

Zika Virus Causing Encephalomyelitis Associated With Immunoactivation

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Brazil has experienced a Zika virus (ZIKV) outbreak with increased incidence of congenital malformations and neurological manifestations. We describe a case of a 26-year-old Brazilian Caucasian man infected with ZIKV and diagnosed with encephalomyelitis. Brain and spinal cord images showed hyperintense lesions on T2 and fluid-attenuated inversion recovery (FLAIR), and levels of proinflammatory cytokines in the cerebrospinal fluid showed a remarkable increase of interleukin (IL)-6 and IL-8. The observed pattern suggests immune activation during the acute phase, along with the neurological impairment, with normalization in the recovery phase. This is the first longitudinal report of ZIKV infection causing encephalomyelitis with documented immune activation.

Keywords. encephalomyelitis; neurological manifestations; proinflammatory cytokines; Zika virus.

In the last 11 months, Brazil has experienced a Zika virus (ZIKV) epidemic along with increased incidence of congenital malformations and neurological manifestations, such as acute myelitis and Guillain-Barré syndrome, supposedly associated with the infection. The presence of the virus in several fluids and tissues (blood, urine, cerebrospinal fluid [CSF]) has reinforced ZIKV infection causality [1, 2]. However, the spectrum, severity, and pathophysiological aspects of the nervous system involvement remain uncertain [2, 3].

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METHODS

A 26-year-old Brazilian Caucasian man was admitted to Hospital Federal dos Servidores do Estado, at Rio de Janeiro, Brazil, in January 2016, with fever, malaise, and hands paresthesias. The informed consent was obtained to use the patient's data in this Brief Report.

The patient was submitted to physical and neurological examinations regularly, including the following imaging exams: computed tomography [CT] and brain and spinal magnetic resonance imaging [MRI]. Brain MRI exams were performed using standard protocol, including axial, coronal, and sagittal FLAIR, T2-weighted (T2), diffusion, and volumetric T1-weighted (T1) sequences and pre- and postgadolinium injection. Spinal cord standard protocol also included sagittal and axial T2, short tau inversion recovery (STIR), T1 and fat-suppressed T1 sequences, and pre- and postgadolinium injection. Cerebrospinal fluid and urine were collected for further molecular and cellular biological analysis.

To evaluate the humoral immune response during ZIKV infection, we investigated the levels of proinflammatory cytokines and chemokines in the CSF samples collected during the acute phase with neurological symptoms (1 week after symptom onset) and in the recovery phase (5 to 6 weeks after symptom onset) associated with a better outcome of the patient. We investigated 27 cytokines, chemokines, adhesion molecules, and growth factors using the Bio-Plex Panel 27-Plex (Bio-Rad, Hercules, CA) (Table 1). Cerebrospinal fluid samples from adult subjects with no history of neurological disorders were used as controls.

RESULTS

The patient had initially noticed malaise and fever, and on the following day he developed paraparesis, lower limbs paresthesias, and urinary retention. On the third day he had tetraparesis, and due to respiratory failure, he required invasive mechanical ventilation. Brain CT was normal, and CSF analysis disclosed 100 white blood cells/mm³ (95% mononuclear), protein of 112 mg/dL, and glucose of 80 mg/dL. Acyclovir and ceftriaxone were started on day 4. The first neurological evaluation was on day 7. Blood pressure was 130 × 80 mmHg, heart rate was 82 beats per minute, oxygen saturation was 99%, and temperature was 39°C. General physical examination was unremarkable. Sedation had been interrupted 12 hours before. The patient had a reduced level of alertness, with eye opening to verbal stimuli; he followed simple verbal commands, but he did not respond properly to the examiner's questions. Nuchal rigidity was observed. Pupils were equal and reactive. There was horizontal gaze palsy,

Table 1. Proinflammatory Cytokines, Chemokines, and Growth Factors Levels in CSF Samples Harvested During the Acute and Recovery Phases

Cytokines	Controls (n = 4)	Acute Phase		Recovery Phase	
	Cytokine Level, pg/mL, Average (SD)	Cytokine Level, pg/mL, Average (SD)	Fold Change (Sample/Control)	Cytokine Level, pg/mL, Average (SD)	Fold Change (Sample/Control)
Inflammatory					
IL-1ra	8.08 (±0.15)	36.01 (±6.63)	4.46	15.05 (±4.79)	1.86
IL-6	9.06 (±6.24)	47.35 (±2.90)	5.22	7.32 (±1.29)	0.81
IL-2	1.88 (±0.15)	0.28 (±0.40)	0.14	0.84 (±0.10)	0.45
IL-5	1.21 (±0.15)	0.52 (±0.10)	0.43	1.41 (±0.57)	1.17
IL-10	2.59 (±0.15)	1.00 (±0.95)	0.39	1.73 (±2.04)	0.67
IL-12	5.45 (±0.1)	0.97 (±1.37)	0.17	2.71 (±2.90)	0.50
IL-13	15.08 (±7.12)	5.84 (±0.95)	0.39	8.39 (±1.05)	0.56
IL-17a	23.48 (±21.25)	5.95 (±3.93)	1.12	13.47 (±3.78)	0.57
IL-4	6.19 (±2.21)	5.35 (±3.00)	0.86	8.87 (±4.02)	1.43
IL-1β	0.84 (±0.10)	0.54 (±0.38)	0.64	0.69 (±0.31)	0.82
IL-9	5.81 (±6.46)	6.06 (±0.10)	1.04	10.70 (±3.51)	1.84
IL-15	2.92 (±2.12)	3.83 (±1.23)	1.31	2.74 (±0.10)	0.94
IFN-γ	34.77 (±16.85)	36.54 (±25.23)	1.05	53.90 (±16.08)	1.55
TNF-α	6.26 (±0.15)	3.52 (±2.72)	0.56	4.30 (±2.77)	0.69
Chemokines					
IL-8	31.00 (±4.36)	135.45 (±1.12)	4.37	57.60 (±8.96)	1.86
MIP-1α	1.04 (±0.59)	2.11 (±0.41)	1.93	2.66 (±0.54)	2.56
RANTES	4.25 (±0.15)	-	-	13.36 (±4.00)	3.14
Eotaxin	33.25 (±20.12)	40.09 (±0.10)	1.50	59.11 (±24.90)	1.78
IP-10	3174.25 (±918.30)	4427.65 (±182.4)	1.39	2317.95 (±25.1)	0.73
MCP-1	151.50 (±52.29)	149.08 (±0.37)	0.98	145.98 (±3.19)	0.96
MIP-1β	11.06 (±3.93)	15.39 (±0.23)	1.39	20.22 (±0.51)	1.83
Adhesion Molecules and Growth Factors					
G-CSF	6.48 (±3.20)	64.24 (±19.48)	9.91	20.00 (±3.57)	3.09
IL-7	8.97 (±7.82)	9.04 (±4.59)	1.00	29.88 (±3.15)	3.33
FGF Basic	15.55 (±11.89)	- ^a	-	3.06 (±0.28)	0.20
VEGF	18,20 (±0.15)	11.72 (±0.74)	0.64	16.73 (±4.14)	0.92

Abbreviations: FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; IFN-γ, interferon γ; IL, interleukin; IP, IFN-gamma-inducible protein; MCP, monocyte chemoattractive protein; MIP, macrophage inflammatory protein; SD, standard deviation; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor.

^aIndeterminate.

particularly to the left, and bilateral facial palsy, with preserved corneal reflexes. Muscle strength was grade 0 in lower limbs and 2 in upper limbs. Deep tendon reflexes were increased in upper limbs, patellar reflexes were normal, and ankle jerks were absent. Plantar responses were neutral. There was a T5 sensory level. On the same day, ampicillin/sulbactam, azithromycin, and methylprednisolone were added to the prescription, and a new CSF had an opening pressure of 49 cmH₂O, 122 cells/mm³ (90% mononuclear), glucose of 77 mg/dL, and negative bacterial and fungi assays. Polymerase chain reaction technique to amplify herpes simplex virus, cytomegalovirus, and Epstein-Barr virus was negative. Human immunodeficiency virus serology and rheumatologic screening panel were also negative. A new brain and spinal CT were normal. On day 12, methylprednisolone 1 gram qd for 5 days was prescribed, and the patient had partial improvement of neurological symptoms: normalization of eye movements and diminished facial palsy, but unchanged muscle strength. The dose is 0.4g of immunoglobulin per kg of the patient per day was prescribed for 5 days, with further benefits. There was marked

strength improvement on upper limbs – grade 3 to 4, and the patient could be weaned from mechanical ventilation; however, paraplegia persisted. Urine and CSF collected 1 week after the initial symptoms were positive for ZIKV reverse transcription-PCR. Brain imaging within 3 weeks of symptoms onset showed multiple patchy areas of hyperintensity on FLAIR and T2 images at both white matter hemispheres, affecting deep and subcortical regions, resembling the projection of corticospinal tract, and, specifically, on the left corona radiata and centrum semiovale, at the posterior limb of both internal capsule, as well as on the left thalami. A remarkable high signal on FLAIR/T2 and low signal on T1 images was found on both medial cerebellar peduncles (Figure 1A). Cortical mantle and basal ganglia showed normal signal intensities, and no pontine or cerebellar lesions were found. An elongated cranial-caudal hyperintensity area after pyramidal tract location could be noted. No abnormal gadolinium enhancement was noted in the parenchyma or meninges. Cervical-thoracic transition of spinal cord showed continuous hyperintensity vertical band on T2/STIR images,

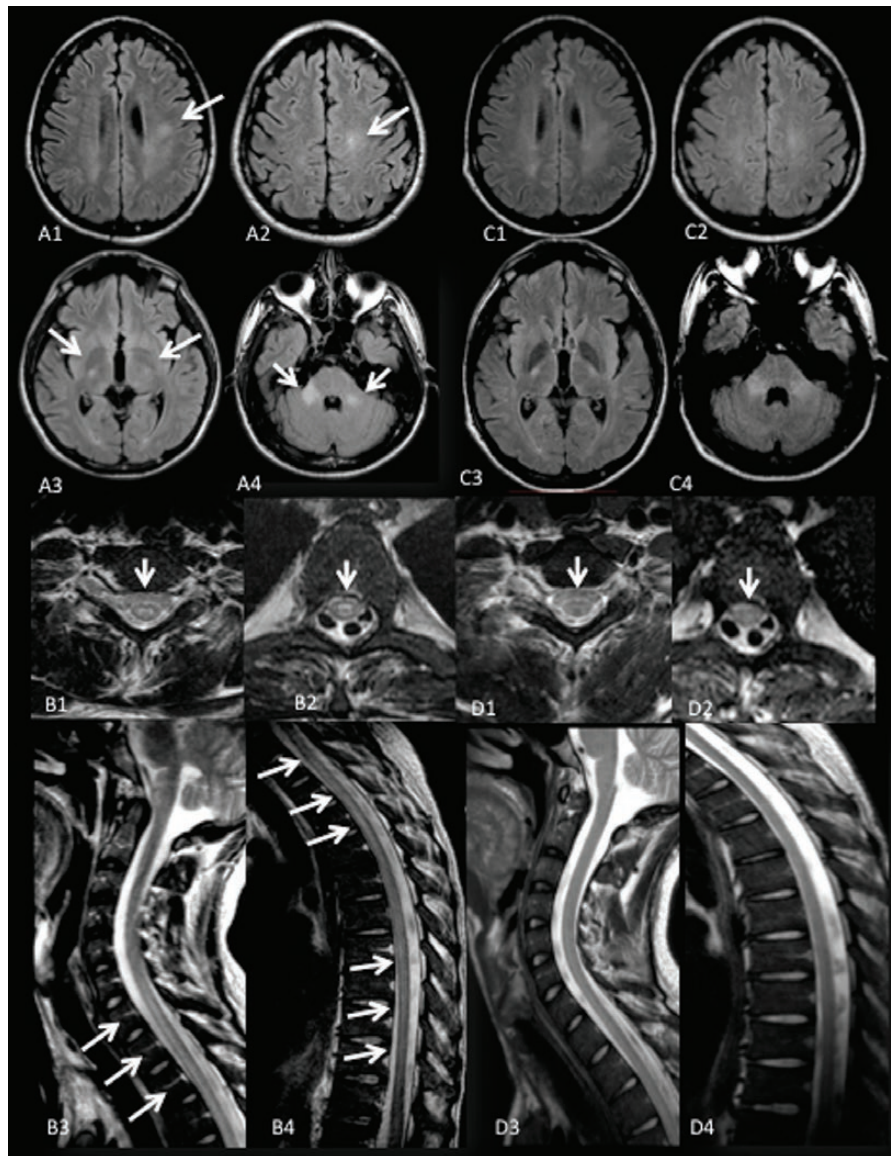


Figure 1. Magnetic resonance of the brain and spinal cord. (A) First brain exam. Fluid-attenuated inversion recovery (FLAIR) images show multiple hyperintensities on the white matter, most evident on the topography of the corticospinal tract, specifically on the corona radiata (A1) and centrum semiovale (A2), thalamus/internal capsule (A3 arrows), and both medial cerebellar peduncles (A4 arrows). (B) First spinal cord exam. The T2-weighted image (T2WI) shows a continuous hyperintensity signal band vertically oriented along cervical (B1 and B3) and thoracic (B2 and B4) segments, with central and ventral distribution (arrows). (C) Second brain exam (15 days later). All patchy areas remain visible on FLAIR images but show decreased signal intensity in comparison to the first exam. No abnormal contrast enhancement or restricted diffusion areas were identified on both exams (data not shown). (D) Second spinal cord exam (15 days later). The T2WI shows no signal abnormalities. Postcontrast T1-weighted image shows no abnormal enhancement, and medullary cone signal seems to be normal on both spinal cord exams (data not shown).

extending from C7 to almost the entire spinal length, mostly perceived on ventral horns, which were slightly tumescent from C7 to T6 levels, confirmed with transversal plane images (Figure 1B). The damage patterns of brain and spinal cord share some key features with the West Nile virus [4, 5] or Japanese encephalitis virus (JEV) neurological involvement, the latter with more severe thalamus lesions [6, 7]. Follow-up brain and spinal MRI performed 5 weeks after symptom onset revealed significant improvement of the brain abnormalities previously described, although discrete hyperintensities were still visible on

FLAIR/T2 images (Figure 1C). Spinal cord exhibited improvement with remarkable remission of signal intensity on images (Figure 1D). No other macroscopic lesions emerged during this period. Follow-up neurological examination revealed improved upper limbs strength (grade 4) and increased deep tendon reflexes, but the patient persisted with flaccid paraparesis with a T5 sensory level. Two weeks later, there was an additional improvement, defined by grade 4 muscle strength in the upper limbs and grade 2 in the lower limbs. Sphincter control was absent, but the patient no longer had urinary retention.

DISCUSSION

The analysis of humoral immune response during the acute ZIKV infection showed no differences between the levels of growth factors granulocyte (G)-macrophage CSF and platelet-derived growth factor-BB between acute and recovery phases. However, a remarkable increase of the inflammatory cytokines interleukin (IL)-6 and IL-8 was found in the CSF during this phase and with the patient presenting tetraparesis and respiratory failure. The same was not observed during the recovery phase when the IL-6 and IL-8 levels returned to normal levels (Table 1). High levels of both cytokines have been shown to be associated with a poorer outcome in patients infected with JEV [8]. These cytokines have also been associated with activation of microglial cells, from which inflammatory products were related to neuronal damage. Indeed, the production of IL-6 and IL-8 could enhance the recruitment of peripheral immune cells to ZIKV-infected sites and contribute to the disruption of the blood-brain barrier, possibly facilitating the dissemination of the virus within the central nervous system. The levels of G-CSF were also increased in the acute phase (up to 9-fold) with a slight decrease in the recovery phase. Granulocyte CSF serves as an autocrine protective signaling mechanism in response to neural injury working as an antiviral cell response that remains in the recovery phase.

CONCLUSIONS

Brazilian ZIKV has already been phylogenetically associated with JEV [9]. Our clinical, morphological, and biochemistry data support the tropism of ZIKV for the nervous system. Finally, this is the first longitudinal observation of an encephalomyelitis case associated to ZIKV that recapitulates other

encephalitis viruses with up-regulation of proinflammatory cytokines that could damage neuron cells. Further studies are needed to better understand the neuropathogenesis of ZIKV infection.

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