



Communication/Comunicação

Humoral immune response of patients bitten by the snake *Bothrops erythromelas*

Resposta imune humoral em pacientes picados pela serpente *Bothrops erythromelas*

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ABSTRACT

Introduction: Snake envenomings are a health problem in rural areas of tropical and subtropical countries, but little is known regarding the immune response presented by bitten individuals. The IgM production of patients bitten by *Bothrops erythromelas* snake was analyzed to identify the effectiveness of treatment in this type of envenomation. **Methods:** *Bothrops erythromelas* venom was submitted to electrophoresis and transferred to a nitrocellulose sheet, following incubation with patients' sera. **Results:** A 38 KDa protein was detected before and 24 h after therapy. **Conclusions:** The result suggests that this protein could be used as a marker for individuals envenomed by *Bothrops erythromelas*.

Key-words: Snake venom. Western blotting. IgM. *Bothrops erythromelas*.

RESUMO

Introdução: Envenenamentos ofídicos consistem problema de saúde pública em áreas rurais de países tropicais e subtropicais, mas pouco sabe-se sobre a resposta imune apresentada pelos indivíduos picados, por isso a avaliação da produção de IgM por pacientes picados por *Bothrops erythromelas* identificando a eficácia do tratamento nesse tipo de envenenamento. **Métodos:** O veneno de *Bothrops erythromelas* foi submetido a eletroforese e transferido para nitrocelulose, seguindo incubação com soro de pacientes. **Resultados:** Foi observada proteína de 38KDa antes e 24 horas após o tratamento. **Conclusões:** Os resultados sugerem que essa proteína poderia ser utilizada como marcador para indivíduos envenenados pela serpente *Bothrops erythromelas*.

Palavras-chaves: Veneno ofídico. Western blotting. IgM. *Bothrops erythromelas*.

Snakebites occur worldwide and are a public health problem, victimizing around 421,000 people every year and involving 20,000 deaths¹. The *Bothrops* genus is responsible for 90.6% of the reported envenomings². In Northeastern Brazil, the endemic species is *Bothrops erythromelas*, locally known as *jararaca-da-seca*. *Bothrops* venoms induce local and systemic hemorrhaging, coagulopathy, edema, local necrosis and can lead to death and/or permanent disabilities³⁻⁵. Since little is known regarding the isotype and specificity of antibodies produced by patients bitten by the *Bothrops erythromelas* snake, this

paper describes the immune response in three groups of patients bitten by this snake in the City of Campina Grande, Paraíba State, Brazil.

Serum samples before treatment, 12 and 24 h posttreatment were collected from patients bitten by the snake *Bothrops erythromelas*. At the hospital, these patients were separated into three groups, according to the severity of envenomation: mild⁴, moderate⁴ and severe³. All patients were submitted to serum therapy at the same hospital. Those who agreed to participate in this study signed a term of free, informed consent. This study was approved by the Ethics Committee of the Universidade Estadual da Paraíba (UEPB). Blood was collected immediately after patient admittance to the hospital. Serum was collected, centrifuged and stored at -20°C till the moment of analysis. Antigens of *Bothrops erythromelas* venom that react with patient antibodies were analyzed by western blotting, following Towbin et al⁶.

To determine whether patient antibodies reacted to venom antigens showing an IgM production pattern, pools of serum from all the patients collected before treatment were analyzed. In all patients, whether classified as mild, moderate or severe envenomation, the main 38KDa protein was detected.

To evaluate the pattern of antibodies recognizing IgM after 24h of treatment, all patients were again analyzed and the same protein was detected (**Figure 1**).

It should be emphasized that despite serum therapy, the antibody response to *Bothrops erythromelas* followed a humoral immune response which is classic for many diseases: primary IgM production. This IgM production was previously observed using *Bothrops jararaca* venom with patients bitten by this snake⁷.

In this work, the main 38KDa protein was observed before and 24h after therapy. It is important to highlight that this could serve as a marker for *Bothrops erythromelas* envenomation, since this protein is not well neutralized by the commercial antivenom used in Brazil, perhaps because the antivenom pool does not include the *Bothrops erythromelas* species. The pool of antivenom used in Brazil may have a low efficacy for *B. erythromelas* venom neutralization.

In another study, the authors had previously observed that *Bothrops jararaca* venom is not well neutralized by the commercial antivenom, since patients analysed showed different patterns of recognition, which could implicate the genetic background of each patient or the degree of absorption of venom components⁷. In this study, the authors elucidated a clearer understand regarding the humoral immune response elicited by patients bitten by *Bothrops erythromelas* as a way of developing a potential new therapy approach.

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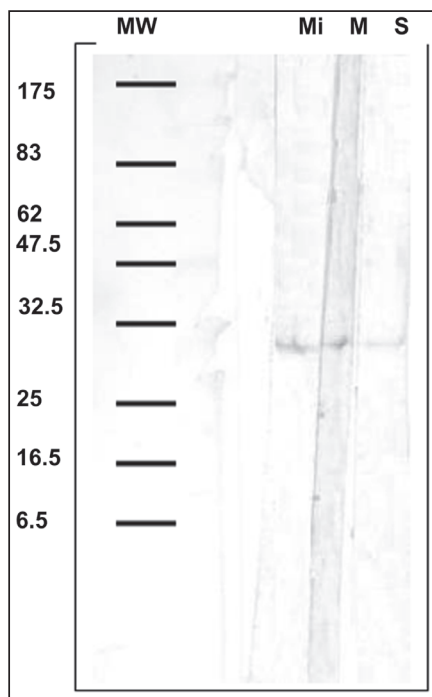


FIGURE 1 - Immunoblotting showing the reaction of *Bothrops erythromelas* venom and serum of patients after 24 hours treatment; 25µg of *Bothrops erythromelas* venom diluted 1:100 were electrophoresed on 10% SDS-PAGE under non reducing conditions and transferred to a nitrocellulose paper, blocked with 5% milk containing 0,05% Tween 20 and incubated with patients serum under 4°C overnight. Estimated molecular markers are shown.

MW: molecular weight, Mi: mild, M: moderate, S: strong envenomation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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