Infection of the central nervous system with dengue virus 3 genotype I causing neurological manifestations in Brazil

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ABSTRACT
A case of dengue virus 3 (DENV-3) genotype I infection with neurological manifestations occurred in Belo Horizonte, Minas Gerais in October 2012. The serotype was detected by PCR, and the genotype was assessed by sequencing and phylogenetic analysis of the C-prM region. The virus causing neurological manifestations clustered with other sequences of DENV-3 genotype I. Because neurological manifestations of DENV are possibly misdiagnosed in Brazil, this study serves as an alert of the importance of DENV diagnoses in CNS infections.

Keywords: Central nervous system. Neurologic manifestations of dengue virus. Dengue virus 3 genotype I.
zoster virus) (VZV), Human herpesvirus 5 [cytomegalovirus (CMV)] and viruses of the genus Enterovirus was negative. Bacterial culture was also negative. For the next two weeks, the patient exhibited confusion and behavioral alterations. She left the hospital on October 27 (Figure 2).

Aiming to characterize the etiological agent, the patient’s CSF was sent to our laboratory. RNA was extracted (Viral RNA QIAamp Extraction Kit; Qiagen, Valencia, USA), followed by reverse transcription using random primers (MMLV; Promega, Madison, USA). The cDNA was used as the template in the quantitative PCR (qPCR) with primers designed to amplify the NS5 region of the viruses from the genus Flavivirus (11). qPCR was performed with a commercial mix (SYBR® Green PCR Master Mix, Life Technologies, Carlsbad, USA), and specific amplification with a melting temperature of 79.5°C was observed. The primers used for amplification were forward, 5′-GTGTCCAGCCGCGGTCATCACG-3′, and reverse, 5′-TACAACATGATGGGAAAGCGAGAAAA-3′ (11). PCR failed to detect HSV-1, HSV-2, VZV, CMV, and viruses of the genus Enterovirus.

To identify the Flavivirus that was the etiological agent of this infection, a specific PCR for DENV was performed, targeting the C-prM region (forward, 5′-TCAATATGCTGAAACGCGCGAGAAACCG-3′, and reverse, 5′-TGCAACAAACGACATGGCTTCCAGG-3′) (12). Amplification of a 511-bpDNA fragment was observed, confirming
Onset signs and symptoms
dengue

Generalized seizures and
hospitalization

started anticonvulsant

Seizures
intensive care

HSV-1 and CMV = PCR negative

Seizures
Electroencephalogram
= irritation in frontal region

Withoout seizure
confusion and behavior
alterations
left Hospital

1’ Day
7’ Day
9’ Day
10’ Day
13’ Day
27’ Day

DISCUSSION

Although data concerning the role of viral agents in CNS infections have increased in recent years, DENV has not been included in diagnostic assays because it is not considered a classical neuropathogenic virus \(^{(2)}\) \(^{(10)}\) \(^{(15)}\). The most frequently described neurological symptom associated with dengue is mental confusion, which is often interpreted as a consequence of high fever. CNS DENV-3 infections have been described in humans and are also reproducible in animal models \(^{(9)}\) \(^{(15)}\).

Our report describes a DENV infection in the CNS caused by DENV-3 genotype I, which has also been detected in human cases and mosquitos \((Figure 1)\) \(^{(6)}\) \(^{(7)}\) \(^{(8)}\). As DENV-3 genotype I was detected in different years in the same city (Belo Horizonte: 2002, 2003, and 2004), this genotype has been circulating in this region since that time \(^{(9)}\). Our previous studies demonstrated that DENV-3 genotype I had neurovirulent potential in a murine model \(^{(9)}\). We have now confirmed the presence of DENV-3 genotype I in the CSF of a patient with generalized seizures and behavioral changes, emphasizing the importance of this genotype to public health in Minas Gerais \(^{(3)}\). Because DENV infections in the CNS and the associated neurological manifestations are possibly misdiagnosed in Brazil (e.g., meningoencephalitis and encephalitis), this report serves as an alert regarding the importance of DENV diagnosis in CNS infections. Furthermore, because this viral pathogen is not identified in most cases of CNS viral infection in Brazil, the number of cases and impact on public health might be more important than suspected, and the number of dengue infections in the CNS might be underestimated \(^{(2)}\) \(^{(10)}\). Finally, this study
provides new information regarding the symptoms and signs of dengue infection in the CNS with neurological manifestations and an update to the DENV-3 genotype I epidemiology in Brazil.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

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**REFERENCES**


