Dengue: profile of hematological and biochemical dynamics

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Aim: *The objective of this study was to correlate laboratory tests during the evolution of dengue fever, comparing frequencies between the different clinical forms in order to use test results to predict the severity of the disease.*

Methods: *This is an observational, descriptive and retrospective study of 154 patients with clinical and serological diagnoses of dengue fever who, in the period from January to May 2008, were admitted in a tertiary state hospital in the city of Fortaleza that is a referral center for infectious diseases. The patients were allocated to two groups according to age: under 15 years old (n = 66) and 15 years or older (n = 88). The tests analyzed were blood count, platelet count, and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations.*

Results: *Thrombocytopenia and elevated transaminases were observed in patients with classic dengue fever. The main laboratory abnormalities found in dengue hemorrhagic fever were thrombocytopenia, hemoconcentration and elevated transaminases, similar to severe dengue with the exception of hemoconcentration. Most laboratory abnormalities started on the 3rd day but were more evident on the 5th day with restoration of values by the 11th day; this was more prominent in under 15-year-olds and with the more severe clinical forms.*

Conclusion: *These results are relevant in assessing the disease because they can be used as markers for more severe forms and can help by enabling the adaptation of the therapeutic conduct to the needs of individual patients.*

Keywords: *Dengue/blood; Dengue hemorrhagic fever/diagnosis; Dengue virus; Prognosis; Clinical laboratory techniques; Hematologic tests*

Introduction

Dengue is caused by one of the four serotypes of the dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4) also referred to as an arbovirus (arthropod-borne viruses) that belongs to the genus *Flavivirus* of the family *Flaviviridae*.[1,2] It is a disease with a wide clinical spectrum and a wide variety of presentations, ranging from asymptomatic to an undifferentiated fever (viral syndrome) to the more severe forms such as severe dengue (SD) or Dengue hemorrhagic fever (DHF).[3]

Transmission to humans occurs by the bite of the female *Aedes aegypti* mosquito infected by one of four serotypes of the virus. This mosquito, a domestic species adapted to urban conditions, is the main vector in Brazil.[4]

The period of transmission from humans to mosquitoes begins one day before the start of fever up to the sixth day of illness corresponding to the viremia phase. After a female bites an individual in the viremia phase, viral replication (extrinsic incubation) begins in the vector in from eight to twelve days. In humans, the incubation period ranges from 3 to 15 days (intrinsic incubation) with an average of 5 days.[4,5]

According to estimates of the World Health Organization (WHO), about 50 million cases of dengue fever occur annually worldwide and 2.5 billion people live in risk areas.[6] In 2005, the World Health Assembly, through WHA Resolution 58.3, in a review of the International Health Regulation (IHR), included dengue fever as an emergent public health disease, with implications for health safety due to the spread of the epidemic beyond national boundaries.[7]

In Brazil, there have been reports of dengue fever epidemics since 1846 in the cities of São Paulo and Rio de Janeiro. In 1990 the serotype DEN-2 was first isolated in Brazil, in the city of Niterói, RJ. With the circulation of two viral serotypes (DEN-1 and DEN-2) came the first reports of DHF. During the first two years of the 1990s, the incidence of the disease remained almost entirely restricted to the states of Rio de Janeiro, Ceará, Pernambuco and Alagoas, but in subsequent years it rapidly spread into other states of Brazil.[6-8]

The diagnosis of dengue fever is carried out based on clinical, epidemiological and laboratory data. Among laboratory tests, both non-specific [blood count, platelet count, tourniquet test, prothrombin time (PT), activated partial thromboplastin time (APTT), liver function tests and serum albumin concentration] and specific tests (viral isolation tests and serology for antibody examination) are used.[9,10]

Leukopenia is the most prominent hematological change, sometimes with counts of less than 2 x $10^3/\mu L$. However, there are reports of mild leukocytosis at the onset of the disease, with neutrophilia. Lymphocytosis is a common finding, with the presence of atypical lymphocytes. The hematocrit concentration should be monitored according to the days of illness, remembering that, with the progression to DHF, there will be a 20% increase in hematocrit from the patient's baseline, associated with thrombocytopenia (< 100 x $10^9/L$).[11,12]

Of biochemical variables, the most frequent changes occur in liver function tests such as in serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), Gamma-glutamyl transpeptidase and alkaline phosphatase levels, and serum albumin concentrations.[9]

In this context, the present study aimed to assess the biochemical and hematological dynamics of patients with dengue fever in order to increase the sensitivity of the screening by healthcare professionals in the most serious cases and to try to identify laboratory markers that may indicate this evolution.

Methods

This is a descriptive observational retrospective study of secondary data obtained from the medical records of 154 male and female patients aged from 2 to 85 years who had serological diagnoses of dengue fever in a state hospital in Fortaleza (*Hospital São José de Doenças Infecciosas*) that is a referral center for infectious and contagious diseases in the period from January to May 2008. The study included all patients diagnosed with positive serology for dengue using the ELISA IgM capture method. This study was approved by the Ethics Committee of the hospital (# 061/2009).

Some clinical presentations of dengue fever and laboratory findings are different in adults compared to children,[13] so the study population was divided into two groups (Table 1): under 15-year-old patients and those aged 15 years old or more.

Patients were classified into classic dengue fever (CD), dengue hemorrhagic fever (DHF) according to WHO criteria (2009), and SD according to the Brazilian Ministry of Health.[3,14] The first day of the disease was considered the onset of symptoms related to dengue fever and the laboratory profile was evaluated for the first 12 days. The variables selected were: hemoglobin (Hb), hematocrit (Ht), leukocytes, lymphocytes, platelets, ALT and AST.

Table 1 - Patients and the clinical classification of dengue

Type	Age < 15 years n (%)	Age ≥ 15 years n (%)	Total
Classic dengue fever	4 (2.60)	16 (10.39)	20
Severe dengue fever	18 (11.69)	26 (16.88)	44
Dengue hemorrhagic fever	44 (28.57)	46 (29.87)	90
Total	66 (42.86)	88 (57.14)	154

A p-value = 0.06 (chi-square test) shows that there is no significant difference in the proportion of clinical forms of dengue fever between under 15-year-old patients and older patients .

The Wilcoxon method (nonparametric) and the Student t-test (parametric) were used for statistical analysis. Descriptive statistics were compared using the chi-square test for the variables of gender, age and the clinical forms of dengue. The Graph Pad PRISM 5.0 computed program was used with the results being presented as boxplots; the significance level was set for an α-error = 5%.

Results

The results of 154 patients with clinical and laboratory diagnosis of dengue fever were analyzed; 82 (53.25%) were female and 72 (46.75%) were male (Table 2). The ages ranged from 2 to 85 years with 33.7% aged from 0 to 9 years (Figure 1). Regarding the clinical form of the disease, 20 (13%) had CD, 44 (28.6%) had SD and 90 (58.4%) had DHF. There was a predominance of women with SD (p-value = 0.01).

In relation to the values of Hb, we found that for the group aged 15 years or over with CD, there was a greater variation in the course of the disease; however, there were no significant differences between groups. For DHF we observed a greater variation of Hb in both groups which was most evident from the 4th to 6th days with the highest

Table 2 - Patients grouped by gender and the clinical classification of dengue

Type	Female n (%)	Male n (%)	Total
Classic dengue fever	10 (06.50)	10 (06.50)	20
Severe dengue*	29 (18.83)	15 (09.74)	44
Dengue hemorrhagic fever	43 (27.92)	47 (30.51)	90
Total	82 (53.25)	72 (46.75)	154

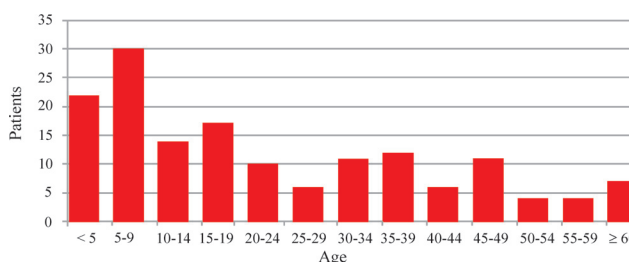* p-value = 0.01 (chi-square test)

Figure 1– Distribution of study population according to age (n = 154)

values in the group of patients aged 15 years old or more. For SD, there is a slight variation between the groups (Figure 2).

Regarding Ht, we found homogeneous and lower values in the under 15-year-old age group for all forms but with a greater variation in DHF (Figure 3).

Leukocytosis was observed in patients with the CD in the first days of the disease, followed by leukopenia, which was more pronounced in the under 15-year-old age group.

For the other forms of the disease, the values were similar throughout the evolution (Figure 4).

Lymphocytosis was observed in all forms, especially in the under 15-year-old age group, during the course of the disease, but this was more pronounced in CD from the 4th to 6th days (Figure 5).

In CD, thrombocytopenia was observed in both age groups from the 4th day of the disease. The DHF and SD
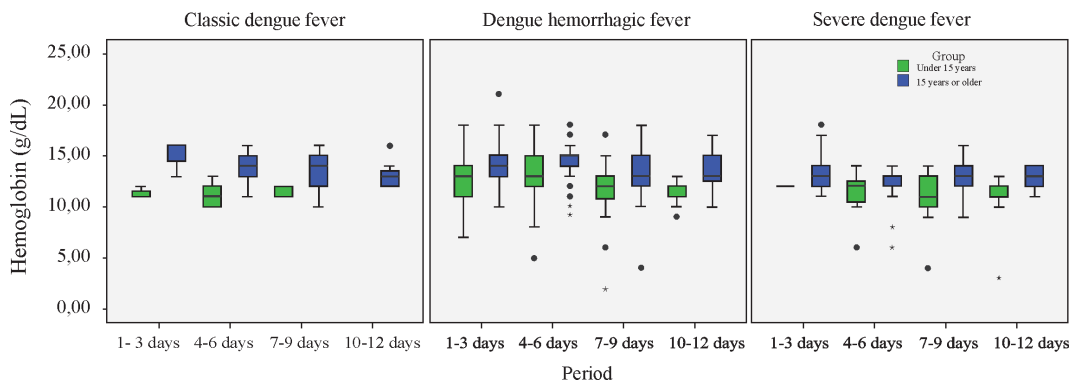

Figure 2 – Dynamics of hemoglobin levels in patients with dengue fever according to age group (< 15 years and ≥ 15 years) and clinical form
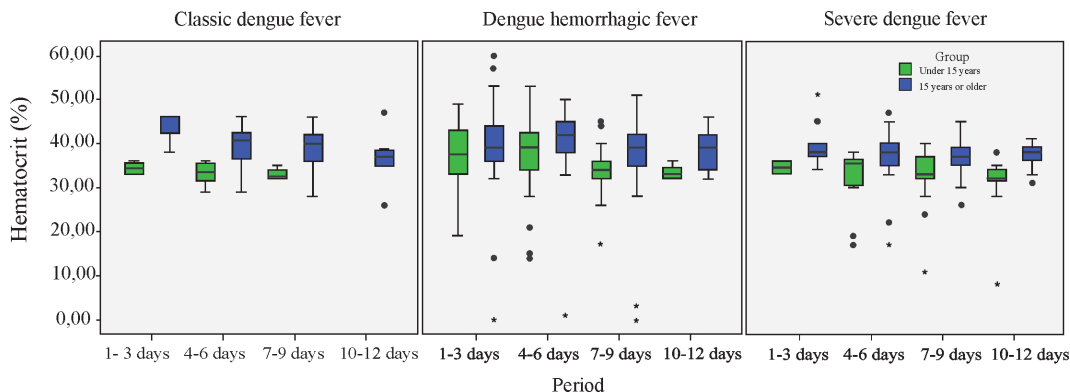

Figure 3 – Dynamics of hematocrit levels in patients with dengue fever according to age group (< 15 years and ≥ 15 years) and clinical form
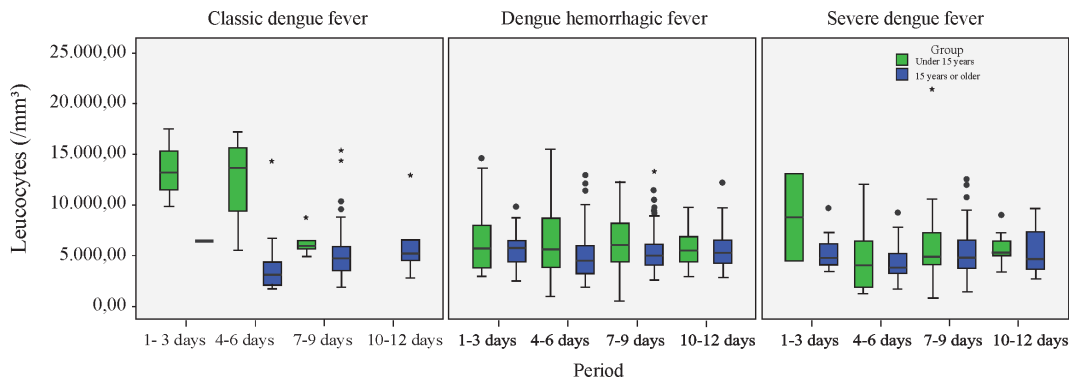

Figure 4 – Dynamics of the overall leukocyte count in patients with dengue fever according to age group (< 15 years and ≥ 15 years) and clinical form
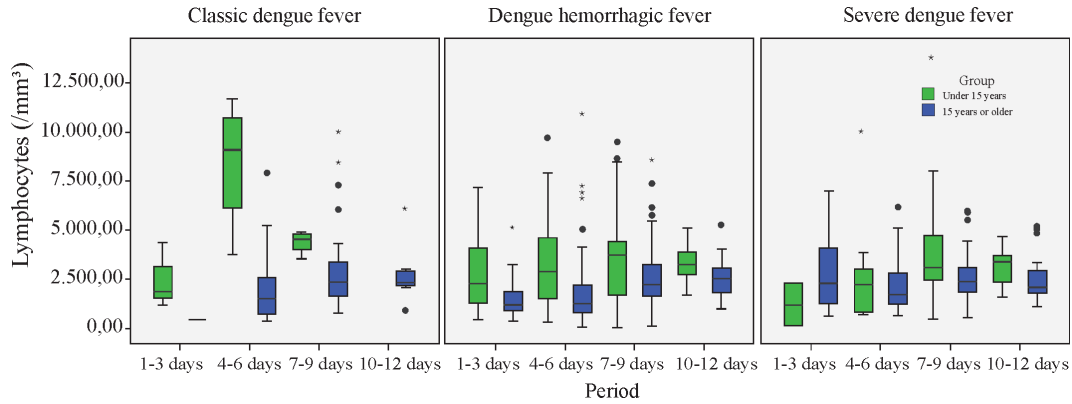
Figure 5 – Dynamics of the absolute number of lymphocytes in patients with dengue fever according to age group (< 15 years and ≥ 15 years) and clinical form
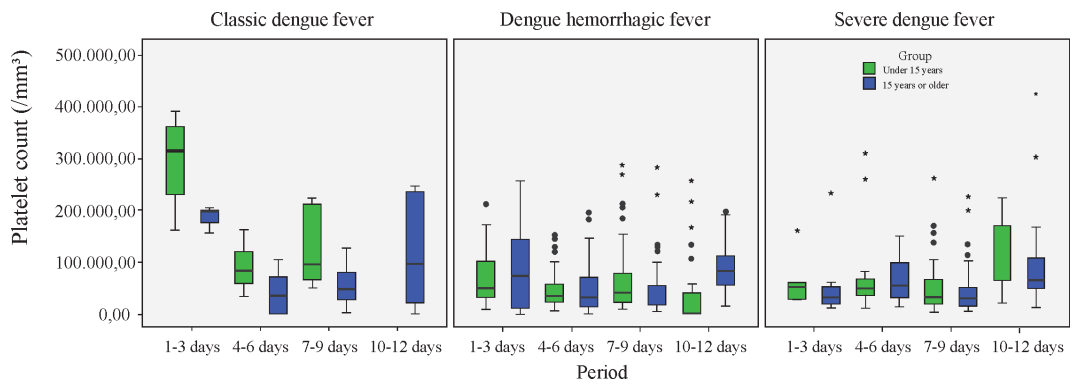

Figure 6 – Dynamics of platelet count in patients with dengue fever according to age group (< 15 years and ≥ 15 years) and clinical form
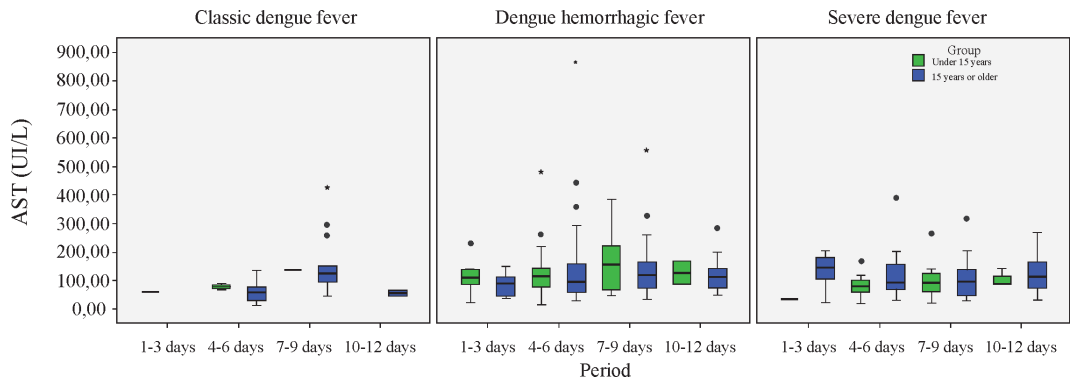

Figure 7 – Dynamics of serum aspartate aminotransferase in patients with dengue fever according to age group (< 15 years and ≥ 15 years) and clinical form

forms started with thrombocytopenia and pronounced variations were recorded throughout the evolution in both age groups (Figure 6).

Increases in the AST enzyme occurred at the beginning of the disease and remained stable for all clinical forms; this was more pronounced in the under 15-year-old age group. Increases in ALT were detected from the 7th day in CD and DHF, which remained high throughout the course of the disease mainly in the under 15-year-old age group. In SD the increase in ALT was recorded at onset in under 15-year-old patients (Figure 7)

Discussion

Dengue fever is an infectious disease which is difficult to distinguish from other viruses prevalent in our region as there are no specific markers that can diagnose the disease early. Because it is a disease that can evolve with serious

consequences and even be fatal, this study aimed at analyzing clinical and epidemiological data and laboratory dynamics in order to try to identify biomarkers that are predictive of severity.

In our study, the worst clinical forms, DHF and SD, were prevalent possibly because the patients were admitted in a tertiary hospital specialized in infectious diseases. Oliveira et al.,[15] in a study on outpatients, showed a predominance of the classical form of the disease.

The frequency of dengue fever in the study was higher in the group aged 15 years old or over. These results are similar to those of Rocha & Tauil[16] in an epidemiological study conducted in Manaus, AM. There was a slight predominance of women in this study; in most published studies, there is no significant difference in the proportions by gender.[16] The correlation between gender and the clinical form showed a significant difference for SD, with a predominance of women, a result that is in disagreement with the literature.[12,13]

Regarding the clinical forms of dengue, only DHF showed peak elevations in Hb and Ht during the course of the disease, a change most likely attributed to hemoconcentration, which can lead to hypovolemic shock.[4,5,17]

It was found that CD began with leukocytosis and leukopenia appearing later. Leukopenia was more pronounced in the CD and DHF clinical forms and in patients of 15 years old or older, similar to other published results.[13] There was a decrease in lymphocytes at the onset of dengue fever, with an increase as the disease progressed; this was statistically significant in all three clinical forms for under 15-year-old patients.

In the hemorrhagic and severe forms, thrombocytopenia occurred from the onset of symptoms and remained stable throughout the progression of the disease. This was more evident in the older age group. In CD, thrombocytopenia started late. This result is in agreement with the literature, which reports moderate or severe thrombocytopenia in DHF.[11,12,15] The inflammatory responses to dengue are attributed to immune complex formation, complement activation and the release of cytokines into the circulation in a phase prior to the most serious forms of the disease. The mechanisms underlying the bleeding in DHF are multiple including vasculopathy, thrombopathies and DIC. Thrombopathy consists of thrombocytopenia and platelet dysfunction.[18]

AST levels increased at the onset of symptoms in all clinical forms and remained at varying but high levels during disease evolution; this was particularly prominent in under 15-year-old patients. ALT started with above normal values in the severe form and remained steady throughout the course of the disease; in the classic and hemorrhagic forms, the increases occurred progressively.

Similar results were obtained by Chen et al.,[19] who showed that both AST and ALT exhibited higher-than-

average values in under 15-year-old patients with DHF. Chau et al.[20] found a significant increase in transaminases, especially AST, in children with dengue when compared to a control group with other febrile (non-dengue) illnesses.

For Chacko & Subramanian,[21] an increase in ALT (≥ 40 IU) in children with dengue fever can be considered a predictive marker for shock syndrome. The liver is one of the target organs for dengue and clinical manifestations of hepatic dysfunction can occur during the course of this disease.[22] The liver is deprived of oxygen leading to lesions of the parenchyma, in which the injured hepatocytes release transaminases that is detectable in the peripheral blood.[23] In most cases, the high levels of transaminases show the degree of hepatocellular injury, prolonging the clinical course of the disease; however, there is no correlation with prognosis.[24-26]

Conclusion

Dengue fever evolves with laboratory alterations starting on the 3rd day and becoming most evident on the 5th day with values restored to normal by the 11th day. The disease was more severe in individuals aged 15 years and older with a more pronounced and persistent presence of liver abnormalities (AST, ALT) and hemoconcentration. The study results are relevant in the characterization of biological markers in the evolution of the disease and can be used as markers for the most severe forms thereby enabling early help with the adaption of therapeutic conduct for specific patients.

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