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

D. B. Oliveira¹, G. Machado¹, G. M. F. Almeida¹, P. C. P. Ferreira¹, C. A. Bonjardim¹, G. de Souza Trindade¹, J. S. Abrahão¹ and E. G. Kroon¹

¹) Laboratório de Vírus, Departamento de Microbiologia and 2) Hospital Risoleta Tolentino Neves, Universidade Federal de Minas Gerais, Minas Gerais, Brazil

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

Original Submission: 29 October 2014; Revised Submission: 16 June 2015; Accepted: 18 June 2015
Available online 4 July 2015

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/mm³. 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
The identity among the nucleotide sequences of the case samples and ENV sequences deposited in 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.

Acknowledgements

We thank J. Rodrigues dos Santos, G. Cirilo dos Santos and their colleagues from the Laboratório de Vírus, Universidade Federal de Minas Gerais, for their excellent technical support.

Financial support was provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG). DBO received fellowships from CNPq and GMFA from CAPES. EGK, CAB, GST and PCPF are researchers from CNPq.

References

[1] King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ. Virus taxonomy: classification and nomenclature of viruses: ninth report of the International Committee on Taxonomy of Viruses. San Diego, CA: Elsevier Academic Press; 2012.
[2] Pallansch M, Roos L. Enteroviruses: polioviruses, coxsackievirus, echoviruses and newer enteroviruses. In: Knipe DM, Howley PM, editors. Fields virology. Philadelphia, PA: Lippincott Williams & Wilkins; 2007. p. 840–93.
[3] Rhoades RE, Tabor-Godwin JM, Tsueng G, Feuer R. Enterovirus infections of the central nervous system. Virology 2011;411:288–305.
[4] Solomon T, Lewthwaite P, Perera D, Cardosa MJ, McElhinney P, Ooi MH. Virology, epidemiology, pathogenesis, and control of enterovirus 71. Lancet Infect Dis 2010;10:778–90.
[5] Dierssen U, Rehren F, Henke-Gendo C, Harste G, Heim A. Rapid routine detection of enterovirus RNA in cerebrospinal fluid by a one-step real-time RT-PCR assay. J Clin Virol 2008;42:58–64.
[6] Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 1977;74: 5463–7.

[7] Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 2007;24: 1596–9.

[8] Khetsuriani N, Lamont-Fowlkes A, Oberst S, Pallansch MA. Enterovirus surveillance—United States, 1970–2005. MMWR Surveill Summ 2006;55:1–20.

[9] Oliveira DB, Campos RK, Soares MS, et al. Outbreak of herpangina in the Brazilian Amazon in 2009 caused by Enterovirus B. Arch Virol 2013;159:1155–7.