Myocardial injury induced by scorpion sting envenoming and evidence of oxidative stress in Egyptian children

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ABSTRACT

In the present study, 45 children in Upper Egypt (less than 16 years old) were admitted to the Pediatric Intensive Care Unit for scorpion envenomation (SE). They were compared with 30 apparently healthy children of matching age and sex as controls. Out of the studied victims, 35 children (78%) showed signs of severe envenomation, while 10 victims (22%) showed signs of mild envenomation. The case fatality was 33%. The serum levels of cardiac markers, cardiac troponin T (cTnT) and I (cTnI), as well as the enzymatic activities of creatine kinase-MB (CPK-MB) and lactate dehydrogenase (LDH) were determined for both victims and controls. In addition, the serum levels of oxidative stress markers, nitric oxide (NO), malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH) and zinc (Zn) were measured. Electrocardiography and echocardiography were done. All the envenomed victims showed significantly higher mean values of cTnT, cTnI, CPK-MB and LDH than control group. These cardiac markers were elevated in severe cases and in non survivors in comparison with mild cases and survivors respectively. Furthermore, the serum levels of NO and MDA were significantly higher while the serum levels of SOD, GSH and Zn were significantly lower in all envenomed victims than the controls ($p < 0.05$ for all). There were no significant differences in the serum levels of oxidative stress markers among severe and mild cases or between survivors and non survivors victims. There were no significant correlations between the serum levels of cardiac markers and the oxidative stress markers in envenomed victims. In conclusions, oxidative stress occurs in scorpion envenomed children, but does not determine prognosis. Cardiac markers, but not the oxidative stress, remain the most important determining factor for the severity and the outcome of SE.

1. Introduction

Scorpion envenomation (SE) is a frequent health hazard, especially in tropical and subtropical countries such as Upper Egypt, and considered as an important risk factor for morbidity and mortality particularly among children (Mohamad et al., 2014; El-Deek et al., 2017). Various components of the scorpion venom cause adverse pathological effects particularly in children, if untreated. The annual number of scorpion stings worldwide is estimated to be 1.2 million with 2.3 billion people at risk (Chippaux and Goyffon, 2008; Khatony et al., 2015). In the united states, the highest exposure indices occurred in Phoenix (up to 677 per 100 000 population) and Tucson (584), both in Arizona (Kang and Brooks, 2017).

Scorpion induced-cardiac injury manifests clinically mainly as left ventricular dysfunction of varying degrees with possible progression into acute pulmonary edema. It is associated with increased serum levels of cardiac markers, as well as electrocardiogram (ECG) and echocardiography (ECHO) abnormalities (Meki et al., 2002; Sagarad et al., 2012a,b, 2013).

The pathogenesis of myocardial damage and cardiac dysfunction resulting from scorpion envenomation has been a subject of debate in the past with catecholamine mediated cardiac injury the most accepted hypothesis (Al et al., 2014).

It has been reported that generation of free radicals, hypoxia, high cytokines levels and nitric oxide following SE, may induce the onset of multiple organ dysfunction (Meki et al., 2003a). Accordingly, the present study aimed to assess various oxidative stress biomarkers and investigate their relationship with the myocardial injury biomarkers in

children with SE of various severities and outcomes.

2. Patients and methods

2.1. Study design and participants

This prospective study has been conducted on 45 children (30 males and 15 females), presenting to the pediatric intensive care unit of Qena University Hospitals-Upper Egypt, with history of SE, presence of sting mark and sting or scorpion seen in vicinity of the child by parents or near family members with clinical and/or investigatory evidence of myocardial injury. *Buthus occitanus* is the main scorpion species in Upper Egypt region, with *Leiurus quinquestriatus* (the Egyptian scorpion or the Death stalker) also present (Lucas and Meier, 1995; Meki et al., 1995, 2003b). The severity of SE in relation to cardiac injury was classified by many investigators (Meki et al., 2003b; Petricevich, 2010; Cupo, 2015) into: mild cases (only mild symptoms of envenomation), moderate cases (moderate manifestations of envenomation without clinical evidence of carditis; and severe cases (severe envenomation with clinical signs of toxic myocarditis). They were compared with 30 apparently healthy children of matching age and sex as controls. All children involved in our study were at age from 1 to 16 years. The study period was from March 2016 to March 2018.

Victims with mild evenomation recieved 3 ampoules (each I ml) given intravenously, using Ringer or glucose 5% solution to be infused over two hours. For moderate cases, the number of ampoules increased to five, while in severe envenomation, it is recommended to give 10 ampoules and can be increased up to 20 ampoules using two intravenous lines, one in each hand, at admission, and was under supervision for 48 h. The anti-venom contains antibodies that neutralize the venom from the following scorpions: *Buthus occitanus*, *Leiurus quinquestriatus*, *Androctonus amoreuxi*, *Androctonus aeneas* and *Androctonus crassicauda* (Meki et al., 2003a).

The study was approved by the ethics committee of Faculty of Medicine, South Valley University, Qena-Egypt and written informed consents from parents of the included children were taken.

Exclusion criteria: Patients with pre-existing congenital or valvular heart diseases, diabetes mellitus, chronic kidney disease, any systemic disorder or those on regular medications, were excluded from the study.

2.2. Data collection

Our cases have been subjected to history taking and thorough clinical examination. Routine investigations in the form of chest X-ray, ECG: ECG tracing was analyzed for signs of myocardial involvement according to the criteria proposed by Jedeikin et al. (1983), echo-cardiographic measurements mainly, left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), % fractional shortening (% FS), left ventricular ejection fraction (LVEF) and cardiac chambers dilatation, using two-dimensional (2D) cardiovascular ultrasound system transthoracic (Vivid 3, 2005, Germany). Also, blood counts and arterial blood gases were analyzed for all children.

2.3. Laboratory workup

Venous blood sample (10 ml) was withdrawn on plane tubes, from every child involved in the study, and was left at room temperature for 30 min to allow clotting. After centrifugation at 3500 rpm for 10 min, separated sera were divided into aliquots, using 1 ml cryotubes and were stored at −80 °C until measurement of the studied biochemical parameters.

Biomarkers of cardiac injury and oxidative stress were determined using commercial kits. Serum levels of cTnT and I were performed as instructed by manufacturers using commercial automated chemiluminescent microparticle immune assay (CMLA) utilizing Chemi-Flex

Technology (Architect i2000, Abbott diagnostics, USA). Spectrophotometric assays were performed of the following biochemical parameters, using T60 UV visible spectrophotometer (PG INSTRUMENTS LIMITED, Alma park, Leicestershire, England. Serial No. 20-1650-01-0010):

A) Serum CPK-MB assay was done using kit supplied by CAL- TECH Diagnostics, Inc. (Chino, CA, 91710, USA), according to Gerhardt et al. (1977).
B) Serum LDH assay was determined using kit supplied by Stanbio LDH (UV- Rate), according to Buhl and Jackson (1978).
C) Oxidative stress markers were determined using chemical methods as mentioned in a previously published work (El-Masry et al., 2018). Serum NO was measured according to Ding et al. (1988), using Griess reagent (1% sulfanilamide and 0.1% n-(1-naphthyl) ethylenediamine dihydrochloride in $5\%H_3PO_4$. Serum MDA was determined according to Wills (1969), depending on the ability of thiobarbituric acid (TBA) to react with MDA in an acidic medium at 95 °C for 30 min to form thiobarbituric acid reactive product. Serum SOD assay was done according to Misra and Fridovich (1972), based on the capacity of SOD to inhibit autoxidation of adrenaline to adrenochrome. Serum reduced glutathione (GSH) was measured according to Ellman (1959), using water soluble (at pH 8) aromatic disulfide [5,5′-dithiobis (2-nitrobenzoic acid)] for determination of the sulfhydryl groups.

Serum zinc levels were estimated by atomic absorption spectrophotometry (EEL240), according to Lee and Jacob (1974).

2.4. Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows version 22. Quantitative data were expressed as means ± standard deviation. Qualitative data were expressed as number and percentage. Numerical data were tested for normality using Kolmogrov–Smirnov and Shapiro-Wilk tests. Mann–Whitney test was used to compare between two groups. Spearman's rank correlation coefficient was used to assess the correlation between variables. A two-tailed $p < 0.05$ was considered statistically significant.

3. Results

3.1. Demographic data of the included children

The present study has been conducted on 45 scorpion envenomed children (SEC) (with male to female ratio 2:1) and 30 healthy matched controls (14 males and 16 females). The mean ± standard deviation of their ages (years) and weights (kg) were (5.13 ± 0.81, 4.62 ± 1.13) and (17.96 ± 1.86, 12.06 ± 1.99) respectively, with no significant difference between the two groups. Children with severe envenomation were 35 (78%) and the remaining envenomed children were mild, 10 (22%). As regards the outcome, survivors represent 30 (67%), while non-survivors envenomed victims were 15 (33%).

3.2. Clinical and cardiac imaging findings of the included scorpion envenomed children

Regarding the relative frequency (number, %) of the cardiac manifestations among cases, there were dyspnea (21, 47%), orthopnea (9, 20%), raised jugular venous pressure (33, 73%), lower limb edema (27, 60%), congested liver (18, 40%) and cardiomegaly (32, 73%). As regard the frequency of ECG abnormalities, 15 (33.3%) of cases had normal ECG, 15 cases (33.3%) had abnormal Q wave (grade IV), 3 children (6.66%) had flat T wave (grade I) while, inverted T wave and depressed ST segment were present in equal proportions, 6 cases (13.34%) each.