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Serum level of scorpion toxins, electrolytes and electrocardiogram alterations in Mexican children envenomed by scorpion sting

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ABSTRACT

The scorpion *Centruroides limpidus limpidus* (*C.l.l.*) is endemic in México, producing hundreds of accidents in humans; children being one of the most susceptible targets. Few studies reported that severe envenoming by scorpion venom induces cardiac damage and electrolytes abnormalities in children, but the relationship of envenoming severity and toxic blood levels is unknown. The aim of this study was to determine the relationship among clinical status of envenoming, serum electrolyte, electrocardiographic abnormalities, and serum toxin levels in 44 children stung by scorpion over a period of 6 months in the State of Morelos, Mexico. The patients were said to be asymptomatic, when they presented just local symptoms, and were said to be symptomatic when showing local symptoms and at least one systemic symptom. The clinical status was evaluated at the admission at the emergency room of the Hospital, and 30 min after the administration of polyspecific F(ab')2 anti-scorpion therapy to symptomatic children. Forty-one percent of the children were asymptomatic and 59% symptomatic. Potassium and sodium imbalance and an elongation of the QT interval were detected; the rate of hypokalemia was higher in symptomatic than on asymptomatic children (50% and 6%, respectively). Hypokalemia persisted in 19% in symptomatic patients, whereas sodium reached normal levels 30 min after anti-venom therapy. The hypokalemia statistically correlated with elongation of the QT interval. The concentration of the toxic components of *C.l.l* in serum was significantly higher in symptomatic than asymptomatic children, and the serum levels of the toxic component significantly decreased to undetectable levels after the application of anti-venom therapy. Despite the small size of the sample, this study establishes that severity of envenoming was statistically related to potassium imbalance in serum, QT interval and the concentration of toxic components in serum, which decreased at undetectable levels after specific treatment with the anti-scorpion venom, correlating with clinical disappearance or greatly reduction of symptoms of envenomation.

1. Introduction

Human accidents caused by scorpion stings are a severe health problem in numerous tropical and subtropical areas; annual global incidence of envenoming by scorpion is estimated to be about 1.5 million events, with 2600 fatal cases (Chippaux and Goyffon, 2008). Although the frequency is higher in adults, the severity in children is greater (Chippaux and Goyffon, 2008). In Mexico 281 species of scorpions exist and eight scorpion species from the genus *Centruroides* are of medical relevance. This includes *Centruroides limpidus limpidus* (*C.l.l.*) distributed in several states at the central and southern regions of Mexico (Santibañez-López et al., 2016). In the year 2015, 265,749 scorpion envenomation cases were reported. In the state of Morelos, where this study was conducted, 31,541 cases were registered (México, 2015). *Centruroides* venoms contain

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different polypeptide toxins, but the main clinical symptoms and lethal effects observed in scorpions are the result mainly of toxins binding to Na^+ channels and to a lesser extent to those acting on K^+ channels, Ca^{2+} and Cl^- channels; a recent review on the subject can be found in Quintero-Hernández et al. (2013). All these toxins to ion channels are in the Fraction II obtained from fractionation of *Centruroides* venom by gel filtration in Sephadex G-50. The fraction II of *C.l.l.* contains about 65% of the venom proteins, and 100% of these have a lethal potency to mammals (Alagón et al., 1988; Ramírez et al., 1994). It is described that scorpion toxins to Na^+, K^+, and Ca^{2+} channels, cause prolongation of the action potential of the cell membrane, so that increases the secretion of acetylcholine and catecholamines at sympathetic and parasympathetic level, causing neurological and cardiac effects (Del Brutto, 2013). In children with severe envenoming by scorpion stings, electrolyte abnormalities occurred, being the hyperkalemia and the hyponatremia the most frequently observed (Osnaya-Romero et al., 2008). The envenoming by scorpion venom is also related to cardiac abnormalities. An experimental model of scorpion sting envenoming conducted in dogs reported damage in the left ventricle, suggesting that this is due to coronary hypoperfusion and ST-segment changes (Cordeiro et al., 2006). Other authors have reported cardiogenic shock and pulmonary edema (Mohamad et al., 2014). In children envenomed by scorpion sting, González et al. (1991) and Tarek et al. (2006) described the presence of myocarditis (52%), electrocardiographic changes (10%), intraventricular conduction disorders (13%), arrhythmias (11%), and increased of creatine phosphokinase-MB level. Different authors determined that the clinical severity of envenomation in humans stung by several scorpions of different species is related to the concentration of scorpion venom. Using ELISA to determined venom concentration in plasma or serum, they showed that serum scorpion venom levels correlate with clinical severity of envenoming by *Tityus serrulatus* (de Rezende et al., 1996), *Androctonus australis* garzonii and *Buthus occitanus tunetanus* (Hammoudi-Triki and Laraba-Djebari, 2003; Krifi et al., 1998), and *Centruroides sculpturatus* (Chase et al., 2009).

To the best of our knowledge, there is no evidence of the probable association between serum levels of the scorpion toxins from *C.l.l.*, and the clinical behavior (serum electrolytes and electrocardiogram alterations) in Mexican children envenomed by scorpion sting. Thus, the objective of this study was to determine the relationship among clinical status of envenoming by *C.l.l.*, electrolytes, and electrocardiographic abnormalities, and serum toxins levels, before and after administration of anti-venom immunotherapy.

2. Methods

The study was conducted with 44 children, from 6 to 182 months. These children were medicated at the Emergency Room (ER) of the "Hospital del Niño Morelense" (HMM) over the period of 6 months (April to September 2004). The patients presented a history of a scorpion sting and compatible clinical symptoms of envenoming by the scorpion sting during the first 2 h of intoxication. Children with a clinical history of heart disease, neuropathy, and renal pathologies were excluded from the study. Signed informed consent from parents of patients was obtained, to be included in this study. The protocol was approved by the Ethics Committee of the Instituto Nacional de Pediatría, México City, México. Patients did not undergo risks in this study, and only their evolution was observed. Patients were provided with anti-venom immunotherapy and life support and the volume of blood extracted to the patients did not jeopardize hemodynamic function. All the patients of this study survived the treatment.

2.1. Classification of patients

Children were classified as asymptomatic if said to have been stung by a scorpion, presented pain, erythema and paresthesia localized into the site of the stung. Asymptomatic patients were aged from 6 to 174 months old. Children were classified symptomatic if, in addition to all the criteria of asymptomatic patient, presented at least one of the following signs or symptoms: irritability, nasal itching, foreign body sensation in the throat, sialorrhea, dysphagia, irritability, tachycardia, tachypnea, respiratory distress based on clinical classification Silverman/Major Anderson 3, fever or hypothermia, nystagmus, saturation by oximeter pulse less than 92%, cyanosis, drowsiness, stupor, coma or seizures (Barros et al., 2014; López, 2014). The age of symptomatic patients varied from 16 to 182 months old.

2.2. Anti-venom therapy

Commercially available polyspecific F(ab')2 anti-scorpion antivenom was from Alacramyn® (Instituto Bioclon, México City, México). The anti-scorpion immunoglobulin was obtained from the serum of horse hyperimmunized with a mixture of venoms from the most poisonous scorpion species of Mexico: *Centruroides noxius*, *Centruroides limpidus limpidus* and *Centruroides suffusus suffusus*.

2.3. Blood sample

From each patient a blood sample of 3 mL was taken at the admission in ER and 30 min after application of anti-venom immunotherapy. Blood samples were used for: 1) electrolytes determination, and 2) serum separation by centrifugation for 5 min at 3000 rpm at room temperature; the serum was recovered and distributed in aliquots that were frozen and kept at −20 °C until the scorpion toxins quantization was made.

2.4. Serum electrolytes

Measurements of sodium and potassium were made in the Bayer 644 (Bayer, Polony) and Miraplus DCL COBAS (Roche Diagnostic Systems, Switzerland) electrolyte analyzers. The values of serum electrolytes were considered in the normal range when potassium was from 3.5 to 5.5 mEq/L, and the sodium values between 135 and 145 mEq/L (Suárez and Uribe, 2014).

2.5. Quantization of toxic components of C.l.l. scorpion venom in serum

The quantization of toxic components present in the serum from envenomed children were established by quantitative ELISA (antigen capture format), developed in our laboratory. For obtaining immunoglobulins against toxic components to mammalian, fraction II from scorpion *C.l.l.* was used (Ramírez et al., 1994). Briefly, the fraction II was obtained from whole soluble venom obtained from telsons of scorpion *C.l.l.* applied to a Sephadex-G50 column. This fraction corresponds to approximately 76% of soluble venom and is toxic to mammalian according to the mouse lethality test, whereas other three fractions obtained from Sephadex-G-50 were not toxic for mammalian in a range of 5–100 μg (Ramírez et al., 1994). This preparation was used to obtain specific immunoglobulins from New Zealand rabbits according to method described by Calderón-Aranda et al. (1999). Briefly, rabbits were immunized subcutaneously five times with the antigen, at ten-day intervals. After the last immunization, rabbits were bled and sera were used for purifying specific immunoglobulins to *C.l.l.*-FII on a column of Sepharose-4B