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STANDARDIZATION OF AN ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETECTING CIRCULATING TOXIC VENOM ANTIGENS IN PATIENTS STUNG BY THE SCORPION *TITYUS SERRULATUS*

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SUMMARY

The sensitivity and specificity of an enzyme-linked immunosorbent assay (ELISA) for the detection of circulating antigens from toxic components of *Tityus serrulatus* scorpion venom was determined in patients stung by *T. serrulatus* before antivenom administration. Thirty-seven patients were classified as mild cases and 19 as moderate or severe cases. The control absorbance in the venom assay was provided by serum samples from 100 individuals of same socioeconomic group and geographical area who had never been stung by scorpions or treated with horse antisera. The negative cutoff value (mean + 2 SD) corresponded to a venom concentration of 4.8 ng/ml. Three out of the 100 normal sera were positive, resulting in a specificity of 97%. The sensitivity of the ELISA when all cases of scorpion sting were included was 39.3%. When mild cases were excluded, the sensitivity increased to 94.7%. This study showed that this ELISA can be used for the detection of circulating venom toxic antigens in patients with systemic manifestations following. *T. serrulatus* sting but cannot be used for clinical studies in mild cases of envenoming since the test does not discriminate mild cases from control patients.

KEYWORDS: Immunodiagnosis; Scorpion envenoming.

INTRODUCTION

Enzyme linked immunosorbent assay (ELISA) for detecting and assaying snake venom and venom antibody was first described by THEAKSTON et al [15]. Thereafter, immunoenzymatic assays were developed for detection of venoms antigens from different animal species [1, 4, 7, 14, 19, 21]. These assays have been used in diagnosis, epidemiological studies [17] and monitoring of antivenom treatment in patients following snake bite [2, 16, 18, 20].

Recently, an ELISA was developed for the detection of antigens from toxic components of *Tityus serrulatus* venom [6]. This test detected toxic venom antigens at a whole venom concentration of 0.1 ng/well. Venom antigens were also detected in the sera of mice inoculated with *T. serrulatus* venom and in the sera of patients with systemic manifestations following *T. serrulatus* sting. The test showed a high specificity, assessed in mice, by the absence of cross-reactivity

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with snakes (*Bothrops jararaca, Crotalus durissus terrificus*), spider (*Phoneutria nigriventer*) and honey bee (*Apis mellifera*) venoms but failed to distinguish *T. serrulatus* from *T. bahiensis* venom.

In this paper we evaluated the sensitivity and specificity of this ELISA for detecting circulating venom antigens in patients stung by the scorpion *T. serrulatus*.

PATIENTS AND METHODS

Patients who presented to Hospital João XXIII, Fundação Hospitalar do Estado de Minas Gerais, Brazil, between January 1992 and December 1993 after being stung by scorpions were considered for the study if the scorpions were brought and identified as *Tityus serrulatus*. Patients who had been treated with scorpion antivenom before their admission to the Hospital were excluded from the study. Informed consent was obtained from patients or their relatives.

History and results of physical examination were recorded on standard forms on admission and at 6-hourly intervals until discharge from hospital. According to clinical and laboratory features, patients were classified as mild, moderate and severe cases [5]. Mild cases presented with only local pain at the site of the sting. Moderate cases had, in addition to local pain, systemic involvement that included at least one of the following clinical and laboratory manifestations: vomiting, profuse sweating, tachycardia, tachypnea, psychomotor agitation, prostration, leucocytosis (white blood cell count> 15000/mm ^{3}), hyperglicaemia (blood glucose > 120 mg/dl) and increased serum amylase enzyme activity (>160 mU/ml). Patients were classified as severe cases when they also had at least one of the following clinical features: profuse vomiting, bradycardia, arterial hypertension or hypotension, shock, cardiac failure or pulmonary edema.

Venous blood was sampled on admission for biochemical, haematological and immunological tests. Venous blood samples were also taken from 100 patients who had never been stung by scorpions or never been treated with horse antisera. These control patients were from the same socioeconomic groups, lived in the same geographical area and were admited to Hospital João XXIII for reasons other than bites and stings. Sera from control, mild, moderate and severe cases were blinded assayed for scorpion venom antigenemia using the enzyme immunoassay technique described by CHAVÉZ-OLORTEGUI et al. [6] Briefly: Immunoplates (Hemobag) were coated overnight with 100 µl / well of 2.5 µg/ml solution in carbonate buffer of the IgG F(ab') 2 fraction of anti- *T. serrulatus* venom antiserum. This was produced against the venom Tst FG-50 fraction, obtained by gel-filtration in Sephadex G-50. After blocking and washing plates, sera at 1:2 in dilution buffer from both patients and controls were incubated in the plates for 30 min at room temperature. The plates were washed again and incubated for 30 min at room temperature with peroxidase-coupled IgG F(ab') 2 anti-Tst FG50. The wells were washed and the assays were stopped after 10 min by addition of 20 µl of 2M sulfuric acid. Absorbance values were determined using a Titerk Multiscan. A reference curve was obtained using dilutions of known concentrations with 0.048 to 25 ng/well of crude venom from *T. serrulatus*. The mean plus 2 standard deviation (SD) optical density at 492 nm from the sera of the 100 control patients was considered as the cutoff value of the assay.

Sensitivity was defined as the percent of patients stung by the scorpion *T. serrulatus* who had a positive ELISA and specificity as the percent of control patients with a negative test.

RESULTS

Fifty-six patients aged 1 to 40 years old were recruited to the study among the 1122 patients stung by scorpions who presented to Hospital João XXIII during the two year period 1992 to 1993. Thirty-seven patients were classified as mild cases and 19 as moderate or severe cases.

Using the control absorbance of 0.074 as the cutoff value (mean + 2 SD), which corresponds to a venom concentration of 4.8 ng/ml, 3 of the 100 normal sera were positive, resulting in a specificity of 97%.

The sensitivity of the ELISA when all cases of scorpion sting were included (37 mild cases and 19 moderate or severe cases) was 39.3%. When mild cases were excluded, the sensitivity increased to 94.7%.

DISCUSSION

The diagnosis of scorpion envenoming is based upon a history of scorpion sting, the identification of the offending animal and the signs and symptoms of

envenoming [5, 8, 9, 10, 11, 12]. Occasionally the scorpion is not identified and the clinical and laboratory features are insufficient to support the diagnosis of scorpion envenoming. In these cases, ELISA could help to establish the correct diagnosis of scorpion envenoming by identifying circulating venom antigens.

The first ELISA for the detection of *T. serrulatus* scorpion venom was reported in 1991 [3] but the assay was not standardized for clinical use. To be clinically useful the ELISA should possess both a high sensitivity and specificity and should provide rapid results [13]. To stablish the sensitivity and specificity of an assay one must first of all determine the positive:negative threshold by testing a large number of appropriate normal controls as well as known positive cases [13, 18, 19, 20]. The control patients should be chosen within the same population as scorpion sting patients to be studied. History of these control patients must be taken to ensure that there has been no past envenoming [13, 20].

Considering these details of assay standardization, the ELISA described by CHAVÉZ-OLORTEGUI et al. [6] showed specificity of 97% and a sensitivity of 39.3% to detect circulating venom toxic antigens in patients stung by the scorpion *T. serrulatus*. The sensitivity increased to 94.7% when mild cases were excluded as this ELISA did not discriminate these cases from control patients. This ELISA was not sufficiently rapid since the assay took at least 2 hr. A delay of 120 min would be inacceptable in a severely envenomed patient. However, this study showed that because of its high sensitivity and specificity the ELISA could be a useful tool for quantification of circulating toxic venom antigens in patients with systemic envenoming by *T. serrulatus*. The study of kinetics of envenoming and serotherapy could provide a more rational approach towards the use of antivenom in patients with systemic envenoming by *T. serrulatus*.

RESUMO

Padronização de um teste imunoenzimático (ELISA) para detectar antígenos tóxicos circulantes do veneno em pacientes picados pelo escorpião *Tityus serrulatus*

Neste trabalho foram determinadas a sensibilidade e a especificidade da técnica imunoenzimática (ELISA) desenvolvida por CHAVÉZ-OLORTEGUI et al. para detectar antígenos circulantes de veneno em pacientes picados po *Tityus serrulatus*. A média mais dois desvios padrão da observância do soro de 100 pacientes controles foi utilizada como limite entre teste positivo e teste negativo ("cutoff"). A especificidade do ELISA foi igual a 97,0%. A sensibilidade do método, quando incluidos pacientes classificados como casos leves, moderados e graves de escorpionismo, foi de 39,3% e aumentou para 94,7% quando considerados apenas os casos moderados e graves. Estes resultados mostram que o ELISA pode ser utilizado para detecção de antígenos tóxicos circulantes em pacientes com manifestações sistêmicas de envenenamento escorpiônico mas não deve ser empregado no estudo de pacientes que apresentam apenas dor no local da picada (casos leves). O tempo necessário para a realização do ELISA é superior a 1 hora. Portanto, o teste tem sua utilização limitada para o diagnóstico de envenenamento, mas pode constituir um instrumento útil para o estudo da cinética de neutralização do veneno pelo antiveneno específico.

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