Arq Neuropsiquiatr 2008;66(2-A):234-237

OLIGOSYMPTOMATIC DENGUE INFECTION

A potential cause of Guillain Barré syndrome

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Abstract – Background: Dengue infection may cause neurological manifestations such as encephalitis, myelitis, mononeuropathies, acute disseminated encephalomyelitis, and Guillain Barré syndrome (GBS). In endemic regions, the infection course can be oligosymptomatic making difficult the diagnosis of the neurological picture associated with dengue infection. **Objective:** To report dengue infection and GBS association, even in oligosymptomatic cases of this infection. **Method:** During the dengue epidemic in Rio de Janeiro city we looked for GBS cases, testing IgM antibodies for dengue and dengue polymerase chain reaction (PCR) in the cerebrospinal fluid (CSF) and serum. **Results:** We report seven cases (46.6%), presenting dengue positive IgM in serum but with poor or without clinical symptoms of the previous infection. Two of them had also positive IgM antibodies in CSF. **Conclusion:** These data show that search for dengue infection should be a routine in GBS cases living in endemic areas.

KEY WORDS: dengue, polyradiculoneuritis, radiculoneuropathy, CSF, Guillain Barré syndrome.

Dengue oligossintomática: causa potencial de síndrome de Guillain Barré

Resumo – Introdução: O vírus da dengue pode determinar várias manifestações neurológicas como: encefalite, mielite, mononeuropatias, encefalomielite disseminada aguda e a síndrome de Guillain Barré (SGB). Em regiões endêmicas a infecção pode ser oligosintomática dificultando o diagnóstico neurológico associado a infecção por dengue. **Objetivo:** Relatar a associação entre SGB e o vírus da dengue, principalmente em formas oligosintomáticas da infecção. **Método:** Durante epidemia pelo vírus da dengue na cidade do Rio de Janeiro, casos de SGB foram selecionados e tiveram suas amostras de soro e líquido cefalorraqueano (LCR) testadas para anticorpos dengue IgM e PCR para dengue. **Resultados:** Descrevemos sete casos (46,6%) com diagnóstico de SGB, apresentando IgM positiva para dengue no soro, porém com poucos ou nenhum sintoma infeccioso prévio. Dois pacientes também apresentavam IgM positiva no LCR. **Conclusão:** Estes dados sugerem que a pesquisa para o vírus da dengue deve ser realizada em todos os casos de SGB em áreas endêmicas.

PALAVRAS-CHAVE: dengue, polirradiculoneurite, radiculoneuropatia, líquido cefalorraqueano e síndrome de Guillain Barré.

Dengue fever is a viral infection caused by an arbovirus from *Flaviviridae* family and transmitted in urban areas by *Aedes egypti* mosquito. It is endemic in tropical and subtropical countries, especially in Southeast Asia, Caribbean basin, South America and Pacific Islands[1]. It is one of the most common arboviruses in the world, considering it is the unique arbovirus fully adapted to the human host and its environment[2]. Furthermore, it is the second cause of infection in travelers[3]. The last outbreak of dengue epidemic in Rio de Janeiro city, Brazil occurred in the summer of 2002. A total of 106721 cases were estimated in the period[4]. During this dengue epidemics, neurological manifestations as encephalitis, myelitis and Guillain Barré syndrome (GBS)were described in people with clinical picture and positive serology for the virus[5]. GBS is the most common cause of acute neuromuscular paralysis in the developed world. It is characterized by a rapidly ascending paralysis, determined by an inflammatory demyelinating

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Received 25 October 2007, received in final form 22 January 2008. Accepted 26 February 2008.

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or axonal polyneuropathy[6]. In general, the cerebrospinal fluid (CSF) exam shows a protein-cytologic dissociation. Electromyography (EMG) of GBS demonstrates a slowed conduction velocity, conduction block in motor nerves, prolonged distal latencies and prolonged or absent F responses. Numerous infections preceding its symptoms have been related, as: *Campylobacter jejuni*, HIV, Herpes virus, Varicella zoster and Cytomegalovirus[7,8].

Dengue virus is rarely reported as a causative agent of GBS. In a previous study[5], GBS was accounted for 30% out of the neurological manifestations of dengue infection.

In order to clarify the association between GBS and dengue infection, we studied serum and CSF samples of patients with suspected GBS during and out of the period of dengue epidemics in Rio de Janeiro city, Brazil. CSF analysis and the clinical data are discussed.

METHODS

Specimens studied

In this retrospective study, samples from patients with medical hypothesis of GBS[9] were selected from January to April of 2002, in a reference CSF laboratory (Neurolife Laboratory). This period was chosen because dengue epidemic occurred between January and April of 2002, in Rio de Janeiro city. Nineteen patients were submitted to CSF examination based on the initial hypothesis of GBS. Four of them were excluded after verifying their medical history and final diagnosis in the medical record (one of them presented vasculitis causing polyneuropathy, one presented encephalitis, and two cases had brainstem stroke). Only one of the remaining 15 cases had dengue infection symptoms. The studied data included: sex, age and history of infection previous to GBS. Considering that it was a retrospective study of medical records, we could not access the EMG results (but a medical description that they were compatible with GBS were found: motor-sensitive demyelinating polyradiculoneuritis). Unfortunately, more details about the exams could not be found. All the EMG exams were done in the first week of GBS symptoms.

As a control group, we studied 14 samples with GBS diagnosis[9] according the medical suspect, which had also their serum and CSF collected at the same laboratory, out of the epidemic period (January to April of 2006).

CSF analysis

CSF and serum samples were collected at the same time and analyzed in a reference CSF laboratory. The samples were stored at –20°C. Total cells count in CSF was determined with a Fuchs-Rosenthal chamber and differential cytology counts by cytosedimentation chamber (Pleocytosis: >5 cels/mm^3). The CSF protein (normal range: 20–40mg/dL) and glucose levels (normal range: 50-80mg/dL) were determined by spectroscopy. The albumin concentration was determined in serum and CSF by immunoprecipitation nephelometry (Dade Bhering). The albumin quotient (Alb Q=CSF albumin/serum albumin) was used to evaluate the integrity of the blood-CSF barrier following previously described protocols[10,11]. A high Alb Q ($\geq 8.0 \times 10^{-3}$), indicates a blood-CSF barrier dysfunction.

Screening for IgM antibodies for dengue

The stored CSF and serum of all patients were screened for anti-dengue IgM antibodies by enzyme-linked immunosorbent assay (ELISA) (PANBIO, Brisbane/Australia). In the sense of increasing the sensitivity of the test in CSF, it was diluted (1:2)[5]. Serum was diluted (1:100) according to the manufacturer's recommendation.

Screening of polymerase chain reaction for dengue in CSF and serum

Investigation of dengue virus RNA was performed using a reverse transcriptase-polymerase reaction following the procedure described by Kumaria and Chakravarti in all IgM positive serum samples[12].

RESULTS

Characterization of the samples

From the 15 samples from patients with GBS (during dengue epidemic) studied, there were 10 males and five females, with ages ranging from 6 to 79 years old. From the 14 samples of control, there were four females and ten males, with ages ranging from 2–93 years old. Seven out of 15 (46.6%) serum from patients with GBS diagnosis studied during dengue epidemic had dengue IgM positivity. In comparison, only one sample (7.1%) of the control group had dengue IgM antibody in the CSF and serum (p>0.005, *Fisher test*). The specificity of the test in serum is 100% and sensitivity was 94.7%, according to the kit manual. The CSF sensitivity was 46% and the specificity, 100%[5].

Characterization of the seven positive dengue cases

Clinical characteristics – The seven Dengue IgM positive cases were the focus of the study, occurring during the Dengue epidemic. Four out seven had oligosymptomatic infectious pictures and there was no history of dengue infection previously. In only one case (case 5) dengue infection was suspected. Surprisingly, two patients (cases 3 and 7) denied any infectious symptoms preceding GBS. Clinical findings are showed in the Table.

CSF analysis – Cytology counts were normal in all seven patients. High protein (>40mg/dL) concentration in CSF was found in six patients with positive IgM for dengue in serum, confirming typical protein-cytology dissociation. Five cases had a high Alb Q (average of 9.7 and standard deviation of 3.6). Two CSF samples were positive to dengue IgM (cases 3 and 6). Both showed the highest values of albumin quotient (12.9 and 13.5x10). Immunological test for syphilis (VDRL) was negative as well the HTLV-I, Herpes simplex, Varicella zoster, cytomegalovirus and HIV1/2 antibodies (ELISA).

Table 1. Clinical characteristics of seven positive dengue patients.

Patients	Time from GBS onset to LP	Time from infection to neurological symptoms	GBS symptoms	Treatment	Evolution after treatment, during hospitalization
1	3 days	Respiratory infection 15 days before GBS	Acute areflexic tetraparesis, dysphonia	Prednisone 60 mg/day (5 days)	Tetraparesis
2	7 days	Viral infection 25 days before GBS	Acute areflexic flaccid paraparesis, paresthesias	Unknown	Unknown
3	6 days	Without infectious symptoms	Facial diplegia, progressive areflexic paraparesis, paresthesias	Immunoglobulin (400 mg/kg/day 5 days)	Asymptomatic
4	7 days	Urinary infection + hematuria 5 days before GBS	Lumbar pain, areflexic paraparesis	Immunoglobulin (400 mg/kg/day 5 days)	Paraparesis
5	2 days	Dengue concomitant to GBS	Acute areflexic tetraparesis,	Immunoglobulin (400 mg/kg/day 5 days)	Paraparesis
6	24 days	Intestinal infection three months before GBS	Right facial paralysis, Hyporeflexic tetraparesis,	Immunoglobulin (400 mg/kg/day 5 days)	Asymptomatic
7	6 days	Without infectious symptoms	Acute areflexic quadriplegia	Immunoglobulin (400 mg/kg/day 5 days)	Asymptomatic

LP, lumbar puncture.

PCR results – Serum and CSF samples were negatives for RNA dengue virus by PCR.

Comparison between CSF results in dengue positive samples and controls – Concerning cytology and protein, all values of positive IgM cases were allocated between the respective variation limits of controls. These data suggest that this group was homogeneous.

DISCUSSION

Unusual manifestations of dengue infection including neurological syndromes have been described each year, mainly during epidemics. Encephalitis, meningitis, myelitis are associated with acute infection. Postinfection disorders include: Bell's palsy, acute disseminated encephalomyelitis, maniac psychosis, dementia, epilepsy, and Guillain Barré syndrome[1]. From all of them, encephalitis is the commonest neurological manifestation. Guillain Barré syndrome has been described by isolated reports. In our literature revision we found seven cases reports of GBS associated to dengue infection[13-18]. There were three children and six adults, ages ranging from one and half to 46 years old. Neurological symptoms developed between four to nineteen days after the onset of dengue picture. All of them presented tetraparesis and, except for the case described by Santos et al.[16], had good recovery. The infection was suspected and confirmed in all of them, but only in one case the immunological test to dengue in the CSF was done[14], and it was negative. A case of Miller Fisher syndrome, a variant form of GBS, caused by dengue was already described[19].

Our report is the first description of dengue and GBS association without typical symptoms or suspicion of this infection. It shows that dengue infection can be underestimated as a causative agent of GBS and should not be considered a coincident association as demonstrated by our control results. During the period out of epidemic the number of GBS cases with a positive serology was significantly lower (7.1%) than during the epidemic (46.6%). Neurological picture of our cases was similar to that described in literature about GBS related with other infections: the ascendent paraparesis as principal manifestation. Improvement after treatment was obtained in almost all cases. It was evaluated during the hospitalization and a continuous improvement of neurological picture might have been followed. These data show that GBS determined by dengue infection has similar characteristics and prognosis to that caused by other infections. This information is very important, since there are only reports of cases described in the literature in which these conclusions could not be demonstrated.

Contrary to the reported cases in the literature, six of our patients did not have the characteristic symptoms of dengue infection but presented positive IgM serologies to this infection. Two of them had also a positive dengue IgM in CSF (cases 3 and 6) with the highest values of Albumin Q of the group. This finding could be due to a dysfunction of the blood-CSF barrier. Despite this fact, their evolution were excellent, suggesting that dengue IgM in CSF is not a prognostic factor. Also, there were no differences in the

course of the disease in these patients. It is known that serum dengue IgM appears to peak about 2 weeks after the primary infection, declining to undetectable levels over 2–3 months whereas its persistence in CSF is about one month[20,21].

PCR is usually positive in the first week of infection[20]. This test was negative in all of our cases probably due the longer time necessary to develop GBS after an infection; frequently more than one week. The only exception would be case 5 presenting GBS concomitant to dengue infection. However the PCR was also negative. Despite de evidence of a positive dengue IgM we could not exclude other concomitant infection determining GBS in this case. In the other cases, IgM positivity was the best test to detect dengue infection and we could not justify GBS caused by other infections.

Evidences suggest that the clinical manifestations of GBS are the result of a cell-mediated immunologic reaction, with a complement dependent antibody targeted, attacking on Schwann cells. This reaction is precipitated by activated T cells, crossing the vascular endothelium (blood–nerve barrier), and recognizing an antigen in the endoneurial compartment. They produce cytokines and chemokines which open the blood–nerve barrier and allow the egress of antibodies. The term "blood-nerve barrier dysfunction" refers to the altered protein permeability of the vascular endothelium in nerve tissue[22].

Several dengue infection studies have demonstrated abnormal immune responses including cytokine and chemokine production, complement activation and immune cell activation[23]. In addition, autoimmune responses may be involved, mainly in dengue haemorrhagic fever pathogenesis. Dengue patients can produce antibodies which cross-reacted with human platelets and endothelial cells, for example. Dengue nonstructural protein 1 antibody (Anti-NS1) produced after dengue infection is described to be responsible, at least in part, for the cross reactivity to endothelial cells[23]. These mechanisms probably have great importance in the development of the neurological disease.

As a retrospective study, we had limitations in the study: the small number of cases, the difficulty to obtain more details about the histories, such as symptoms of previous infections. Concerning this last point we can conclude they were tenuous to the typical dengue fever, but may be part of the oligosymptomatic dengue picture.

Dengue infection can determine since a catastrophic dengue haemorrhagic fever picture to an oligosymptomatic case[21]. This last condition underestimates the number of cases with neurological manifestations in association with the infection. In GBS it can be worse as shown by the long time taken between the onset of infection and neurological symptoms. Even so, in our cases CSF tests to other infections were negatives, reinforcing dengue diagnosis. In endemic areas, dengue infection should be tested as a possible etiological agent in cases of GBS.

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