Article/Artigo

Clinical and laboratory findings in patients with dengue associated with hepatopathy

Achados clínicos e laboratoriais em pacientes com dengue associada à hepatopatia

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ABSTRACT

Introduction: Hepatic disorders caused by dengue infection may progress to severe manifestations, including mortality and morbidity. Cytokines are involved in it, such as the migration inhibitory factor of macrophages (MIF), tumor necrosis factor (TNF), natural killer cells (NK), B lymphocytes, and macrophages. **Methods:** This study was carried out from January to April 2007 at a public hospital from the Federal University of Mato Grosso do Sul, Campo Grande, Brazil. Sixty-eight patients were studied concerning hepatic alterations, with 56 reported having classic dengue, 6 with hemorrhagic dengue grade I, and 6 with hemorrhagic dengue grade II. **Results**: Among the 56 with classic dengue, 83.3% had aspartate aminotransferase (AST) alterations, and 69.6% had altered alanine aminotransferase (ALT). For those with hemorrhagic dengue grade I, 100% had AST alterations, and 83.3% had altered ALT. All the patients with hemorrhagic dengue grade II had AST and ALT alterations. AST variations reached 22.0 and 907.0, with an average value of 164.6. For ALT, we found variations between 25.0 and 867.0, with an average value of 166.07. There had been statistical significance between dengue clinical shapes and hepatic function markers. **Conclusions:** We conclude that the infection was predominant in adults, females, and in those with low income and education. The liver enzymes were of larger amount in hemorrhagic dengue, but there was weak statistical evidence of the clinical manifestations and transaminases. Major signs and clinical symptoms were fever, headache, myalgia, arthralgia, weakness, severe pain behind the eyes, and rashes.

Keywords: Dengue. Hemorrhagic dengue. Hepatitis.

RESUMO

Introdução: Afecções hepáticas causadas pela infecção da dengue podem evoluir para quadro grave, incluindo mortalidade e morbidade. O mecanismo de lesão do fígado está relacionado com a exacerbação da resposta imune. As citocinas estão envolvidas nele como fator inibidor da migração de macrófagos (MIF), fator de necrose tumoral (TNF), células natural *killer* (NK), B linfócitos e macrófagos. **Métodos:** Este estudo foi realizado em um hospital público da Universidade Federal do Mato Grosso do Sul. As alterações hepáticas pelo dengue podem evoluir com quadros graves e potencialmente letais. Foram avaliados exames de 68 pacientes atendidos e confirmados com dengue, onde 56 foram classificados como dengue clássico, seis, como dengue hemorrágico grau I e seis como dengue hemorrágico grau II. **Resultados:** Do dengue clássico, 83,3% tiveram alterações de aspartato aminotransferase (AST) e 69,6% alterações para alanino aminotransferase (ALT). No dengue hemorrágico grau I, AST elevou-se 100% e para ALT 83,3%. No dengue hemorrágico grau II observou-se 100% de alterações tanto para AST, quanto para ALT. A variação de AST ficou entre 22,0 e 907,0 com média de 164,6. A alanino aminotransferase variou entre 25,0 e 867,0 com média de 166,07. Houve significância entre formas clínicas do dengue e marcadores de função hepática. **Conclusões:** Conclui-se que a infecção predominou em adultos do sexo feminino, de baixa renda e escolaridade. As enzimas hepáticas elevam-se mais no dengue hemorrágico, fraca evidência estatística entre as manifestações clínicas e as transaminases. Os mais prevalentes sinais/sintomas clínicos foram febre, cefaléia, mialgia, artralgia, fraqueza, dor retrorbitária e exantema.

Palavras-chaves: Dengue. Dengue hemorrágico. Hepatite.

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INTRODUCTION

Dengue is an acute febrile viral disease of short duration, but nowadays, it is considered reappearing and is distributed on continental proportions. It is caused by an arbovirus, *Flaviviridae* family, *Flavivirus* gender, divided into four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. Man is the only infection host, but there are the wild cycles that keep the viral circulation[1,2]. The disease is transmitted through *Aedes* gender mosquito bite, with *Aedes aegipty* being the main vector, which has strong ability to adapt to the peridomiciliar environment[3,4].

There is a wide viral circulation in Brazil of three serotypes (DENV-1, 2, and 3), triggering the expansion of the epidemic[5,6]. Currently, all Brazilian states have detected the presence of dengue virus, with some imported cases as in State of Santa Catarina[7].

DENV-1 was isolated in Mato Grosso do Sul in 1987, and the first epidemic occurred in 1990 with 9,700 cases reported and an incidence rate of 1,470 cases per 100,000 inhabitants[8]. In 1996, there was the introduction of DENV-2 with another epidemic, and in 2002, the introduction of DENV-3 led to an incidence rate of 1,920 cases per 100,000 inhabitants in another epidemic, on the same year[9].

Dengue does not usually evolve seriously in most cases, but the disease may present atypical clinical manifestations[10,11]. Hepatic involvement is one of the forms of these events.

The main objective of this work is to describe liver alterations resulting from the infection by dengue virus, which is also described as unusual, and to check possible relations with clinical manifestations.

METHODS

This is a descriptive study. Data were collected from secondary sources through the analyses of medical records, from the database of the Day-public Hospital Professor Esterina Corsini (HDEC) of Mato Grosso do Sul Federal University (UFMS) referring to the epidemic cases that had occurred from January to April 2007.

Five hundred and thirteen medical records of patients who were examined with suspected dengue at the hospital during the epidemic period were analyzed. Statistical analysis was performed using Epi-Info (version 3.5.2). Information regarding the serological test results, clinical examinations, and laboratory tests (biochemistry) were extracted from the medical records, and the information were recorded in a form designed for the research.

The trial included all the medical records of the patients who fulfilled the clinical requirements with the signs and symptoms of dengue (fever, headache, myalgia, prostration, severe pain behind the eyes, arthralgia, and rashes) determined by the World Health Organization in 2005 and the following laboratory criteria: those individuals with positive serology for enzyme method ELISA capture of IgM capture (Panbio Diagnostics kit, Australian), negative serology for B hepatitis and C hepatitis (Bio-Rad Laboratories kits), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (AP), total protein (TP), and albumin (ALB) on at least one collection. The biochemical tests were carried out on automated Dimension RXL equipment, and reagents from Labtest Diagnostic SA were used. All values above normality for liver enzymes ([AST>37U/I, ALT>65U/I, GGT>182U/I, AP>136U/I] and [TP<6.4U/I, ALB<3.4U/I]) were considered altered; the reference values on International Units (U/I) were adopted at the university hospital of UFMS.

The criteria for exclusion were determined: information gaps referring to the studied variables, acute infectious or chronic comorbidities, and those taking medications that are potentially hepatotoxic.

Ethical considerations

As it is a research with secondary data collection, the preparation is dispensed as a term of informed consent, and a waiver request was sent to the ethics committee of the Federal University of Mato Grosso do Sul, Campo Grande, Brazil. In compliance with the recommended resolution 196/96, an associated term commitment to the use of information was obtained from medical records as well as an authorization to access the records.

RESULTS

Serology results

All the 68 patients indicated positive serology for dengue by IgM-ELISA capture; of that total, 85.3% (58/68) did not have confirmed dengue before or did not have signs/typical symptoms of the illness. All of them were submitted to the serological markers detection for hepatitis B (HBsAg and anti-HBc IgM and IgG) and for hepatitis C by anti-HCV detection.

Clinical manifestations presented

Wide variations on signs and symptoms were observed on clinical manifestations; they were obtained during the appointment or during patient hospitalization. The most common symptoms observed in at least 60% of the patients were fever, headache, myalgia, nausea, and vomiting (**Table 1**).

Biochemical changes

The biochemical changes observed on these patients were of significant values (**Table 2**). From the 68 patients, 83.8% (57) presented values above normal for AST, and 73.5% (50) reached

TABLE 1 - Percentage of patients admitted at The Day-Hospital Esterina Corsini from January to April 2007 according to observed signs and symptoms.

Signs and symptoms	Yes n	Yes %	No n	No %	Unknown n	Unknown %
Anorexia	20	29.4	46	67.6	2	2.9
Arthralgia	37	54.4	28	41.2	3	4.4
Headache	60	88.2	7	10.3	1	1.5
Abdominal pain	35	51.5	30	44.1	3	4.4
Severe pain behind the eyes	34	50.0	31	45.6	3	4.4
Fever	64	94.1	3	4.4	1	1.5
Palpable liver	9	13.2	54	79.4	5	7.4
Asthenia	36	52.9	31	45.6	1	1.5
Hypotension	9	13.2	55	80.9	4	5.9
Myalgia	60	88.2	7	10.3	1	1.5
Nausea/vomiting	47	69.1	20	29.4	1	1.5
Rash	35	51.5	31	45.6	2	2.9
Dizziness	22	32.4	42	61.8	4	5.9

Note: The patients could present some or all the signs/symptoms.

TABLE 2 - Percentage of patients admitted at The Day-Hospital Esterina Corsini from January to April 2007 according to dengue clinic forms and biochemical change examinations.

Change examinations	CD (n=56) n	CD (n=56) %	HD I (n=6) n	HD I (n=6) %	HD II (n=6) n	HD II (n=6) %	P
AST	45	80.3	6	100.0	6	100.0	0.25
ALT	39	69.6	5	83.3	6	100.0	0.24
GGT	24	42.8	3	50.0	5	83.3	0.17
AP	15	26.7	1	16.6	2	33.3	0.80
TP	27	48.2	3	50.0	4	66.6	0.69
ALB	17	30.3	3	50.0	4	66.6	0.15

CD: classic dengue; **HD I**: hemorrhagic dengue grade I; **HD II**: hemorrhagic dengue grade II; **AST**: aspartate aminotransferase; **ALT**: alanine aminotransferase; **GGT**: gamma glutamyl transferase; **AP**: alkaline phosphatase; **TP**: total protein; **ALB**: albumin.

above-normal values for ALT. The AST values of the patients ranged between 22.00 and 907.00, with an average value of 164.63±178.59 (median=96.00). The ALT values ranged between 25.00 and 867.00, with an average value of 166.07±158.31 (median=105.00).

For GGT, 47% (32/68) of the patients reached values above normal, and for AP, 26.5% (18/68) had values above normal. ALB values were observed below the reference value in 35.3% (24/68) of the patients, while TP values were below normal in 50% (34/68) of the patients.

Patients with classic dengue syndrome (n=56) showed alterations in liver function, as measured by AST and ALT. In this group, 83.3% and 69.6% of the patients showed altered levels of AST and ALT, respectively. In the hemorrhagic dengue grade I group (n=6), 100% and 83.3% of the patients showed altered levels of AST and ALT, respectively. In patients with hemorrhagic dengue grade II (n=6), there were elevations for both AST and ALT (**Table 2**).

The AST and ALT dosage on patients with classic dengue and those who had hemorrhagic dengue (**Table 3**).

There was a significant linear correlation between AST dosage and patient age; however, it was weak (Pearson linear correlation test, $p=0.02$, $r=0.29$, $r^2=0.08$). The same was observed between

TABLE 3 - AST e ALT dosage presented on an average ± deviation average pattern, according to dengue clinic forms on patients of The Day-Hospital Esterina Corsini, from January to April 2007.

Variable	AST	ALT
Dengue clinic form		
classic	147.46±185.28	152.80±163.48
hemorrhagic I	236.00±142.27	259.83±126.11
hemorrhagic II	253.50±103.16	196.17±111.10
p value	0.007 significance	0.046 significance
	HD II > CD	

Note: *p* value: Kruskal-Wallis test followed by Dunn post-test (clinic form). **AST**: aspartate aminotransferase; **ALT**: alanine aminotransferase; **CD**: classic dengue; **HD II**: hemorrhagic dengue grade II.

ALT dosage and age (Pearson linear correlation test, p=0.03, r=0.26, r2=0.07). On the other hand, there was a strong linear correlation between AST and ALT (Pearson linear correlation test, p<0.001, r=0.84, r^2=0.70) (**Figure 1**).

Correlation analysis was performed between dengue clinical forms with AST and ALT changes. Generally, there was significant difference between the types of dengue in relation with AST dosage (Kruskal-Wallis test, p=0.007). The AST dosage on patients with dengue type II was higher than the one observed on patients with classic dengue (A-D) (post-Dunn test, p<0.05). The ALT dosage on patients with classic dengue (A-D) was 152.80±163.48. Among those with dengue type I, it was 259.83±126.11, and it was 196.17±111.10 among those with dengue type II. We observed a significant difference between the types of dengue in relation with ALT dosage (Kruskal-Wallis, p=0.046), but no difference was observed between them on the multiple comparison test (post-test Dunn, p>0.05) (**Figure 2**).
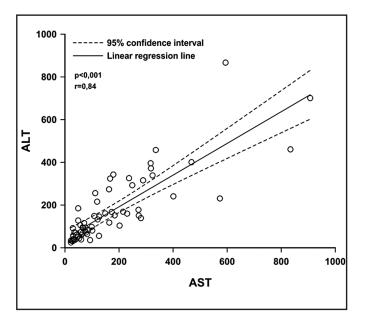

FIGURE 1 - Linear correlation between AST and ALT.
Each symbol (circle) represents the AST and ALT dosage for a single patient (Pearson linear correlation test).
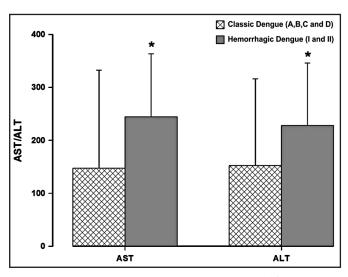**AST**: aspartate aminotransferase, **ALT**: alanine aminotransferase.

FIGURE 2 - AST and ALT dosage according to dengue clinical form.
The columns represent the average values, and the bars represent the standard deviation.
*Significant difference as compared with the classical form of dengue (Mann-Whitney test; AST: p=0.002, ALT: p=0.0014).
AST: aspartate aminotransferase, **ALT**: alanine aminotransferase.

DISCUSSION

In relation with the clinical manifestation investigation, which could be associated with hepatic function disorders (nausea and vomiting, abdominal pain and hepatomegaly), there were no significant differences among those patients with altered enzymes (AST and ALT) and those who had enzyme dosage within the normal range. These results agree with those observed in other studies[1,4,11-13].

Regarding the changes in the hepatic function markers, similar values were observed as to that of the other authors' in terms of clinical and epidemiological importance, although there were no statistically significant results[8,14-16]. Both proteins and enzymes, GGT and AP, are scarcely reported on studies relating dengue and hepatic alterations in Brazil. Among those authors cited, only Uehara et al., in 2006, described changes of 40% (8/20) for GGT, 18% (4/22) on alkaline phosphatase, 30.3% (10/33) on total protein, and 48.4% (16/33) of dosage alteration on serum albumin on a study of 41 patients with hemorrhagic dengue. In our study, these values were similar to those found by the author.

As there has been no significant difference among patients who took or not took potentially hepatotoxic drugs, in relation to aminotransferase elevations, it is possible that these increases have occurred due to dengue infection. The transaminase level may remain normal or elevated regardless of the medication.

The same situation was observed on patients with or without chronic disease. There was no significant relationship between the presence and absence of the disease and the elevation and normality of the transaminase levels.

Many results in this work do not have statistical significance, and many others are justified by the fact that there are three main factors that determine the differences between the samples. The sample size, the size differences among them, and the variability between individual observations of each sample are big problems at the moment in statistical calculations. In our case, we can observe a high variability of AST and ALT, leading to a very large standard deviation and, thus, statistically insignificant results within the confidence interval when compared with clinical proposals.

The number of the studied patients was relatively low for this type of research, but they strictly followed the proposed criteria for inclusion and exclusion. At the beginning of the proposed research, there were 513 patients, in which 445 were excluded because they did not fulfill the inclusion criteria.

Liver is one of the target organs of dengue virus, and it can be diagnosed hepatomegaly and an increased hepatic protein in serum after infection[14], therefore, by the range release of inflammatory mediators such as cytokines, which can both protect and damage the body according to individual variations or numbers of infections[17]. These are able to perform their actions by acting on specific receptors present in the membranes of the target cells. The receptor binding is made with high specificity on the extracellular portion, from which there is an intracellular signaling involving proteins of the hepatocyte cytosolic portion[18].

Several studies have demonstrated that the *macrophage migration inhibitory factor* (MIF) cytokine has an important role as mediator in inflammatory case[19]. This was first described as a lymphokine released from activated T cells, after finding out that it was responsible for the random migration inhibition of macrophages to the injury-hit site. Thus, these cells started to be addressed in an orderly manner under the MIF guidance. The alpha tumor necrosis factor (TNF), a cytokine that is capable of inducing the production of inflammatory mediators in the local and distant organs, promoting a neutrophil recruitment to the injured areas[20], has already shown an important role in the unchaining of the inflammatory response induced by dengue virus infection in models with testing animal[12]. Active cells of the immune system are also targets of dengue virus infection; such cells include dendritic cells, *natural killer* cells (NK), T and B lymphocytes, macrophages, and monocytes.

The high percentage of cases with alterations on hepatic enzymes found in this study highlights the importance of hepatic appraisal on patients with dengue and hemorrhagic dengue. However, we still have much to understand on the interrelationships between DENV and liver. The DENV action in the hepatic cell and its evolution with the illness on previously healthy patients and those with chronic diseases not related to the liver are issues that need to be elucidated in future studies.

The need for a pre-established protocol in conducting this kind of studies is justified once more. On attending patients with suspected dengue, a standardized list of examinations must be requested.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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