to four species known to infect humans (EV-A, B, C, and D) have been identified.

Aseptic meningitis, defined as an acute infectious disease with cerebrospinal fluid (CSF) negative for bacteria, is most frequently due to viral infection (Han et al., 2016). Specifically, aseptic meningitis is associated with various viruses, among which EVs are the most common etiology (Khetsuriani et al., 2006). Various studies over several decades have shown that most EV types are the causal agents for this disease, and a number of EVs associated meningitis outbreaks have been described (Oberste et al., 1999a; Wang et al., 2006). Shandong is a coastal province in Eastern China. Several outbreaks have been observed in that region in the years of 2003, 2008 and 2009. Echovirus (E) −30, coxsackievirus (CV) -B3, and CV-B5 have been the dominating EV types, respectively (Chen et al., 2013; Tao et al., 2012a, 2012b; Wang et al., 2006). However, since aseptic meningitis has not been classified as a notifiable disease in mainland China, the information on the EVs causing this disease in the population is limited.

In this study, a population-based EV surveillance was conducted in six sentinel hospitals from the cities of Jinan, Linyi, and Yantai of Shandong Province. The aim was to characterize the epidemiology, clinical symptoms and EV types of aseptic meningitis in our geographic region, and the molecular phylogenetic analysis of three predominant EV types was carried out.

2. Results

2.1. Demographic analysis

During the study period, a total of 927 patients confirmed by the clinical physicians were obtained from six sentinel hospitals. Fig. 1 showed the age distribution for all the cases. Overall, the age distribution ranged from 1 month to 79 years old (median age, 6 years) with a male-to-female ratio of 1.88:1. Of these, 442 patients (47.7%) were ≤ 5 years old and 711 (76.7%) were ≤ 15 years old. When this

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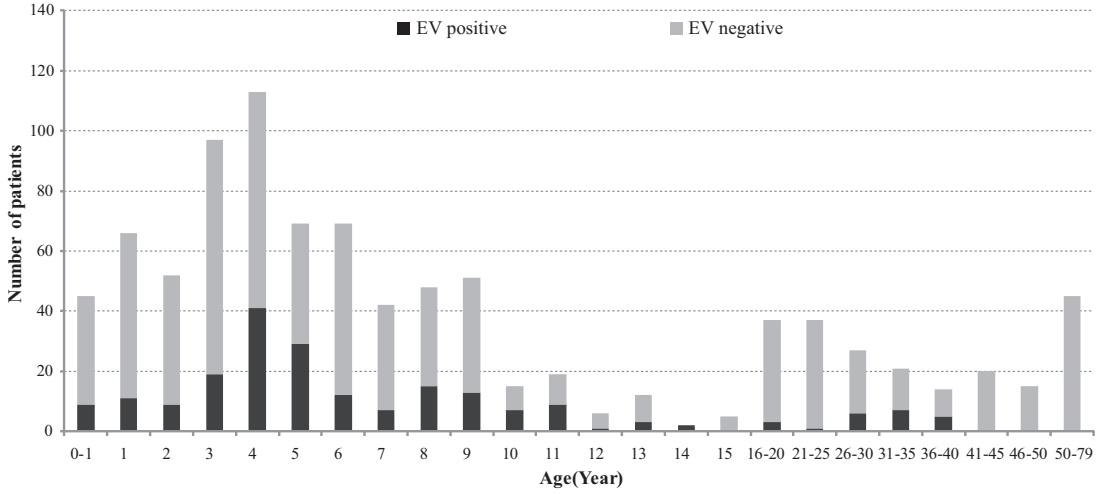

Fig. 1. Age distribution in years of 927 aseptic meningitis patients with positive and negative results of EV infection.

two population groups were analyzed individually, the median age was 3 years and 4 years, respectively, with a male-to-female ratio of 2.16:1 and 2.24:1, respectively. Out of the 927 patients in this study, only 209 (22.5%) were positive for EV on cell culture (157) and RT-snPCR (52), and a similar gender ratio of 2.21:1 (144/65) was detected. For EV-positive patients, the age ranged from 2 month to 37 years (median age, 5 years), of which 118 patients (56.5%) were ≤ 5 years old and 187 (89.5%) were ≤ 15 years old. However, the male-to-female ratio showed no significant difference between EV-positive patients and the negative patients ($P > .05$).

The distribution of aseptic meningitis patients by month was shown in Fig. 2. Although the patients were observed throughout the year, most patients (65.7%, 609/927) were more prevalent during the warm season (May-September). The peak calendar month was July with a total of 159 patients (17.2%). Similarly, most of the EVs (84.7%, 177/209) were detected between May and September (18 in May, 33 in June, 60 in July, 48 in August and 18 in September).

2.2. Clinical characteristics

The clinical characteristics were presented in Table 1. Fever, vomiting, and nausea were the most common clinical symptoms reported for aseptic meningitis patients and EV-positive patients (98.6%, 72.7% and 41.1%, respectively). There were several differences between EV-positive patients and the negative patients in clinical symptoms. The positive patients were more likely than the negative patients to suffer from fever, vomiting, nausea, headache, pharyngitis and have meningeal irritation sign on exam ($P < .05$). However, more frequencies were observed for the occurrences of convulsion (11.6%) and unconsciousness (11.4%) in EV-negative population ($P < .05$), while the relative ratios in EV-positive patients were only 3.3% and 1.4%. The fact that the clinical symptoms between EV-positive patients and EV-negative patients exhibited dramatic differences suggested enterovirus meningitis had special symptoms and might provide clues to the differentiation and diagnosis of enterovirus meningitis.

2.3. Enterovirus isolation and phylogenetic analysis

A total of 157 EV isolates were identified based on virus isolation, and another 52 EV sequences were determined by RT-snPCR analysis for the CSF samples that were negative for virus isolation. Molecular typing based on the VP1 region of these 209 EV sequences revealed 203 viruses belonged to EV-B (CV-B5 (n = 84), E-6 (n = 62), E-30 (n = 45), E-7 (n = 5), E-25 (n = 3), E-3 (n = 2), E-9 (n = 1) and E-14 (n = 1)), with only 6 belonging to EV-A (CV-A2 (n = 3), CV-A4 (n = 2) and CV-A10 (n = 1)). For the three predominant types of CV-B5, E-6, and E-30, the peaks of monthly isolation occurred in July (CV-B5), August (E-6), and July (E-30), respectively (Fig. 2). In addition, the clinical symptoms were not significantly associated with infections by CV-B5, E-6 or E-30 (Table 1). Furthermore, the whole VP1 coding sequences of CV-B5, E-6, and E-30 isolates in this study were phylogenetically compared with global reference sequences previously reported. Shandong CV-B5 isolates in 2014 segregated into a single cluster alongside with several former Chinese isolates and one foreign isolate from France

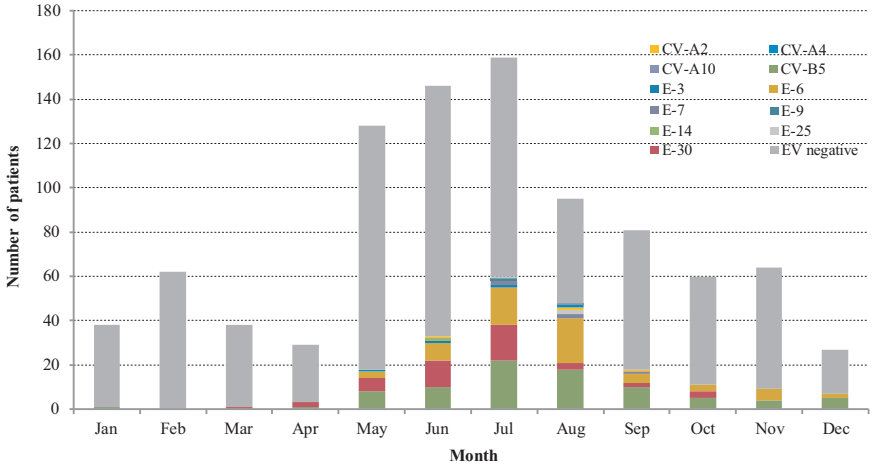

Fig. 2. Monthly distribution of the aseptic meningitis patients with different EV types and the negative results in CSF samples.