Isolation of dengue 2 virus from a patient with central nervous system involvement (transverse myelitis)

Isolamento de dengue 2 de paciente com comprometimento do sistema nervoso central (mielite transversa)


Abstract

A dengue fever case is described in a 58-year-old male patient with febrile illness and thrombocytopenia complicated by neurological involvement characterized by transverse myelitis followed by weakness of both legs and flaccid paralysis. Muscle strength was much diminished and bilateral areflexia was observed. Dengue 2 (DEN-2) virus was isolated and the patient sero-converted by hemagglutination-inhibition and IgM-ELISA tests. The RT-PCR test was positive to DEN-2 in acute phase serum and culture supernatant, but negative in the cerebrospinal fluid. After three weeks of hospitalization the patient was discharged. No other infectious agent was detected in the blood and cerebrospinal fluid samples. The patient had full recovery from paralysis six months after the onset of DEN-2 infection.

Key-words: Dengue fever. Transverse myelitis. Dengue 2. Virus isolation. RT-PCR.

Resumo

Um caso de febre clássica de dengue é descrito em paciente masculino de 58 anos de idade que desenvolveu quadro febril acompanhado de trombocitopenia complicado com envolvimento neurológico caracterizado por mielite transversa, a qual se seguiu de fraqueza de ambos os membros inferiores e paralisia flácia. A força muscular do paciente ficou bastante diminuída e arreflexia bilateral também foi observada. O sorotipo dengue 2 (DEN-2) foi isolado e o paciente também soro-converteu em testes de inibição de hemaglutinação e IgM-ELISA. Da mesma amostra de soro da fase aguda da qual o vírus foi isolado, teste de RT-PCR foi positivo para DEN-2. RT-PCR foi igualmente positivo utilizando o sobrenadante da cultura positiva, mas foi negativo usando-se o líquido cefalo-raquidiano (LCR) obtido com 12 dias de doença. Nenhum outro agente infeccioso foi isolado ou identificado em testes sorológicos utilizando as amostras séricas e o LCR. O paciente foi hospitalizado por três semanas, tendo recebido alta do quadro de dengue ainda com problemas da paralisia que foi sanada após seis meses do início da infeção pelo DEN-2.


Dengue fever (DF) and dengue hemorrhagic fever (DHF) are caused by dengue (DEN) virus serotypes 1 to 4, transmitted by bites of Aedes aegypti mosquitoes, and represent important infectious diseases in tropical and subtropical countries4 5 23. In Brazil, from 1986 through 1999, more than one million and a half cases have been reported23. Few cases of DHF have been diagnosed, and the serotypes DEN-1 and DEN-2 have been responsible for epidemics in the whole country, including the city of Belém16 23 from which the case reported here came. This case is an unusual presentation of DF with neurological complications, caused by dengue 2 virus.
CASE REPORT

The patient, a 58-year-old man, born in Japan and living in Belém, Brazil, for 10 months, presented for medical consultation with a history of febrile illness for three days. When seen (18th January 2000), the symptoms were fever of sudden onset, weakness and malaise for three days, accompanied in the last two days by mild headache, diarrhea and rash on the legs and arms. During the interview, the patient gave a history of moderate use of alcohol, bilateral occurrence of renal lithiasis, angina pectoris, a transient cerebrovascular ischemic stroke in 1995, and diverticulosis in 1999. On examination he appeared mildly ill, and reported nasal and gingival hemorrhages. An erythematous fine pruriginous macular rash was observed on the arms and legs, where several petechiae were also detected. No tenderness was found in the abdomen, costovertebral angles or spines. Neurologic examination was negative, the extremities were perfusing, and the axillary temperature reached 38°C.

Specimens of blood were obtained and showed: leucogram 2,800/mm³, lymphocytes 25%, platelets 60,000/mm³, ALT: 66U/mm³ and AST: 99U/mm³. From this serum sample virus isolation was attempted. The presumptive diagnosis was dengue fever (DF) with reduction of platelets, and the patient was sent home.

Two days later (20th January, 2000) he returned with fever, muscle pains, anorexia, reporting acute urinary retention, and although the leucocyte count had returned to normal values (6,100 cells/mm³), he was admitted.

On admission, a Foley's bladder probe was used and an evaluation by an urologist was made that did not find alterations. Blood samples were taken for biochemical examination (Table 1). Blood and urine cultures were also performed. The platelets were 50,000/mm³ and leucocytes remain normal, but aminotransferases were still showing a slight increase (Table 1), but the patient felt much improved and physical examination was unchanged. On 24th January (9th day after onset) the platelet count remained low but was showing a discrete increase reaching to 116,000/mm³, and γ-GT was highly increased (Table 1). Clinically the patient exhibited sleepiness and confusion in answering verbal inquiries, but the fever improved and the rash was slowly fading. Cardiologic evaluation was done, and apparently good conditions were observed excluding the possibility of myocarditis. The patient exhibited difficulties in walking and answering questions, and had cough, malaise, and hiccups. Chest X-ray was normal. On the next day, 25 January (10th day of disease), the patient showed a better disposition and accepted oral nutrition.

Table 1 - Main biochemical results of exams carried out in a patient with dengue and transverse myelitis.

<table>
<thead>
<tr>
<th>Exam</th>
<th>18 Jan</th>
<th>20 Jan</th>
<th>22 Jan</th>
<th>24 Jan</th>
<th>27 Jan</th>
<th>29 Jan</th>
<th>8 Feb</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes</td>
<td>2,800</td>
<td>6,300</td>
<td>9,100</td>
<td>9,500</td>
<td>18,000</td>
<td>8,600</td>
<td>7,500</td>
<td>5-10 X 10³/mm³</td>
</tr>
<tr>
<td>eosino</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2-4%</td>
</tr>
<tr>
<td>mono</td>
<td>3</td>
<td>2</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>4-8%</td>
</tr>
<tr>
<td>neut</td>
<td>69</td>
<td>74</td>
<td>70</td>
<td>54</td>
<td>82</td>
<td>69</td>
<td>69</td>
<td>36-65%</td>
</tr>
<tr>
<td>bands</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>0-5%</td>
</tr>
<tr>
<td>lympho</td>
<td>25</td>
<td>22</td>
<td>21</td>
<td>42</td>
<td>11</td>
<td>26</td>
<td>25</td>
<td>25-44%</td>
</tr>
<tr>
<td>Platelets</td>
<td>60,000</td>
<td>50,000</td>
<td>60,000</td>
<td>116,000</td>
<td>160,000</td>
<td>204,000</td>
<td>230,000</td>
<td>150-450 X 10³/mm³</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>43%</td>
<td>47%</td>
<td>45%</td>
<td>42%</td>
<td>45%</td>
<td>45%</td>
<td>41%</td>
<td>42-47%</td>
</tr>
<tr>
<td>ALT</td>
<td>66</td>
<td>123</td>
<td>-</td>
<td>80</td>
<td>62</td>
<td>79</td>
<td>47</td>
<td>10-34 U/dl</td>
</tr>
<tr>
<td>AST</td>
<td>99</td>
<td>199</td>
<td>-</td>
<td>82</td>
<td>94</td>
<td>49</td>
<td>28</td>
<td>9-43 U/dl</td>
</tr>
<tr>
<td>Urea</td>
<td>15</td>
<td>16</td>
<td>-</td>
<td>25</td>
<td>33</td>
<td>34</td>
<td>-</td>
<td>15-40 mg/dl</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.2</td>
<td>1.1</td>
<td>-</td>
<td>0.9</td>
<td>1.0</td>
<td>0.9</td>
<td>-</td>
<td>0.4-1.3 mg/dl</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>1.3</td>
<td>-</td>
<td>-</td>
<td>0.2-1.0 mg/dl</td>
</tr>
<tr>
<td>direct</td>
<td>-</td>
<td>0.3</td>
<td>-</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.05-0.3 mg/dl</td>
</tr>
<tr>
<td>Glycemia</td>
<td>104</td>
<td>105</td>
<td>-</td>
<td>144</td>
<td>126</td>
<td>140</td>
<td>100</td>
<td>70-110 mg/dl</td>
</tr>
<tr>
<td>γ-GT</td>
<td>-</td>
<td>745</td>
<td>423</td>
<td>-</td>
<td>233</td>
<td>-</td>
<td>-</td>
<td>7-45 U/l</td>
</tr>
</tbody>
</table>

- Not found or not performed

Two days later, 27th January (12th day after onset), flaccid paraparesis was observed, the fever returned and leucocytes reached 18,000/mm³ (84% neutrophils). Cerebrospinal fluid (CSF) showed 21 cells/mm³ (100% mononuclear cells), a slight increase of protein (89mg/dL), but chlorides and glucose had normal values. A CT scan (brain, column and chest) showed bilateral pneumonia, while magnetic resonance of CNS did not detect abnormalities. Ceftriaxone was used. From 28 January through 6 February clinical examination was unchanged. On 7th February the patient reported diminution in the field of vision, especially in the right eye, and on ophthalmologic examination chorioretinitis was diagnosed compatible with residual toxoplasmosis.

DEN-2 virus was isolated in C6/36 cells from the blood obtained on 19 January. Serology for dengue in paired samples showed serologic conversion (acute sample negative, collection on 18th January) and...
convalescent positive (obtained on 25th January) for IgM-ELISA; by HI titres were < 1:20 in acute and > 1:1280 [secondary response for all flaviviruses tested (DEN-1, DEN-2, DEN-3, DEN-4, yellow fever, Ilheus, Pocoio and Saint Louis Encephalitis viruses)], and negative in both samples for alphaviruses (Eastern Equine, Western Equine and Venezuelan Equine (Subtype III - Mucambo) encephalitis, and Mayaro viruses), and bunyaviruses (Oropouche, Tacaiuma, Guaroa, Caraparu, Catu and Maguari viruses). The CSF test was negative for dengue, syphilis, cysticercosis, and toxoplasmosis, as well as cultures for bacteria, mycosis and viruses. RNA was detected by RT-PCR to DEN-2 from acute phase serum and from culture supernatants, but was negative in the CSF.

Other specific exams (for differential diagnosis) were carried out from blood collected on different days of the illness and showed the following results: Serology - IgM-ELISA were negative to toxoplasmosis (IgG positive), CMV (IgG positive), EBV and other herpes viruses (IgG negative), rubella (IgG positive), HIV (IgG negative), HTLV I (IgG negative), HTLV II (IgG negative), HAV (anti HAV IgM), HBV (HbsAg and anti HBc IgM), and HCV (anti HCV IgM). Hemoculture (three samples) was negative, and culture of urine (two samples) was also negative. Exams for systemic lupus erythematosus (antineuclear factor, anti DNA antibodies, SSB/LA, SM and SSA/RO) were negative.

Dengue viruses have been striking the inhabitants of Belém from 1996 to date. The occurrence of cases increases annually during the rainy season that occurs between December and May. The case reported occurred in January, when the rains are daily, and the vector population (Aedes aegypti) increases.

The DEN-2 serotype was isolated from a blood sample and RNA of the virus was recovered from acute phase serum and from culture supernatants by RT-PCR. Moreover, serologic conversion to flaviviruses was obtained. Thus, the etiology of the illness was the DEN-2 virus. The question is if the neurological involvement reported in the case was DEN-2 associated or if it resulted from metabolic alterations.

The pathogenesis of DHF and DF is complicated and still little understood. The main hypotheses are not compatible for neurological involvement, and cases with central nervous system (CNS) involvement are generically included as dengue with unusual manifestations.

Thus, the occurrence of neurological complications during dengue infections is debatable and not well understood. Frequently dengue infection has been associated with encephalopathy followed by shock and hypoxemia of CNS, but articles published elsewhere have shown that in some cases the criteria for breakdown of the blood-brain barrier of the CNS have been fulfilled. Effectively, Lum et al. found dengue virus antigens in Virchow Robin space, both articles support or point to the hypothesis that dengue viruses can infect the CNS.

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