

## The Impact of Dengue on Liver Function as Evaluated by Aminotransferase Levels

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The objective of this study was to evaluate the impact of dengue virus infection on liver function by measuring aminotransferase in blood samples from patients serologically diagnosed by according to two MAC-ELISA protocols. Degrees of liver damage were classified according to aminotransferase levels: grade A – normal enzyme levels; grade B – increased levels of at least one of the enzymes; grade C – increased, with at least one of the enzymes being at levels higher than three times the upper reference values; grade D – acute hepatitis, with aminotransferase levels at least ten times their normal values. Of the 169 serologically confirmed cases of dengue at the dengue referral center in Campos dos Goytacazes in the state of Rio de Janeiro, Brazil, 65.1% had abnormal aminotransferase levels: 81 cases being classified as grade B, 25 as grade C and 3 as grade D. A further 34.9% of cases had normal enzyme levels and were classified as grade A. Liver damage is a common complication of dengue infection and aminotransferase levels are a valuable marker for monitoring these cases.

**Key-Words:** Dengue, dengue hemorrhagic fever, aminotransferases, hepatitis.

Dengue is an acute febrile disease of viral etiology, the evolution of which is benign in its classic form, and serious when presenting as dengue hemorrhagic fever / dengue shock syndrome (DHF/DSS). The etiologic agent (DENV) belongs to the *Flaviviridae* family and to the *Flavivirus* genus, with 4 serotypes. Today, this disease represents a major public health issue, principally in tropical countries where environmental conditions favor the development and proliferation of *Aedes Aegypti*, the principal vector of this zoonosis. This disease is transmitted by mosquito bites in a human-*Aedes aegypti*-human cycle. After ingestion of infected blood, the mosquito is able to transmit the virus following an extrinsic incubation period of 8-12 days. In the state of Rio de Janeiro, serotypes 1, 2 and 3 have already been isolated [1-3]. Dengue virus type 3 (DENV-3) was isolated for the first time in December 2000 and was the principal agent of the 2002 epidemic.

The symptomatology of dengue varies, generally consisting of the mild forms found in cases of classic dengue, the principal manifestations of which are high fever, arthralgias, myalgias, cephalgia and gastrointestinal disorders. Atypical forms have been described in Brazil as a whole and in the state of Rio de Janeiro, in particular [4,5].

AST and ALT are liver enzymes involved in amino acid metabolism. The inflammatory process resulting from infection by the dengue virus leads to a parenchymatous lesion that releases these markers into the blood. In the acute phase of the disease, an increase occurs in aminotransferases, the levels of which subsequently decrease as the liver recovers. AST

may be found in high concentrations in the heart muscle, liver cells and skeletal muscle and, in lower concentrations, in the kidney and pancreas. Together with other enzymes, this test is useful for the diagnosis of myocardial infarction and liver disease. Patients with acute kidney disease, skeletal muscle disease, pancreatitis or trauma may have transient high levels. In turn, ALT is mainly found in the liver and in lower concentrations in the kidney, heart and skeletal muscle. High levels are found in infectious and toxic hepatitis, pancreatitis, cirrhosis, biliary obstruction, hepatocellular carcinoma and dengue.

In view of the wide range of symptomatology, the diagnosis of dengue should be based both on clinical and laboratory data. Complementary exams aid in evaluating the severity of the disease and the complications resulting from this pathology.

Virus isolation is the most specific laboratory test for the diagnosis of dengue. In this case, a blood sample should be collected prior to the 6<sup>th</sup> day of symptoms, the viremic period. Immunoenzymatic tests (ELISA) are an alternative for confirmation of the diagnosis and are available in the laboratories of the public healthcare network. These tests permit confirmation in samples taken from the 7<sup>th</sup> day of the disease onwards in around 80% of cases, achieving as high as 100% of cases after two weeks [6].

In a previous study [5], the liver was found to be affected in around 60% of cases according to a rapid strip test. In the present study, the enzymatic method was used during an epidemic of dengue in the municipality of Campos dos Goytacazes in 2003.

### Materials and Methods

#### Study Sample

A total of 336 patients receiving care at the Dengue Referral Center for Diagnosis and Treatment were included in this study between May and July 2003. Dengue was suspected when two or more of the following symptoms were present: fever,

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cephalea, retroorbital pain, myalgias, arthralgias, skin rash, nausea, vomiting, prostration and hemorrhagic manifestations.

Patients were either followed up as outpatients or were hospitalized in accordance with the standard protocol of the institute during the period in which symptoms were present or while laboratory tests remained abnormal. Complete blood counts and aminotransferase determinations were carried out.

#### Definition of Cases of Dengue Hemorrhagic Fever (DHF)

The diagnosis of DHF was established according to the adapted World Health Organization (WHO) criteria: thrombocytopenia  $<100,000/\text{mm}^3$ , hemoconcentration and hemorrhagic manifestations such as spontaneous petechiae, or a positive tourniquet test. Hemoconcentration was defined as hematocrit  $>45\%$  in men,  $>40\%$  in women and  $>38\%$  in children under 12 years of age. The criterion of a 20% increase in hematocrit was not applicable for this study population because in the majority of cases, previous, recently performed blood counts were not available for comparison.

#### Isolation of the Virus

The virus was isolated in cell culture and the serotype was identified in the Flavivirus Laboratory of the Oswaldo Cruz Institute/FIOCRUZ, as previously described by Nogueira et al. [2].

#### Serology

Tests for the detection of anti-dengue antibodies were carried out in blood samples collected between the 7<sup>th</sup> and 11<sup>th</sup> days following the onset of symptoms.

These samples were simultaneously submitted to two serological tests: immunoenzymatic assay IgM-Dengue-Biomanguinhos and immunoenzymatic assay IgM-Dengue-PanBio, in accordance with the manufacturer's instructions. When the results of both tests were positive, patients were considered to be currently infected by the dengue virus, while cases in which the results of both samples were negative were considered unconfirmed. Whenever there was discordance, patients were tested according to the CDC protocol, which was considered the gold standard.

#### Degree of Liver Damage

The degree to which the liver was affected was evaluated in these patients and classified into four groups according to AST and ALT levels during the period of infection. Grade A comprised patients with normal AST and ALT levels. The laboratory reference values of AST and ALT for males were 59 IU/L and 72 IU/L, respectively, and for women reference values were 36 IU/L and 52 IU/L, respectively. Grade B was composed of patients in whom the level of at least one of the aminotransferases was increased but no higher than three times the normal value. When the values of at least one of the enzymes were between 3 and 10 times the reference values, patients were classified as Grade C. Patients in whom there was an increase in one or both enzymes to levels more than 10

times the reference values were classified as Grade D, thereby defining the presence of hepatitis caused by DENV.

In this study, the patients with increases in aminotransferases greater than 10 times the reference values were tested for hepatitis viruses A, B and C using polymerase chain reaction (PCR). In cases in which a diagnosis of dengue was eliminated, other diagnostic possibilities were investigated on an individual, case by case, basis.

#### **Results**

Of a total of 336 patients, dengue was confirmed in 169 cases by both immuno-enzymatic tests. Of these, 97 patients (57.4%) were female and 72 (42.6%) were male. Mean age of patients was 34.5 years (range 7-78 years).

A total of 127 cases (75.1%) were classified as classic dengue and 42 (24.9%) as DHF (Table 1). DENV-3 was isolated in 3 cases, 2 of which were classified as classic dengue and 1 as DHF. In 34.9% of the population studied, no changes in aminotransferases were detected (Grade A), whereas aminotransferase levels were high in 65.1% of patients, 48.5% classified as Grade B, 14.8% as Grade C and 1.8% as Grade D. In the cases classified as classic dengue, aminotransferases were high in 61.4%, while in cases of DHF, aminotransferases were abnormal in 76.2% of cases (Table 1).

In cases considered reinfection, i.e. patients in whom IgG tested positive, liver enzyme abnormalities were present in 61.4% of patients, whereas in cases of primary infection, 66.7% of patients had abnormal aminotransferase levels (Table 1).

#### **Discussion**

In Brazil, atypical manifestations of dengue infection, such as hepatitis, myocarditis and encephalitis, have been observed during epidemics that occurred in Ceará in 1994, in the state of Pernambuco in 1997 and in Campos in 2005 [4,7,8].

The extent to which the liver is affected by DENV ranges from mild lesions to fulminant hepatitis [5,9-13].

Liver involvement may be characterized by manifestations such as pain in the right hypochondrium, hepatomegalia, varying degrees of jaundice [14], choloria and an increase in liver markers, principally ALT and AST, similar to those found in acute hepatitis caused by the A, B, C, D and E viruses.

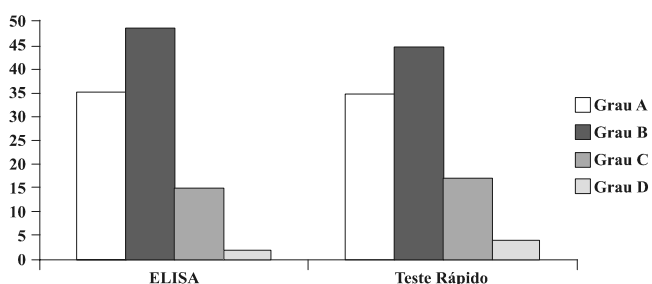
Kuo et al. [15] reported that approximately 90% of the patients in that study had abnormal AST levels, while abnormal levels of ALT, bilirubin, alkaline phosphatase and gamma-glutamyl transferase (GGT) were found in 80%, 7%, 16% and 83%, respectively, of patients with classic dengue. Liver involvement occurred through an inflammatory process in the parenchyma provoked directly or indirectly by the virus, reducing the diameter of the lumen of the biliary canaliculus, causing obstruction and leading to bilirubinemia or even jaundice, as reported in 3 cases of DHF [9,14].

In this study, patients with aminotransferase levels more than 10 times the reference values had negative results for hepatitis A, B and C according to polymerase chain reaction (PCR).

**Table 1.** Clinical presentation of dengue, type of infection and degree of liver involvement according to AST and ALT levels

	Grade A	Grade B	Grade C	Grade D	Total
Dengue					
Classic	38.6% (49)	45.7% (58)	15% (19)	0.8% (1)	75.1% (127)
Hemorrhagic	23.8% (10)	57.1% (24)	14.3% (6)	4.8% (2)	24.9% (42)
Sex					
Male	45.8% (33)	38.9% (28)	13.9% (10)	1.4% (1)	42.6% (72)
Female	26.8% (26)	55.7% (54)	15.5% (15)	2.1% (2)	57.4% (97)
IgG*					
Positive	38.6% (22)	42.1% (24)	17.5% (10)	1.8% (1)	34.5% (57)
Negative	33.3% (36)	51.9% (56)	13.9% (15)	0.9% (1)	65.5% (108)
Total	34.9% (59)	48.5% (82)	14.8% (25)	1.8% (3)	100% (169)

Grade A – no change; Grade B – = 3 times the reference values; Grade C – 3-10 times the reference values; Grade D – > 10 times the reference levels. \* Cases in which IgG was positive were considered sequential infections. Four patients failed to undergo IgG serology for dengue.

**Figure 1.** Percentage of patients distributed according to the degree of liver damage caused by the dengue virus in two studies in which different diagnostic methods were used for diagnostic confirmation.

Source: De Souza, Luiz J. et al. 2003 [5].

In the group of patients in whom dengue was not confirmed, 2 cases of leptospirosis and 1 case of hepatitis A were found, emphasizing the importance of a differential diagnosis.

It is important to emphasize that none of the patients included in the study had previous active liver disease and the abnormal aminotransferase levels attributed to the dengue infection returned to normal within 45 days following the onset of symptoms.

In a study carried out in Bangkok [11], 104 patients with a clinical and serological diagnosis of dengue were classified according to severity into: classic dengue, dengue hemorrhagic fever and dengue shock syndrome. Liver function tests showed that the most severely ill patients had higher levels of aminotransferases and lower levels of globulin, whereas increases in albumin, alkaline phosphatase, bilirubin and prothrombin were unrelated to the severity of the clinical status. In children, 74% had hepatomegaly, and aminotransferase and alkaline phosphatase levels were between 80 and 87% higher compared to normal values. The reduction in serum globulin is an important factor in fluid loss into the third space, which is indicative of severity in dengue due to a reduction in the gradient of intra- and extravascular pressure. These findings have been confirmed by other

authors in cases of hepatitis in dengue shock syndrome [10,12]. Therefore, AST, ALT and globulin are valuable parameters for the evaluation of the severity of the infection.

In a study carried out by Nguyen et al. [16], AST values were reported to be frequently abnormal, reaching values higher than those of ALT, around 97.7% and 37.3% above normal levels, respectively.

In a previous study carried out during a dengue epidemic in 2001-2002, using the rapid test method (immunochromatography) to confirm diagnosis of dengue, results comparable with those obtained in the present study were found, showing that during epidemics rapid methods may be reliable for confirmation of cases. Nevertheless, in isolated cases, specificity is higher with immunoenzymatic methods [5] (Figure 1).

False-positive results were found with the use of the rapid strip test in the initial sample. In one case, serology was positive for hepatitis A and another case tested positive for leptospirosis.

In the present study sample, the DEN-3 virus was isolated in 12 cases and DEN-2 in 1 case, all in the classic form of the disease.

With respect to the pharmacological treatment of the symptomatology of dengue, the association of previous pathologies and the liver toxicity of this virus should be taken into consideration, as well as the possibility of avoiding the use of any drugs with hepatic metabolism and clearance.

## Conclusion

Dengue is normally associated with a moderate increase in aminotransferases and, less frequently, acute hepatitis. In this present study, liver damage was more frequent among women, in patients with a primary infection and in those with DHF. In all the cases observed, dengue was found to be self-limiting, and there were no cases of liver failure.

DENV may, therefore, provoke varying degrees of damage to the hepatic parenchyma, ranging from mild increases in aminotransferases to increases of up to 30 times the reference values. Therefore, the use of liver tests to evaluate the degree

of liver damage is of great importance, and markers such as AST and ALT may be used as parameters to evaluate severity.

In complicated classic dengue, dengue hemorrhagic fever and in dengue shock syndrome, concomitant deficiency of coagulation factors and thrombocytopenia should be considered in the pathophysiology of the disease as factors that tend to aggravate hemorrhagic status.

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