RESEARCH OPEN ACCESS

Dengue and chikungunya infection in neurologic disorders from endemic areas in Brazil

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Abstract

Objective

To detect the frequency of *dengue virus* (DENV), *Chikungunya virus* (CHIKV), and *Zika virus* (ZIKV) in adult patients with suspected viral infection of the CNS or postinfectious syndromes living in the state of Rio de Janeiro, Brazil.

Methods

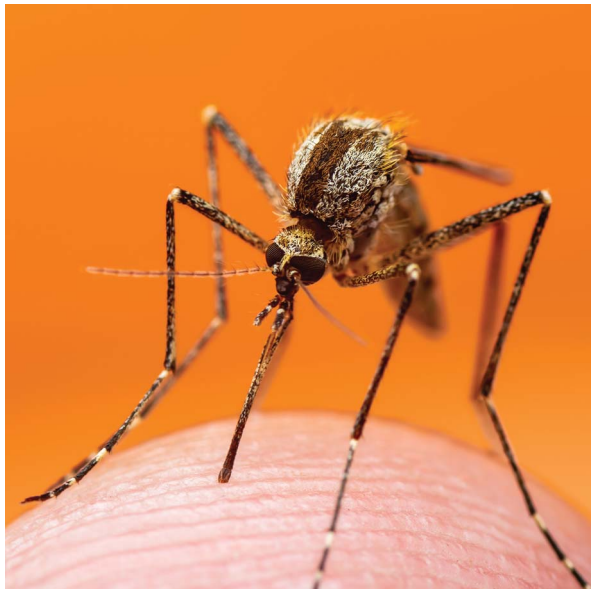
DENV, CHIKV, and ZIKV RNA by reverse transcription PCR (RT-PCR) and specific IgM antibodies were investigated in 47 CSF and serum samples of 36 adult patients suspected with viral infection or postinfectious neurologic diseases. In addition, intrathecal synthesis of anti-DENV and anti-CHIKV IgG antibodies was also evaluated using a specific antibody index.

Results

Of the total group, neuroinvasive arbovirus was confirmed in 31% (11/36) of the cases: 6 (55%) by RT-PCR in CSF and/or serum, 1 (9%) by RT-PCR in CSF and/or serum and specific IgM in CSF, and 4 (36%) by specific IgM in CSF. Five cases had DENV infection, and 6 patients were positive for CHIKV. No sample amplified for ZIKV. In addition, 3 of 7 (42%) tested cases had intrathecal synthesis of DENV or CHIKV antibodies. The neurologic complications included encephalitis (7), *Guillain-Barré* syndrome (2), optic neuritis (1), neuromyelitis optica spectrum disorder (1), polyneuropathy, (1) and myelitis (1).

Conclusion

DENV and CHIKV are a frequent cause of emerging and reemerging infections. It increases the number of cases with neurologic complications worldwide. We demonstrated that the combined use of molecular and immunologic tests in CSF/serum might support more widely the diagnosis of neurologic disorders caused by arbovirus in endemic areas. The detection of intrathecal synthesis of specific IgG antibodies may be promising for the retrospective diagnosis of neuroinvasive disorders caused by arbovirus.



Dengue virus (DENV), *Zika virus* (ZIKV), and *Chikungunya virus* (CHIKV) are arthropod-borne viruses (arboviruses) transmitted by infected *Aedes* species mosquitoes. These

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arboviruses are present in the subtropical and tropical regions of the world. There are around 4 billion people living in endemic areas including more than 120 countries. Although most cases are asymptomatic, the development of hemorrhagic conditions and hypovolemic shock because of these febrile diseases may occur.[1] In addition, there has been an increase in the occurrence of severe neurologic manifestations associated with these viruses, such as encephalitis, meningitis, Guillain-Barré syndrome (GBS), and congenital disorders.[1–5] It represents a serious public health problem because of the high morbidity, mortality, and underestimated diagnosis and is also a challenge for neurology.

Neurologic symptoms are not specific for these arboviruses, making diagnosis difficult.[1,5,6] Laboratorial diagnosis with the detection of ZIKV, CHIKV, and DENV nucleic acid by reverse transcription PCR (RT-PCR) in any samples confirms the infection. The specific IgM (ELISA) in CSF is also a confirmatory test because other arboviruses endemic in the area had been excluded.[1,7,8] However, in cases of negative results for ZIKV and DENV by RT-PCR with positive IgM for both viruses, confirmatory neutralizing antibodies and/or paired acute and convalescent serum samples are often needed to confirm recent arboviral infection because of the possibility of cross-reaction among viruses within the same viral genus.

Intrathecal synthesis of specific antibodies has been used as an important tool for the diagnosis of inflammatory disorders of the CNS.[5,6,9]

We evaluated the frequency of arbovirus infection in adult patients with suspected viral infection of the CNS or post-infectious syndromes living in the state of Rio de Janeiro, Brazil, based on the combined use of molecular and immunologic specific tests in CSF/serum and intrathecal synthesis of specific IgG antibodies.[10,11]

Methods

Patient sample and data collection

This is a retrospective, descriptive study with a cross-sectional design, including 47 samples (36 CSF with 11 paired serum) obtained in the course of routine diagnostic testing during July 2014–July 2016. The samples were from 36 adult patients with suspected viral infection of the CNS or postinfectious syndromes living in the state of Rio de Janeiro, Brazil.[10,11] The patients were 23–75 years old with a predominance of women (66%). The neurologic manifestations included meningitis (9), encephalitis (9), encephalopathy (4), myelopathy (3), neuropathy (5), GBS (3), neuromyelitis optica spectrum disorder (1), and acute disseminated encephalomyelitis (1).

Screening arbovirus investigation

The presence of specific antibodies for DENV and CHIKV was determined in CSF and serum samples using commercial kits: Panbio Dengue IgM Capture ELISA, Panbio Dengue IgG Indirect ELISA (Standard Diagnostics, Republic of Korea), Anti-Chikungunya Virus ELISA (IgG), and Anti-Chikungunya Virus ELISA (IgM) (EUROIMMUN, Germany). For ZIKV, the immunochromatographic test ZIKV IgG/IgM RDT kit (GenBody Inc, Korea) was used. The amplification of the viral nucleic acid DENV, ZIKV, and CHIKV was performed by RT-PCR, as described in the literature.[7,8]

CSF and serum analysis

All CSF samples underwent routine diagnostic analysis, which included specific and global cell count and protein and glucose dosage and examinations for bacteria, fungi, mycobacteria, and syphilis. CSF and paired serum samples were evaluated for total IgG/albumin concentrations by nephelometry. Intrathecal synthesis of total IgG was calculated based on the intrathecal IgG fraction (IgG IF), which represents the percentage of total IgG concentration produced in the CSF.[9] Blood-CSF barrier function was calculated by albumin quotient (CSF/serum). All cases had blood tests negative for antibodies against hepatitis B and C, T-cell lymphotropic virus type 1 (HTLV-1), HIV, *Cytomegalovirus*, Epstein-Barr virus, and herpes simplex virus (HSV). CSF examinations were negative for bacteria, fungi, mycobacteria, and syphilis.

Specific antibody index

Intrathecal synthesis of specific IgG was quantitatively calculated using the specific IgG antibody index (AI IgG) and using commercial kits such as Panbio Dengue IgG Indirect ELISA (Standard Diagnostics, Republic of Korea) and Anti-Chikungunya Virus ELISA (IgG) (EUROIMMUN, Germany). It is based on the level of specific antibodies in serum, CSF, and blood-CSF barrier function.[9] Specific AI IgG ≥1.5 is a criterion of intrathecal synthesis of specific antibodies.[9]

Diagnosis criteria

Laboratorial diagnosis of arbovirus was based on the detection of viral nucleic acid by RT-PCR in serum or CSF or specific IgM by ELISA assay in CSF. The presence of intrathecal synthesis of specific antibodies (specific AI) was considered as an additional support to the laboratorial diagnosis. Pleocytosis (>4 cells/mm^3), hyperproteinorrhachia (>40 mg/dL), blood-CSF barrier dysfunction (albumin quotient CSF/serum >8 × 10^{-3}), intrathecal synthesis of

total IgG (IgG IF > 0), or intrathecal synthesis of specific IgG (specific AI IgG ≥1.5) were indicative of the inflammatory reaction in CSF.[9]

Standard protocol approvals, registrations, and patient consents

This study was approved by the Ethical Review Board of HUGG/UNIRIO (54448216.2.0000.5258) and HUCFF/UFRJ (58626816.9.000.5257).

Data availability

All data used in this study are anonymized and will be shared by request from any qualified investigator.

Results

Clinical findings

Table 1 shows the clinical characteristics of the 11 patients with a confirmed diagnosis (RT-PCR/IgM in CSF) of neuroinvasive arbovirus and 2 others who present only intrathecal synthesis of specific antibodies. In total, DENV was associated with neurological alteration in seven cases, and CHIKV in six cases. There was no case of ZIKV. Encephalitis was the most common neurologic manifestation (7 cases). Four of them were associated with DENV and 3 of them with CHIKV infection.

Higher frequency of PCR detection

The virologic study presented amplification for viral RNA in 19% (9/47) of serum and/or CSF, with 5 samples of DENV and 4 samples of CHIKV. The immunologic tests for specific IgM were reactive in 13% (6/47) of samples: 1 for DENV and 5 for CHIKV. No samples were reactive for anti-ZIKV IgM (table 2). Specific reactive IgM in CSF and/or the presence of viral RNA in serum and/or CSF were detected in 15 of 47 (32%) of the samples and in 31% (11/36) of the cases.

Specific AI

Three of 7 studied cases had intrathecal synthesis of specific IgG (table 2). Two of them were negative for arboviruses by RT-PCR or specific IgM: 1 case of DENV encephalitis (DENV AI IgG = 3.3) and 1 case of DENV associated with optic neuritis (DENV AI IgG = 4.9). CHIKV encephalitis was diagnosed using a specific AI (CHIKV AI IgG = 7.2) in a patient with reactive IgM CHIKV in serum and CSF diagnosed as encephalitis.[6]

CSF analysis

There were no red blood cells in CSF of the studied cases. Inflammatory CSF occurred in 54% (7/13) of the positive arbovirus cases (pleocytosis and hyperproteinorrhachia): 3 had DENV infection (2 with encephalitis and 1 with neuromyelitis optica spectrum disorder) and 4 cases of CHIKV infection (encephalitis, myelitis, GBS, and neuropathy) (table 2). In addition, 3 cases (encephalitis, GBS, and polineuropathy) associated with CHIKV had protein-cytological dissociation. Hypoglycorrhachia was found in another patient with DENV-2 encephalitis. None of the cases had intrathecal synthesis of total IgG.

The intrathecal synthesis of specific IgG antibodies may represent an inflammatory biomarker for neurologic involvement associated with DENV and CHIKV.

Discussion

In this study, markers of arbovirus infection (DENV and CHIKV) were detected in 11 (31%) cases with neurologic manifestations associated with infectious or postinfectious syndromes by RT-PCR in CSF/serum or IgM in CSF. Most of these (81%) were confirmed by viral detection. In addition, 2 others cases (encephalitis and optic neuritis) had only intrathecal synthesis of DENV antibodies. The patients were seen in the city of Rio de Janeiro during arbovirus epidemic from July 2014 to July 2016, a period of low prevalence of ZIKV in adults. The laboratorial diagnosis was based on immunology and molecular biology techniques in CSF and/or serum. The detection of DENV IgM in CSF has a high specificity (97%) for the neurologic diagnosis.[5] In accordance with the criteria for defining neuroinvasive arbovirus cases by the Brazilian Ministry of Health and by the Centers for Disease Control and Prevention, we consider as confirmed cases of neuroinvasive arbovirus those that meet the following criteria: viral detection by RT-PCR in serum or CSF and/or detection of IgM antibodies in CSF by enzyme immunoassay (ELISA) with exclusion of endemic arbovirus.[10,11] According to the Brazilian Health Ministry, the neuroinvasive arboviruses of major epidemiologic interest and public health importance in our country are genera *Flavivirus*, *Alphavirus*, and *Orthobunyavirus*, especially DENV, CHIKV, and ZIKV.[10] All our studied cases were screened for the 3 arboviruses not only by immunologic but also by molecular analysis. The patients also had clinical symptoms compatible with CHIKV infection (data not shown). We had no coinfections. Based on these data, we confirmed that 31% of our patients had neuroinvasive arbovirus (DENV or CHIKV).

In addition, we performed the detection of intrathecal synthesis of specific IgG using the specific AI.[5,6,9] The specific IgG AI discriminates fractions of immunoglobulins produced in blood from those synthesized in the CNS against an agent.[9] It may be useful in the differential diagnosis of neurologic disorders associated with arboviruses. The analysis of specific intrathecal synthesis of antibodies has been used to support the retrospective diagnosis of HSV, varicella zoster virus, measles, rubella, neuroborreliosis, and human T-cell leukemia virus type 1 nervous system infection.[5,6,9]

Among the neurologic diseases associated with arboviruses, similar to previous reports, encephalitis was the most

Table 1 Demographic data and clinical manifestations in 13 positive cases of DENV and/or CHIKV infection: 11 confirmed cases and 2 possible cases (15 and 30)

Case	Age/sex	City	Diagnosis/virus	Signs and symptoms	Evolution
11	63/M	Rio de Janeiro	Encephalitis/DENV	Fever, adynamia, hiporexia, weakness, tetraparesis, paresthesia in upper limbs, ptosis eyelid, diplopia and mydriasis on the left, and holocranial headache	Hospital release
12	28/F	Fortaleza	Encephalitis/DENV	Cognitive decline, tonic-clonic seizure, dysarthria, and spastic tetraparesis	Unknown
14	64/F	Rio de Janeiro	Encephalitis/CHIKV	Fever, myalgia, arthralgia, asthenia, lethargy, hypokalemia, hyponatremia, hypophosphatemia, and tonic-clonic seizure;	Hospital release
15	45/F	Unknown	Encephalitis/DENV	Behavior change (disinhibition), abnormal gait, disorientation, and memory loss.	Unknown
16	63/F	Rio de Janeiro	Encephalitis/CHIKV	Fever, arthralgia, edema in the lower limbs, prostration, cognitive alterations, skin rash, pruritus.	Hospital release
17	75/M	Campina Grande	Encephalitis/CHIKV	Fever, prostration, myalgia, diffuse maculopapular rash, oliguria, disorientation, lethargy, and reduced GCS	Hospital release
18	27/F	Rio de Janeiro	Encephalitis/DENV	Diplopia, nausea, vomiting, asthenia, headache, ataxic march, Romberg sign, multidirectional nystagmus, and head-shaking maneuver positive.	Hospital release
26	59/F	Rio de Janeiro	GBS/CHIKV	Constipation, ascending paraparesis, dysphonia, facial diparesis, cervical paresthesia, global areflexia, bilateral tactile and painful bilateral hypesthesia in the lower limbs and hands, and tetraplegia.	Hospital release
28	48/F	Rio de Janeiro	GBS/DENV	Severe chest pain, dry cough, herpes zoster, arthralgia, pneumonia, breathlessness, and cardiopulmonary arrest	Death
30	22/M	Unknown	Optic neuritis/DENV	Subacute loss of both visual fields	Unknown
33	52/F	Rio de Janeiro	Polyneuropathy/CHIKV	Paresthesia in the lower limbs and hands, paraplegia, deep bilateral upper limb flexure, hypotonia and bilateral areflexia in the upper limbs, hyperalgesia to mild plantar stimulation, and superficial and deep hypesthesia.	Hospital release
34	64/M	Rio de Janeiro	Myelitis/CHIKV	Romberg sign, urinay retention, paraplegia, spasticity in the lower limbs, reduced segmental proprioception, tactile and painful hypesthesia at the T2 level, and hiporreflexia in the upper limbs.	Hospital release

Abbreviations: CHIKV = *Chikungunya virus*; DENV = *Dengue virus*; F = Female; GBS: Guillain-Barré syndrome; M = Male; NMOSD = neuromyelitis optica spectrum disorder.

frequent manifestation. It included 7 cases (54%): 4 by DENV (DENV-2, DENV-3) and 3 by CHIKV. We detected two cases of GBS (18%): 1 by DENV and 1 by CHIKV infection. In addition, it was found that one case of myelitis and one of polyneuropathy caused by CHIKV as well as a case of autoimmune disorder and optic neuritis were associated with DENV. Amplification occurred in paired serum and CSF of 2 patients. One of them by DENV-3 and another by CHIKV.

In DENV, it is estimated that 0.5%–21% of patients develop neurologic manifestations.[2] DENV-2 and DENV-3 are the most commonly found in cases of myelitis, meningitis, and encephalitis.[2] Regarding CHIKV, since the 1960s, it has been known that the infection affects the CNS.[3] During the 2006 epidemic in India, 16.3% of cases of neurologic manifestations were caused by CHIKV. In 2016, the cohort study conducted at *La Réunion Island* was associated with CHIKV infection in 42% of cases of encephalitis.[4]

The detection of viral RNA and specific IgM in CSF are indicative of the viral infection in the CNS. The intrathecal synthesis of specific IgG antibodies may represent an inflammatory biomarker for neurologic involvement associated with DENV and CHIKV. In addition, the combined use of CSF and serum

Table 2 CSF findings of 13 cases with neurologic disorders associated with DENV or CHIKV infection: 11 confirmed cases and 2 possible cases (15 and 30)

Case	Neurologic manifestation	CSF analysis[a]			Positivity criteria
		Cell count/mm^3	Protein (mg/dL)	Glucose (mg/dL)	
11	Encephalitis	1	32	79	Amplification for DENV-3 in CSF
12	Encephalitis	**7**	**77**	58	Reactive IgM for DENV in CSF
14	Encephalitis	1	**77**	98	Amplification for CHIKV in CSF and serum. Reactive IgM for CHIKV in CSF
15	Encephalitis	1	35	64	AI IgG for DENV ≥1.5
16	Encephalitis	1	29	40	Reactive IgM for CHIKV in serum and CSF. AI IgG for CHIKV ≥1.5
17	Encephalitis	**70**	**67**	71	Reactive IgM for CHIKV in CSF
18	Encephalitis	**24**	**81**	34	Amplification for DENV-2 in CSF
26	GBS	**9**	**216**	82	Amplification for CHIKV in CSF
28	GBS	1	28	56	Amplification for DENV-3 in CSF and serum
30	Neuritis	1	23	63	AI IgG for DENV ≥1.5
33	Polyneuropathy	**8**	**240**	63	Amplification for CHIKV in CSF
34	Myelitis	**32**	**59**	43	Reactive IgM for CHIKV in CSF
35	NMOSD	**120**	**35**	78	Amplification for DENV-1 in serum

Abbreviations: CHIKV = *Chikungunya virus*; DENV = *Dengue virus*; GBS = Guillain-Barré syndrome; NMOSD = neuromyelitis optica spectrum disorder. There were no red blood cells in CSF.
[a] Reference values: Pleocytosis >4 cells/mm^3; hypoglycorrhachia <40 mg/dL; hyperproteinorrhachia >40 mg/dL; specific AI IgG ≥1.5 = intrathecal synthesis of specific antibodies.

for the calculation of the AI IgG increases the diagnostic accuracy of neurologic diseases caused by DENV and CHIKV, which can support the retrospective diagnosis, especially during epidemics, avoiding possible sequels and improving the quality of life of patients. Cases of encephalitis, myelitis, aseptic meningitis, and postinfectious neurologic syndromes should be screened for neuroinvasive arbovirus in endemic areas.

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Disclosure

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Appendix Authors

Name	Location	Contribution
Cíntia da Silva Mello, MSc	Universidade Federal do Rio de Janeiro (UFRJ); and Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Rio de Janeiro, Brazil	Concept, acquisition and interpretation of data, and performed the laboratorial analysis and critical revision
Mauro Jorge Cabral-Castro, PhD	Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil	Concept, acquisition interpretation of the data, and performed the laboratorial analysis and critical revision
Luiz Claudio Silva de Faria, BSc	Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil	Concept, acquisition interpretation of the data, and performed the laboratorial analysis and critical revision
José Mauro Peralta, MD, PhD	Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil	Concept, acquisition interpretation of the data, and critical revision

Continued

Appendix *(continued)*

Name	Location	Contribution
Marzia Puccioni-Sohler, MD, PhD	Universidade Federal do Rio de Janeiro (UFRJ); Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Rio de Janeiro, Brazil	Concept, acquisition interpretation of the data, drafted the manuscript, and critical revision

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