PROSPECTIVE CASE-CONTROL STUDY OF ENCEPHALOPATHY IN CHILDREN WITH DENGUE HEMORRHAGIC FEVER

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Abstract. We present a prospective case-control study of 27 serologically confirmed dengue hemorrhagic fever (DHF) patients with severe central nervous system symptoms. Dengue associated encephalopathy accounted for 0.5% of 5,400 patients admitted with DHF. The mortality rate among children with encephalopathy was 22%, with the survivors experiencing a complete recovery. Liver enzymes and bilirubin were significantly elevated in the study group. In analysis of the cerebrospinal fluid (CSF), reverse transcriptase-polymerase chain reaction revealed dengue-3-specific RNA in one evaluated case. Dengue-specific immunoglobulin M was detected in CSF in 14 of 22 assessable patients, indicating a localized infection. Magnetic resonance imaging scans showed cerebral edema in the majority of patients, although encephalitis-like changes were less common. There was an equal distribution of primary and secondary infections. On the basis of previous reports and of the findings of our study, DHF probably encompasses an expanding clinical spectrum that infrequently involves encephalitis due to a direct neurotropic effect of dengue virus.

INTRODUCTION

Dengue viruses are single-stranded RNA arboviruses and members of the Flaviviridae family. Infection by any of the 4 serological types of dengue viruses (dengue-1–4) causes dengue fever and dengue hemorrhagic fever (DHF), which represents an increasingly important public health problem in Western Pacific regions and in Southeast Asia. Unlike most other arboviral diseases, dengue virus does not usually cause neurologic symptoms. In recent years, we and others have occasionally received DHF patients with symptoms of encephalopathy. The pathophysiology of the neurologic symptoms in the reported cases have been attributed to cerebral edema, anoxia, hemorrhage, hyponatremia, hepatic failure, microcapillary hemorrhage, and release of toxic substances. Animal and clinical studies have recently suggested a neurotropic potential of dengue virus leading to encephalitis in some cases.

We present a prospective case-control study of 27 serologically confirmed DHF patients with severe central nervous system (CNS) symptoms. The patients and the controls underwent clinical and paraclinical examinations; the information we gathered helped us elucidate contributing factors in dengue-associated encephalitis.

PATIENTS AND METHODS

A prospective case-control study was carried out at Children’s Hospital No. 1, Ho Chi Minh City, Vietnam, October 1997–October 1999. Informed consent was obtained from parents or legal guardians; the hospital ethical review board approved the project. Pending parental consent and a stable hemodynamic condition, children aged <16 years with serologically verified DHF and CNS symptoms admitted at the intensive care unit were enrolled in the study. Possible CNS symptoms included convulsions, spasticity, focal neurological signs, and altered level of consciousness for >8 hr (i.e., drowsiness, lethargy, agitation, or coma). The next patient admitted to the hospital without CNS symptoms but with the same age and sex and an identical grade of DHF was included in the control group. Children with chronic liver or renal failure, purulent meningitis, cerebral malaria, cerebral palsy, or epilepsy were excluded.

When the patient was included in the study, we established the grade of DHF and the presence of hemorrhagic symptoms, and we also took a medical history. A neurologist evaluated the level of consciousness according to the Glasgow Coma Score from Days 1–6. The neurological status was evaluated until total recovery and for a minimum of 3 months. Paraclinical tests included glucose, electrolytes, carbamide, creatinine, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), ammonium, and prothrombin. Serological tests, including enzyme-linked immunosorbent assay and hemagglutination inhibition tests for both dengue virus and Japanese encephalitis virus, were performed. A lumbar puncture was carried out in members of the study group, and the cerebrospinal fluid (CSF) was analyzed for the presence of protein, glucose, cells, and dengue virus—and Japanese encephalitis virus-specific antibodies. Reverse transcriptase-polymerase chain reaction (RT-PCR) and virus isolation were performed at the Pasteur Institute, Ho Chi Minh City. The study group underwent magnetic resonance imaging (MRI) scans (evaluated by the same radiologist), and children aged <1 year were additionally subjected to a cerebral ultrasound scan. All patients in the study and control groups received treatment according to the World Health Organization guidelines for dengue fever.

RESULTS

Clinical characteristics. During the 2-year study period, 5,400 patients with DHF were admitted to the hospital, of whom 224 were referred to the intensive care unit. A total of 27 patients, 10 girls and 17 boys, with dengue encephalopathy were enrolled in the study (incidence of 0.5%). The median age was 7 years (range, 8 months to 15 years), in- cluding 6 (22%) infants aged <1 year and 19 (70%) children aged ≥6 years. When classifying the patients, 33% had DHF grade II, 52% had DHF grade III, and 15% had DHF grade IV. The control group matched the study group for all these parameters. The time from onset of disease to development of neurological symptoms showed a median of
Results showed that infiltration of virus-infection and CSE in other studies were associated with decreased CSE. A growing number of case reports regarding possible neurologic involvement in DHF prompted us to investigate this relationship in a prospective case-control study. During the 2-year study period, patients with dengue-associated encephalopathy accounted for 0.5% of all patients admitted with DHF. In a retrospective survey, Hendarto and Hadinegoro found an incidence of 6.2%, but contrary to the patients included in our study, the reported patients appeared to be only mildly affected, with just 14% in a state of stupor or coma. In addition, a number of patients with DHF and concurrent neurologic symptoms have been described, mainly as case reports or as part of minor series of patients with unusual manifestations of DHF.11,12 A survey describing 9 patients with encephalitis in association with dengue infection detected involvement of the CNS in 4 patients by dengue viral antigens were detected in CSF and serum.1 Finally, dengue viral antigens were detected in CSF and serum.16,19

In previous reports of neurologic involvement in dengue infections, the observed encephalopathy was thought to be due to prolonged DHF with fluid extravasation, cerebral edema, hyponatremia, and liver failure, renal failure, or both, as opposed to encephalitis defined by a localized invasion of the CNS. Recent reports, however, have demonstrated a possible direct neurotropic effect of dengue virus. Animal studies in mice showed a virus-induced, cytokine-mediated breakdown of the blood-brain barrier.11 A survey describing 9 patients with encephalitis in association with a dengue infection detected involvement of the CNS in 4 patients by the presence of virus or IgM antibodies in CSE. In another study, dengue virus was observed in the CSE in 5 of 6 patients presenting with encephalitis, indicating that virus may cross the blood-brain barrier and directly invade the brain.13 Search for viral RNA or viral isolation was negative in the remaining patient, but specific IgM was found in the CSE.14 Detection of IgM in CSE is indicative of viral replication in CNS, but the titer is generally lower and short-lived when compared with serum.15 Finally, dengue viral antigens were demonstrated by immunohistochemistry in CNS biopsies from 5 fatal cases of dengue infection associated with encephalopathy.6 Results showed that infiltration of virus-infected macrophages could be one of the pathways by which the virus may enter the brain in dengue encephalitis.6

**DISCUSSION**

Dengue virus is a member of the Flaviviridae family, which includes a number of neurotropic viruses such as Japanese encephalitis virus, St. Louis encephalitis virus, and tick-borne encephalitis virus. A growing number of case reports regarding possible neurologic involvement in DHF prompted us to investigate this relationship in a prospective case-control study.

During the 2-year study period, patients with dengue-associated encephalopathy accounted for 0.5% of all patients admitted with DHF. In a retrospective survey, Hendarto and Hadinegoro found an incidence of 6.2%, but contrary to the patients included in our study, the reported patients appeared to be only mildly affected, with just 14% in a state of stupor or coma. In addition, a number of patients with DHF and concurrent neurologic symptoms have been described, mainly as case reports or as part of minor series of patients with unusual manifestations of DHF.11 A survey describing 9 patients with encephalitis in association with dengue infection detected involvement of the CNS in 4 patients by dengue viral antigens were detected in CSF and serum.1 Finally, dengue viral antigens were detected in CSF and serum.16,19

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**Microbiological findings.** All patients in the study and control groups had an acute dengue infection as evidenced by the finding of a positive hemagglutination inhibition test.1 Finally, dengue viral antigens were detected in CSF and serum.16,19

**Laboratory findings.** Because of severe coagulation disorders or convulsions, lumbar puncture was performed in only 22 patients; the control group was not subjected to this procedure for ethical reasons. Protein, glucose, and cells in the CSF were all normal. All liver enzyme (AST, ALT, and alkaline phosphatase) and bilirubin levels were significantly decreased in the control group (Table 1). Platelets, serum potassium, ionized serum calcium, creatine, and ammonia showed no statistical differences between the 2 groups of patients.

**Follow-up.** The mortality rate was 22%, with the survivors experiencing a complete recovery of consciousness and neurologic symptoms within a maximum of 7 days.

**Comparison between the 14 CSF IgM-positive and 8 CSF IgM-negative patients within the study group showed no statistical differences regarding clinical characteristics, MRI scans, laboratory findings, or outcome.**

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**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter (normal range)</th>
<th>Study group</th>
<th>Control group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>31.4 ± 7.7</td>
<td>35.5 ± 5.5</td>
<td>0.027</td>
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<tr>
<td>Leukocytes (mm³)</td>
<td>8,378 ± 4,244</td>
<td>5,648 ± 2,440</td>
<td>0.005</td>
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<tr>
<td>Platelets (10⁹/L)</td>
<td>76 ± 54</td>
<td>60 ± 26</td>
<td>0.168</td>
</tr>
<tr>
<td>Glycemia (75–105 mg %)</td>
<td>84 ± 43</td>
<td>106 ± 21</td>
<td>0.024</td>
</tr>
<tr>
<td>Serum sodium (135–145 mmol/L)</td>
<td>135.3 ± 5.9</td>
<td>131.8 ± 3.5</td>
<td>0.011</td>
</tr>
<tr>
<td>Uremia (15–40 mg %)</td>
<td>48 ± 25</td>
<td>35 ± 15</td>
<td>0.021</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>60 ± 41</td>
<td>50 ± 27</td>
<td>0.346</td>
</tr>
<tr>
<td>Aspartate aminotransferase (31–38 U/L)</td>
<td>2,751 ± 3,885</td>
<td>234 ± 485</td>
<td>0.001</td>
</tr>
<tr>
<td>Alanine aminotransferase (32–40 U/L)</td>
<td>984 ± 777</td>
<td>81 ± 152</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bilirubin total (1 mg %)</td>
<td>1.22 ± 0.97</td>
<td>0.35 ± 0.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alkaline phosphatase (&lt;350 U/L)</td>
<td>279 ± 227</td>
<td>161 ± 59</td>
<td>0.015</td>
</tr>
<tr>
<td>Prothrombin (70–100%)</td>
<td>42 ± 25</td>
<td>61 ± 23</td>
<td>0.005</td>
</tr>
<tr>
<td>D-dimer (positive)</td>
<td>17</td>
<td>6</td>
<td>0.007</td>
</tr>
</tbody>
</table>

* Values are expressed as mean ± standard deviation. Student's t-test was used for calculating all probabilities except D-dimer, for which the chi-square test was used.
accordance with these recent reports, we found 14 (64%) of 22 patients had dengue-specific IgM in the CSF, indicating a localized infection in the CNS. One patient also exhibited dengue RNA when examined by RT-PCR. Interpretation of PCR results in viral infections may be difficult, but patients with a positive PCR result in CSF have a very high probability ratio for a possible diagnosis of viral infection of the CNS. On the basis of previous reports and of the findings of our study, DHF probably encompasses an expanding clinical spectrum that infrequently involves encephalitis due to a direct neurotropic effect of dengue virus.

Among members of Flaviviridae, antigenic cross-reactivity appears to involve a group-reactive antigen shared by all members. In people with previous nondengue flavivirus infection such as Japanese encephalitis, these circulating low-titered antibodies may show cross-reactivity to dengue virus. Accordingly, dengue-specific IgM was detected in CSF in 14 of 22 assessable patients, whereas Japanese encephalitis IgM was found in 7 of the 14 dengue-positive patients, but at a lower titer. This suggests that detection of both specific dengue and Japanese encephalitis antibodies should be carried out in regions where these 2 viruses co-circulate.

The severity of neurologic disease caused by the different dengue serotypes has been examined in a number of studies. In conclusion, serotypes 2 and 3 have primarily been reported to cause neurologic symptoms, and these patients may experience both primary and secondary infections. In our study group, there was an equal distribution of primary and secondary infections. Four patients with primary infection were infants in whom maternal antibodies may have caused immune enhancement. We did not determine the dengue serotype, but at the time of the study, dengue-2 and dengue-3 were the most prevalent in the community.

Computed tomography has been used to demonstrate cerebrospinal edema in a few patients with dengue-associated encephalopathy. Magnetic resonance imaging is superior to computed tomography scans when demonstrating most CNS lesions. Present, for the first time, MRI scans in a cohort of patients with dengue encephalopathy. The majority of patients had cerebral edema; encephalitis-like changes were less common. Possibly, the neurologic symptoms we observed may only infrequently be caused by bleeding, because just one patient had intracranial hemorrhage.

Abnormal levels of liver enzymes in patients with DHF are a common finding and may reflect liver dysfunction. It is reasonable to assume that DHF in some cases may cause a hepatic encephalopathy, as reported numerous times. As seen in Table 1, we found a significant elevation of liver enzymes (AST and ALT) that probably was a major contributing factor in the development of the observed neurologic symptoms. It is equally true, however, that we found evidence of viral replication in CNS in a large proportion of the patients we studied, which in our view justifies the use of the term "dengue-associated encephalitis." Unfortunately, we do not know whether DHF patients without neurologic symptoms have evidence of viral replication in the CNS.

In conclusion, dengue encephalitis seems to be a true, albeit rare, entity. The patients are characterized by marked depression of consciousness, significantly elevated liver enzymes, and a substantial mortality; the survivors, on the other hand, experienced complete recovery. Pathophysiologic findings include cerebral edema as well as specific IgM and RNA in CSF.

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