

Flaviviruses as agents of childhood central nervous system infections in Brazil

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Abstract

Flaviviruses are agents of a major emerging human public health issue and members of this genus have been associated with central nervous system (CNS) infections. In Brazil, a country endemic for some arboviruses, the most clinically relevant neurotropic flaviviruses include Dengue virus and Zika virus. Flaviviruses cause diseases ranging from mild or sub-clinical infections to severe cases as CNS infections. There is a lack of data about the incidence of flaviviruses in the CNS of children in Brazil. In this review, we provide a general overview of several flaviviruses that cause CNS infections in Brazilian children and explore the importance of epidemiological surveillance of CNS infections in cases of flavivirus infections.

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Introduction

Aseptic meningoencephalitis is an important cause of mortality and morbidity in children and the virus more frequently associated with central nervous system (CNS) infections may vary depending on the geographic region [1]. Enteroviruses and herpesviruses are the viruses most associated with infections in the CNS of children in the USA whereas in Asia the Japanese encephalitis virus is found most often [2,3].

Japanese encephalitis virus, and some representatives of the Flavivirus genus (family *Flaviviridae*) are transmitted by mosquitoes and classically classified as neurotropic viruses, including West Nile virus (WNV) and Saint Louis encephalitis virus (SLEV) [4]. However, in recent years, other members of

this genus, primarily Dengue virus (DENV) and Zika virus (ZIKV), have been associated with CNS infections [4–6].

In Brazil, flaviviruses considered neurotropic agents, such as as WNV and Rocio virus, have been described as circulating but without case reports in children [7,8]. Some other potential neurotropic flaviviruses, including DENV and ZIKV, circulate in epidemics; however, there are few reports of these viruses as aetiological agents of CNS infections in children. Therefore, the incidence of flaviviruses causing this type of infection in children is unknown. Our goal was to review information about childhood CNS infections caused by the main flaviviruses circulating in Brazil.

Transmission

Flaviviruses are transmitted to humans primarily through the bite of infected mosquitoes. Some flaviviruses, including ZIKV and DENV, have two distinct transmission cycles: an enzootic, sylvatic cycle, where the virus circulates in *Aedes* spp. mosquitoes and non-human primates; and a human or urban cycle, between humans and peridomestic/domestic *Aedes* spp. [9].

Both WNV and SLEV are transmitted to humans and other mammals by *Culex* spp. [10]. and humans are usually dead-end hosts, as they generally do not generate sufficient viraemia to infect mosquitoes. WNV can also be transmitted between humans via organ transplants, blood transfusions and infectious maternal milk [11].

Some findings have suggested that ZIKV can be transmitted from mother to fetus during pregnancy [12] and an ability to cross the placental barrier was also previously suggested for WNV [13]. However, the mechanism by which these viruses cross the placental barrier remains unclear.

Flaviviruses as aetiological agents of CNS infections

Dengue virus

Dengue virus causes high morbidity and mortality in children living in tropical and subtropical areas of the world [14]. This virus is endemic in Brazil and, up to October, 203 157 probable cases of DENV infection had been notified in 2018 [15]. The number of cases in children is unknown.

In 2017, the Brazilian Ministry of Health [16] reported 7237 notified cases of meningitis probably caused by viruses. Our group investigated 299 cases of children with suspected meningoencephalitis from 2014 to 2018 in Minas Gerais State, Brazil and analysed cerebrospinal fluid (CSF); DENV RNA was detected in approximately 6% (unpublished data). Viruses commonly detected in childhood CNS infections, such as enteroviruses and herpesviruses, were found at frequencies of 4% and 3%, respectively, similar to those of flaviviruses (unpublished data). Therefore, our data demonstrate the importance of these viruses as causes of childhood CNS infections in Brazil.

Few studies in Brazil have related DENV to CNS infections in children. Marinho *et al.* [17] (Table 1) reported seven cases where DENV RNA was found in CSF samples from children with meningoencephalitis tested during the 2014/2015 epidemic in Minas Gerais State, Brazil. A previous study (2010–2013) using samples from 70 children from the same

hospital detected DENV by PCR or CSF serology in 11.7% of cases [18]. A study of 209 suspected viral meningitis/meningoencephalitis patients in Ceará State from 2005–2008 found three children positive for DENV in CSF by serological tests [19]. Other studies in Rio de Janeiro [20] and Espírito Santo [21] analysed samples from individuals with suspected DENV infection. The study carried out in Rio de Janeiro involved 13 IgM-seropositive patients who exhibited neurological manifestations during the course of their infections. One of these patients was an 11-year-old girl with a diagnosis of encephalitis and positive serum IgM [20]. In Espírito Santo State, Domingues *et al.* described two children infected with DENV and with CNS involvement. In both cases, DENV-3 serotype was detected by PCR in the serum and CSF [21].

A case of post-infection acute disseminated encephalomyelitis was reported in a child by de Miranda de Sousa *et al.* in 2006 [22]. The patient was an 11-year old girl born in Porto Velho (Rondônia, Brazil), who presented with anti-dengue IgM antibodies in the serum and CSF, indicating that neuromyelitis optica may be caused by DENV.

Recently a study carried out in the Amazon region looking for DENV in CSF samples found five children positive for DENV by serology or PCR and diagnosed with meningitis/encephalitis. All children presented cytological CSF characteristic of viral meningoencephalitis [23].

Zika virus

The circulation of ZIKV has been described in Brazil since 2015 [24] and the Brazilian Ministry of Health notified 7071 cases in 2018 [15].

Infections of the CNS related to ZIKV have been described, not only in fetuses [4,6,25]. In Brazil, only a single case of encephalitis has been described in an 8-year-old child with ZIKV infection and reactivation of varicella zoster virus. All CSF parameters were normal and this sample was positive for ZIKV by PCR [26].

In our study with 299 CSF samples (unpublished data), 3% of CSF samples from children with suspected meningoencephalitis were ZIKV positive by PCR. Although studies have primarily focused on congenital infections caused by ZIKV, acquired childhood infections should also be carefully monitored, as our

TABLE 1. Main flavivirus associated with central nervous system infections in children in Brazil

Virus	Number of cases	Viral diagnosis	CNS manifestation	State	References
Dengue virus	1	serological	encephalitis	Rio de Janeiro	Soares <i>et al.</i> 2006 [20]
	1	serological	acute disseminated encephalomyelitis	Rondônia	Miranda de Sousa <i>et al.</i> 2006 [22]
	1	PCR	encephalitis	Espírito Santo	Domingues <i>et al.</i> 2007 [21]
	3	serological	meningitis	Ceará	Araujo <i>et al.</i> 2012 [19]
	7	PCR and serological	meningoencephalitis	Minas Gerais	Marinho <i>et al.</i> 2017 [17]
	5	PCR and serological	meningitis	Minas Gerais	De Oliveira <i>et al.</i> 2017 [18]
Saint Louis encephalitis virus	5	PCR and serological	Encephalitis and meningitis	Amazonas	Bastos <i>et al.</i> 2018 [23]
	3	PCR	meningoencephalitis	São Paulo	Mondini <i>et al.</i> 2007 [32]
Zika virus	1	PCR	encephalitis	—	Silva Vieira <i>et al.</i> 2018 [26]

data suggest that the virus is an important causative agent of meningoencephalitis in paediatric patients.

Saint Louis encephalitis virus

Saint Louis encephalitis virus was first isolated in 1933 during a major epidemic in St Louis, Missouri, USA. Currently, this virus is widespread in the Americas and has been detected from Canada to Argentina [27]. In Brazil, SLEV was first isolated in 1969 in the northern region from a pool of *Sabethes belisarioi* mosquitoes in Pará State [28] and was first isolated from a human sample in São Paulo in 2004 [27].

No outbreaks of SLEV have been described in Brazil; however, serological evidence has demonstrated its circulation in buffaloes in Pará State [29] and equines in Minas Gerais and São Paulo [30,31].

SLEV RNA has been detected in Brazil; during a large dengue outbreak in 2006, 54 serum samples that were negative for DENV and Yellow fever virus were analysed and SLEV was found in three samples from paediatric patients, and all had diagnoses of viral meningoencephalitis. In two of these children viral RNA was found in the CSF, but in one of them only in the patient's serum [32].

Neuropathogenesis

Studies using mouse and hamster models have helped to elucidate how flaviviruses that may enter into the CNS; however, the pathways used by these neurotropic flaviviruses remain to be elucidated. Petersen *et al.* [33] reviewed some of the potential pathways for WNV entry into the CNS, including direct infection of the vascular endothelium; viral passage through the vascular endothelium due to disruption of blood–brain barrier integrity by vasoactive cytokines; and a Trojan horse mechanism, whereby infected monocytes are trafficked into the CNS.

Neurons have been described as the main targets for flavivirus infection such as Japanese encephalitis virus in CNS in mice model and they described that activation of astrocytes and microglia may further contribute to neuronal damage [34]. Ramos *et al.* identified, in a human brain autopsy from a fatal case of dengue haemorrhagic fever, immunoreactivity in neurons, astrocytes, microglia and endothelial cells [35].

Studies suggest that the flavivirus neuropathogenesis is related to the apoptosis of infected neuronal cells and/or the immune response generated by the cells of the immune system, as microglia that can produce and release factors that may be toxic to neurons [34,36]. Souza *et al.* [37] studying DENV 3

neurovirulence in mice found that increased levels of nitric oxide synthase 2 could be the cause of the death as it correlates with increased NOS2 and cytokine expression and virus in brain. In NOS2^{-/-} mice no clinical signs of infection were observed and cytokines were expressed at low levels, with the exception of interferon- γ [37].

Clinical manifestations

Approximately 80% of individuals infected with flavivirus are asymptomatic. Most of the signs and symptoms of these viral infections, such as skin rash, fever, arthralgia, myalgia, headache, retro-orbital pain and conjunctivitis, are common to other arbovirus infections. Symptoms appear to be similar in both children and adults [11,23], with rash observed in approximately 50% of patients, particularly children [11].

Neurological manifestations associated with DENV and ZIKV were recently reviewed by Li *et al.* [14]. The neurological manifestations described in children were seizures or tonic-clonic seizures, convulsions [5,14,26]; meningitis signs with acute onset of fever, headache, vomiting, nuchal rigidity [19]; encephalitis signs of cerebral involvement with altered consciousness or cognition [14,23], increase of intracranial pressure [18,19], drowsiness, tremors [19].

Paediatric patients with CNS flavivirus infections often do not exhibit the classic disease presentation [6]. Hence, laboratory diagnosis is of paramount importance for identification of the aetiological agent.

Laboratory diagnosis

Diagnosis of CNS viral infection, even as flavivirus, is a combination of clinical findings, molecular and serological assays in CSF, neuroimaging and chemocytological analysis of CSF [1].

Diagnosis of neuroinvasive disease caused by flaviviruses can be made by detection of RNA in the CSF as described for WNV, for example; however, this technique can be of low sensitivity unless it is performed early in the course of infection. Serology for the detection of virus-specific IgM in CSF is used as an adjunct method [10]. Serology is commonly used to diagnose viruses in the CNS, including flaviviruses, because virus-specific IgM in CSF shows intrathecal synthesis and is considered to reflect viral target antigens within the CNS, as IgM does not cross the blood–brain barrier [4,33].

Chemocytological analysis of CSF can accurately distinguish between a wide range of CNS diseases that can otherwise be difficult to diagnose. In the case of CNS viral infections, characteristics such as pleocytosis with cell counts up to 200 cells/mm³,

predominance of lymphocytes and high level of protein could be detected [1,14]. These parameters will however not always be altered in cases of CNS infections by flavivirus. Soares *et al.* [20] had 57% of the cases of meningitis caused by DENV with normal chemocytological CSF. Other studies also found positivity for DENV and SLEV without changes in CSF [17,18,21,32], showing that the CSF chemocytological analysis is not enough to characterize CNS infection by flavivirus.

Samples such as urine and saliva have been used to detect flaviviruses, including ZIKV and WNV. This sample collection is non-invasive and may improve tests for routine surveillance and research involving infants and young children [38]. Further, ZIKV RNA has been detected in urine at higher levels and for an extended period time, up to 36 days after disease onset [39]. The choice of diagnostic test will depend on time of sample collection and the goal of the assay.

Although for some flaviviruses such as DENV, no specific changes in magnetic resonance imaging or tomography are seen, they are also important for a general analysis of the areas affected by CNS viral infection [40].

Conclusion

Flaviviruses have a large public health impact across the world, including in Brazil. Although congenital cases, primarily caused by ZIKV, have been the focus of much attention in recent years, cases of flaviviruses acquired in childhood should not be neglected, particularly during outbreaks in endemic areas. These viruses cause CNS infections and should be considered in differential diagnosis of children presenting with aseptic meningoencephalitis. Studies of the incidence of these viruses in the CNS of children are of paramount importance, to provide an accurate idea of their impact on public health in Brazil, as well as to facilitate monitoring of the emergence of other flaviviruses that are circulating in the country and could cause outbreaks. To ensure proper understanding of the contribution of flaviviruses to childhood CNS disease in Brazil, these viruses must be considered in all relevant clinical contexts.

Conflict of interest

There is no conflict of interest.

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