Dengue and Dengue Hemorrhagic Fever among Adults: Clinical Outcomes Related to Viremia, Serotypes, and Antibody Response

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Background. Clinical manifestations of dengue vary in different areas of endemicity and between specific age groups, whereas predictors of outcome have remained controversial. In Brazil, the disease burden predominantly affects adults, with an increasing trend toward progression to dengue hemorrhagic fever (DHF) noted.

Methods. A cohort of adults with confirmed cases of dengue was recruited in central Brazil in 2005. Patients were classified according to the severity of their disease. Associations of antibody responses, viremia levels (as determined by real-time polymerase chain reaction [PCR]), and serotypes (as determined by multiplex PCR) with disease severity were evaluated.

Results. Of the 185 symptomatic patients >14 years of age who had a confirmed case of dengue, 26.5% and 23.2% were classified as having intermediate dengue fever (DF)/DHF (defined as internal hemorrhage, plasma leakage, manifested signs of shock, and/or thrombocytopenia [platelet count, ≤50,000 platelets/mm3]) and DHF, respectively. The onset of intermediate DF/DHF and DHF occurred at a late stage of disease, around the period of defervescence. Patients with DHF had abnormal liver enzyme levels, with a ~3-fold increase in aspartate aminotransferase level, compared with the range of values considered to be normal. Overall, 65% of patients presented with secondary infections with dengue virus, with such infection occurring in similar proportions of patients in each of the 3 disease category groups. Dengue virus serotype 3 (DV3) was the predominant serotype, and viremia was detected during and after defervescence among patients with DHF or intermediate DF/DHF.

Conclusions. Viremia was detected after defervescence in adult patients classified as having DHF or intermediate DF/DHF. Secondary infection was not a predictor of severe clinical manifestation in adults with infected with the DV3 serotype.
and the number of DHF-associated hospitalizations during the past decade [9, 11, 12]. These data indicate a shift from mild illness toward a more severe clinical manifestation that could be interpreted as an epidemiologic transition pattern. In Brazil, DF and DHF/DSS are predominantly reported among adults; in contrast, in other countries in the Americas and in Southeast Asia, children are the most affected subpopulation [9, 13–16].

Several studies have explored the risk factors that may predict progression of disease from DF to DHF and/or DSS [17–20]. Issues related to immune status, population genetics, viremia titers, distinct serotypes, and the proinflammatory cytokines elicited were investigated predominantly among children in Southeast Asian countries [20–22]. Age at infection and the presence of secondary DV infection due to heterologous serotypes have been associated with disease severity, with the latter risk factor known as the “antibody-dependent enhancement theory” [23]. However, predictors of disease severity are not consistent across all settings [5, 22, 24]. In the present study, we assessed the association of disease severity with primary or secondary DV infections, viremia titers, and DV serotypes among adults during the months when dengue is at its peak in central Brazil. The objective of the study was to determine whether secondary infection or viral titers were related to the clinical outcomes in a cohort of adult patients with dengue.

**METHODS**

**Study population and setting.** A clinical cohort of patients with suspected cases of dengue was recruited in the city of Goiânia (population, ~1.2 million) in the midwestern region of Brazil, during an outbreak occurring from January 2005 through July 2005. The first outbreak of infection due to DV1 in the region was reported in 1994, with sequential introduction of DV2- and DV3-associated outbreaks noted in 1999 and 2002, respectively. According to regional laboratory surveillance, there was a shift in predominance of infection with DV1 to infection with DV3 in the city, with cocirculation of 3 serotypes [25]. Patients were enrolled in the reference infectious diseases hospital (Hospital of Tropical Diseases), in one private hospital, and in primary health care units in the same catchment area. The study protocol was approved by the institutional ethics review board. Signed, informed consent was obtained from all participants or legal guardians.

**Design and data collection.** The inclusion criteria were age ≥15 years and laboratory-confirmed dengue. We prospectively collected baseline demographic and clinical information from all patients, by use of a standard study protocol. Data on age, sex, previous dengue episodes, and key warning signs of illness (e.g., hypotension, intense abdominal pain, and significant bleeding) were recorded. Clinical data and laboratory test results were recorded in each patient’s file and were checked by the clinical coordinator. The duration of follow-up for hospitalized patients was defined as the period from the first medical visit to either the discharge date or death. The duration of follow-up for outpatients was measured as the interval between the first and second blood collections performed during the convalescent phase of disease (~15 days).

**Dengue classification.** DF was defined by the presence of acute febrile illness and ≥2 of the following symptoms: headache, retro-orbital pain, myalgia, arthralgia, rash, and hemorrhagic manifestations. DHF was defined as fever with thrombocytopenia (platelet count, ≤100,000 platelets/mm³), any hemorrhagic manifestation or positive tourniquet test result, and evidence of plasma leakage (as denoted by a ≥20% increase in the hematocrit from the baseline value or by the presence of pleural or abdominal effusion or hypoalbuminemia). DHF was classified into 4 grades of severity, according to definitions of the World Health Organization (WHO): for grade 1, a positive tourniquet test result was the only hemorrhagic manifestation; for grade 2, spontaneous bleeding was noted; for grade 3, hypotension, a narrow pulse pressure (≤20 mm Hg), restlessness, and a rapid, weak pulse were noted; and, for grade 4, shock was identified but blood pressure or pulse was undetectable [1]. Intermediate DF/DHF was defined as internal hemorrhage, plasma leakage, manifested signs of shock, and/or thrombocytopenia (defined by a platelet count of ≤50,000 platelets/mm³). This group comprised patients who did not fulfill the criteria for DHF/DSS. This intermediate DF/DHF category is in concordance with current case definitions for clinical management published by the Brazilian Control Program Guideline [26]. To grade disease severity, other studies have adopted similar clinical groups [17, 27, 28]. Cases of dengue were confirmed by (1) DV isolation or DV RNA detection by multiplex polymerase chain reaction (PCR) or real-time PCR or (2) IgM antibody capture (MAC) ELISA in the first or second paired samples. Primary and secondary infections were identified using an IgG avidity test [29].

**Definition of variables.** The first 5 days after onset of symptoms were considered to denote the early, acute phase of illness. Illness “day 1” was defined as the day of onset of symptoms. “Day 0” was defined as the day of defervescence, “day –1” was defined as the day before defervescence, and “day +1” was defined as the day after defervescence. Hospitalized patients remained within a hospital ward for at least 24 h. Patients receiving day care were patients who stayed in the hospital for intravenous fluid replacement for <24 h. Ambulatory patients were defined as those patients who attended outpatient clinics without requiring any hospitalization during the current episode of illness.

**Virologic and serologic assays.** Blood samples (10 mL) were collected at the initial clinical visit and ~15 days after the onset of symptoms. Serologic testing of serum samples collected during the acute and convalescent phases of illness was performed using in-house dengue MAC ELISA [30]. For specimens collected until day 5 after the onset of symptoms, virus isolation...
was performed using a monolayer of C6/36 Aedes albopictus cells [31], and DV isolates were identified using an indirect fluorescent antibody test that used serotype-specific monoclonal antibodies. Serologic and virologic tests were performed at the regional referral laboratory (Laboratório Central de Saúde Pública de Goiás [LACEN-Go]), according to the recommendation of the Dengue Control Program [25]. All samples were also evaluated by use of an IgG avidity test, real-time PCR, and multiple PCR performed in the virology laboratory at the Institute of Tropical Medicine, University of São Paulo, São Paulo, Brazil [32]. All tests were performed without the investigators having previous knowledge of the epidemiologic and clinical data.

The IgG avidity test was performed on acute- and convalescent-phase samples that were IgG positive and were obtained within 30 days after the onset of symptoms, by use of an “in-house” ELISA. In brief, antigens were prepared with A. albopictus C6/36 cells infected with DV1–4 and disrupted by sonication. The avidity index, expressed as a percentage, was calculated by determining the ratio of optical density with urea to the optical density without urea and then multiplying that value by 100 [29]. A receiver-operating characteristic curve analysis performed using Analyze-it software (version 1.73; Analyze-it Software) was used to evaluate the ability of the avidity test to distinguish between primary and secondary dengue infections. The cutoff point was defined as the highest sum of the estimates of sensitivity and specificity. Secondary infection was defined by an IgG avidity index cutoff point of 30%.

**RNA extraction.** RNA was extracted in duplicate from 140 µL of plasma, by use of the Qiagen Viral RNA Kit (Qiagen). Elution was performed in 60 µL of elution buffer, according to the manufacturer’s instructions.

**cDNA synthesis and multiplex RT-PCR.** cDNA was synthesized and multiplex PCR performed as described below. In brief, 5 µL of cDNA was added to 20 µL of a PCR mix consisting of primers D1, TS1, and TS2 at 0.5 mol/L; 3 mmol/L MgCl₂; 1 × PCR buffer; 200 µmol/L dNTPs; and 1.25 U of Platinum Taq polymerase (Invitrogen). Fragments of different lengths were obtained from dengue serotypes: a 482-bp fragment was obtained from DV1, a 119-bp fragment from DV2, a 290-bp fragment from DV3, and a 389-bp fragment from DV4 [33].
Real-time PCR. Duplicates of 10 μL of RNA eluate were directly applied to a dengue real-time commercial kit (RealArt; artus/Qiagen) in a final volume of 25 μL and were analyzed on ABI 7300 real-time equipment (Applied Biosystems). The mean value of the duplicate was adopted. A previous study evaluated the use of real-time PCR for the diagnosis of dengue in samples collected from all enrolled patients [32].

Laboratory values. Hematocrit and platelet analyses were performed for all patients with dengue, as recommended by the Brazilian Clinical Guideline for Management [26]. Hematocrit and platelet data for hospitalized patients were collected at least once per day. Data on serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and albumin were obtained and recorded at study entry for hospitalized and day care patients; data were obtained again when clinical manifestations were noted, and nadir values were reported. The reference values for normality were a serum AST level of ≤45 U/L, a serum ALT level of ≤43 U/L, and an albumin level of 3.5–5.0 g/dL. Chest radiography and ultrasonography were performed for patients with suspected pleural effusions or ascites, for further documentation of plasma leakage.

Statistical analysis. The positive predictive accuracy (PPA) of the clinical diagnosis was defined as the number of laboratory-confirmed cases divided by the total number of suspected cases. We compared the clinical characteristics and laboratory results of the patients, according to health care settings and severity. The χ² test was applied for categorical variables, and the Kruskal-Wallis and Mann-Whitney tests were used for comparison of median values.

Table 2. Antibody response patterns, dengue viremia titers, and serotypes noted for 185 patients with confirmed dengue, according to clinical category of disease.

<table>
<thead>
<tr>
<th>Variable</th>
<th>DHF (n = 43)</th>
<th>Intermediate DF/DHFa (n = 49)</th>
<th>DF (n = 93)</th>
<th>P*</th>
</tr>
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<tbody>
<tr>
<td>Antibody response patternc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary infection</td>
<td>13 (31.0)</td>
<td>14 (29.8)</td>
<td>36 (39.1)</td>
<td>.46</td>
</tr>
<tr>
<td>Secondary infection</td>
<td>29 (69.0)</td>
<td>33 (70.2)</td>
<td>56 (60.9)</td>
<td></td>
</tr>
<tr>
<td>Serum samples positive for viremia,d n/N*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At ≤5 daysf</td>
<td>6/11</td>
<td>15/25</td>
<td>42/61</td>
<td>.79</td>
</tr>
<tr>
<td>At &gt;5 daysf</td>
<td>5/32</td>
<td>1/24</td>
<td>4/32</td>
<td>.40</td>
</tr>
<tr>
<td>Viral load,c median (range), log₁₀ RNA copies/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At ≤5 daysf</td>
<td>4.45 (2.87–6.30)</td>
<td>4.78 (2.36–8.01)</td>
<td>5.62 (2.23–8.98)</td>
<td>.05</td>
</tr>
<tr>
<td>At &gt;5 daysf</td>
<td>3.51 (2.69–5.26)</td>
<td>. .</td>
<td>3.10 (1.38–3.41)</td>
<td>.33</td>
</tr>
<tr>
<td>Serotypesg</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DENV-3</td>
<td>8 (80.0)</td>
<td>10 (100.0)</td>
<td>38 (95.0)</td>
<td>NA</td>
</tr>
<tr>
<td>DENV-2</td>
<td>2 (20.0)</td>
<td>. .</td>
<td>1 (2.5)</td>
<td></td>
</tr>
<tr>
<td>DV2/DV3</td>
<td>. .</td>
<td>. .</td>
<td>1 (2.5)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients, unless otherwise indicated. Dengue hemorrhagic fever (DHF) and dengue fever (DF) were defined according to World Health Organization criteria. NA, not available.

d Dengue characterized by internal hemorrhage, plasma leakage, manifested signs of shock, and/or thrombocytopenia (platelet count, ≤50,000 platelets/mm³).

e The χ² test was used for categorical variables, and the Kruskal-Wallis and Mann-Whitney tests were used for comparison of median values.

f A total of 181 patients had their immune status assessed by use of an IgG avidity test.

g As determined by reverse-transcription polymerase chain reaction (PCR).

h The no. of samples with positive viremia/total no. of samples tested.

t After the onset of symptoms.

i As determined by multiplex PCR.

RESULTS

Patient characteristics. A total of 226 patients ≥15 years of age underwent screening, and 185 of these patients had laboratory-confirmed dengue: 76 (41.1%) had cases confirmed by real-time PCR and/or multiplex PCR and/or viral culture, and 109 (58.9%) had cases confirmed by IgM serologic tests. The ratio of females to males was 1.3:1.0. Almost one-half of the patients were admitted to a hospital ward, 34.6% were monitored in day care settings, and 17.8% were ambulatory patients. The PPA of the suspected clinical diagnosis was ~80%, with similar percentages noted for specific sex and age groups.

Table 1 presents the demographic characteristics of and clinical and laboratory parameters for patients with dengue, according to disease category. Of 185 laboratory-confirmed cases of dengue, 43 (23.2%) were classified as DHF and 93 (50.3%) were classified as DF. Among patients with DHF, 32 (74.4%) pre-
sent with grade 1 or 2 disease, and 11 (25.6%) had hypoten-
sion and/or shock (grade 3 or 4 disease), with 2 deaths occurring. Forty-nine patients were classified as having intermediate DF/ DHF and presented with ≥1 of the following severe manifesta-
tions of dengue: signs of shock (11 patients), internal hemor-
rhage (11 patients), plasma leakage (10 patients); and thrombocytopenia (34 patients). Mean age (±SD) was similar among patients, regardless of disease category, and females were slightly outnumbered by males in all groups. All patients with DHF were hospitalized, except for 4 day care patients. The mean duration of hospitalization (±SD) was 4.3 ± 2.6 days. One-half of the patients with intermediate DF/DHF required hospitalization (mean duration, 3.4 days), and ~25% of patients with DF were also hospitalized (mean duration, 2.6 days). Spontaneous hemorrhage was more frequently observed among patients with DHF (83.7% of patients) and patients with intermediate DF/ DHF (53.1% of patients) than among patients with DF (37.6%) (P < .01). Approximately 70% of patients with intermediate DF/DHF had a platelet count nadir of <50,000 platelets/mm³. Almost one-half of patients with DF had a platelet count of <100,000 cells/mm³. The incidence of bleeding was negatively correlated with the platelet count (P < .01). A hematocrit in-
crease of ≥20% was detected in ~50% of patients with DHF. For the remaining patients, plasma leakage was documented by ei-
ther pleural or abdominal effusion or hypoalbuminemia. The median albumin level was significantly different among patients in different disease groups (P < .01). Liver involvement, as de-
noted by AST and ALT levels, was greater among patients with DHF and severe manifestations than among patients with DF (P < .05). Of patients with DHF, 62.2% had a >3-fold increase in the serum AST level (compared with reference values for lev-
els considered to be normal), whereas patients with intermediate DF/DHF and those with DF had 38.7% and 34.1%, respectively, which was a statistically significant difference (P = .03).

Assignment to primary or secondary infection group. Overall, 65% of patients presented with secondary infections. There were no statistically significant differences in the percent-
age of patients with secondary infection among the groups. Approx-
imately 70% of patients with intermediate DF/DHF and 61% of patients with DF had secondary infections (P = .46) (table 2). There was a trend toward an increase in the develop-
ment of secondary infection according to age group, with 53.3% of patients 15–25 years of age and 80.8% of patients >50 years of age developing such infection.

Viremia and serotypes. Of the samples collected within 5 days of the onset of symptoms, the percentage that were positive for viremia, as determined by molecular tests, was 50%–68% among the disease groups (P = .79). The percentage of samples positive for viremia varied from 4% to 15% for samples collected 5 days after the onset of symptoms, and the percentage was not statisti-
cally significant different among groups (P = .40). Median vi-
remia titers were similar among patients with DHF or inter-
mediate DF/DHF (4.45 and 4.78 log₁₀ RNA copies/mL, re-
spectively) and patients with DF (5.62 log₁₀ RNA copies/mL), for samples collected within 5 days of the onset of symptoms. DV3 was the predominant serotype, as determined by multiplex PCR, and 3 patients had DV2 infection (table 2). Dual infection (with DV2 and DV3) was detected, by means of multiplex PCR, in 1 patient, who demonstrated the following characteristics: mild illness, serologic test results compatible with secondary in-
fec tion, and a viral load of 2.22 log₁₀ copies/mL.

Figure 1 presents the box-plot distribution of the viral load by disease category and by primary and secondary dengue infection, considering samples collected within 5 days of the onset of symptoms. There was no statistical difference between the vire-
mia levels noted in association with primary and secondary in-
fec tions, for each disease category.

Figure 2 shows the viral titers for specimens collected within 5 days of the onset of symptoms, as stratified by disease category and type of attendance according to defervescence day. For ambulatory patients with DF, a median viral titer of 5.51 log₁₀ RNA copies/mL was detected. A similar result (4.84 log₁₀ RNA copies/mL) was found among patients with intermediate DF/DHF who were hos-
pitalized or in day care settings. At defervescence (day 0), median viremia titers were similar among patients with severe cases and patients with DF. Patients with DHF or intermediate DF/DHF had late viremia from days 1–3 after defervescence.
DISCUSSION

Our findings showed that, in the epidemiologic scenario, mild cases of dengue were predominant among adults, and severe cases due to DV3 were not associated with secondary infection. Most patients with DHF or intermediate DF/DHF were >5 days beyond the onset of symptoms at the point-of-care setting. Of interest, plasma viremia remained detectable during the postdefervescence period among patients with severe disease.

In the present study, ~70% of patients with severe illness had secondary infection, a finding similar to the percentage of patients with DF who had secondary infection (60%). These findings are in agreement with the clinical, epidemiologic, and virologic characteristics noted in dengue studies that also showed the lack of association between disease severity and secondary dengue infection in Nicaragua [17, 34]. Of interest, DV3 was the predominant serotype in our setting as well as in the study by Harris et al. [34]; this suggests that primary infections with this serotype may lead to a severe clinical outcome in adults. These findings support the role of virus virulence as a potential risk factor for disease severity [14, 20, 35, 36].

Our findings in adults contrast with prospective studies conducted in Asia among children [20, 23]. In these studies, an increased relative risk for severe disease was noted among children with evidence of previous DV infection, in accordance with the established immune enhancement theory. Fatal cases were reported in the course of primary infection in Brazil [37]. In a retrospective study in Thailand, secondary dengue infection was significantly associated with DHF outcomes in children, but not in adults [18]. In Cuba, epidemiologic studies documented that the majority of overt cases (DF or DHF cases) were secondary dengue infection with DV1 and/or DV2 serotypes [38]. In our

Figure 2. Levels of dengue viremia, by “fever day,” in plasma samples collected within 5 days of the onset of symptoms, according to whether the patient was receiving care in a hospital/day care setting or was an ambulatory patient receiving outpatient care. Fever day 0 was defined as the day that defervescence occurred, day −1 was defined as 1 day before defervescence, and day +1 was defined as 1 day after defervescence. Dashed lines denote median levels of viremia. For each disease category shown in the key, the n/N values denote the no. of samples with positive viremia/the total no. of samples tested. DF, dengue fever; DHF, dengue hemorrhagic fever; intermediate DF/DHF, dengue characterized by internal hemorrhage, plasma leakage, manifested signs of shock, and/or thrombocytopenia (platelet count, ≤50,000 platelets/mm^3).
results, ~30% of primary infections were found in patients with severe and nonsevere cases, in contrast with findings from the Cuban experience. A possible explanation is the more recent circulation of DV in central Brazil, since the first outbreak of DV1 infection occurred in 1994 [25]. In addition, in this study area, a previous household survey conducted in 2000 detected an overall prevalence of 30%, which increased with age, indicating a large susceptible population [6].

The hemagglutination inhibition test or the IgM/IgG ELISA ratio determinations are traditionally performed to define primary and secondary dengue infections [39]. In a previous study, the IgG avidity test exhibited sensitivity and specificity equivalent to those of the IgM/IgG ELISA ratio determinations and the IgG titer assay for discriminating primary from secondary acute DV infection, with all 3 assays maintaining high sensitivity (100%) and specificities (range, 95.7%–97.8%) [40]. The WHO/Special Programme for Research and Training in Tropical Diseases (TDR) report stated that the IgG avidity ELISA can be used to differentiate primary from secondary infection, and it considers this technique to be more useful than the hemagglutination inhibition test for this purpose [5, 41]. The possible identification of secondary flavivirus infection rather than true secondary DV infection cannot be ruled out. However, if a cross-reaction occurred, it would have resulted in overestimation of the secondary infection rates.

A number of studies, mainly those conducted among children in Asia, have correlated viremia with dengue severity [19, 20]. In a study conducted in Taiwan, patients with DHF had a higher viral load and a slower rate of clearance of viremia than did adult patients who had DF with secondary DV2 infection [42]. Our results showed detectable viremia levels from days 1–3 after the defervescence period among groups in the adult population with DHF or intermediate DF/DHF, by use of real-time PCR. The possible explanations for detectable viremia among patients with DHF at defervescence and afterward should consider, in addition to prolonged viremia, differences in the viremia clearance rates and the contribution of primary and secondary infection to the clearance rate [42], although this study was not designed to describe the kinetics of viremia. In the DF group, the fact that few patients were tested for viremia in the defervescence period precludes meaningful comparisons of these patients with patients with severe disease. In our setting, 75% of patients with DHF and intermediate DF/DHF arrived at the point of care while already in the defervescence transition period, whereas patients with DF were mostly seen at the early onset of symptoms. Under the assumption of late onset of DHF/DSS, if our study protocol had included only patients in the early, acute phase of illness (~5 days after the onset of the illness), when viremia is highly detectable, only 25% of the patients with DHF and 50% of the patients with DHF or intermediate DF/DHF would have been recruited. This selective criterion to recruit only patients in the early stages of illness would exclude most adult patients with severe cases of dengue in central Brazil.

For patients with DF and those with intermediate DF/DHF, no differences were observed in the proportion of samples positive for viremia or in the median viremia titers. However, one limitation of the present study is that viremia was measured at a single point in time that corresponded with the time of entry of the patient into the study, and peak viremia might not have been identified. We analyzed viremia levels within 5 days of the onset of symptoms, because, in our previous report [32], there was no significant difference between the median viremia titers detected between days 1–3 and days 4–5 of illness (data not shown).

Approximately 27% of patients with intermediate DF/DHF had ≥1 of the 4 severe manifestations of dengue, including internal hemorrhage, plasma leakage, manifested signs of shock, and/or marked thrombocytopenia, but they did not fulfill the DHF/DSS WHO criteria [1]. Other studies have also suggested that the number of severe cases of dengue may be underestimated by adopting this criterion [17, 27]. Controversies about the application of WHO dengue classification have been extensively discussed in the literature, mainly in relation to the documentation of plasma leakage in adult patients [43–45]. Evidence of hepatic inflammation was demonstrated by high median serum levels of AST and ALT in patients with DHF or intermediate DF/DHF, compared with those noted in patients with DF. The lack of serial daily determinations of laboratory values for day care attendees or outpatients is a limitation that could result in underestimation of AST and ALT values. However, abnormal liver enzyme values have been associated with disease severity in several other reports [46–48].

In conclusion, abnormal liver enzyme levels were associated with a poor outcome of dengue. Detection of viremia after the defervescence period was observed in adult patients classified as having intermediate DF/DHF and DHF. Secondary infection was not a predictor of severe clinical manifestation in adults, who were primarily infected with serotype DV3 in central Brazil.

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References


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