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VISCEROCUTANEOUS FORM OF LOXOSCELISM AND ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEFICIENCY

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SUMMARY

In a period of time of five years, all patients who exhibited viscerocutaneous form of loxoscelism were investigated for erythrocyte glucose-6-phosphate deficiency, and in two patients out of seven it was found this deficiency. This finding suggests that this genetical enzyme deficiency could account for the hemolysis after **Loxosceles** bite, at least in some of the cases.

INTRODUCTION

Loxoscelism is a disease caused by the spider **Loxosceles sp.**bite. The human symptomatology of loxoscelism presents two clinical variants: 1) The cutaneous form. 2) The viscerocutaneous form. The cutaneous form of loxoscelism is the most commonly observed and develops very painful local edema and or local skin necrosis. The viscerocutaneous form is much more serious and occurs, according to SCHENONE & SUAREZ [22], in an average of 13% of the total cases of loxoscelism. Besides exhibiting local manifestations identical to those seen in the cutaneous form, this clinical variant is composed of hemolytic anemia, jaundice, fever and sensorial involvement usually appearing within the first 6 to 24 hours after the bite. The utmost symptom accompanying this picture is the hemolytic anemia, which may induce to hemoglobinuria and occasional oliguria and anuria [5,8,17,18,20,24].

The fact that only 13% of the cases develop hemolysis lead to the assumption of a possible susceptibility of these individuals. Certainly this greater susceptibility would not be caused by environmental factors, but it would be feasible to relate it to constitutive factors, to genetic factors. These constitutive factors would be represented by the lacking of genetically determined protective mechanisms or by the presence of a predisposition factor. This last hypothesis could be represented by the erythrocyte glucose-6-phosphate dehydrogenase deficiency. This deficiency is a very common genetic polymorphism, with high prevalent rates in several populations [1,3]. The G-6-PD deficient individual would develop the hemolytic crisis after a loxosceles bite. In this paper we have searched for glucose-6-phosphate dehydrogenase deficiency in all patients who developed hemolytic crisis in loxoscelism, in a period of five years in Instituto Butantan, São Paulo, Brazil.

MATERIAL AND METHODS

The clinical and laboratory data are summarised below:

1. **Case M. L.** — A white boy 10 years old was referred to Hospital Vital Brasil 8 hours after a bite in the costal region, presenting a very painful lesion, fever of 38°C and vomiting. He was interned and he was given 10 ampoules of anti-loxosceles serum, injected subcutaneously. The next day, he showed jaundice and dark urine. The spider was classified as **Loxosceles gaucho.** Laboratory data: hemoglobin: 11.0 g/dl; di-

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rect bilirubin: 0.58 g/dl; indiret bilirubin: 2.35 mg/dl; he was considered cured 7 days after and he was discharged in good condition.

2. **Case J.B.S.** — A white boy 13 years old was referred to Hospital Vital Brasil two days after a bite in his left ear, by an unidentified insect. The boy declared that 12 hours after the accident he felt very bad and the urine turned dark. He was given 12 ampoules of anti-loxosceles serum, sub-cutaneously. The laboratory data showed: hemoglobin: 5.8 g/dl; direct bilirubin: 0.6 mg/dl; indirect bilirubin: 2.4 mg/dl; creatinine: 2.3 mg/dl; BUN: 62 mg/dl. He was heavily hydrated, being discharged 16 days after.

3. **Case J.P.L.F.** — A white boy 11 years old was examined at Hospital Vital Brasil 24 hours after a spider bite on the left thigh. At Hospital he exhibited painful edematours-echymotic lesion, besides jaundice, dark urine and oliguria. He was given 10 ampoules of anti-loxosceles serum sub-cutaneously. The laboratory data showed: hemoglobin: 6.7 g/dl; direct bilirubin: 1.4 mg/dl; indirect bilirubin: 3.6 mg/dl. After 18 days of adequate hydration, he could be discharged.

4. **Case C.D.C.** — A white boy 6 years old was referred to Hospital Vital Brasil two days after a bite on the left arm by an unidentified insect, in bad state, presenting jaundice and hemoglobinuria. The local lesion was typical of loxosceles bite, i.e., very painful with necrotic area and multiple ecchymosis. 10 Ampoules anti-loxosceles serum was given intravenously. Laboratory data showed: hemoglobin: 9.1 g/dl; BUN: 57 mg/dl; direct bilirubin: 0.7 mg/dl; indirect bilirubin: 2.9 mg/dl; creatinine: 1.0 mg/dl; 16 days after he was discharged in good condition.

5. **Case G.S.** — A white woman 42 years was interned at the Hospital seven days after a bite on the right thigh. She had not seen the animal. She exhibited ecchymotic necrotic lesion on her right-thigh as well as slight jaundice. The laboratory data revealed: hemoglobin: 9.1 g/dl; direct bilirubin: 0.6 mg/dl; indirect bilirubin: 2.35 mg/dl. Three days after she was discharged without symptoms.

6. **Case G.C.M.** — A white man 39 years old was referred to Hospital Vital Brasil 7 hours after having been bitten in the right shoulder by a small spider which was killed afterwards and neglected. He presented scarlatiniform exantema, fever, and an ecchymotic edematous lesion with central vesicle. He was given 10 ampoules of anti-loxosceles serum sub cutaneously. The day after he bite, jaundice and dark urine were noticed. The laboratory data showed: hemoglobin: 13.1 g/dl; direct bilirubin: 0.6 mg/dl; indirect bilirubin: 2.9 mg/dl. Three days after the bite, he was well and could be discharged.

7. **Case J.D.S.** — A white mulatto, 19 years old was seen at Hospital Vital Brasil 24 hours after having been bitten in the right shoulder by a small spider which was killed and neglected. She exhibited jaundice, exantema, dark urine, and 38,5°C fever. He was given 110 ampoules of anti-loxosceles serum sub-cutaneously. The laboratory data revealed: hemoglobin: 7.5 g/dl; direct bilirubin: 0.8 mg/dl; indirect bilirubin: 4.0 mg/dl; BUN: 106mg/dl. The patient was hydrated, and seven days after the bite he was discharged cured.

Blood was collected by venous puncture in ACD, kept at 4°C, and the enzyme assays were performed within 4 to 5 days. The red cells were washed in saline at 4°C, hemolysed in deionized water and centrifuged at 15.000 g. The glucose-6-phosphate dehydrogenase activity was done in the supernatant according to BEUTLER [2], in Gilford 2451 recording spectrophotometer at 37°C.

RESULTS

The glucose-6 phosphate dehydrogenase activities are shown in Table I.

DISCUSSION

The pathogenesis of hemolysis in loxosceles envenomation has been exaustively studied in the last years, but it has been facing great problems, as the determination of its chemical structure. SUAREZ et al. [21] suggested a glycoprotein nature for the component of **L. laeta** venom, and at least two toxic fractions were separated by ODELL et al. [18]. Some enzymes have been

T A B L E I

Erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD) activity in hemolytic form of loxoscelism

Patients	G-6-PD i.u./g Hb/min/37°C
Normals	12.12 ± 2.09(02)
M.L.	11.0
J.B.S.	18.4
J.P.L.F.	11.3
C.D.C.	10.2
G.S.	4.8
G.C.M.	10.1
J.D.S.	5.3

described in extracts of the venoms of some species, but not linked to the toxic fractions. Thus, the enzymes adenosine triphosphatases [21], hialuronidase [4] were found in **L. laeta,** and alkaline phosphatase [11], esterase and hialuronidase [24], lipase [16], protease [24] in **L. reclusa**, although GEREN et al. [9] did not find protease activity in this last specie.

The proteases could be a red cell lytic factor, but the contradictory data obtained do not help to keep up this hypotesis. The phospholipases A, C and D which could account for the hemolytic process were not found in **L. laeta** and **L. reclusa** [10,16,21]. The hyaluronidase would have a predisposition role in venom penetration in the tissues, but not in hemolysis [24]. KNIERIM et al. [12] noticed that the venom itself did not cause hemolysis **in vitro** in red cells of several species, but abdominal wall, hemolymph, abdominal content, eggs and cephalo-torax extracts did lead to hemolysis.

An hypothesis to be considered would be the activating effect on the complement, which leads to hemolysis. The importance of hemolytic serum complement was first suggested by KNIKER & MORGAN [13], and KNIKER et al. [14]. They were able to show that very small amounts of brown recluse spider venom interacted with complement. Further studies [6] demonstrated that venom reacted with several of the components of human complement and anactivated them. MORGAN et al. [15] have shown that washed human erythrocyte were altered in the presence of small quantities of venom in such a way that subsequent addition of fresh and non heat inactivated blood group compatible serum lead to lysis. FUTRELL et al. [7] subsequently developed a quantitative hemolytic test based on this observation. They verified that hemolysis was quantitatively related to individual sera rather than to differences in erythrocytes and also noticed that swine erythrocytes behave as human ones. This was not observed in rabbit, guinea pig and dog erythrocytes. BIGGEMAN [4] verified that rats (**Rattus norvegicus**) did not present any venom serum inhibitor, and demonstrated that the venom binds strongly to the human erythrocyte membrane.

So, there are many doubts and few certainties on the pathogenesis of the hemolysis in Loxoscelism. As hemolysis occurs in a minute percentage of all cases, genetic factors could account for this tendency, and this hypothesis is stronly supported by the fact that venom does not lead to hemolysis in all mammals. Among the possible genetic factors the presence of a venom inborn inhibitor which would be absent or decreased in some individuals could be considered. Another hypothesis would be a genetic deficiency of a protective enzyme, in such a way that the deficiency individual would develop hemolysis when in contact with the venom.

The role of glucose-6-phosphate dehydrogenase (G-6-PD) deficiency in the pathogenesis of hemolysis was first investigated by NANCE [16], who found normal levels of activity enzyme in one tested patient who inhibited a hemolytic form of loxosceles. On our series, we studied 7 patients with a viscerocutaneous type of loxoscelism, and we could detect 2 out of them with G-6-PD deficiency. The mechanism of hemolysis in G-6-PD deficiency seems to be related to an oxidative effect of a group of chemicals, as sulphonas and sulphonamides, primaquine and related compounds, nitrofurantoins, bacterial or virotic infections [3]. In loxoscelism, the hemolysis in some patients could be related to an oxidant substance present in the venom. Although we had in five years only seven cases, what does not allow for an analysis with statistical purposes, it could suggest that the G-6-PD deficiency would be another factor, among others, leading to hemolysis in some individuals.

RESUMO

Forma viscerocutânea de loxoscelismo e deficiência de glicose-6-fosfato desidrogenase

Em um período de cinco anos, todos os pacientes que exibiram a forma viscerocutânea do

loxoscelismo foram investigados para a deficiência de glicose-6 fosfato desidrogenase eritrocitária, e em dois pacientes em um total de sete, esta deficiência foi encontrada. Este achado sugere que esta deficiência enzimática genética poderia ser uma das causas da hemólise que ocorre em decorrência da picada por **Loxosceles.**

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