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A prospective, comparative study of severe neurological and uncomplicated hand, foot and mouth forms of paediatric enterovirus 71 infections

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ABSTRACT

Objectives: In this study, we document the clinical characteristics and investigated risk factors for uncomplicated and severe forms of EV-A71 disease in Cambodian children.
Methods: From March to July 2014 inclusive, all patients with suspicion of EV-A71 infection presenting to Kantha Bopha Hospitals in Phnom Penh and Siem Reap and confirmed by the Virology Unit at the Institut Pasteur du Cambodge were prospectively enrolled in this study. Throat swabs, rectal swabs and serum samples were collected from all consecutive patients with suspected EV-A71 infection. In addition, CSF was also collected from patients with suspected EV-A71 associated encephalitis. A total of 122 patients (29 with uncomplicated disease and 93 with severe disease) with confirmed EV-A71 infection with all available demographic and clinical data for clinical classification and further analysis were included in the study.
Results: In this prospective EV-A71 study in Cambodia, we confirmed the previously reported association of male gender and absence of mouth or skin lesions with severe disease. We also highlighted the strong association of neutrophils in blood, but also in CSF in patients with pulmonary oedema. More importantly, we identified new putative nutrition-related risk factors for severe disease.
Conclusions: EV-A71 is an important cause of encephalitis in the Asia-Pacific region. Further studies to determine the risk factors associated with severe EV-A71 disease are needed.

Introduction

Enterovirus 71 (EV-A71) is a genotype within the species Enterovirus A, genus Enterovirus, family picornaviridae. The virus is one of the most common aetiologies of hand, foot and mouth disease (HFMD) in children. EV-A71 associated disease is usually mild with children typically recovering within 4-6 days. However, the virus has been associated with fulminant disease during large outbreaks in many parts of the World, including Bulgaria (Shindarov et al., 1979), Hungary (Nagy et al., 1982), Malaysia (Chan et al., 2000), Taiwan (Huang et al., 1999), Singapore (Chong et al., 2003), and China (Li et al., 2012; Liu et al., 2014; Huang et al., 2014). A meta-analysis has estimated case-fatality rates for hospitalized cases of HFMD associated with EV-A71 at 1.7% (Zhao et al., 2015).

In 2012, an outbreak of EV-A71 in Cambodia associated with cardiovascular collapse and pulmonary oedema gained international attention following the deaths of nearly 100 young children

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over a short period (Duong et al., 2016). Although this was the first EV-A71 epidemic reported in Cambodia, retrospective seroepidemiological testing showed that there was widespread circulation of the virus for at least a decade prior to the outbreak (Horwood et al., 2016). The pathogenetic and epidemiological mechanisms of how this virus intermittently causes large deadly outbreaks remains unclear.

We report factors associated with clinical characteristics of 122 Cambodian pediatric patients during an EV-A71 outbreak in 2014. We focus on environmental, clinical and biological risk factors for severe neurological and/or pulmonary disease associated with confirmed EV-A71 infection.

Methods

Clinical methods

During an EV-A71 disease outbreak in Cambodia from March to July 2014 inclusive, all patients with confirmed EV-A71 infection presenting at either of two Kantha Bopha Hospitals (one in Cambodia's southeastern capital Phnom Penh and the other in Siem Reap in the north-west of the country) were enrolled in the study. The clinical definitions used throughout the study are listed in Table 1. Patients with uncomplicated EV-A71 HFMD were compared to patients with severe forms of infection, including isolated encephalitis without pulmonary oedema (ECP) and pulmonary oedema with or without neurological involvement (PO). Demographic and clinical data were recorded for further analysis on a case report form (CRF) that was specifically designed for the study. Clinical outcome was not available for the study and patients were not followed-up.

Virological methods

Throat swabs, rectal swabs and serum samples were collected from all patients presenting to Kantha Bopha Hospitals with suspected EV-A71 infection. In addition, cerebrospinal fluid (CSF) was also collected from patients with suspected EV-A71 associated encephalitis. Nucleic acids were extracted from all samples using the QIAamp Viral RNA Minikit (Qiagen, Hilden, Germany), as outlined in the manufacturer's instructions. EV-A71 infections were detected by testing all clinical samples using an EV-A71 specific qRT-PCR assay (Khanh et al., 2012) or culture isolation of EV-A71 from original clinical material in Vero E6 cells. EV-A71 infection was confirmed if PCR or culture was positive in any of the tested samples. Severe EV-A71 cases with neurological symptoms were confirmed by the detection of EV-A71 by qRT-PCR or culture from CSF or blood; and/or detection of EV-A71 in both throat and rectal swabs, as previously recommended (Jain et al., 2014). Laboratory test results were integrated in the light of clinical findings to provide diagnosis (Figure 1).

EV-A71 phylogenetic analysis

EV-A71 isolates were randomly selected from throughout the outbreak period for sequence analysis of the VP-1 region. Amplicons (~500 bp) of the VP-1 region were produced using RT-PCR (Nix et al., 2006) for EV-A71 isolates associated with uncomplicated HFMD (n = 6) and severe EV-A71 infection (n = 5). The amplicons were sequenced at a commercial facility (Macrogen, South Korea) by Sanger method. Contiguous sequences were assembled using CLC Workbench (CLC bio) and compared to representative EV-A71 sequences downloaded from the NCBI GenBank database. Neighbour-joining trees were constructed with MEGA5 (Tamura et al., 2011) and bootstrap values were calculated and expressed as a percentage from 1,000 replicates.

Data collection and analysis

Data was collected at the bedside using a specifically designed CRF that was focused on socio-demographic variables as well as general and neurological clinical findings. Demographic and clinical data were collected for age, sex, hospital of inclusion, province, fever, peak body temperature, and neurological and extra neurological symptoms and signs at presentation. While in hospital, bodyweight, heart rate, blood pressure, body temperature, mental status, general and neurological examination and treatment were assessed. Weight-for-age z scores were computed for all patients and compared with the World Health Organization child growth standards (www.who.int/childgrowth).

Laboratory data including blood-cell count and differentiated white-blood-cell count, haemoglobin, platelet count, creatinin, liver enzymes, and blood glucose were collected from all patients. Cell count, differentiated cell count, glucose, and protein were also collected from the cerebrospinal fluid of patients with neurological symptoms. Chest radiography, brain computed tomography, and brain magnetic resonance imaging scans were reviewed by radiologists and recorded. Data was entered using standardized paper forms and then exported into an Excel® spreadsheet.

Statistical analysis

Data was analysed with the SPSS statistical package (Version 22; IBM Corp. Armonk, NY: USA). The Student's t-test for

Table 1
Clinical definitions used in this study.

- Suspected EV-A71 infection was defined as an acute illness with either systemic (e.g., fever), respiratory, neurological or skin signs and symptoms suggestive of viral infection.
- Confirmed EV-A71 infection was defined as a suspected EV-A71 infection plus the isolation of EV-A71 virus or molecular detection of EV-A71 RNA in a rectal swab, throat swab, serum or cerebrospinal fluid sample.
- Uncomplicated Hand Foot Mouth Disease patients (HFMD) were defined as patients with a confirmed viral infection presenting with the classical signs and symptoms of HFMD and without neurological symptoms.
- Skin lesions were defined as vesiculo-papular or maculo-papular rash (mostly present over the hands, soles and/or buttocks).
- Mouth lesions were defined as oral ulceration usually observed on anterior tonsillar pillars, soft palate, buccal mucosa, or uvula.
- Neurological symptoms were defined as Glasgow Coma Scale (GCS) score (or modified GCS in less than 2 years old) less than 14 or clinical rhombencephalitis or limb weakness.
- Neurological involvement was defined as neurological symptoms and/or CSF white cell count >5/mm3 and/or abnormal brain imaging (computed tomography scan or magnetic resonance imaging) suggestive of encephalitis.
- Pulmonary oedema was defined as respiratory symptoms and bilateral alveolar congestion on chest radiography.
- Encephalitis patients (ECP) were defined as patients with neurological involvement without pulmonary oedema.
- Pulmonary oedema patients (PO) were patients with pulmonary oedema with or without neurological involvement.
- ECP and PO were termed severe patients.