First fatal case of CNS infection caused by *Enterovirus A* in Brazil

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Abstract

We describe what is to our knowledge the first fatal case of central nervous system *Enterovirus* infection in Brazil. Molecular and phylogenetic characterization revealed that *Enterovirus A* was the aetiologic agent of this case.

Keywords: CNS, *Enterovirus*, *Enterovirus A*, Picornavirus, Virus
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The genus *Enterovirus* (ENV) (*Picornaviridae* family) consists of 12 species, of which seven are common human pathogens with a worldwide distribution (*Enterovirus A, Enterovirus B, Enterovirus C, Enterovirus D, Rhinovirus A, Rhinovirus B and Rhinovirus C*) [1,2]. ENVs have been associated with many human diseases, including myocarditis, pericarditis, pancreatitis, chronic inflammatory myopathy, viral conjunctivitis and infections in central nervous system (CNS) [3,4]. Infections can lead to serious illness, particularly in infants and immunocompromised patients. Currently, the number of cases in which *Enterovirus A* (ENV-A) infection leads to severe disease is increasing, in particular ENV-71 [3,4]. Severe CNS infection is not the classic course of diseases related to ENV infection [1,2]. In this report, we describe a fatal case of ENV-A infection of the CNS.

The patient, a 28-year-old woman, sought care at a community health center in Belo Horizonte city, Brazil, and received a diagnosis of a suspected dengue virus infection. On day 1, the first symptoms were fever, headache, myalgia and clinical signs of meningitis. On day 3, the patient exhibited generalized seizures and was hospitalized. On the following day, the patient experienced sensory decrease (Glasgow Coma Scale = 7) and seizures. Biochemical data for cerebrospinal fluid were as follows: protein 48 mg/dL; glucose 89 mg/dL; cells 2/mm^3. On day 13, the patient experienced three separate events of respiratory arrest and died (Fig. 1A).

Our laboratory received samples of the patient's cerebrospinal fluid collected during the acute phase of the disease (7 days after onset of symptoms) with the aim of identifying the aetiologic agent. The study followed the rules of the ethics committee of Universidade Federal de Minas Gerais. DNA extraction was performed on the samples, followed by PCRs targeting DNA viruses. PCRs for human herpesvirus 1, 2 and 5 failed to identify the aetiologic agent. Attempts of virus isolation in Vero cells and tests for bacterial identification also failed. RNA was extracted from the sample (RNAQIAampExtraction, Qiagen, USA), followed by reverse transcription using random primers (MMLV, Promega, USA). The cDNA was used as the template in a PCR designed to amplify the 5′ untranslated region (UTR), a relatively conserved region in the genomes of ENVs [5]. ENV-specific amplification in the real-time PCR assay was observed in the sample tested (cycle threshold 28.7). The virus load of the sample was determined to be 100 PFU/mL (based on the positive control virus load). To identify and confirm the aetiologic agent responsible for this case, the amplicons were directly sequenced in both directions using a Mega-BACE sequencer (GE Healthcare, UK) [6]. The optimal alignment of the 5′ UTR using ClustalW (MEGA) showed high

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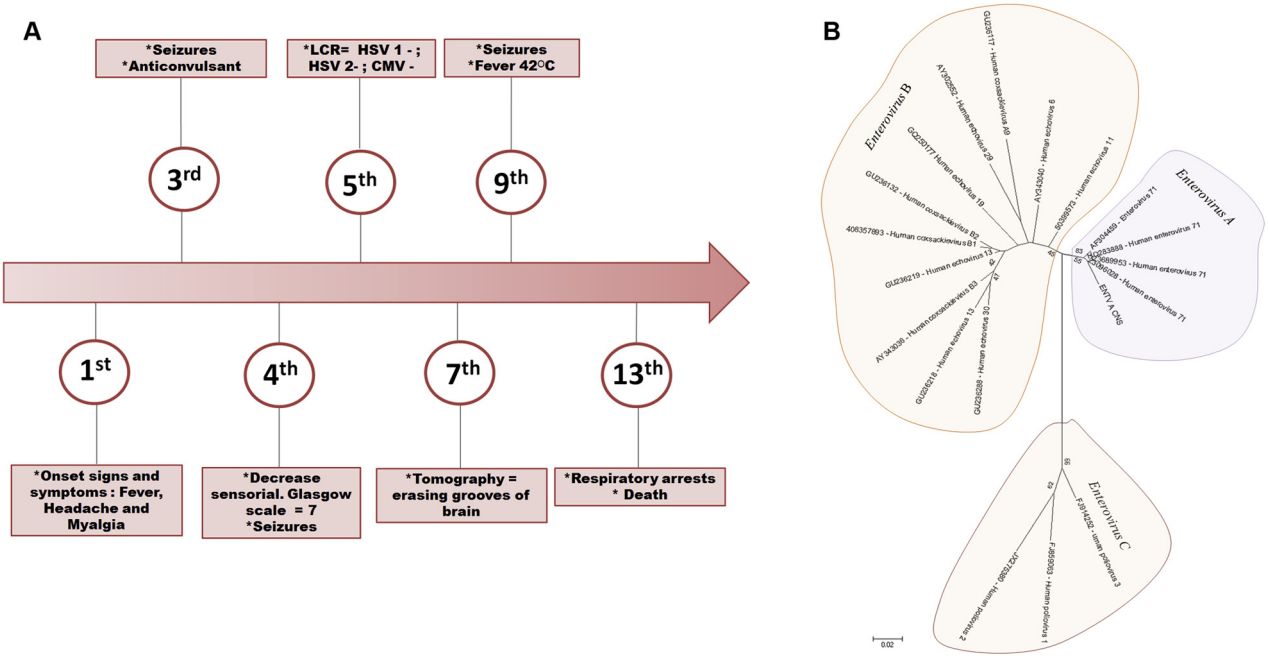

FIG. 1. (A) Timeline describing disease course. (B) Phylogenetic analysis of the 5′ untranslated region (UTR) of *Enterovirus A* (ENV-A) (110 bp). Other ENV sequences deposited in GenBank. Alignments were analyzed using the neighbour-joining statistical method with the Kimura two-parameter method in MEGA4 (http://www.megasoftware.net). Rate of variation among sites was modeled with gamma distribution (shape parameter = 1). Bootstrap confidence limits (from 1000 replicates) are indicated at each node.

identity among the nucleotide sequences of the case samples and ENV sequences deposited in the GenBank database [7–9]. The identity among the studied sample and available ENV-A isolates ranged from 98.3% to 98.7%. A phylogenetic tree of the 5′ UTR region (Fig. 1B) showed that the obtained sample cluster with ENV-A. The *Flavivirus* PCR also failed to amplify specific fragments.

Our report describes the first fatal case of CNS infection caused by ENV-A in Brazil. In most Brazilian states, the laboratory diagnosis of viral CNS infections is not performed. This case emphasizes the importance of the diagnosis of viral infection in the CNS.

Conflict of interest

None declared.

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