Journal of Clinical Virology 89 (2017) 22–29

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Comparative analysis of viral shedding in pediatric and adult subjects with central nervous system-associated enterovirus infections from 2013 to 2015 in Switzerland

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ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form 25 January 2017
Accepted 28 January 2017

Keywords:
Enterovirus
CNS infection
Viral shedding
Epidemiology

ABSTRACT

Background: Several enterovirus (EV) genotypes can result in aseptic meningitis, but their routes of access to the central nervous system remain to be elucidated and may differ between the pediatric and adult populations.
Objective: To assess the pattern of viral shedding in pediatric and adult subjects with acute EV meningitis and to generate EV surveillance data for Switzerland.
Study design: All pediatric and adult subjects admitted to the University Hospitals of Geneva with a diagnosis of EV meningitis between 2013 and 2015 were enrolled. A quantitative EV real-time reverse transcriptase (rRT)-PCR was performed on the cerebrospinal fluid (CSF), blood, stool, urine and respiratory specimens to assess viral shedding and provide a comparative analysis of pediatric and adult populations. EV genotyping was systematically performed.
Results: EV positivity rates differed significantly between pediatric and adult subjects; 62.5% of pediatric cases (no adult case) were EV-positive in stool and blood for subjects for whom these samples were all collected. Similarly, the EV viral load in blood was significantly higher in pediatric subjects. Blood C-reactive protein levels were lower and the number of leucocytes/mm^3 in the CSF were higher in non-viremic than in viremic pediatric subjects, respectively. A greater diversity of EV genotypes was observed in pediatric cases, with a predominance of echovirus 30 in children ≥3 years old and adults.
Conclusion: In contrast to adults, EV-disseminated infections are predominant in pediatric subjects and show different patterns of EV viral shedding. This observation may be useful for clinicians and contribute to modify current practices of patient care.

1. Background

Enterovirus (EV) is the major cause of aseptic meningitis [1,2] with an incidence peak documented during the warm season in the northern hemisphere [3–7]. In most cases, EV meningitis is self-limited with a low rate of complications or sequelae. Neonates represent the most susceptible population in terms of morbidity and mortality rates [2], and a rapid and accurate diagnosis is of the utmost importance in terms of clinical management to reduce the length of hospital stay, costs, and antibiotic use [8–11]. At present, EV meningitis is mainly diagnosed based on the analysis of cerebrospinal fluid (CSF) with EV-specific real-time reverse transcription (rRT)-PCR assays. The viral load observed in the CSF is relatively low and several studies have demonstrated that some cases of EV-associated meningitis could be missed if only CSF is

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http://dx.doi.org/10.1016/j.jcv.2017.01.008

investigated and they recommend to screen blood specimens in combination [12–14].

The central nervous system (CNS) invasion remains an unclear process. The two most likely possibilities are that it occurs either as a consequence of systemic infection by crossing the blood–brain barrier or via retrograde axonal transport [2]. The fecal–oral route is the most common mode of EV transmission and the primary sites of replication are the gastrointestinal or respiratory tracts. It can be postulated that a significant viral replication in the primary site can then be followed by viremia, thus leading potentially to CNS or other organ infections. This hypothesis is essentially based on animal studies [15–18], and it has not been investigated systematically for EV infection in humans.

Many EV genotypes circulate worldwide and are responsible for EV-associated meningitis, with a high prevalence of echovirus 30 (E30) identified in recent outbreaks observed in several European countries [5,19–23].

2. Objectives

To assess the pattern of viral shedding in the CSF, blood, stool, nasopharyngeal and urine specimens in a prospective manner in human cases. The respective quantitative viral load in the different putative sites of replication or viral shedding in pediatric and adult subjects with acute EV meningitis was investigated over a three-year period, enabling also to investigate potential intra-host EV variability. We provide also an evaluation of the etiology of EV meningitis in the western part of Switzerland during the same period.

3. Study design

3.1. Patients

The study was conducted from January 2013 through December 2015. All pediatric (<16 years old; $n = 37$) and adult (≥16 years-old; $n = 23$) patients admitted to the University Hospitals of Geneva (Geneva, Switzerland) with a diagnosis of EV meningitis were considered for study inclusion. Five additional pediatric patients from the University Hospital Center in Lausanne (Switzerland) were also enrolled in 2015. Only patients with a clinical diagnosis of EV-associated meningitis or meningo-encephalitis confirmed by a positive EV-specific rRT-PCR in CSF by the routine laboratory were included (negative direct CSF examination and/or culture, negative by r(RT)-PCR for any other virus requested by the physicians such as herpes simplex virus, varicella zoster virus and/or human parechovirus). After obtaining signed informed consent, the following specimens were collected specifically for the study, if not normally collected for clinical care: upper respiratory specimens (nasopharyngeal swabs or aspirate), stool or anal swab, and urine. Blood specimens were not specifically collected for the sole purpose of this study and only leftover plasma or serum specimens collected for medical reasons were used. Patients had the possibility to refuse the specific collection of respiratory, anal and/or urine specimens.

3.2. Quantitative rRT-PCR

For each collected specimen, the viral genome was extracted individually from 200 μL using the NucliSENS easyMAG (bioMérieux, Geneva, Switzerland) nucleic acid kit, with an elution volume of 25 μL. The quantitative EV-specific rRT-PCR (Entero/Ge/08) that detects all known EV genotypes was performed as previously described [24,25]. As an internal control, 10 μL of standardized Canine Distemper Virus was added to each sample before extraction as previously described [26].

3.3. EV typing

Extracted RNA from the CSF were reverse transcribed with random hexamers (Roche, Indianapolis, IN, USA) using the reverse transcriptase SuperScript II (Invitrogen, Carlsbad, CA, USA). The VP1 (primers AN88 and AN89 [27]) and VP4/VP2 (primers F848 and adapted R1126 [28]) viral capsid regions were then amplified by PCR and amplicons were purified with the MSB Spin PCRapace kit (Stratec, Birkenfeld, Germany) before sequencing on a ABI Prism 3500XL DNA Sequencer (Applied Biosystems, Rotkreuz, Switzerland). Sequences were analyzed with the Geneious Pro 6.1.8 analysis software (Biomatters Ltd, Auckland, New Zealand). EV typing analysis was performed from blood or stool specimens when the viral load in the CSF or CSF and blood were too low to obtain VP1 or VP4/VP2 amplicons.

3.4. Phylogenetic analysis

Sequence alignment was constructed using the MAFFT v7.017 multiple sequence alignment program [29]. Maximum-likelihood trees for the VP1 and VP4/VP2 capsid regions were built using the PhyML method [30] with one thousand bootstrap replicates.

3.5. Statistical analysis

Differences between groups were tested using the Mann–Whitney U tests for continuous variables and the χ^2 test or Fisher's exact test for categorical variables using GraphPad Prism version 6.00 (GraphPad Software, La Jolla, CA, USA). A two-sided p value of <0.05 was considered significant.

4. Results

4.1. Demographic analysis

During the three-year study period, a total of 60 EV-associated meningitis cases (37 pediatric and 23 adults) confirmed by rRT-PCR were detected at the University Hospitals of Geneva. Overall, the age distribution ranged from 5 days to 40 years old (median age, 4 months; Supplementary Fig. 1a) with a male-to-female ratio of 1.1:1. When the pediatric and adult populations were analyzed individually, the median age was 1 month and 29 years old, respectively, with a male-to-female ratio of 1.6:1 and 0.6:1, respectively. The monthly distribution showed that positive cases were more prevalent during the warm season (April–September) (Supplementary Fig. 1b).

4.2. Viral shedding in pediatric versus adult subjects

Of a total of 42 pediatric patients (37 from Geneva; 5 from Lausanne), stool, blood, upper respiratory and urine specimens were obtained for 32, 37, 25 and 24 cases, respectively (Table 1). Among the 23 adult patients (all from Geneva), stool, blood, upper respiratory, and urine specimens were obtained for 12, 21, 15 and 12 cases, respectively (Table 1).

Urine and respiratory specimens showed a weak positive rate that did not significantly differ between the pediatric and adult populations (Table 1). By contrast, the detection rate in stool and blood specimens was significantly different between the two populations. Positive rates in pediatric cases reached up to 91% and 68%, respectively, whereas they were only 25% and 29%, respectively, in adults. CSF, blood, and stool specimens were obtained for 32 pediatric cases and 12 adults. Out of 32 pediatric patients (62.5%), 20 were positive in stool and blood in addition to the CSF (Fig. 1), 9 were positive only in the CSF and stool samples, and