

Cardiac Biomarkers Assay for Early Detection of Toxic Myocarditis Following Scorpions Stings ExposureHeba Youssef Mohamed¹, Rasha Emad Badr², Mohamed Seif El-din³ and Omneya, I. Youssef⁴¹Forensic Medicine and Clinical Toxicology department Faculty of medicine Port Said University²Clinical Pathology department Faculty of medicine Port Said University³Information decision support center, Critical care department, Ministry of Health & Population, Egypt⁴Pediatric Cardiology unit, Pediatric department Faculty of medicine - Ain Shams University Hospitalsdrheba_sayed@med.psu.edu.eg

Abstract: Background: Scorpion envenomation is an important health hazard in tropical regions. Envenomation by scorpions can result in a wide range of clinical manifestations, but the most dangerous are the cardiotoxic effects. **Objective:** The aim of this study was first to evaluate the sociodemographic variables and clinical manifestations among patients exposed to scorpions stings. Second to assess the validity of serum cardiac biomarkers for early detection of acute toxic myocarditis. **Subjects & Methods:** The study was carried out in the period from March to December 2015 where sixty two patients of both sexes with different ages diagnosed as acute chest pain following scorpion sting. The patients were divided into three groups according to the severity of scorpion envenomation plus twenty four healthy volunteers served as a control group. **Results:** The sociodemographic variables revealed that most of cases were males exposed to stings by yellow colored scorpions at urban areas at night during summer months more at their lower limbs. General examination revealed significant hypertension among group II and III. Bradycardia, tachycardia, tachypnea and bradypnea were more significant among group III. Pan systolic murmur, cardiogenic pulmonary edema and CVS collapse were evident only among group III. Paroxysmal supraventricular tachycardia PSVT, atrial fibrillation and first degree heart block were significant only among group III. Regrouping of patients into cardiac and non-cardiac affected groups revealed two steps of significant increase in the levels of H-FABP and myoglobin from control group to non-cardiac affected group then to cardiac- affected group, in contrary to CK-MB and Troponin I levels showed significant increase in cardiac-affected group only. Analysis of cardiac biomarkers proved that the area under the curves were observed to be higher in Troponin I followed by H-FABP, myoglobin and CK-MB, with marked inter significant difference between them ($P < 0.001$). The area under the ROC showed diagnostic discrimination of biomarkers, at the optimum cut-off value, sensitivity and specificity to be 96 and 47% respectively in H-FABP, 96 and 25% respectively in CK-MB, 96 and 31% respectively in Myoglobin, 92 and 97% respectively in Troponin I. **Conclusion:** Troponin is proved to be the cardiac biomarker of highest specificity but has delayed onset of appearance in the serum. H-FABP becomes positive earlier than troponin and is more sensitive than myoglobin. **Recommendations:** Troponin and H-FABP assay should be done for early diagnosis and better outcome of acute chest pain following scorpion sting exposure.

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Key words: Scorpion sting, Cardiac biomarkers, Toxic myocarditis

1. Introduction

In many tropical and subtropical regions of the world scorpions, crab like arachnids are the most important venomous animals after snakes. Scorpions are eight-legged arthropods; order Scorpionida and class Arachnida. The terminal segment, called the telson (bulb containing a pair of venom secreting salivary glands), contains two venom glands connecting with the curved needle sharp sting that is used either for defense or to obtain food (1). Telson is surrounded by striated muscular layer facilitating and regulating venom ejections which explains the variation of intensity of symptoms and existence of "dry" sting without envenoming. The scorpion venom is a mixture of various active substances namely

polypeptides and enzymes. Venom consists of: hemolysins, agglutinins, hemorrhagins, leukocytolysins, coagulins, lecithin, cholesterolin, cardiotoxins, nephrotoxins, hyaluronidases, phosphodiesterase, phospholipases, glycosaminoglycan, histamine, tryptophan and cytokine releasers and neurotoxin, which act on the cardiac muscle, vasomotor centers, respiratory nerve terminals and motor end plates(2). Also it contains a number of free amino acids and serotonin (3). The scorpion venom toxins, contain peptides that block the sodium channels (beta-toxins). There is massive release of endogenous catecholamines into the circulation due to delayed activation of sodium neuronal channels by the venom (4). The length of

adult scorpions varies from 2 cm to about 20 cm but the length does not relate to its toxicity (some of the most dangerous scorpions are only 2–4 cm long) (5). It takes 6–10 months for the newborn scorpions to become poisonous. In spite of advances in pathophysiology and therapy the mortality remains high in sandy areas and areas devoid of poison control centers (6), the issues that necessitate early management with antiscorpion venom and close follow up to predict toxic myocarditis which is considered the most severe of its complications.

Cardiac biomarkers play an important role in the diagnosis of acute coronary syndrome (ACS). The commonly used methods are to quantify blood levels of troponin-I (TnI), myoglobin, myocardial-bound creatine kinase (CK-MB), and most recently heart-type fatty acid-binding protein (H-FABP). Cardiac biomarkers, however, have advantages and disadvantages due to different time-dependent features for the evaluation and management of patients with suspected ACS (7). An ideal marker is the one which can predict the onset of the disease thus could aid in reducing the deaths due to ACS.

Aim of the work

So the aim of this study was first to evaluate the sociodemographic variables and clinical manifestations among patients exposed to scorpion stings. Second to assess the clinical validity of serum cardiac biomarkers namely H-FABP, CK-MB, myoglobin, and (TnI) levels, for early diagnosis of acute toxic myocarditis in those patients.

2. Methodology

Subjects and Methods

A) Subjects:

A clinical prospective descriptive study was performed on the patients manifested with severe chest pain following scorpion sting admitted to emergency department of Port Said general hospital in the period from March to December 2015. Entry criteria included 62 patients of both sexes with different ages diagnosed as acute chest pain (ACP) following scorpion sting plus 24 healthy nonsmoker volunteers (matched age and sex) served as a control group. Patients with history of cardiac or pulmonary diseases and patients who were recently resuscitated by DC shock were excluded from the study.

Grouping: The patients were divided into 3 groups according to the severity of scorpion envenomation according to Bouaziz et al. (8).

Group I: Included 20 patients with mild symptoms and signs.

Group II: Included 15 patients with moderate symptoms and signs.

Group III: Included 27 patients with severe symptoms and signs.

Group IV: Included 24 healthy nonsmoker volunteers served as a control group.

Mild group are those patients who had only localized effects of scorpion. **Moderate group** included patients who had also systemic manifestations in form of moderate chest pain, dyspnea, tachycardia, tachypnea, nausea, vomiting, tachyarrhythmia and diarrhea. **Severe group** included patients with severe cardiorespiratory manifestations, mainly cardiogenic shock, pulmonary edema, severe neurological and parasympathetic manifestations.

Ethical consideration:

Written informed consent was obtained from patients or from their next kin according to the guidelines of Ain Shams University ethical committee of scientific research. Also a written approval form for this study was taken from the general director of Port Said General Hospital and from the director of Information decision support center, Critical care department, Ministry of Health & Population, Egypt.

B) Methods:

Study area:

Port Said governorate is a city that lies in north east Egypt extending about 30 kilometers along the coast of the Mediterranean Sea, north of the Suez Canal. Port Said city has six major regions (South, East, Elzohor, ElManakh, El Arab and Port Fouad). Port Said climate is classified as a hot desert climate according to Köppen climate classification, but blowing winds from the Mediterranean Sea greatly moderates the temperatures, typical to the northern coast of Egypt, making its summers moderately hot and humid while its winters mild and moderately wet. January and February are the coolest months while the hottest are July and August.

The estimated population of the city was 603,787 inhabitants in 2010, with 74.2% living in the urban zones and 25.8% in rural areas. The economic base of the city is based on the industrial sector, which hosts a large number of national and multinational companies. Also fishing and trading. Within the city are increasing investments in areas such as fish farming, agribusiness and rural production. In the South of the city, there are huge increase in cattle, logging and cultivation of crops mainly cotton and rice. While in El Zohor district there is increasing slums areas with abundant cattle and accumulation of rubbish.

The following parameters were studied in all groups:

(I) Socio-demographic variables:

Including age, sex, place of exposure, occupation, timing of sting (day or night and season), color of scorpion and site of sting.

(II) Clinical assessment:

i) History Taking: Full medical history with particular emphasis on delay time of intoxication

ii) Physical Examination which included:

1) Local signs of scorpion sting: all patients were examined for swelling, redness, tenderness at site of sting mark.

(2) General Examination:

All patients were generally examined on admission to obtain vital data (blood pressure, pulse, temperature and respiratory rate). Regarding reference values, hypothermia was diagnosed at a temperature below 36.5°C, fever was diagnosed at a temperature above 37.2°C and normal temperature was between 36.5°C -37.2°C(9). Tachypnea was diagnosed at a respiratory rate of more than 18 breath /minute; bradypnea was diagnosed at a respiratory rate of less than 12 breath/minute and normal respiratory rate considered between 12-18 breath/minute. A normal breathing rate for an adult at rest is 8 to 16 breaths/minute and for a child, a normal rate is up to 44 breaths/minute (10). For infants and children

respiratory rate were compared to normal values for age (11). Bradycardia for adult patients was diagnosed at a heart rate of less than 60 beats /minute and tachycardia at a heart rate of more than 90 beats /minute (12). Hypotension was considered if the patient systolic blood pressure reading was lower than 90 mmHg, hypertension was considered if the patient's blood pressure was more than 140/90 mmHg and normal blood pressure considered if systolic blood pressure not more than 140 mmHg and diastolic blood pressure not less than 60 mmHg (12). As regards infants and children blood pressure and heart rate values were compared to age and gender centiles (13).

(3) Systemic Examination:

- **Central nervous system CNS Examination:** thorough neurological examination and assessment of the level of consciousness of patients according to Reed's classification (14).

Table (1): Reed's classification of the level of consciousness.

Stage	Confusion level	Pain response	Reflex response	Respiration	Circulation
Stage 0	Asleep	Arousable	Intact	Normal	Normal
Stage I	Comatose	Withdraws	Intact	Normal	Normal
Stage II	Comatose	None	Intact	Normal	Normal
Stage III	Comatose	None	Absent	Normal	Normal
Stage IV	Comatose	None	Absent	Cyanosis	Shock

Irritability, drowsiness, hallucination, agitation, and seizures were also looked for.

- **Gastrointestinal Examination;** for vomiting, diarrhea and abdominal tenderness.
- **Genitourinary system Examination;** for Polyuria, incontinence, loss of bladder control and priapism.
- **Parasympathetic manifestations:** for lacrimation, salivation, bronchospasm, bronchorrhea, miosis, piloerection and generalized muscle weakness.
- **Respiratory system Examination:** Air entry and abnormal sounds on auscultation were examined.
- **Cardiovascular Examination:** heart rate or heart sounds were examined. As routine measures all patients subjected standard electrocardiograph (ECG). An ECG was recorded to some subjects under study using Cardiomax, FKODA model 2011. Twelve leads ECG analysis for estimation of heart rate, rhythm, QRS, P-R and corrected QTc intervals as well as presence of any detectable dysrhythmias or conduction defects.

(III): Cardiac Biomarkers laboratory assay

Within 30min of patient arrival at the emergency room one venous blood sample (10 ml) was withdrawn by sterile venous puncture and clean dry syringes from the cubital vein of every investigated subject into plain tubes (without anticoagulants) and

allowed to clot for 1/2 an hour. After centrifugation blood serum was separated from the clot and liquated under complete aseptic condition. The serum obtained was kept frozen at -20°C till the analysis of serum H-FABP.

First: Serum Heart type fatty acid binding protein (H-FABP):

The quantitative determination of serum H-FABP was done using commercially available enzyme-linked immunosorbent assay ELISA Kit supplied by Bio Vendor Gmbh, Heidelberg, Germany (15).

1. Seventy microliter (μ l) of buffer solution was placed in an eppendorf tubes. Then 70 μ l of standard solution or sample was added to each eppendorf. The eppendorfs were agitated gently on a mixer.

2. The H-FABP antibody coated wells were prepared. One hundred μ l of the mixed solution prepared in procedure 1 was added to the H-FABP antibody coated wells using a multichannel pipette.

3. The microtiter wells were incubated for 30 min at 15-25°C.

4. The microtiter wells were washed three times with wash buffer 300 μ l /.

5. Residual solution in each well was removed by turning the wells upside down and tapping on the paper towel after the final wash.

6. H-FABP antibody –enzyme conjugate (100 µl) was added to the microtiter wells and wells were agitated for 30 sec.

7. The microtiter wells were incubated for 30 min at 15-25°C.

8. Steps 4 and 5 were repeated.

9. One hundred aliquot of substrate solution was added to each well with a multichannel pipette row to assay the HRP enzyme activity.

10. The microtiter wells were shielded from light with aluminum foil and incubate for 15 min at 15-25°C.

11. The HRP enzyme reaction was terminated by addition of 100 µl of stop solution to each well with multichannel pipette row by row at the same interval as the addition of substrate solution then agitated for 30 sec.

12. The absorbance of each well was measured at 450 nm (main wave length) and 620 nm (reference wave length) using microplate reader within 3 hours.

13. The concentration of H-FABP mass was calculated in each sample by reference to the standard curve obtained from the seven points of standard solution expressed as ng of H-FABP protein /ml. of serum.

Second Other Cardiac Biomarkers Assay:

Serum Troponin I (TnI) and myoglobin was determined by Pathfast assay (LSI Medicine Corporation, Tokyo, Japan). Measurement of Creatine Kinase CK-MB by Microparticle Enzyme Immuno Assay using Cobas 300 (Roch Diagnostic Indianapolis, USA). Upper reference limit (99th percentile) for (TnI) concentration is 0.011 ng/ml.

Levels > 4.99 ng/ml for CK MB, and > 48.8 ng/ml for myoglobin are abnormal.

(IV): Statistical analysis

Results are expressed either as mean ± SD or as median and interquartile range. Differences in clinical characteristics between groups were analyzed by Student's t-test, x 2 test, or Fisher's exact test, as appropriate. Differences in serum cardiac biomarkers concentrations are expressed in mean ± SD, and for comparison by the nonparametric Mann–Whitney U-test, or Kruskal–Wallis H-test, as appropriate. The 95% confidence intervals (CI) and ROC curves were used to assess the discriminatory ability of indicators. A univariate Z-test was used to compare the areas under the ROC curves of indicators. Differences in sensitivity, specificity, and predictive accuracy between indicators were evaluated by the sign test. $P < 0.05$ was considered statistically significant.

3. Results

I: Sociodemographic variables:

Table 2 shows the number and percentage of sociodemographic variables among cases exposed to scorpions stings admitted to Port Said general hospital from March to December 2015. Most of cases were males exposed to stings at urban areas. Their occupation varied in each group. In group I (40% were students, 25% farmers, 15% fishermen, 10% manual workers and 10 % house wives). In group II (60% were students, 26.6% manual workers and 13.3% farmers). In group III (48% were manual workers, 33.3% students, 11.11% farmers and 7.4% fishermen). Most patients exposed to the yellow color scorpions sting during night time mainly in summer months (June to September) at their lower limbs.

Table (2): Number and percentage for sociodemographic variables among studied groups of patients exposed to scorpions stings from March to December 2015.

Sociodemographic Variables	Group I N=20		Group II N=15		Group III N=27	
	N	(%)	N	(%)	N	(%)
Gender						
Male	17	85	13	86.6	26	96.3
Female	3	15	2	13.3	1	3.7
Place of exposure						
Urban	20	100	10	66.6	22	81.5
Rural	-	-	5	33.3	5	18.5
Occupation						
Fisherman	3	15	-	-	2	7.4
Manual worker	2	10	4	26.6	13	48.1
Student	8	40	9	60	9	33.3
Farmer	5	25	2	13.3	3	11.11
Housewife	2	10	-	-	-	-
Time of sting						

Day time	5	25	6	40	8	29.6
Night time	15	75	9	60	19	70.4
March	2	10	-	-	1	3.7
April	1	5	-	-	1	3.7
May	1	5	1	6.6	1	3.7
June	1	5	3	20	6	22.2
July	5	25	3	20	8	29.6
August	4	20	3	20	4	14.8
September	4	20	4	26.7	6	22.2
October	1	5	1	6.6	-	-
November	1	5	-	-	-	-
December	-	-	-	-	-	-
Color of scorpion						
Yellow type	10	50	10	66.6	23	85.2
Black type	10	50	5	33.3	4	14.8
Site of sting						
Upper limb	3	15	1	6.6	4	14.8
Lower limb	14	70	11	73.3	21	77.8
Other sites	3	15	3	20	2	7.4

Figure 1 shows the most common age categories were those below 18 and more than 60 years among

studied groups of patients exposed to scorpions' sting from March to December 2015.

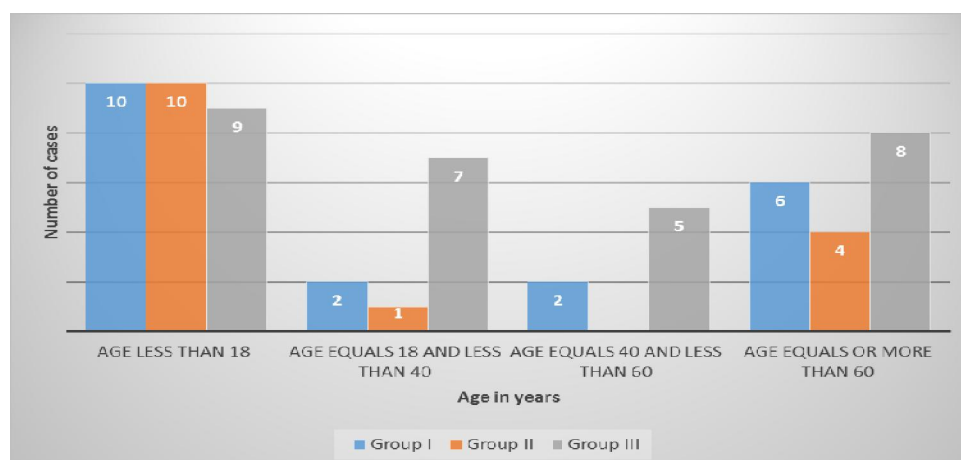


Figure (1): Age distribution among studied groups of patients exposed to scorpions stings from March to December 2015

II- Clinical assessment Results:

Table (3) shows significant difference was noticed regarding delay time between the three studied groups.

Table 4 shows that the studied groups of patients exposed to scorpions stings from March to December 2015 have non-significant differences in the occurrence of local effects at site of sting between studied groups. General examination reveals significant hypertension among group II and III patients. Bradycardia, tachycardia, tachypnea and

bradypnea were more significant among group III. CNS examination was significant only among group III. GIT manifestations show significant occurrence of nausea, vomiting and diarrhea among studied groups. Urinary manifestations show significant loss of bladder control and priapism among group III only. Also parasympathetic signs were significant among group III only in form of lacrimation, salivation, bronchospasm, bronchorrhea, miosis, piloerection and generalized muscle weakness.

Table (3): Kruskal-Wallis test analysis for the delay time in minutes from exposure to scorpions' stings till hospital attendance among studied groups of patients from March to December 2015

Delay time /minutes	Group I N = 20	Group II N = 15	Group III N = 27	P value
Mean \pm SD	48 \pm 20	98 \pm 54	167 \pm 45	.000

Data are expressed as mean and standard deviation. P values < 0.05 is significant

Table 5 shows highly significant ECG changes regarding sinus bradycardia and sinus tachycardia with ST-T changes in the three studied groups. There was significant Paroxysmal supraventricular tachycardia PSVT, atrial fibrillation and first degree heart block among group III only.

III –Cardiac Biomarkers Results

Table 6 shows a comparison in the serum levels of H-FABP among studied groups where there were two steps of significant increases of mean H-FABP (ng/ml), the first is from control group (4 \pm 1.3) to group I (17.4 \pm 5.4) and group II (18.2 \pm 10.2) where there was no significant values between group I and II. The second significant increase from group II to group III (11.77 \pm 5.60). The same as for the levels of myoglobin it showed two steps significant increase of mean myoglobin (ng/ml), the first from control group (33.3 \pm 10.3) to group I (60.2 \pm 12.2) and group II (65.1 \pm 11.1) where there was also no significant values between group I and II, and second a border line significant increase from group II to group III (72.1 \pm 7.9). As regard comparison of the levels of CK-MB showed no significant increases of mean CK-MB (ng/ml) in control group (3.0 \pm 1.0), group I (3.5 \pm 1.5) and group II (3.6 \pm 1.2), but significantly increase in group III (5.7 \pm 1.7). Troponin I showed no significant increases of mean (TnI) (ng/ml) in control group (0.095 \pm 0.01), group I (0.098 \pm 0.015) and

group II (0.112 \pm 0.019), but significantly increase in group III (0.15 \pm 0.032).

Table 7 shows specifying the results by grouping of patients into cardiac- affected groups that comprise 26 patients diagnosed by symptoms, signs and ECG changes and non-cardiac affected group that comprises 36 patients, reveals two steps of significant increase in the levels of H-FABP and myoglobin from control group to non-cardiac affected group then to cardiac- affected group, in contrary to CK-MB and (TnI) levels where there is no significant increase from control group to non-cardiac affected group and significant increase in cardiac-affected group.

Figure 2 shows cardiopulmonary manifestations in form of ACP, dyspnea, tachyarrhythmia among studied groups. While pan systolic murmur, cardiogenic pulmonary edema and CVS collapse were evident only among group III.

Figure (3) demonstrate the comparison of ROC analysis of H-FABP, CK-MB, myoglobin and Troponin I levels as a risk determinant in patients with positive cardiac-affected vs non cardiac-affected patients exposed to scorpions stings. The area under the curves were observed to be higher in (TnI) followed by H-FABP, myoglobin and CK-MB, by 0.97, 0.95, 0.90 and 0.83 respectively with marked inter significant difference between them ($P < 0.001$).

Table (4): Chi square and Fisher's exact tests as appropriate for categorical variables for the clinical manifestations among studied groups of patients exposed to scorpions' stings from March to December 2015

Clinical Manifestations	Group I N=20	Group II N=15	Group III N=27	P value
1)Local effects				
Swelling, redness, sting mark, tenderness at site of sting N (%)	20 (100)	15 (100)	27 (100)	NS
2)General Examination Signs				
Blood pressure/mmHg				
Hypertension N (%)	-	7 (46.6)	22 (81.5)	S
Hypotension N (%)	-	5 (33.3)	5 (18.5)	S
Normal N (%)	20 (100)	3 (20)	-	S
Pulse/min				
Bradycardia N (%)	3 (15)	4 (26.6)	12 (44.4)	S
Tachycardia N (%)	1 (5)	5 (33.3)	14 (51.8)	S
Normal N (%)	16 (80)	6 (40)	1 (3.7)	S
Respiratory rate /min				
Tachypnea N (%)	-	4 (26.6)	14 (51.8)	S
Bradypnea N (%)	-	6 (40)	11 (40.7)	S

Normal	N (%)	20 (100)	5 (33.3)	2 (7.4)	S
Temperature /°C					
Hyperthermia	N (%)	-	3 (20)	21 (77.7)	S
Subnormal	N (%)	-	6(40)	6(22.2)	S
Normal	N (%)	20(100)	6(40)	-	S
3) Systemic Manifestations					
CNS					
Normal Consciousness level	N (%)	20 (100)	15 (100)	12(44.4)	S
Coma	N (%)	-	-	15(55.6)	S
Restlessness	N (%)	-	-	22(81.5)	S
Confusion	N (%)	-	-	15(55.6)	S
Delirium	N (%)	-	-	15(55.6)	S
Seizures	N (%)	-	-	9(33.3)	S
GIT					
Nausea	N (%)	20(100)	10(66.6)	20(74)	S
Vomiting	N (%)	10(50)	5(33.3)	20(74)	S
Diarrhea	N (%)	10(50)	5(33.3)	22(81.5)	S
Urinary					
Loss of bladder control	N (%)	-	-	14(51.9)	S
Priapism	N (%)	-	-	19(70.4)	S
Parasympathetic					
Salivation	N (%)	-	-	19 (70.4)	S
Lacrimation	N (%)	-	-	19(70.4)	S
Sweating	N (%)	-	12 (80)	24 (88.8)	S
Bronchospasm	N (%)	-	-	20 (74)	S
Bronchorrhea	N (%)	-	-	20 (74)	S
Diarrhea	N (%)	-	-	19(70.4)	S
Miosis	N (%)	-	-	19 (70.4)	S
Piloerection	N (%)	-	-	19(70.4)	S
Generalized weakness	N (%)	-	-	13(48.1)	S

** $P < 0.01$ Highly Significant; N = number of cases. S/NS = significant and nonsignificant relation done by Chi square and Fisher's Exact tests as appropriate for analysis of categorical variables regarding clinical manifestations.

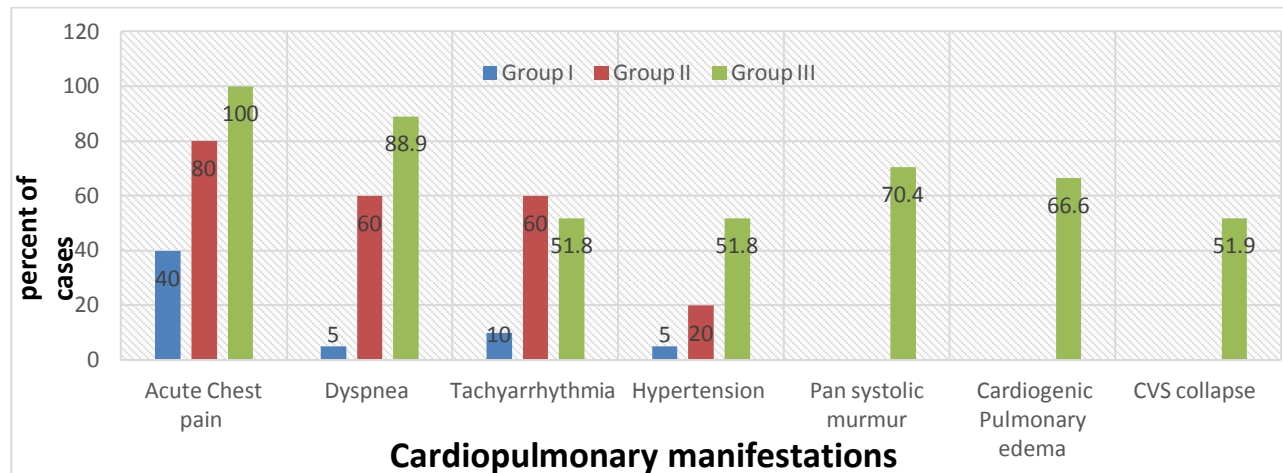


Figure (2): Cardiopulmonary manifestations among studied groups of patients exposed to scorpions' sting from March to December 2015

Table 5: Fisher's exact test for ECG changes among studied groups of patients exposed to scorpions stings from March to December 2015

ECG changes	Group I No= 20		Group II No= 15		Group III No= 27		S/NS	P
	N	%	N	%	N	%		
Sinus Bradycardia	3	15	4	26.6	12	44.4	S	0.000*
Sinus Tachycardia with ST_T changes	1	5	5	33.3	14	51.8	S	0.000*
Paroxysmal Supraventricular Tachycardia PSVT	-	-	2	13.3	6	22.2	S	0.007*
Atrial fibrillation	-	-	3	20	5	18.5	S	0.001*
First degree heart block	-	-	2	13.3	3	11.1	S	0.000*

** $P < 0.01$ Highly Significant; S/NS = significant and nonsignificant relation done by Fisher's exact tests; N = number of cases

Table 6: Mann-Whitney and/or Kruskal-Wallis test for Comparison of serum levels of H-FABP, CK-MB with Myoglobin and Troponin I among studied groups of patients exposed to scorpions stings from March to December 2015.

Serum levels of Cardiac Biomarkers	Control Group (n = 24)	Group I (n = 20)	Group II (n = 15)	P Value Control vs I&II	P Value I vs II	Group III (n = 27)	P Value II vs III
	Mean \pm SD	Mean \pm SD	Mean \pm SD			Mean \pm SD	
H-FABP	4 \pm 1.3	17.4 \pm 5.4	18.2 \pm 10.2	0.000	0.521	331 \pm 10.2	0.001
CK-MB	3.0 \pm 1.0	3.5 \pm 1.5	3.6 \pm 1.2	0.257	0.780	5.7 \pm 1.7	0.000
Myoglobin	33.3 \pm 10.3	60.2 \pm 12.2	65.1 \pm 11.1	0.000	0.419	72.1 \pm 7.9	0.020
Troponin I	0.095 \pm 0.01	0.098 \pm 0.015	0.112 \pm 0.019	0.107	0.064	0.15 \pm 0.032	0.000

Data are expressed as mean and standard deviation. Nonparametric Mann-Whitney U-test and/or Kruskal-Wallis test are used for statistical analysis as appropriate. * $P < 0.05$ is significant.

Table 7: Mann-Whitney tests for comparison of serum levels of H-FABP, CK-MB, Myoglobin and Troponin I among studied groups of patients exposed to scorpions stings from March to December 2015.

Serum levels of Cardiac Biomarkers (ng/ml)	-ve (n = 36)	+ve (n = 26)	Control Group (n = 24)	P Value -ve vs +ve	P Value Control vs -ve
H-FABP	16.7 \pm 6.2	35.1 \pm 9.5	4 \pm 1.3	.000	.000
CK-MB	3.4 \pm 1.4	6.0 \pm 1.4	3.0 \pm 1.0	.000	.434
Myoglobin	61.6 \pm 11.7	73.6 \pm 5.9	33.3 \pm 10.3	.000	.000
Troponin I	0.102 \pm 0.013	0.15 \pm 0.03	0.095 \pm 0.01	.000	.052

Data are expressed as mean and standard deviation, (-ve): are negative cardiac-affected patients after exposure of scorpions' sting. (+ve): are positive cardiac-affected group, nonparametric Mann-Whitney test was used and * $P < 0.05$ is significant

Table 8 shows the comparative analysis of the resulted sensitivity, specificity, positive predictive value (PPV), Negative Predictive Value (NPV), area under the curve (AUC), 95% confidence interval (CI) and optimal cutoff values of the biomarkers, followed by the adjusted (A) sensitivity and A specificity at this optimal cutoff value. The optimum cut-off values above which H-FABP, CK-MB, myoglobin and (TnI) can be considered positive were found to be 18.2 ng/ml, 2.4 ng/ml, 58.5 ng/ml and 0.115 ng/ml respectively. The area under the ROC also summarizes the diagnostic discrimination of a biomarker, at the optimum cut-off value, sensitivity and specificity were

found to be 96 and 47% respectively in H-FABP, 96 and 25% respectively in CK-MB, 96 and 31% respectively in myoglobin, 92 and 97% respectively in (TnI).

Figure 4 shows a positive correlation 0.759 in the distributions of serum levels of Troponin I ng/ml (the most specific indicator of cardiac affection) in relation to time of presentation in minutes among patients after their exposure to scorpions stings with a significant ($P < 0.001$) value, and the magnitude of relation (coefficient of determination) is 86%. Also the first positive case is observed in 120 minutes after admission.

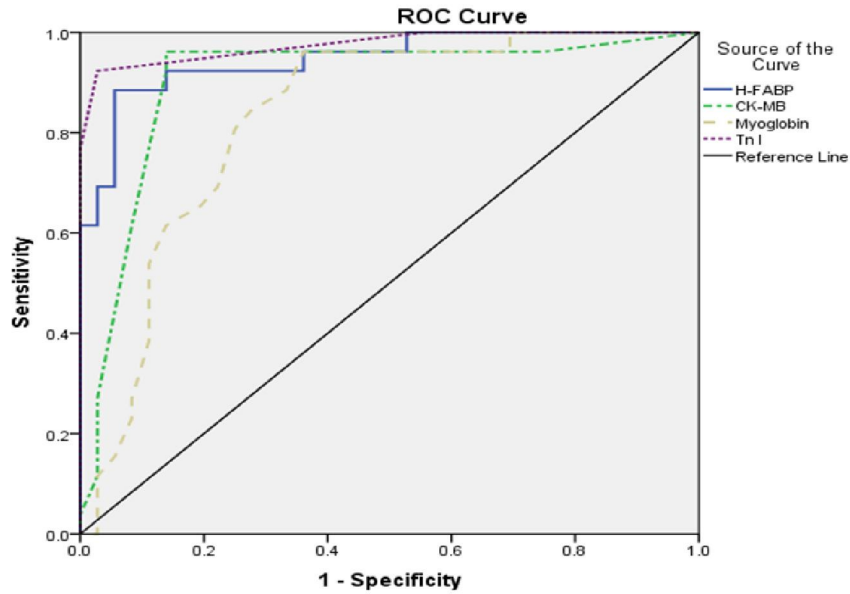


Figure 3. Correlation of H-FABP, CK-MB, myo

of H-FABP,

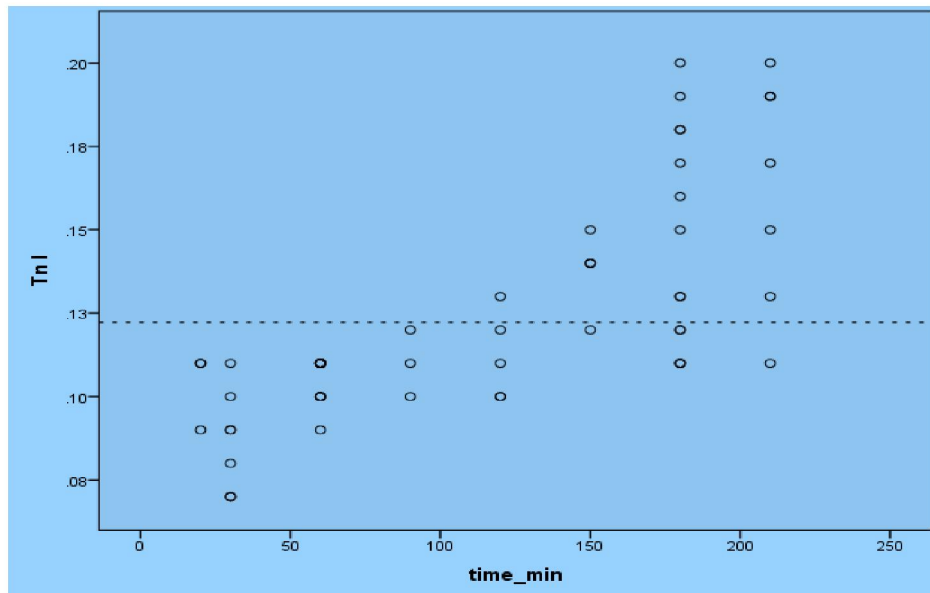


Figure 4. Distributions of Serum levels of Troponin I ng/ml in relation to time of presentation in minutes with reference cutoff value line among patients exposed to scorpions' stings from March to December 2015.

Discussion

Most of the stings in the present study occurred in the summer months (June to September) since cities and countries with tropical and subtropical climates like Port Said governorate are places where scorpion stings most commonly occur (14). High temperatures make scorpions more active and therefore increase their proximity to humans in contrast to low temperatures which make these animals hyphenate (16).

The sociodemographic criteria of patients in the current study are in agreement with Jahan *et al.* (17)

study who reported that males were more liable for exposure to scorpion sting than females. A similar results were obtained from another study in Saudi Arabia, in which male to female ratio was 2.6/1 (18). Also Pardal *et al.* reported the majority (83.3%) of victims were males in Brazil (19).

On the other hand, it was reported in two different studies made in USA and Australia that the females were stung more frequently than the males (20 & 21). Females are more susceptible than males to the same amount of scorpion venom due to their lower body weight.

Scorpions are nocturnal creatures; they hunt during the night and hide in crevices and burrows during the day to avoid the light. Thus, accidental human stinging occurs when scorpions are touched

while in their hiding places, with most of the stings occurring on the extremities especially on lower limbs (22).

Table 8: Sensitivity, specificity, positive predictive value, negative predictive value, area under the curve, and 95% confidence interval of H-FABP, CK-MB, myoglobin and Troponin I at the optimum cut-off value obtained from the ROC followed by the adjusted sensitivity and specificity at this optimal cutoff value for patients with cardiac affection after scorpion envenomation.

Parameter	H-FABP	CK-MB	Myoglobin	Troponin I
Sensitivity	100	92	100	92
Specificity	0	86	8	97
PPV	42	83	44	96
NPV	0	94	100	95
AUC	.95	.90	.83	.97
95% CI	0.893 - 1.0	0.814 - 0.991	0.728 - 0.935	0.939 - 1.0
Cutoff value (ng/ml)	18.2	2.4	58.5	0.115
Sensitivity A	96	96	96	92
Specificity A	47	25	31	97

This was in agreement with many study which revealed that scorpion sting most common to occur at night between 6:00 pm and 12:00 am (80%) and a second peak from 6:00 am to 12:00 pm (20%). Both of these peaks coincides maximum human activity with maximum scorpion activity (23).

The ends of the lower limbs were the most affected anatomical regions, this is in agreement with previous results from the literature (24 & 25). This necessitate wearing a personal protective equipment as a preventive measure for the protection of the body with the use of boots and gloves for populations at high risk during work is suggested. Workers' health in this region needs more attention from policy-makers. The most common occupations observed in the present study were manual workers, fishermen and farmers who were more liable for exposure to scorpion while they were performing their jobs.

Although Port Said governorate is an urban town but some of its areas such as the south and El Zohor districts contain some slums areas. In these regions, there are huge recent increase of cattle, logging and farming activities, including cultivation of rice. In this context, the destruction of the natural habitat of scorpions could lead to increased contact between these animals and humans during work activities (26).

Scorpions have different colors according to their species. Black and yellow scorpions are the most frequent and suggested to be most dangerous, and well-known species. In the studies by Brennan, Kumar and Jaggarao (27) black scorpion was the most commonly seen species, whereas Dittrich et al. most frequently encountered with yellow species (28).

In current study, the most frequently seen species was yellow scorpions. In terms of venom lethality, the

venom of *Leiurus quinquestriatus* (yellow colored scorpion) is the most toxic (29).

The present work revealed that the most common age categories were those younger than eighteen and older than sixty years.

This is attributed to the fact that young children and teenagers are more likely to develop a more rapid progression, increased severity of symptoms, and higher mortality because of their lower body weight, with a global rate of 10 deaths per 1000 cases (30). Furthermore, elderly persons are more susceptible to stings because of their decreased physiologic reserves and increased debilitation (31).

Different studies have shown varied age distribution for scorpion's stings. Duddin *et al.* reported the mean age of scorpion sting cases in Brazil as 33.6±18.3 (32). In Qassim, Saudi Arabia, the mean age was reported smaller (23.1 ±16.8) (33). Another study on scorpion stings in Mexico reported that 77% of all accidents occurred among persons less than 30 years of age (34). A hospital-based study from the Kingdom of Saudi Arabia mentioned that ages in scorpion sting cases ranged from 2 months to 101 years and 70.6% of the cases were under 18 years of age (35). Dehesa-Davilla and Possanireported 36% of the cases in Morocco, being under 15 years (36). In contrast, Jahan, Al Saigul and Hamedreported most victims in Qassim, to be 50 years of age or older (37).

The current study revealed a significant difference was noticed regarding delay time between the three studied groups.

Delay time is the duration elapsed between scorpion sting and hospital admission. It is probably a key factor for better outcome. Early medical attention, and administration of scorpion antivenin plus avoiding

conventionally used harmful drugs like steroids, antihistaminics and other cocktail of sedatives may reduce complications and mortality. Also timely diagnosis of pulmonary edema and early initiation of ventilatory and inotropic support in ICU led to better outcome (38).

This is in accordance with many medical reports from different regions of Brazil (39), where the delay time was less than 3 hours in 69.6% of cases. Most of the severe cases (79.4%) also received treatment in less than 6 hours after the sting, probably contributing to preventing higher lethality in the Amazon. This may be related to the location of most cases in urban areas or rural areas in the southern region of the state with roads and less isolated human settings which would facilitate access to specialized medical treatment.

All patients in the present study showed non-significant difference in the occurrence of local effects at the site of scorpion sting in the form of swelling, redness, sting mark and tenderness. General examination reveals significant hypertension among group II and III patients. Bradycardia, tachycardia, tachypnea and bradypnea, CNS manifestations, loss of bladder control and priapism were more significant among group III. GIT manifestations show significant occurrence of nausea, vomiting and diarrhea among all patients. Also parasympathetic signs were significant among group III only in form of lacrimation, salivation, bronchospasm, bronchorrhea, miosis, piloerection and generalized muscle weakness.

The clinical presentation of scorpion sting is known as "Scorpion sting syndrome" or "Scorpionism" which defined by Neale (40). The typical manifestation of scorpionism can be described as local pain, with proximal radiation often with tenderness, swelling and redness followed by systemic symptoms which most often include hypertension and or tachycardia, often with anxiety, nausea and epigastric pain (41).

These manifestations are secondary to scorpion venom which contains multiple toxins and other compounds. The venom is composed of varying concentrations of neurotoxin, cardiotoxins, nephrotoxins, hemolytic toxin, phosphodiesterases, phospholipases, hyaluronidases, glycosaminoglycans, histamine, serotonin, acetyl choline, tryptophan, and cytokine releasers. The most important clinical effects of envenomation are local tissue effects, neuromuscular, cardiopulmonary and autonomic manifestations. The primary targets of scorpion venom are voltage-dependent ion channels, of which sodium channels are the best studied. Venom toxins alter these channels, leading to prolonged neuronal activity. Many end-organ effects are secondary to this excessive excitation. Autonomic excitation leads to

cardiopulmonary effects observed after some scorpion envenomation. Somatic and cranial nerve hyperactivity results from neuromuscular overstimulation. Additionally, serotonin may be found in scorpion venom and is thought to contribute to the pain associated with scorpion envenomation (42). Scorpionism usually occur within a few minutes and cease in 72 hours. After sympathetic involvement, hyperthermia, tachycardia, tachypnea, hypertension, pulmonary edema and hyperglycemia are predominantly seen (43). Bronchoconstriction, bradycardia, hypotension, increased secretion and miosis may be seen in parasympathetic involvement (44).

In some studies, it has been reported that localized effects were the commonest presentation especially pain and tenderness at site of sting. However, other studies reported systemic effects such as fatigue, nausea, vomiting, hypo- or hypertension, shock, pulmonary edema, myocardial dysfunction and unconsciousness (2, 6 & 45) in moderate to severe envenomation. In agreement with these studies, localized effects at sting mark and systemic manifestations were also more commonly seen in the current study among group II and more severe among group III patients. In severe envenomation serious health problems may occur; ranging from acute anginal chest pain, bleeding disorders (46), neuropsychiatric manifestations including mental abnormalities (47), cerebral edema, seizures (48) stroke (49) paresthesia, and paralyzes (48) allergic reactions, anaphylaxis (49), infections including infective endocarditis (50) toxidrome syndromes and also hypertension, myocardial ischemia, and pulmonary edema (24, 30, 31) by means of different toxins and mechanisms. The polymorphism and severity of symptomatology of scorpion envenomation is due to its enormous content of toxins, neurotransmitters, enzymes, inflammatory mediators and cytokines (51). In Bouaziz *et al.* study, 61.5% of patients had a pulmonary edema, while 20.5% patients had a cardiogenic shock (8). The main clinical features of scorpion envenomation are localized reactions that occur in up to 97% of affected people and they were evident in 100% of patients in the present study.

Group III patients in the present study were manifested with neurological manifestations which were considered as an indicator of the severity of scorpion sting. These signs are observed at 2/3 of hospitalized patients in previous studies (52 & 53). These manifestations are variables, going from the simple hyperthermia (54) to severe neurological manifestations (miosis, coma and/or convulsions) (55). In the severe cases hyper sweating, and priapism

can be observed and they are secondary to parasympathetic and muscarinic syndrome (56).

The incidence of gastrointestinal manifestation varies from 11% to 100% according to the type of the scorpion and age of the patients (57). They are relatively more frequent in children than in adult patients (58). In Bouaziz *et al.* study, almost 74% of their patients have experienced gastrointestinal manifestations on admission (8). The most observed clinical manifestations are vomiting (37), diarrhea, hypersalivation, gastric distension, and dysphagia (43). Hematemesis and a melena are rarely observed (44). More rarely, scorpion envenomation can lead to acute pancreatitis (45). In current study, no patient with severe gastrointestinal manifestation was assessed. Just only vomiting and diarrhea were observed.

Cardiopulmonary manifestations in the current results were in form of ACP, dyspnea, tachyarrhythmia among studied groups. While pan systolic murmur, cardiogenic pulmonary edema and CVS collapse were evident only among group III.

Cardiovascular manifestations are due to direct effect of excess circulating catecholamines and cholinergic neurotransmitters from autonomic hyper stimulation. Severe envenomation can lead to hypotension, left ventricular failure, cardiogenic shock and pulmonary edema. Cardiac dysfunctions was attributed to catecholamine-induced myocarditis and increases in myocardial metabolism oxygen demand (leading to myocardial ischemia-induced myocardial hypoperfusion) as well as to the direct effects of the toxin (leading to myocarditis and myocardial conduction interference) (58).

Respiratory failure may occur secondary to diaphragm paralysis, alveolar hypoventilation, and bronchorrhea (59). Transient apical pansystolic murmur is consistent with papillary muscle damage (60). Pulmonary edema with hemoptysis are secondary to a direct toxin-induced increased pulmonary vessel permeability effect and is also secondary to catecholamine-induced effects of hypoxia and intracellular calcium accumulation, which leads to a decrease in left ventricular compliance with resultant ventricular dilation and diastolic dysfunction (61).

Cardiovascular collapse occurs secondary to toxin induced myocarditis, biventricular dysfunction and profuse loss of fluids from sweating, vomiting, diarrhea, and hypersalivation (62).

The present results revealed highly significant ECG changes regarding sinus bradycardia, sinus tachycardia with ST-T changes in the three studied groups. There was significant Paroxysmal supraventricular tachycardia PSVT, atrial fibrillation and first degree heart block among group III only.

ECG changes with sinus rhythm disturbances are not dose-dependent but are related to the venom composition. Sinus tachycardia which considered the most common rhythm, followed by QTc prolongation, ST changes, T-wave inversion, ventricular repolarization abnormalities, bundle-branch block and first-degree block. A possible sequence of ECG changes has been noted. This sequence starts with bizarre, broad-notched, biphasic, peaked T waves with a beat-to-beat variation. This bizarre T wave is followed by the appearance of tiny Q waves and then atrioventricular dissociation with an accelerated junctional rhythm (63).

So the aim of this study was to evaluate the clinical validity of plasma cardiac biomarkers such as H-FABP, CK-MB, myoglobin and troponin I levels, for early detection of acute toxic myocarditis in victims exposed to scorpion sting. Acute toxic myocarditis (ATM) is the main cause of mortality following severe scorpion envenomation. Early and correct diagnosis is of great importance to enable immediate and intensified treatment which consequently reduces mortality (64).

In the current work the mean serum levels of H-FABP and myoglobin among studied groups revealed two steps of significant increase the first from control group to group I and II. The second significant increase is from group II to III, while there were no significant values between group I and II, which indicates the early positive influence of envenomation in the levels of those factors in absence of cardiac involvement in group I and II, with the start in cardiac affection in group III cause another significant increase, the border line significant increase in myoglobin level in group III indicates its less specificity and later influence than H-FABP.

This is in agreement with Açıkalın *et al.* (65) that concluded that the high levels of H-FABP occurred secondary to the muscle injury following the scorpion bite. The early detection of level H-FABP and its specificity over myoglobin were proved in many studies by Ibrahim Elmadbouh *et al.* (66) and Ishii *et al.* (67).

As regard the mean serum levels of CK-MB and troponin I (ng/ml) showed no significant increases between control, groups I and II, but significantly increases in group III, this proved the later detection of those markers and higher selectivity than myoglobin, as proved by Priya Gururajan. (68) and Açıkalın *et al.* (65).

The present study specified the results by grouping of patients into cardiac and non-cardiac-affected group, showed two steps of significant increase in the levels of H-FABP and myoglobin from control group to non-cardiac affected group then to cardiac affected group, in contrary to CK-MB and

Troponin I levels were there is no significant increase from control group to non-cardiac affected group and significant increase in cardiac-affected group.

Multiple studies based on ROC analysis for comparison between cardiac biomarkers of patients admitted to emergency room with typical cardiac chest pain. Study by Glatz *et al.* (69 & 70) demonstrated increased area under the ROC for H-FABP (0.945) than myoglobin (0.892) in patients admitted after 3–6h after symptom onset. Also Priya Gururajan (68) discovers area under the ROC for H-FABP (0.965) followed by CK-MB (0.798) and (TnI) (0.765). In contrary to our study that is based on the effect of post scorpion envenomation induced (ATM), the area under the curves were observed to be higher in (TnI) followed by H-FABP, myoglobin and CK-MB, by 0.97, 0.95, 0.90 and 0.83 respectively with marked inter significant difference between them ($P<0.001$).

Studies that assess the diagnostic accuracy of cardiac biomarkers to predict AMI in situations other than envenomation, showed that H-FABP has earliest diagnostic value in patients with suspected AMI than does (TnI), CK-MB or myoglobin within 4 h following onset of acute chest pain rather than at a later 6 h. concludes the early sensitive. A study by Ruzgar *et al.* (71) showed 38% sensitivity on troponin, 76% sensitivity on CK-MB and 95% sensitivity on H-FABP in patients admitted within six hours of chest pain onset. Within 6–24 hours, sensitivity of troponin and CK-MB increased to 100 and 90% respectively, while that of H-FABP was 91%. After 24 hours, the sensitivity of H-FABP drastically decreased to 27.3% while that of (TnI) and CK-MB remained the same.

Another study observed 89% sensitivity of H-FABP within two hours of chest pain where troponin had a sensitivity of 22% (72). Our study focused on biomarkers levels at admission rather than kinetic studies of the marker, as well as the influence of envenomation in the results. Our results showed conditional agreement as regards sensitivity but completely contradict in specificity being highest in troponin 97% and very low in other markers, the optimal cutoff value was triple the normal value of H-FABP at 18.2 ng/ml giving 47% specificity.

Cardiac troponin I (TnI) assessment has become gold standard in the assessment of acute coronary syndromes. Cardiac troponin I may be elevated in non-ischemic conditions. Its measurement and benefits in scorpion sting envenomation has been reported by Meki *et al.* (73). In their study on 41 children, elevated (TnI) levels were noted in 17 severe cases and 14 moderate cases. The level was normal in 10 children with mild symptoms. Cardiac troponin I showed 100% specificity and sensitivity for the diagnosis of myocardial injury in relation to the Echo

finding in the envenomed victims (74). In severe cases, (TnI) was found to be positively correlated with left ventricular ejection fraction (LVEF). Based on their findings, they had concluded that (TnI) was a specific marker for the diagnosis of myocardial injury in scorpion envenomation. In the current study also, all group III patients with (ATM) had significant elevated (TnI) serum levels, which further confirmed the findings of Meki *et al.* (73). The normal levels of (TnI) in patients without an evidence of myocarditis is an important indicator, as it will help the clinicians when echocardiography is not available.

The current results proved that (TnI) have been the preferred biomarker due to its highest sensitivity and specificity but due to its delayed appearance in the serum, there is a still in need to other less sensitive marker for earlier diagnosis. Heart fatty acid binding protein (H-FABP) is a new cardiac biomarker with a low molecular weight which indicates early myocardial tissue injury (75). Various studies have shown that H-FABP becomes positive earlier than troponin and is more sensitive than myoglobin (76). But, H-FABP are found in skeletal muscle is approximately 10-20% of the concentration in comparison to heart muscle. Although H-FABP is present in both the heart and skeletal muscle tissue, its concentration in skeletal muscle is 10-fold lower than that in the myocardium, compared with myoglobin, which is 2-fold higher in skeletal muscle than in myocardium (77). Comparative studies for the diagnosing of acute myocardial injury (AMI) have demonstrated that quantitative measurement of H-FABP is more sensitive than both myoglobin and CK-MB measurements, and more specific than myoglobin (78). Clinical investigations of the release kinetics of H-FABP and other cardiac biomarkers have revealed that H-FABP is elevated above the cut-off level within 3 h of onset, reaching a peak value at 4 h, and its release into the blood is approximately 24–36 h after onset of AMI (79). However, a quantitative assay using the sandwich-ELISA method requires an analysis time of 90 min or more and requires the use of laboratory equipment, so whole blood rapid panel tests have been developed for use in emergency situations. The rapid H-FABP test detects concentrations of H-FABP >6.2 ng/ml, the cut-off level for diagnosing AMI, in whole blood samples within 15min. The introduction of the rapid (TnI) test for cardiac emergencies was revolutionary because it facilitated rapid bedside evaluation and risk stratification in patients with suspected acute coronary syndrome (80). However, previous study, which evaluated the diagnostic efficacy of the rapid (TnI) test, revealed that positive test results could only be obtained more than 3–4 h after onset because of the time-lag in the release kinetics of (TnI), and therefore

the diagnostic sensitivity was temporally limited for patients assessed in the early acute phase (81). The limitation in the diagnostic sensitivity of the (TnI) test in the early acute phase is mainly due to its detection in serum within 3 h of onset, which is the most beneficial time-frame for administration of anti-scorpion venom and stabilization of the general condition of the patients plus starting coronary reperfusion therapy to salvage the toxic myocarditis and thus improve long-term prognosis (82).

Previous studies for cardiac biomarkers detection after acute myocardial injuries in hours, revealed that onset of detection of myoglobin (1-4 hours with peak reached after 6-7 hours and lasts for 18-24 hours. CK-MB onset of detection in serum ranged from 3-12 hours with peak level after 18-24 hours and lasted for 24-36 hours. Troponin I started to appear after 2-12 hours with peak level reached after 18 -24 hours and lasted for 7-10 days. The earliest cardiac biomarker to be detected in cardiac affected patients was H-FABP which has early onset of 1-1.5 hours with peak level after 4-6 hours and lasted for 24 hours (84& 84).

Conclusion

Troponin (TnI) is the most specific indicator of cardiac affection biomarker for acute toxic myocarditis following scorpion envenomation due to its highest specificity. However its delayed appearance in the serum, demands detection to other less sensitive biomarker for earlier diagnosis.

H-FABP can detect early toxic myocardial injury following scorpion sting within golden one hour of envenomation.

H-FABP can be useful in the early diagnosis of acute chest pain in comparison with other traditional biomarkers CK-MB, Tn-I and myoglobin.

Emergency departments are under increasing pressure to manage patients in less time. Therefore, a test that stratifies the risk of patients with chest pain more rapidly would be valuable, facilitating earlier discharge.

Recommendation

The planning of urbanization and farming activities, in parallel with the awareness of workers at risk for scorpion stings and the need for personal protective equipment use should be considered as public policies for preventing scorpionism.

It is recommended to use troponin I in combination with H-FABP to provide early diagnosis of ATM for better outcome after therapy.

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