Advance Publication by J-STAGE

Japanese Journal of Infectious Diseases

Meningoencephalitis associated with Zika virus and Chikungunya virus infection

Gabriela de Almeida Oliveira Evangelista, Rejane Hughes Carvalho,
Gabriela Sant'Ana menezes, Yara Carvalho de Abreu, Silvia Ines Sardi, and
Gubio Soares Campos

Received: December 28, 2020. Accepted: April 5, 2021.

Published online: April 30, 2021.

DOI:10.7883/yoken.JJID.2020.1000

Advance Publication articles have been accepted by JJID but have not been copyedited
or formatted for publication.

Meningoencephalitis associated with Zika virus and Chikungunya virus infection

Gabriela de Almeida Oliveira Evangelista^{1,2}

Rejane Hughes Carvalho¹

Gabriela Sant'Ana menezes¹

Yara Carvalho de Abreu¹

Silvia Ines Sardi¹

Gubio Soares Campos¹

¹ Laboratory of Virology, Health Sciences Institute, Universidade Federal da Bahia. Av. Reitor Miguel Calmon, s/n - Canela, Salvador - BA, 40231-300, Brazil.

² Hospital Insituto Couto Maia. Rua Cel. Azevedo 2, Cajazeiras, Salvador - BA, 41332-010, Brazil.

Corresponding author: Rejane Hughes Carvalho

Email: hughescv@gmail.com

Phone: +55 71 3283-8899

Keywords: Meningoencephalitis, Zika virus, chikungunya virus, etiology

Running head: Zika and Chikungunya virus meningoencephalitis

Summary

Etiology of viral meningoencephalitis is frequently unidentified. Chikungunya virus (CHIKV) and Zika virus (ZIKV) have been known to affect the central nervous system, and should, therefore, be considered for diagnosis of meningoencephalitis since its outcome may be influenced by the etiologic agent, age and immunological condition of the patient. In this study we aimed to determine if CHIKV and ZIKV were the etiological agent of viral encephalitis in meningoencephalitis patients admitted to the main hospital of infectious disease in the city of Salvador, Brazil. From 1049 patients with neurological symptoms who were admitted to the hospital during the time of this study, 149 were enrolled and 20 (13.34%) tested positive for ZIKV (12%) or CHIKV (1.34%). No specific clinical manifestations were observed to be associated to the ZIKV or CHIKV infection. Determination of the etiology of meningitis' and encephalitis' causing agent is important for patient management and correct treatment.

Meningoencephalitis of viral etiology, while common, goes frequently without determination of its causative agent. Although its clinical presentation often evolves to a benign outcome, it may present severe symptoms whose gravity depends mainly on the etiologic agent, age and immunological condition of the patient and correct diagnosis. Most cases of viral meningoencephalitis are known to be caused by herpes simplex viruses, enteroviruses and varicella zoster virus (1). Viral encephalitis caused by arboviruses are being increasingly reported, with its etiology mostly being St. Louis encephalitis virus, West Nile Virus and Japanese encephalitis virus, from the flaviviridae family and the Equine Encephalitis Viruses from the togaviridae family (2).

Zika virus (ZIKV) and chikungunya virus (CHIKV) are respectively members of the flaviviridae and togaviridae family of viruses, and are among the most important arboviruses in Brazil. They are both transmitted by the same vectors *Aedes aegypti* and *Aedes albopictus*, and although the diseases caused by them have similar signs and symptoms, they also present specific clinical manifestations (3). ZIKV infection during pregnancy may lead to the development of microcephaly in the fetus (4), and some cases may evolve to more severe cases affecting the nervous system such as Guillain-Barré syndrome (GBS), and other neurological complications such as transversal myelitis and meningitis (5). On the other hand, besides chronic arthritis, complications of CHIKV infection may include flaccid paralysis and cranial nerve palsy (6).

ZIKV associated encephalitis has been more frequently reported than CHIKV. The first case of encephalitis caused by CHIKV in Brazil was reported in Salvador (7). Although it is less frequent than neuro-infections caused by other arboviruses, more severe cases of neuro-chikungunya have been arising, and it may be age related, being observed more frequently in neonates and elderly (6). Identification of the etiology in cases of viral encephalitis are scarce, with most cases remaining undiagnosed. A study

carried out by Soares in 2011 (8) reported that between 2006 and 2008, dengue virus was associated to almost 50% of the viral meningoencephalitis cases in Rio de Janeiro. As for Dengue virus, ZIKV and CHIKV are endemic to Salvador, but their diagnosis in cases of viral encephalitis is nonetheless still an unusual practice, except during outbreaks. This it is not only detrimental to the epidemiological surveillance of meningoencephalitis, but it may also be detrimental to patient management. This study aimed, therefore, to investigate whether ZIKV or CHIKV was the etiological agent of meningoencephalitis in patients with clinical signs of neurological disease admitted to the Couto Maia Hospital, the reference hospital for infectious disease in the state of Bahia. The study was approved by the Ethical Committee of the Couto Maia Hospital under the number CAAE 68180017.60000.0046. Informed consent was obtained from all participants in this study.

Samples of cerebrospinal fluid (CSF) were obtained from 1049 patients with neurological symptoms. All CSF presenting physicochemical properties indicative of infectious disease were tested. Samples that presented positive for bacterial or fungal infection were discarded from the study. A total of 149 samples that had suspected etiology of viral infection were included in the study. Physicochemical analysis was performed in all 149 samples by color and appearance by cell count using microscopy and via automated spectrophotometry to test biochemical properties. Presence of ZIKV and CHIKV in CSF samples were investigated by RT-qPCR. CSF samples were aliquoted into 200 μ l and stored at -70° C till tests were performed. Viral RNA extraction was carried out from 140 μ l of CSF samples using the Qiaamp Viral RNA Mini Kit (Qiagen®) following manufacturer's instruction. After extraction, RNA was eluted in 60 μ l of buffer AVE, aliquoted into 20 μ l and stored at -70° C. The RT-qPCR was carried out using primers and probes as previously described (9, 10): ZIKV 1086/1162c/1107-FAM and CHIKV 6856F/6981c/6919-FAM. Reaction were performed using GoTaq® Probe 1-Step

RT-qPCR system (Promega, Madison, USA) with 5 µl of RNA and 20 µl final volume. Samples were considered positive when cycle threshold were below 37.5 and 38.5 for CHIKV and ZIKV respectively.

From the 149 samples included in the study, 18 were positive for ZIKV (12%) and two for CHIKV (1.34%). All 18 CSF samples had clear and transparent appearance with low cell count (<500 cells/mm³) and predominantly mononuclear cells (Table 1). Glucose varied from normal to slightly altered (reference value (RV): 45 – 80 mg/dL), and the same was observed for protein levels (adult RV: < 40mg/dL; 0 – 180 days RV: 40- 120 mg/dL). Global cell count varied between 2 to 93 cells/mm³, with one sample presenting no cell. For both, ZIKV and CHIKV, clinical manifestations were similar. General manifestations were fever, headache, motor and sensory loss. All symptoms were consistent with the clinical manifestations of viral meningoencephalitis. Among the 18 positive patients, five presented previous diagnosis for HIV and two others had previously being diagnosed with neuro-syphilis. From those, six were co-infected with ZIKV (case Ids 3, 89, 92, 94, 98 and 100) and one with CHIKV (case ID 4). For case ID 89, no HIV viral load was detected though serology was positive. Clinical manifestations separated by age group are shown on Table 2.

The detection of ZIKV and CHIKV in CSF of patients that presented symptoms of encephalitis corroborates reports that those viruses infect central nervous system and may be the etiological agent causing meningoencephalitis. In addition, studies on viral meningoencephalitis report that most patients affected by this infection are under the pediatric and young adult age range (11). In our study we have found that 8 of the 18 cases were in the age groups of <1 to 15 years, confirming those findings.

In a study reported by Vieira *et al.* (12), more than half of the ZIKV infected patients presented, besides GBS, other neurological complication, such as those involving

meninges, cerebral parenchyma, spinal cord and peripheral nerves. They investigated the ZIKV negative patients and found the presence of CHIKV in a parcel of those patients, indicating that CHIKV is also a significant agent for manifestation of neurological diseases.

In this study, both CSF cytological and biochemical analysis showed compatibility with infection of viral etiology in all cases, except for case ID 5, which presented predominance of polymorphonuclear cells. This finding may be due to cellular deterioration or as a result of the small volume of the analyzed sample.

In the cases of ZIKV/HIV and CHIKV/HIV co-infection, the immunodeficient state of the patients may have contributed to the development of the viral encephalitis. There have been few studies regarding complications related to co-infection with those viruses and HIV (13), but HIV infection is a pathological condition that may interfere with the effect of the etiological agent of meningoencephalitis (14).

One of the patients enrolled in this study was being treated with antibiotics, regardless of the negative diagnostics for bacterial meningitis. After the diagnosis of ZIKV in his CSF, course of treatment was changed and short thereafter his condition improved enough for him to be discharged from the intensive care unit.

Diagnosis of viral meningoencephalitis plays crucial role in patient management, reducing use of antibiotics and time of patient care in hospital. In addition, it increases data for epidemiological surveillance on meningoencephalitis, improving public health policies.

Acknowledgment

We thank CAPES (Zika Fast Track – CAPES project n° 88887-116628/2016) and FAPESB (project n° APP0076/2016) for the financial support of this work.

Conflict of interest

None to declare.

Reference

1. Kennedy PG. Viral encephalitis: causes, differential diagnosis, and management. *J Neurol Neurosurg Psychiatry*. 2004. Mar;75(Suppl 1):i10-5. Review.
2. Salimi H, Cain MD, Klein RS. Encephalitic Arboviruses: Emergence, Clinical Presentation, and Neuropathogenesis. *Neurotherapeutics*. 2016 Jul;13(3):514-34
3. Zanutto PMA, Leite LCC. The Challenges Imposed by Dengue, Zika, and Chikungunya to Brazil. *Front Immunol*. 2018 Aug 28;9:1964
4. Nunes ML, Carlini CR, Marinowic D, et al. Microcephaly and Zika virus: a clinical and epidemiological analysis of the current outbreak in Brazil. *J Pediatr (Rio J)*. 2016 May-Jun;92(3):230-40
5. da Silva IRF, Frontera JA, Bispo de Filippis et al. Neurologic Complications Associated With the Zika Virus in Brazilian Adults. *JAMA Neurol*. 2017 Oct 1;74(10):1190-1198
6. M R, Gerardin P, de Brito CAA, Soares CN, Ferreira MLB, Solomon T. The neurological complications of chikungunya virus: A systematic review. *Rev Med Virol*. 2018 May; 28(3): e1978.
7. Bandeira AC, Campos GS, Sardi SI, et al. Neonatal encephalitis due to Chikungunya vertical transmission: First report in Brazil. *IDCases*. 2016 Jul 25;5:57-9
8. Soares CN, Cabral-Castro MJ, Peralta JM, et al. Review of the etiologies of viral meningitis and encephalitis in a dengue endemic region. *J Neurol Sci*. 2011 Apr 15;303(1-2):75-9

9. Lanciotti RS, Kosoy OL, Laven JJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis.* 2008 Aug;14(8):1232-9
10. Lanciotti RS, Kosoy OL, Laven JJ, et al. Chikungunya virus in US travelers returning from India, 2006. *Emerg Infect Dis.* 2007 May;13(5):764-7
11. Neshar L, Hadi CM, Salazar L, et al. Epidemiology of meningitis with a negative CSF Gram stain: under-utilization of available diagnostic tests. *Epidemiol Infect.* 2016 Jan;144(1):189-97
12. Vieira MADCES, Costa CHN, Linhares ADC, et al. Potential role of dengue virus, chikungunya virus and Zika virus in neurological diseases. *Mem Inst Oswaldo Cruz.* 2018 Oct 29;113(11):e170538
13. Rothan HA, Bidokhti MRM, Byrareddy SN. Current concerns and perspectives on Zika virus co-infection with arboviruses and HIV. *J Autoimmun.* 2018 May; 89:11-20
14. Rajasingham R, Rhein J, Klammer K, et al. Epidemiology of meningitis in an HIV-infected Ugandan cohort. *Am J Trop Med Hyg.* 2015 Feb;92 (2):274-9.

Table 1: Physicochemical properties of the ZIKV and CHIKV CSF positive samples

Case ID	Global Cell count (Cell/mm ³)	Predominant cell type	Glucose (mg/dl)	Protein (mg/dl)	ZIKV (Ct)	CHIKV
2	72	Mononuclear	49	20	+ (36,39)	-
3	5	Mononuclear	43	56	-	+ (35,84)
4	0	-	42	30	-	+ (35,29)
5	42	Polymorphonuclear	63	66	+ (34,93)	-
29	2	Mononuclear	47	19	+ (32,48)	-
71	93	Mononuclear	24	126	+ (36,57)	-
72	42	Mononuclear	32	66	+ (34,61)	-
73	25	Mononuclear	88	13	+ (35,62)	-
86	93	Mononuclear	59	35	+ (35,81)	-
89	49	Mononuclear	15	95	+ (33,55)	-
90	17	Mononuclear	82	37	+ (33,61)	-
92	93	Mononuclear	60	37	+ (34,65)	-
93	17	Mononuclear	58	31	+ (32,52)	-
94	49	Mononuclear	62	22	+ (33,96)	-
95	25	Mononuclear	75	20	+ (34,73)	-
96	2	Mononuclear	55	12	+ (36,21)	-
97	5	Mononuclear	42	220	+ (36,95)	-
98	2	Mononuclear	65	15	+ (35,32)	-
100	72	Mononuclear	85	34	+ (35,61)	-
101	2	Mononuclear	51	36	+ (36,43)	-

Table 2: Clinical manifestations

Age group (years old)	ZIKV or CHIKV positive	Clinical manifestations		
		Convulsion	Disorientation	Others (CVA, syphilis, HIV)
< 1 ano	4	1		
1 to 15	4			1
16 to 30	2			2
31 to 50	4	1	1	2
51 to 80	3		1	2
Unknown	1			

CVA= Cerebrovascular accident; HIV= human immunodeficiency virus