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LIVER DYSFUNCTION IN PATIENTS BITTEN BY *CROTALUS DURISSUS TERRIFICUS* (LAURENTI, 1768) SNAKES IN BOTUCATU (STATE OF SÃO PAULO, BRAZIL)

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SUMMARY

Thirty-two patients bitten by venomous snakes sixteen by *Bothrops* spp. and sixteen by *Crotalus durissus terrificus* were studied. The group comprised thirty males and two females, aged eight to sixty-three years (mean 33±15). Bromsulphalein tests were increased in the majority of patients bitten by *Crotalus durissus terrificus*. The correlation coefficient of Spearman was positive between bromsulphalein tests and alanine aminotransferase levels, and between alanine aminotransferase and aspartate aminotransferase levels only in the *Crotalus* group. The only patient who died was bitten by *Crotalus durissus terrificus* and showed hydropic degeneration and mitochondrial injury in the liver. It was concluded that the hepatic damage might have been caused by at least two possible mechanisms: venom effect on liver mitochondria and cytokine effects on hepatocyte, specially interleukin-6.

KEYWORDS: Ophidic accidents; *Crotalus durissus terrificus*; Liver dysfunction; Bromsulphalein test.

INTRODUCTION

Ophidic accidents are a serious health problem for tropical countries [5, 6, 9, 38]. In Brazil about 20,000 ophidic accidents occur every year, 2,000 of them only in the State of São Paulo [22]. About 80% of the accidents occurring in Botucatu, State of São Paulo, are caused by three species of *Bothrops* (*B. jararaca, B. alternatus* and *B. neuwiedi*) and 20% by *Crotalus durissus terrificus* [5, 6, 9].

Hepatic damage has been a concern since 1954 when WAJCHENBERG et al. [37] studied nine patients bitten by *Crotalus durissus terrificus* and described steatosis and liver congestion in two patients and centrolobular necrosis in one of them. Six patients bitten by *Agkistrodon rhodostoma* were studied by REID et al. [27] in Southeast Asia in 1963. Bromsulphalein, alanine aminotransferase and aspartate aminotransferase were evaluated and no alterations were observed. BANCHER et al. [4], in 1973, demonstrated the ability of *Crotalus durissus terrificus* venom to fix preferentially in nervous and hepatic tissues in mice. Hepatic tissue can fix about $15DL_{50}$ of *Crotalus durissus terrificus* venom per gram of tissue, a higher value than that detected in renal and muscle tissue. Hepatic damage may occur after rattlesnake bites, concluded the authors [4], perhaps even more extensively than that observed in renal parenchyma. Hydropic degeneration, steatosis and hepatic necrosis were observed by SALIBA et al.

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[31] in an experimental study on the effect of *Crotalus* venom on cattle. Serum aspartate aminotransferase levels, were respectively, 670 and 1,900 mUI/ml in two patients bitten by *Crotalus* snakes as observed by MAGALHÃES et al. [20]. Hepatic steatosis and increased serum aspartate aminotransferase were reported in two patients by AZEVEDO-MARQUES et al. [2].

BARRAVIERA et al. [10], in 1989, observed serum aspartate and alanine aminotransferase levels, respectively, 1,840 and 360mUI/ml, in one patient who died bitten by *Crotalus durissus terrificus*. An autopsy was carried out, and the histopathological study of his liver showed extensive hepatic necrosis. Hepatotoxicity related to crotalic venom was postulated by the authors [10] at that occasion.

Based on these data, we may hypothesize that hepatic damage frequently occurs in crotalic accidents, although there is no specific research available. The objective of this study is to evalute the hepatic function using sensitive and less expensive methods, comparing patients bitten by *Bothrops* spp. with those bitten by *Crotalus durissus terrificus* snakes.

PATIENTS AND METHODS

Thirty-two patients bitten by venomous snakes, 16 by *Bothrops* spp. and 16 by *Crotalus durissus terrificus*, admitted to the Tropical Diseases Service, School of Medicine of Botucatu were studied. Thirty patients were males and two females, aged 8 to 63 years of age (mean 33±15). Nineteen out of 32 brought the snake and 13 did not. Ten snakes belonged to the *Bothrops* genus and 9 belonged to the *Crotalus durissus terrificus*. Thirteen patients were diagnosed based on clinical symptoms described by several authors [1, 5, 6, 9, 22], in spite of these patients not bringing the snakes. After clinical evaluation all patients were submitted to specific antivenom therapy, according to the severity of each case. Patients from the *Bothrops* group received from 4 to 12 ampules (100 to 300 mg) of specific antivenom and the *Crotalus* group received from 20 to 30 ampules (200 to 300 mg) of specific antivenom. Thirty-one patients were tested for bromsulphalein (BSP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) 48 hours and 30 days after envenomation. Hepatitis B surface antigen (HBsAg) and antibody (anti-HBsAg) were tested in all patients on the first day of the accident. All patients were asked about alcohol abuse.

A 8-year old patient bitten by *Crotalus durissus terrificus* who was hospitalized in Avaré (SP), for 28 hours without specific treatment died. Before his death, because of the deteriorating clinical conditions, (increase of arterial pressure and cardiac beats, anuria and somnolence) the patient was referred to the University Hospital of the School of Medicine of Botucatu - UNESP where he immediately received 15 ampules (150 mg) of specific anticrotalus serum. Physical examination revealed a patient in general bad conditions, somnolent, with bilateral palpebral ptosis, diplopia, agitation, tachypnea and bilateral mydriasis. During antivenom serum application, he had an irreversible cardio-respiratory arrest despite attempts at resuscitation. The following laboratorial tests were performed: hematological, urinary and serum analyses. Immediately after death, the patient was submitted to a liver tissue-biopsy aiming to carry out electron microscopy analyses. Later, an autopsy and a histopathological study were carried out in skeletal muscle, kidneys and liver.

Statistical analyses were performed by the Student's t-test, and the correlation coefficient of Spearman was applied in each group of patients [33].

TABLE 1

Bromsulphalein analyses in patients bitten by *Bothrops* spp. and *Crotalus durissus terrificus*, measured 48 hours and 30 days after the accident. Results are reported as means ± SD

Patients bitten by	* Bromsulphalein test (%)		Statistical analyses
	48 hours after envenomation	30 days after envenomation	
Bothrops spp. (n=16)	5.38 ± 3.30	4.99 ± 3.65	p>0.20
Crotalus durissus terrificus (n=15)	8.53 ± 8.05	4.42 ± 3.68	p<0.05

* Normal value for BSP retention test < 5% according to several authors [21, 25, 32].

RESULTS

Table 1 summarizes bromsulphalein (BSP) determinations obtained 48 hours and 30 days after the accident. BSP tests were increased ($p<0.05$) only in patients of *Crotalus* group.

Tables 2 and 3 summarize bromsulphalein, alanine aminotransferase and aspartate aminotransferase determinations obtained 48 hours and 30 days after the accident.

Bromsulphalein tests were normal in nine (56.25%) non-alcohol-abuse patients of *Bothrops* group with hepatitis B surface antibody-negative (anti-HBsAg). After thirty days, 10 (62.5%) patients presented normal bromsulphalein tests. Four other out of 6 (37.5%) patients presented anti-HBsAg positive and three were alcohol abusers.

Bromsulphalein tests were increased in nine (60.00%) non-alcohol-abuse patients of *Crotalus* group with anti-HBsAg negative. After thirty days, 13 (86.66%) patients presented normal bromsulphalein tests. Two non-alcohol-abuse patients of each group had anti-HBsAg negative and their bromsulphalein tests remained elevated thirty days after the accident.

The correlation coefficient of Spearman shows positive correlation between bromsulphalein and alanine aminotransferase tests only in the *Crotalus* group ($r=0.66$; $\alpha=0.01$). The correlation coefficient of Spearman shows positive correlation between alanine and aspartate aminotranferase tests only in the *Crotalus* group ($r=0.81$; $\alpha=0.01$).

Results of laboratory tests performed in the patient who died.

Hematological findings: hematocrit = 45%, hemoglobin = 15mg%, white blood cells = 28,000/mm³, neutrophils (72%) = 20,160, lymphocytes (9%) = 2,520, basophils (15%) = 4,200, monocytes (4%) = 1,120, eosinophils = zero.

Urine analyses: pH = 6.0, proteinuria = ++, heme-pigment = ++++.

Serum analyses: sodium = 128 mEq/l, potassium = 7.1 mEq/l, calcium = 6.0 mg%, urea = 77 mg%, creatinine = 3.7 mg%, alanine aminotransferase = 300 mUI/ml, aspartate aminotransferase = 1,400 mUI/ml, creatine kinase = 79,000 mUI/ml, clotting time > 60'.

Histological changes:

Skeletal muscle shows strong and homogeneous eosinophilic cytoplasm and nuclear picnosis both indicating hyaline necrosis (rhabdomyolysis);

Kidneys present hydropic degeneration of proximal tubule epithelial cells;

Liver with centrolobular hepatocytic hydropic degeneration observed in Figure 1.

Electron microscopy:

Figures 2 and 3 show disrupted organelles and dilated mitochondria with damage and loss of their

TABLE 2

Bromsulphalein and alanine aminotransferase analyses in patients bitten by *Bothrops* spp. and *Crotalus durissus terrificus*, measured 48 hours and 30 days after the accident.

Patients bitten by	Alanine aminotransferase (ALT) (mUI/ml)	Bromsulphalein test (%)			
		48 hours after envenomation		30 days after envenomation	
		Normal N(%)	Increased N(%)	Normal N(%)	Increased N(%)
Bothrops spp.	Normal	8 (50.00)	*6 (37.50)	9 (56.25)	***6 (37.50)
(n=16)	Increased	1 (6.25)	**1 (6.25)	1 (6.25)	0 (0.00)
Crotalus durissus terrificus	Normal	3 (20.00)	2 (13.33)	11 (73.34)	1 (6.66)
(n=15)	Increased	3 (20.00)	#7 (46.67)	2 (13.34)	1 (6.66)

* Hepatitis B surface antibody (anti-HBsAg) and alcohol abuse positive in three patients.

** Hepatitis B surface antibody-positive (anti-HBsAg).

*** Hepatitis B surface antibody (anti-HBsAg) positive in four patients, three were alcohol abusers.

Alcohol abuse in one patient.

Normal value for BSP retention test <5%

Normal value for alanine aminotransferase activity: 2-18 mUI/ml

Normal value for aspartate aminotransferase activity: 4-20 mUI/ml

TABLE 3

Bromsulphalein and aspartate aminotransferase analyses in patients bitten by *Bothrops* spp. and *Crotalus durissus terrificus* measured 48 hours and 30 days after the accident.

Patients bitten by	Aspartate aminotransferase (AST) (mUI/ml)	Bromsulphalein test (%)			
		48 hours after envenomation		30 days after envenomation	
		Normal N(%)	Increased N(%)	Normal N(%)	Increased N(%)
Bothrops spp.	Normal	3 (18.75)	**6 (37.50)	9 (56.25)	**5(31.25)
(n=16)	Increased	6 (37.50)	*1 (6.25)	*1 (6.25)	1 (6.25)
Crotalus durissus terrificus	Normal	1 (6.66)	1 (6.66)	12 (80.00)	0 (0.00)
(n=15)	Increased	5 (33.34)	***8 (53.34)	1 (6.66)	2 (13.34)

* Alcohol abuse and hepatitis B surface antibody-positive (anti-HBsAg).
** Hepatitis B surface antibody-positive (anti-HBsAg) in three patients, two of them were alcohol abusers.
*** Alcohol abuse in one patient.
Normal value for BSP retention test <5%
Normal value for alanine aminotransferase activity: 2-18 mUI/ml
Normal value for aspartate aminotransferase activity: 4-20 mUI/ml

matrix and cristae. The nucleus loses its sharp details of chromatin and the cytoplasm is represented by flocculent material.

DISCUSSION

Bromsulphalein (BSP) test has remained to date one of the best and most sensitive indicators of liver dysfunction [12, 14, 25, 34]. An abnormal BSP retention represents, therefore, a disorder of one or more liver functions [12, 14, 25, 34]. According to MARTINEZ et al. [21], this test evaluates the hepatocytic function from membrane to membrane, i.e., from vascular to biliary pole, as well as hepatic circulation and canalicular permeability. An elevation of BSP retention has been observed when any alterations in the hepatic perfusion, biliary excretion or impaired hepatocyte physiology occur [12, 34]. Despite these considerations, BSP test is not specific and its clinical application was abandoned due to adverse reactions which might eventually occur [32].

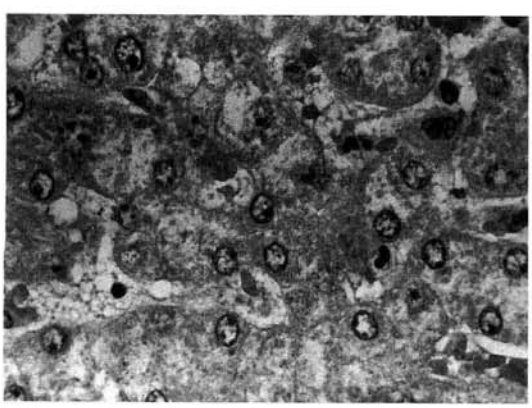

Fig. 1 - Liver with centrolobular hepatocytic hydropic degeneration (HE: 200X).

In this work, patients of *Crotalus* group presented higher BSP retention than those of *Bothrops* group. These results suggest that *Crotalus* venom interacts with hepatocyte producing these modifications.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes are located mainly in the liver, kidneys, heart and skeletal muscle [23, 24]. AST is a mitochondrial enzyme found in different organs in the following proportions [23]: heart (1.56), liver (1.42) and skeletal muscle (1.0). ALT is a cytoplasmatic enzyme observed in the following proportions [23]: liver (9.5), kidneys (4.0), heart (1.5) and skeletal muscle (1.0). The increase of these enzymes in sera suggest injury in different organs. According to AZEVEDO-MARQUES et al. [13] increased AST suggests the myotoxicity caused by ophidic venoms, specially *Crotalus durissus terrificus* venom.

On the other hand, increased ALT is more specific to hepatic damage because this enzyme is found in higher proportion in the liver [23]. According to SHERLOCK [32], increased serum levels of ALT are more specific to hepatocyte damage. In patients of the *Crotalus* group we observed an increase of BSP retention, a positive correlation between BSP retention and

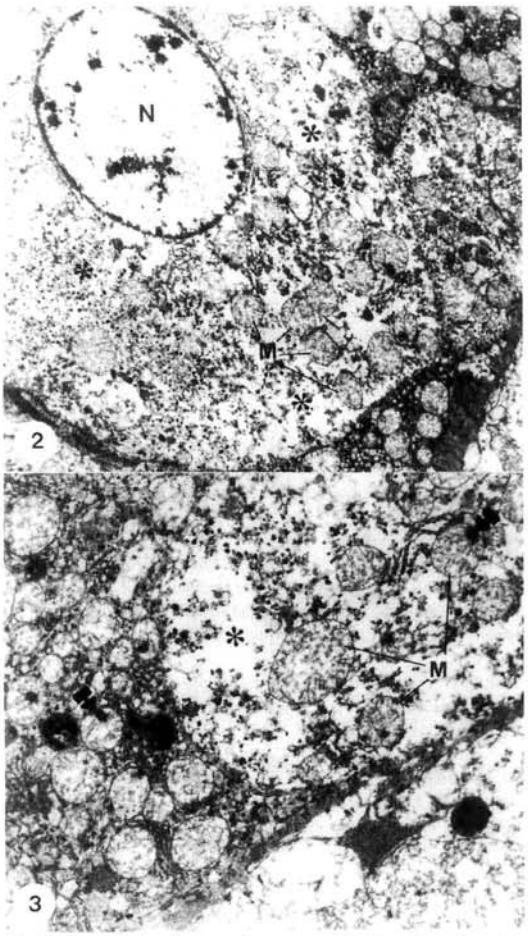

Fig. 2 - Transmission electron microscopy of the liver of a patient who died 28 h. after *Crotalus durissus terrificus* snakebite showing a hepatocyte nucleus with loss of sharp chromatin details (X 8,000).
Fig. 3 - Transmission electron microscopy of the liver of a patient who died 28 h. after *Crotalus durissus terrificus* snakebite showing a hepatocyte flocculent aspect of cytoplasm and enlarged mitochondria (X 13,000).

ALT serum levels, and a positive correlation between AST and ALT serum levels, suggesting that these alterations are related to liver dysfunction.

BARRAVIERA [7] corroborated this hypothesis by experimentally inoculating Wistar rats with *Crotalus durissus terrificus* venom when ALT and AST serum level elevations were observed. Hepatocyte electron microscopy showed mitochondrial damage characterized by their enlargement, swelling, loss of matrix and cristae. These damages were similar to those observed in that patient who died. Based on these data, it is possible suggest, at least, for the crotalic accident, that liver injury might occur partly in the cytoplasm and partly in the mitochondria. BSP retention elevation might be related to lack of energy supply by hepatic mitochondria, since BSP biliary excretion is an active mechanism which involves energy consumption [12, 36]. Furthermore, the literature shows that hydropic degeneration may occur with energy lack because of sodium - potassium pump failure [17, 30]. Cytoplasmatic and mitochondrial lesions observed in the electron microscopy of liver agree with the hypothesis that the elevation of AST and ALT enzymes is derived from liver tissue.

In relation to alcohol abuse by the patients, the literature shows that the continuous use of alcohol, even in small doses, may cause a BSP retention elevation [30, 32, 34]. Acute [19, 26] or chronic [15, 18, 28] hepatitis can also increase BSP retention.

BLASCHKE et al. [13] evaluated BSP retention in 20 normal human volunteers submitted to fever induced by bacterial endotoxin extracted from *Salmonella abortus*. These authors observed a rise of BSP retention in 45% of the patients and suggested that the fever alone is capable of acting on the hepatocyte producing the alterations observed. Nowadays, it is known that bacterial lipopolysaccharide can activate non-specific immune system response [35]. These molecules are able to stimulate macrophages and mononuclear cells to release pyrogenic cytokines, such as interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor (TNF). These cytokines are related to fever mechanism. On the other hand, IL-1, IL-6 and TNF can modify protein synthesis by hepatocytes [16, 29]. Analyzing the paper of BLASCHKE et al. [13] and comparing their results to ours, we can suggest the participation of pyrogenic cytokines in BSP retention elevation.

Recently BARRAVIERA et al. [8, 11] evaluated serum levels of IL-1, IL-6, IL-8 and TNF in patients bitten by *Bothrops* spp and *Crotalus durissus terrificus* snakes. They observed a very important rise of IL-6 and IL-8, specially in patients bitten by *Crotalus durissus terrificus*. Thus, the possible effect of cytokines causing an increase of BSP retention might not be discarded.

In conclusion, we suggest that part of the AST and ALT enzymes released in a crotalic accident is from hepatic tissue, although this venom also presents a myotoxic effect. The increase of BSP retention corroborates these assumptions. Liver dysfunction mechanisms may be due to mitochondrial damage producing energy lack for the hepatocytes besides the cytokine effects on liver, specially IL-6.

RESUMO

Alterações hepáticas em doentes picados por serpentes *Crotalus durissus terrificus* (Laurenti, 1768) na região de Botucatu, Estado de São Paulo, Brasil.

Os autores estudaram 32 doentes picados por serpentes venenosas, sendo 16 picados por *Bothrops* spp. e 16 por *Crotalus durissus terrificus*. Trinta doentes eram do sexo masculino e dois do feminino com idades variando entre 8 e 63 anos (méda 33±15). A prova da retenção da bromosulfaleína apresentou-se aumentada na maioria dos doentes picados por serpentes *Crotalus durissus terrificus*. Houve correlação positiva entre a retenção da bromosulfaleína e os níveis séricos de alanina aminotransferase e entre alanina e aspartato aminotransferase apenas nos doentes do grupo *Crotalus*. Um dos doentes evoluiu para o óbito e apresentou no exame anatomopatológico do fígado degeneração hidrópica e lesões mitocondriais. Os autores concluem que as alterações hepáticas são causadas por pelo menos dois mecanismos a saber: lesão mitocondrial por efeito do veneno crotálico; efeito das citoquinas, especialmente a interleucina-6.

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