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EVALUATION OF PLATELET NUMBER AND FUNCTION AND FIBRINOGEN LEVEL IN PATIENTS BITTEN BY SNAKES OF THE *BOTHROPS* GENUS

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Platelet function and plasma fibrinogen levels were evaluated in 14 patients, 10 males and 4 females, aged 13-59 years bitten by Bothrops *genus snakes. There was a statistical difference ($p < 0.05$) among plasma fibrinogen levels evaluated 24 and 48 hours after envenomation. There was a tendency towards normalization after 48 hours of treatment. The low platelet number was clear in 24-48 hour evaluations with a tendency towards normalization after 48 hours of treatment ($p < 0.05$). When platelet function was stimulated by collagen and epinephrine, it appeared to be within normal values. On the other hand, when it was stimulated by adenosine diphosphate (ADP), platelet function was hypoaggregated by a single micromol concentration until 48 hours after treatment. At a 3 micromol concentration, there were alterations only before specific treatment ($p < 0.05$). Fibrinogen levels and fibrin degradation product (FDP) levels appeared to be altered in 83.33% of patients evaluated. The authors suggest that platelet hypoaggregation is related to decreased fibrinogen and increased FDP levels.*

Key-words: Platelet function. Fibrinogen. FDP. Bothrops.

Envenomations are a serious health problem in tropical countries[1 25]. In Brazil around 20,000 envenomation cases occur every year, 2,000 of them only in the State of São Paulo[1]. About 80% of the cases occurring in Botucatu area are caused by three species of *Bothrops (B. jararaca, B. alternatus* and *B. neuwiedi)*[2].

Local and systemic alterations are occasioned by *Bothrops* venom when inoculated in humans[19 20]. Proteolytic venom fractions[1] result in tissue necroses. Hemorrhagic and coagulant fractions act on the systemic level leading to vessel and blood changes[7 9]. Blood white cells, platelets and coagulant factor alterations are observed[7]. Coagulant factor changes have been demonstrated *in vitro* and *ex vivo* experiments[7 14 16]. Factors II and X are activated with consequent fibrinogen consumption[7 14 16]. Platelet changes have been observed in accidents caused by different snakes[7 9 15 26]. Thomazini et alii[23] demonstrated a decrease of platelet aggregation activity in patients bitten by *Crotalus* snakes. On the other hand, Zingali et alii[27] showed an increase of platelet aggregation activity *in vitro*. Based on these data, the aggregant or anti-aggregant effect caused by different snake venoms is controversial[23 26 27].

The objectives of the present study were to evaluate the number and platelet function , as well as fibrinogen and FDP plasma levels in patients bitten by *Bothrops* snakes.

MATERIALS AND METHODS

Fourteen patients, bitten by *Bothrops* snakes, 10 males and 4 females aged 13-59 years, admitted to the Tropical Diseases Service of the School of Medicine of Botucatu/UNESP were studied. Blood samples were collected immediately after the patients' admission, 24, 48 hours and 8 days after the specific treatment for hemostatic system evaluation. All patients were treated with specific antivenom according to several authors[1 20].

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Fibrinogen plasma levels were measured by Ratnoff and Menzie method[18]. Platelet function was evaluated by stimulating aggregation with 1 micromol (μm) and 3μm adenosine diphosphate , collagen and epinephrine from Helena Laboratories[5] by using a Zenith platelet aggregation for the graphic record. Numerical score of platelets was made utilizing the Newbauer chamber method by phase contrast. Fibrin degradation product (FDP) levels were determined in serum through the latex proof with the American Dade reagents.

Statistical analyses were carried out using the Wilcoxon non-parametric proof or two dependent samples[4].

RESULTS

Table 1 shows fibrinogen plasma levels decreased before and after 24 hours after the specific treatment ($p<0.05$). Forty-eight hours later the level augmented and remained stable until 8 days. The number of platelets was low before, 24 and 48 hours after specific treatment returning to the normal on the 8th day ($p<0.05$).

Table 2 shows platelet stimulation with 1μm and 3μm ADP. When stimulated by 1μm ADP, platelets were hypoaggregant until 48 hours after treatment with a tendency to normalization on the 8th day ($p<0.05$). When ADP concentration was increased to 3μm, the platelets were hypoaggregant in the 24-hour evaluation tending to normalization after 48 hours ($p<0.05$).

Table 1 - Fibrinogen plasmatic levels and platelet count of patients studied. Results are reported as means ± SD.

	A Before (n=14)	B 24hr (n=12)	C 48hr (n=12)	D 8th day (n=8)
Fibronogen (1) Normal: 200 a 400mg%	211.5±70.19	223.83±36.13	261.73±72.79	289±47.02
Platelets (2) Normal: 200 a 400x10^8	158.14±110.35	182.55±71.64	158.25±55.89	194.13±46.71

Statistical analysis:

$A_1 \times B_1 \times C_1 \times D_1$; $A_1 = B_1 < C_1 = D_1$; ($p<0,05$)
$A_2 \times B_2 \times C_2 \times D_2$; $A_2 = B_2 = C_2 < D_2$; ($p<0,05$)

Table 2 - Platelet aggregation activity with 1μm and 3μm ADP of patients studied. Results are reported as means ± SD.

	A Before (n=14)	B 24hr (n=12)	C 48hr (n=9)	D 8th day (n=7)
Stimulated with ADP 1μm (1) (normal > 60%)	17.84±28.69	23.17±28.73	18.90±21.84	37.57±29.09
Stimulated with ADP 3μm (2) (normal > 60%)	35.76±32.98	58.41±23.71	62.66±15.44	66.57±25.04

Statistical analysis:

$A_1 \times B_1 \times C_1 \times D_1$; $A_1 = B_1 = C_1 < D_1$; ($p<0,05$)
$A_2 \times B_2 \times C_2 \times D_2$; $A_2 < B_2 = C_2 = D_2$; ($p<0,05$)

Table 3 shows no alteration when platelets were stimulated by collagen and epinephrine.

Table 4 shows fibrinogen plasma levels and fibrin degradation products (FDP) increased in 5 out of 6 patients studied. Three patients presented values up to 40 micrograms/ml, while the other two presented levels of 10-40μg/ml. The only patient with normal FDP (< 10μg/ml) values showed normal platelet activity and normal fibrinogen values.

DISCUSSION

Blood coagulation changes both *in vitro* and *ex vivo* have been observed in envenomations caused by different venomous snakes[6 7 13 14 20 21 23]. Kornalik[8] working *in vitro* with snake venoms of *Agkistrodon* and *Vipera* genera demonstrated that a fibrinogen bovine solution, when pre-incubated with these venoms, lost its capacity of turning into fibrin. Kamiguti et alii[6], studying patients bitten by *Bothrops jararaca*, noticed fibrinogen consumption. Studies carried out by Thomazini et alii[23] in patients bitten by *Crotalus durissus terrificus*, agree with the results obtained by Kamiguti et alii[6]. Santoro et alii[22] inoculating *Bothrops jararaca* venom in rabbits observed low plasmatic fibrinogen levels and increased levels of fibrin degradation products (FDP). Sano-Martins et alii[21] studied patients bitten by *Bothrops* and found high levels of FDP immediately before antivenom therapy, tending to normalize after a 24-hour treatment.

In this study, immediately and 24 hours after the accident, fibrinogen plasmatic levels showed a decrease, but were still within normal values. These normalized 48 hours after specific treatment. A

Table 3 - Platelet aggregation activity with collagen and epinephrine of patients studied. Results are reported as means ± SD.

	A Before (n=14)	B 24hr (n=12)	C 48hr (n=11)	D 8th day (n=7)
Collagen (1) (5μg/ml) (normal > 60%)	49.64±33.13	74.17±12.92	75.73±13.42	77.43±12.33
Epinephrine (2) (30μm) (normal > 60%)	45.35±30.92	76.25±14.76	67.27±19.50	72.29±17.77

Statistical analysis:

$A_1 \times B_1 \times C_1 \times D_1$; $A_1 < B_1 = C_1 = D_1$; ($p < 0,10$)
$A_2 \times B_2 \times C_2 \times D_2$; $A_2 < B_2 = C_2 = D_2$; ($p < 0,10$)

Table 4 - Fibrin degradation product levels (FDP), fibrinogen plasmatic levels and platelet aggregation activity for six patients studied.

Patients studied (n=6)	FDP levels μg/ml (normal < 10μg/ml)	Fibronogen mg% (normal: 200 a 400)	Platelet activity ADP 1μm
1	> 40	250	hypofunction
2	10 - 40	250	hypofunction
3	< 10	310	normofunction
4	10 - 40	111	hypofunction
5	> 40	136	normofunction
6	> 40	-	hypofunction

possible action of "factor X activator" and "thrombin-like" fraction from *B. jararaca* venom may explain these alterations. "Factor X activator" from *B. jararaca* venom transforms factor X from blood into activated factor X. Prothrombin is transformed into thrombin by activated factor X. Thrombin is capable of producing fibrin by acting on fibrinogen[16].

Thrombin-like fractions act directly on fibrinogen molecule transforming it into fibrin. This effect is similar to that of human thrombin.

In this way, there will be plasmatic fibrinogen consumption and formation of fibrin degradation products(FDP)[10 11 12 22] as final consequences.

Bothrops venom besides acting on fibrinogen, also presents an effect on platelet, both in number and in function[6 7 21 22].

Prado-Franceschi et alii[17] studying rabbits inoculated with *Crotalus durissus terrificus* venom, observed a discreet low number of platelets and an increase in platelet aggregant activity. Zingali et alii[27] described that pure *Bothrops* venom has a fraction that can stimulate platelet aggregation. These authors[27 28] in 1992, described a new component of *Bothrops* venom which might inhibit platelet aggregation when induced by thrombin. Thomazini et alii[23] observed platelet hypofunction in patients bitten by *Crotalus durissus terrificus*, when this was stimulated by 1μm ADP. In this work platelet number decreased until 8 days after specific treatment. Platelets also showed hypoaggregation when stimulated by 1μm and 3μm ADP. Patients who presented high FDP levels were those who had platelet hypofunction. Marcus[10 11 12] demonstrated the anti-aggregant action of fibrin degradation products. According to Santoro et alii[22], platelet hypoaggregation observed may be explained by high levels of circulating FDP, which would have an inhibitory action on platlets due to competition with aggregant agents in platelet receptors. Vargaftig et alii[24] evaluating convulxin fraction effect of *Crotalus durissus terrificus* observed that this fraction presented an aggregant action *in vitro*. Aggregation would occur by stimulating platelet receptors, releasing thromboxane A_2, the platelet enzyme that facilitates platelet adhesion and aggregation mechanisms to the vascular subendothelium[24]. This action would lead to exhaustion of platelet reserves of thromboxane A_2, causing hypofunction. In the present study, we observed normalization of platelet aggregation curves on the 8th day after specific treatment. As platelet medium lifetime is 8 days, it is possible that aggregant function recovery occur, by renewing platelets in blood circulation.

According to Benett and Vilaire[3], platelets need a suitable level of extracellular fibrinogen for perfect functioning. When these levels are decreased, there is a loss of potency of aggregant agents on receptors causing, in the least analysis, an aggregation decrease.

In conclusion, patients bitten by *Bothrops* snakes presented fibrinogen consumption, platelet hypofunction and increased fibrinogen degradation products (FDP), suggesting a relationship among these factors.

RESUMO

Foram avaliadas a função plaquetária e os níveis séricos de fibrinogênio em 14 doentes picados por serpentes do gênero Bothrops, *sendo 10 do sexo masculino e 4 do sexo feminino, com idades compreendidas entre 13 e 59 anos. Houve diferença estatística ($p < 0,05$) entre os níveis séricos de fibrinogênio avaliados 24 e 48 horas após o acidente. Houve tendência à normalização após 48 horas do tratamento. A plaquetopenia foi evidente nas avaliações de 24 e 48 horas. Houve tendência à nomalização no 8^o dia após o tratamento ($p < 0,05$). A função plaquetária, quando estimulada pelo colágeno e epinefrina, apresentou-se dentro da normalidade. Quando estimulada pelo ADP, foi hipoagregante na concentração de 1 micromolar até 48 horas após o tratamento. Na concentração de 3 micromolar houve alteração apenas antes do tratamento específico ($p < 0,05$). Os níveis de produtos de degradação de fibrina (PDF) mostraram-se alterados em 83,33% dos pacientes avaliados. Os autores sugerem que a hipoagregação esteja relacionada com níveis baixos de fibrinogênio e elevados de PDF.*

Palavras-chaves: Função plaquetária. Fibrinogênio. Produtos de degradação de fibrina. Bothrops.

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