Biochemical Alterations as Markers of Dengue Hemorrhagic Fever

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Abstract. We evaluated biochemical alterations as predictors of dengue hemorrhagic fever (DHF). Patients with confirmed infection with dengue virus were prospectively evaluated for the first seven days of disease to determine their final clinical outcome. Serum samples taken 48–96 hours after onset of fever were used for biochemical tests. Of 199 patients, 30 developed DHF. Cases of DHF had higher levels of lactate dehydrogenase (LDH), creatine kinase (CK), and aspartate aminotransferase, and lower levels of albumin, total cholesterol, and triglycerides. Multivariate analysis showed that early alterations of CK (hazard ratio [HR] = 6.98, 95% confidence interval [CI] = 2.34–20.85, P = 0.001), LDH (HR = 3.19, 95% CI = 1.01–10.12, P < 0.05), and albumin (HR = 2.54, 95% CI = 1.09–5.92, P = 0.03) were associated with DHF. Triglyceride levels > 160 mg/dL were negatively associated with developing DHF (HR = 0.07, 95% CI = 0.01–0.59, P = 0.01). Early alterations of biochemical markers can predict DHF in patients with acute fever caused by dengue.

INTRODUCTION

Dengue is the arboviral infection with the largest incidence worldwide.1 Clinical expression of dengue virus infection varies widely from no symptoms to dengue shock syndrome, but this infection more commonly causes dengue fever (DF) and dengue hemorrhagic fever (DHF).1,2 Nearly 100 million cases of DF and between 250,000 and 500,000 cases of DHF are annually reported to the World Health Organization.2,3

The most dramatic increase in dengue in the past decade occurred in South America, mainly in Colombia, Ecuador, Paraguay, Peru, Venezuela, and Brazil.2–5 In Colombia, dengue infection is endemic with cyclical outbreaks in almost all cities less than 1,800 meters above sea level with an at-risk population of 20 million persons.5

Dengue hemorrhagic fever is characterized by thrombocytopenia, spontaneous hemorrhages, and gradual plasma leakage that can lead to shock.1,2 Despite its clinical variability, the acute phase of dengue begins with fever that is indistinguishable from the initial phase of other acute febrile infectious diseases.1,2,6 Thus, acute dengue infection is often unrecognized until the appearance of the more severe forms of the disease. This observation leads to underestimation of the actual incidence, as well as inadequate or late treatment of a disabling and potentially lethal medical condition.7

There is direct and indirect evidence of biochemical alterations related to severity of dengue. Studies have reported that patients with DHF have elevated serum levels of transaminases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]),6–11 amylase,12–13 lactate dehydrogenase (LDH),14,15 and creatine kinase (CK).15 Patients with DHF also have elevated levels of phospholipase A2, a protein whose concentration is correlated with that of C-reactive protein (CRP).17,18 Cross-sectional studies have shown differences in serum levels of cholesterol and triglycerides associated with severe forms of DHF studies.11,12 However, these potential biochemical markers have not been evaluated prospectively in early stages of dengue. Thus, the utility of biochemical alterations for timely identification of patients who will develop DHF is unknown.19 The objective of this study was to evaluate biochemical markers as predictors of dengue severity.

MATERIALS AND METHODS

Setting. This prospective cohort study was conducted in metropolitan Bucaramanga, which includes four municipalities in the Department of Santander in northeastern Colombia. The incidence of dengue in the area has increased in recent years, ranging between 113.4 and 268.7 cases per 100,000 persons.5,20 The study protocol was reviewed and approved by the Medical Ethics Committee of the Universidad Industrial de Santander.

Study participants were more than five years of age and had acute febrile syndrome caused by dengue with a duration of symptoms less than 96 hours. After a physical examination was conducted and informed consent was obtained, blood sample were obtained to determine albumin levels, hematocrits, and platelet counts. Patients with any of the following conditions were excluded: history of concomitant diseases such as diabetes, acquired immunodeficiency syndrome, hematologic disorders, cancer, or cardiac disease, or, at baseline, DHF (see case definition), major bleeding albuminemia (< 3 g/dL), effusions, or shock. All patients were enrolled before the development of DHF.

Participants were followed daily until the seventh day of disease. Data collected included signs and symptoms and daily microhematocrit determinations to facilitate recognition of DHF. We conducted an IgM enzyme-linked immunosorbent assay, viral isolation, and biochemical tests 48–96 hours after onset of fever. Platelet counts were repeated daily in cases with previous counts less than 120,000/mm³ or when the patient had spontaneous hemorrhages, signs of effusion, edema, or a change in the hematocrit greater than 10%. A convalescent blood sample was obtained 7–15 days after the onset of symptoms to detect IgM antibodies to dengue.

Study definitions. We defined dengue virus infection as any of the following: viral isolation; a shift from a negative to a positive IgM test result, or a ≥ 4-fold increase in previously existing levels of antibodies to dengue virus. Patients with negative results in convalescent IgM tests were considered to have other febrile (non-dengue) illnesses.

The DHF cases had a dengue infection and satisfied all of the following criteria: a platelet count ≤ 100,000/mm³, any

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spontaneous hemorrhagic manifestation (or at least one positive tourniquet test result), and evidence of plasma leakage (i.e., pleural effusion, ascitis, hypoalbuminemia, or a variation in hematocrit greater than 10%). A hematocrit value of 10% was chosen (instead of 20%) because initial hematocrits have greater sensitivity in identifying complications in dengue and have been associated with serious symptoms in patients from areas endemic for dengue.

**Biomarkers.** Sera tested were obtained at the first evaluation and stored at −70°C until after follow-up and severity and infection classifications were obtained. Biochemical tests were performed using acute-phase sera (obtained 48–96 hours after onset of symptoms) from all patients with a dengue infection (including those who would develop DHF) and from a random sample of 28 patients with other febrile (non-dengue) illnesses.

We measured levels of CK, LDH, AST and ALT with modified liquid-UV tests; semi-quantitative levels of CRP with a colorimetric rapid test; levels of amylase and albumin with colorimetric tests; and lipid profiles (cholesterol, triglycerides, low-density lipoprotein [LDL] and high-density lipoprotein [HDL]) with a liquid-color test. Positive control human samples were included in all tests.

**Data analysis.** Biochemical test results (except for those for semi-quantitative CRP) were compared using Student’s t-test. We determined the frequency of biochemical alterations using accepted normal upper limits for CK (70 U/L in women and 90 U/L in men), amylase (180 U/L), lipids (HDL = 35 mg/dL, LDL = 80 mg/dL, triglycerides = 160 mg/dL), and LDH (570 U/L); and a three-fold normal upper limit for transaminases (105 U/L) and albumin (4 g/dL) as cut-off values. For CRP, values greater than 6 mg/L were considered increased.

Cox’s multivariate regression analysis was used to identify biochemical markers independently associated with DHF. We computed hazard ratios (HRs) with their 95% confidence intervals (CIs) for each marker, adjusting for other biochemical markers, age (> 15 years), sex, time of disease, and use of intramuscular medications during present illness. An association was considered statistically significant when \( P < 0.05 \). Analyses were computed using STATA software package version 8.0 (Stata Corporation, College Station, TX).

**RESULTS**

Of 682 patients, 174 were excluded during the first clinical evaluation. Four other patients with other known causes of acute febrile syndrome were excluded later in the follow-up (Figure 1). Medical assessment of this cohort included at least three visits, three hematocrit measurements, and one platelet count. Overall, 203 patients had a dengue infection; 30 (15.1%) of these patients developed DHF. Twelve patients with dengue were hospitalized, including four with DF and eight with DHF. Biochemical tests were performed in 199 patients with dengue (169 with DF and 30 with DHF).

There was no significant difference in age, sex, and duration of symptoms between patients with DF and those with DHF. Patients with DF and a severity sign frequently did not meet all criteria for DHF (Table 1). Patients with other febrile conditions had lower levels of LDH, AST, and ALT than patients with DF or DHF. Conversely, patients without dengue infection had higher levels of albumin, cholesterol, and triglycerides than patients with DHF. Patients with DHF had higher levels of LDH, CK, and AST than patients with DF. Levels of albumin, total cholesterol and triglycerides were significantly lower in patients with DHF (Table 2).

![Figure 1](https://via.placeholder.com/150)  
**Figure 1.** Enrollment and study status of patients. *Laboratory diagnosis was performed after the survey was finished. †Biochemical tests could not be performed for four cases.
TABLE 1

Characteristics of dengue patients according to final diagnosis *

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (N = 199)</th>
<th>DHF (n = 30)</th>
<th>DF (n = 169)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, no. (%)</td>
<td>99 (49.8)</td>
<td>16 (53.3)</td>
<td>83 (49.1)</td>
<td>0.67</td>
</tr>
<tr>
<td>Mean ± SD age, years</td>
<td>26.4 ± 17.1</td>
<td>26.3 ± 14.6</td>
<td>26.4 ± 17.5</td>
<td>0.96</td>
</tr>
<tr>
<td>Adults (&gt; 15 years of age), no. (%)</td>
<td>147 (73.9)</td>
<td>24 (80)</td>
<td>123 (72.8)</td>
<td>0.41</td>
</tr>
<tr>
<td>Mean ± SD disease time, hours</td>
<td>73 ± 18.6</td>
<td>70.8 ± 19.5</td>
<td>73.9 ± 18.5</td>
<td>0.84</td>
</tr>
<tr>
<td>Enrolled after three days of disease, no. (%)</td>
<td>117 (58.8)</td>
<td>17 (56.7)</td>
<td>100 (59.2)</td>
<td>0.80</td>
</tr>
<tr>
<td>Severity criteria, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia†</td>
<td>47 (23.6)</td>
<td>30 (100)</td>
<td>17 (10.1)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Hemoconcentration</td>
<td>102 (51.3)</td>
<td>29 (96.7)</td>
<td>73 (43.2)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Pleural effusion or ascitis</td>
<td>6 (3)</td>
<td>5 (16.7)</td>
<td>1 (0.6)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Spontaneous hemorrhage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive tourniquet test result</td>
<td>159/198 (80.3)</td>
<td>28 (93.3)</td>
<td>131/168 (78)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* DHF = dengue hemorrhagic fever; DF = dengue fever.
† Minimum platelet count = 100,000/mm³.

Increased levels of CK were significantly associated with previous intramuscular drug use (odds ratio = 2.1, 95% CI = 1.2–3.8, P = 0.008). However, the association between increased levels of CK and DHF was independent of this intervention and other biochemical alterations (HR = 6.98, 95% CI = 2.34–20.85, P = 0.001). An early increase in LDH (three times the normal value) was an independent predictor of DHF (HR = 3.19, 95% CI = 1.01–10.12, P < 0.05). Triglycerides levels greater than 160 mg/dL and albumin levels greater than 4 g/dL were negatively associated with DHF. Alterations of other biochemical markers were not associated with DHF by multivariate analysis at the level of power of this study (Table 3).

Kaplan-Meier curves of biomarkers (LDH, CK, triglycerides, and albumin) significantly associated with DHF are shown in Figure 2. The relationship between each biomarker and disease outcome was compatible with the proportional hazard assumption (Figure 2). Among DF cases, levels of biomarkers were not significantly associated with hemorrhagic manifestations or hemocoagulation (P > 0.05 for all comparisons).

DISCUSSION

This study supports the association between development of DHF and early alterations of the serum levels of CK, LDH, triglycerides, and albumin. These biomarkers have been proposed as indicators of severity in dengue in retrospective and cross-sectional studies.10,11,13–16 In the present study, the risk of DHF was seven times greater among patients with elevated levels of CK within the first 96 hours of the onset of fever. Additionally, we observed an increase in levels of LDH in DHF patients when compared with DF patients.

Theses alterations may be caused by damage of the skeletal muscle in DHF patients, followed by increases in levels of LDH and CK.10,15,16,24,25 Moreover, liver damage is a frequent problem in dengue that can also be associated with increased levels of LDH.6,10,11 Our results suggest that these biomarkers can predict a more severe form of dengue and could also be indicators of early tissue injury in the acute phase of dengue infection.

Levels of AST and ALT in patients with dengue were higher than those in patients with non-dengue febrile conditions. However, these biomarkers were not independent predictors of severity among patients with dengue infection (Figure 2). Amylase levels did not differentiate patients with DF from those with DHF (Tables 2 and 3). However, in other studies, these biochemical markers have been associated with DHF.10–14 These alterations may appear later in the disease course and may not be useful as early predictors of DHF.

Plasma leakage, which indicates that dengue causes hypalbuminemia, is an indicator of severity.1–3 In our study, albuminemia greater than 4 g/dL was associated with lower risk of DHF. It is probable that high values of albuminemia may reflect the integrity of the vascular endothelium, whereas albumin levels less than 4 g/dL may be an early indicator of

TABLE 2

Results of biochemical tests conducted within 48–96 hours of dengue *

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>OFI (n = 28)†</th>
<th>DF (n = 169)¶</th>
<th>DHF (n = 30)§</th>
<th>P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH (U/L)</td>
<td>449.8 (405.4–494.2)</td>
<td>562.3 (524.9–599.7)</td>
<td>711.6 (612.6–810.6)</td>
<td>0.02 &lt; 0.0001 &lt; 0.003</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>437.4 (149.9–724.9)</td>
<td>298.7 (223.1–374.3)</td>
<td>549.6 (267.5–831.6)</td>
<td>0.21 &lt; 0.57 0.02</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>49.8 (41.5–58)</td>
<td>90.2 (78.3–102.1)</td>
<td>142.7 (106.3–179.2)</td>
<td>0.007 &lt; 0.0001 &lt; 0.001</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>35.0 (28–39.1)</td>
<td>69.7 (58–81.4)</td>
<td>90.8 (57–124.5)</td>
<td>0.01 0.002 0.18</td>
</tr>
<tr>
<td>Amylase (U/L)</td>
<td>190.0 (161.8–219.4)</td>
<td>200.6 (185.3–215.9)</td>
<td>184.1 (157–212.2)</td>
<td>0.02 &lt; 0.74 0.39</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.21 (4.03–4.4)</td>
<td>4.11 (4.05–4.17)</td>
<td>3.95 (3.79–4.11)</td>
<td>0.21 &lt; 0.03 0.049</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>152.9 (134.2–171.5)</td>
<td>144.8 (139.2–150.4)</td>
<td>128.9 (114.8–143.0)</td>
<td>0.31 0.04 0.03</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>35.9 (32.1–39.8)</td>
<td>36.1 (34.6–37.7)</td>
<td>36.5 (32.8–40.2)</td>
<td>0.92 0.83 0.86</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>89.4 (73.3–105.5)</td>
<td>83.1 (78.3–87.8)‡</td>
<td>73.4 (60.1–86.7)</td>
<td>0.35 0.12 0.13</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>137.5 (102.9–172.2)</td>
<td>128.9 (117.1–140.8)</td>
<td>96 (82.1–109.9)</td>
<td>0.60 0.02 0.03</td>
</tr>
</tbody>
</table>

* OFI = Other febrile (non-dengue) illnesses; DF = dengue fever; DHF = dengue hemorrhagic fever; LDH = lactate dehydrogenase; CK = creatine kinase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TG = triglycerides.
† Values are mean (95% confidence interval).
‡ By t-test.
§ n = 167.
vascular permeability alteration. Therefore, this parameter may be an early indicator of plasma leakage and a useful prognostic marker.

Another interesting finding was the negative association between triglyceride levels and DHF. Low levels of triglycerides have been described in patients with severe malaria, although other studies have reported hypertriglyceridemia associated with malaria parasitemia and other causes of acute febrile syndromes. A previous dengue study did not find significant changes in 50 children with different severity levels. Another study that evaluated 66 children with dengue showed lower triglyceride levels in DHF patients with shock. Although the mechanism by which triglyceride levels changes occurs in dengue has not been well established, this alteration can be explained by the interaction between lipids and free radicals, which are increased in patients with den-
guez.10 Nutritional factors (not evaluated in this study) might explain serum levels of triglycerides as well as severity of dengue.11 These issues should be evaluated by including nutritional aspects and dietary habits as well as indicators of oxidative stress. Unfortunately, these variables were not evaluated in our study.

In summary, this study suggests that some biochemical alterations detected between 48 and 96 hours after symptoms can predict a more severe form of dengue infection. These results also imply that early pathogenic changes occurred before complications developed. Therefore, these biochemical alterations may be associated with severe stages of dengue and may be early prognostic markers for monitoring illness and identifying those who may benefit from future therapies.

Application of these findings may help optimize resource allocation, leading to a more opportune and effective care of those patients with dengue in disease-endemic areas. Similar studies will lead to establishment of predictor biomarkers of dengue severity that will help decrease morbidity and mortality caused by this disease.

Received December 12, 2006. Accepted for publication December 19, 2007.

Acknowledgments: We thank Raquel Elvira Ocazionez, Sergio Gómez, and Fabián Cortés (Centro de Investigación en Enfermedades Tropicales) and Karol Patricia Torres (Laboratorio Clínico de la Facultad de Salud de la Universidad Industrial de Santander) for help with laboratory diagnosis and biochemical tests.

Financial support: This study was supported by the Instituto Colombiano para el Desarrollo de la Ciencia y la Tecnología Francisco José de Caldas and the Universidad Industrial de Santander research contract 1102-04-12919.

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