

MID-REGIONAL PRO ATRIAL NATRIURETIC PEPTIDE (MR-PRO-ANP) AS A NOVEL BIOMARKER FOR EARLY PREDICTION OF CARDIAC INJURY FOLLOWING SCORPION STING AMONG CHILDREN.

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ABSTRACT

Background: Scorpion envenomation represents a vital health problem, especially in children. Scorpion venom causes diverse harmful side effects on different body systems; however, the most serious one is considered the cardiotoxic effect. **Aim of the work:** this study aims to predict heart failure in children following scorpion sting using MR-proANP (cardiac biomarker), owing to reduced sensitivity and specificity of currently used biomarkers. **Subjects & methods:** the study was carried out on 87 children patients presented to Minia University Poisoned Control Center (MUPCC) with a history of a scorpion sting (within the first hour of sting) during the period from 1st of June 2018 to the 31st of May 2020. Venous blood samples were drawn from patients for biochemical analysis (MR-proANP and troponin I). **Result:** MR-proANP predicted clinical heart failure (HF) in the first hour of sting (sensitivity was 73.91% and the specificity was 100%) and predicted sub-clinical heart failure after 6 hours of sting (sensitivity was 89.66% and the specificity was 91.67%); however, troponin I predicted HF only after 12 hours of the sting. **Conclusion:** MR-proANP was superior to troponin I in predicting HF due to scorpion envenomation.

Keywords: MR-proANP; Scorpion; Troponin I; Heart failure; Cardiac injury; Cardiac biomarkers.

INTRODUCTION

Scorpion sting represents an actual endemic public health problem in several developing countries. Scorpion envenomation evoked a potent autonomic storm starting with transient cholinergic manifestations and followed by adrenergic stimulation. Myocardial dysfunction, pulmonary edema, and cardiogenic shock may occur later in the course and are considered an integral part of the autonomic storm (Forrester & Stanley, 2004).

As the cardiac injuries represent the most critical dilemma in these patients, several studies were conducted to predict it using cardiac blood biomarkers. The most common biomarkers used were TnI

(troponin-I), (myocardial-bound creatine kinase), CK-MB, and heart-type fatty acid-binding protein (H-FABP) (Meki et al., 2003).

Many studies explored the leading role of MR-proANP in predicting acute & chronic heart failure in cardiac patients (Hausfater et al., 2017; Francis et al., 2016). So, in this study, we aim to investigate the role of MR-proANP in predicting heart failure following scorpion sting.

SUBJECTS & METHODS

The present prospective controlled study was applied on 87 children's patients (39 males "44.8%" and 48 females "55.2%") with age ranged between 5- 17

years (8.3 ± 2.7) presented to Minia university Poison Control Center (MUPCC) with a history of a scorpion sting (within the first hour of sting) during the period from 1st of June 2018 to the 31st of May 2020.

Detailed history and complete clinical examination were performed on all patients. Venous blood samples were drawn for biochemical analyses at the time of admission, 6 hours after the sting, 12

hours after a sting from all patients, immediate centrifugation was done, and the serum samples were stored at $-80\text{ }^{\circ}\text{C}$. Assents were taken from children, but written consents were taken from the patients' guardians for participation in this study. Also, we have obtained approval from Scientific Research Ethics Committee for our study. Patients were classified clinically according to abroug classification in to:

Table (1): Abroug classification of clinical manifestation of scorpion sting

Grade	Clinical Picture		
I	• Local pain.	• Local paresthesia.	• Local erythema.
II	• Shivering.	• Vomiting.	
	• Fever.	• Diarrhea.	
	• Hypertension.	• Excessive sweating.	
	• Nausea.	• Priapism.	
III	Cardiovascular, respiratory, or neurological symptoms:		
	• Cardiogenic shock.	• Altered consciousness.	
	• Pulmonary edema.		

(Abroug et al., 1995).

Echocardiography was done for all patients at the onset of symptoms of cardiac affection or otherwise after 12 hours in

apparently free patients. Measurements of ejection fraction (EF) were interpreted as follows:

Table (2): Normal and abnormal values of ejection fraction

> 55 %	Normal
45- 54 %	Mild (subclinical HF) ↓ in EF%
30- 44 %	Moderate ↓ in EF%
< 30%	Severe ↓ in EF%

(Colombo et al., 2012).

Inclusion criteria

1. Children below 18 years old of both sex

2. History of a scorpion sting (within the first hour of sting) and the presence of manifestations of envenomation.

Exclusion criteria

1. The presence of renal affection as the MR-proANP was excreted from the body through the kidney.

2. Age ≥ 18 years old due to the more significant potential of cardiac affection with younger ages.

3. Patients started antivenin therapy before arrival at the hospital

4. Subjects arrived at the MUPCC after the 1st hour of envenomation

Biochemical analysis

ELISA kit My Bio Source Catalog No. MBS 772481 contains all the necessary reagents required for performing quantitative measurement of (MR-proANP) levels from samples including serum, plasma, culture medium, or other biological fluids in a sandwich ELISA format. (Roberts et al., 2015).

Serum TnI level was determined using Immulite troponin I Kit, which is a solid-phase, two-site chemiluminescent enzyme immuno- metric assay for use with the immulite automated analyzer (Cat No.

LKTI, Diagn. Products Corp, USA). The detection limit of the TnI assay was approximately ≥ 0.1 ng/ml (Paul et al., 2001).

The comparison between MR-proANP and TnI was made, and findings diagnostic of heart failure in echocardiography like wall motion abnormalities including hypokinesia, akinesia or global impairment, and ejection fraction calculation were done and recorded for each patient (Fig 2 A & B).

Statistical method

The Statistical Package for Social Sciences program (SPSS) version 26 was used to code, tabulate, and statistically analyze the collected data. Descriptive statistics were done by mean, Standard deviation (SD), minimum, and maximum range. The **Chi-square test** was used to compare qualitative data between two groups. Analysis of quantitative data was done using **one way ANOVA** test between the three groups, with post-HOC Tukey's correction between every two groups for parametric data and Kruskal-Wallis Test with a pairwise comparison between every two groups for non-parametric data. **Simple and Multiple Binary Logistic regression analyses** were done to evaluate the risk factors predicting heart failure. **Receiver Operating characteristics (ROC) Curve** was done to calculate optimal cutoff point, AUC, sensitivity, specificity, PPV, NPV, and accuracy of risk factors predicting bad prognosis. The significant level was detected at (**p-value < 0.05**)

RESULTS

This study included 87 children patients aged 5- 17 years old. (Table 3). Examining study patients for signs of heart failure revealed that 46 patients developed clinical signs of heart failure, including dyspnea, hypotension, and tachycardia, etc. according to echocardiography, the remaining 41 free patients were further classified into ECHO affected group (29 patients) (Fig 2 A & B) who were

considered having subclinical HF and ECHO free group (12 patients).

According to Abroug classification, all patients with clinical HF were found to have severe symptoms, while patients with subclinical HF were mild to moderate (10 & 23% respectively) (Table 4). Comparing EF in the three groups revealed significant statistical differences (Fig 1).

Kruskal-Wallis Test with a pairwise comparison of age between the three groups found that patients who developed clinical HF were significantly younger than free patients; however, no significant differences were noted compared with patients who developed subclinical HF (Table 5).

The ICU stay duration of patients ranged between one to eight days according to the severity of the manifestation, with a mean of 1.75 in the No heart failure group, 3.07 in the sub-clinical heart failure group, and 4.4 in the clinical heart failure group (patients not discharged from ICU except after becoming clinically free and the EF > 55 %). Eight patients from the clinical heart failure group died, but all other groups were lived. Survivors from patients with clinical heart failure stayed in the ICU for a significantly longer time than patients with subclinical HF and free patients (Table 6).

As shown in (Table 7), a Simple logistic regression test revealed that MR-ProANP predicted clinical heart failure (clinical HF) from the 1st-hour post sting, while troponin I stayed 12 hours to be able to predict it (Table 9). Moreover, MR-ProANP was significantly better than troponin I in predicting clinical heart failure after 12 hours according to multiple logistic regression as shown in (Table 10).

Receiver operating characteristics (ROC) curve analysis detected that sensitivity of MR-ProANP increased from 73.91% at 1st-hour post sting to 100% after 12 hours with a specificity of 100% and 91.67%, respectively, while sensitivity and

specificity of troponin I after 12 hours was 91.3% 91.67% respectively (**Table 15**).

Regarding subclinical heart failure, MR-ProANP was earlier than troponin I in its prediction and superior to troponin after 12hs in subclinical HF prediction according to multiple logistic regression (**Table 12, 13,14**) (**Table**).

The ROC Curve analysis of data concerning subclinical heart failure

detected that the sensitivity of MR-proANP was 89.66 %, and the specificity was 91.67 % after 6 hours of envenomation and increased to 100%, 91.67% respectively after 12 hours, while sensitivity and specificity of troponin I after 12 hours from sting was 89.66%, 91.67% respectively (**Table 16**).

Table (3): Range, mean and standard deviation of ages of patients exposed to scorpion sting (N=87).

	N = 87
Age Range	(5-17)
Mean ± SD	(8.3±2.7)

Table (4): Chi square test analysis of abroug classes of children exposed to scorpion sting regarding development of heart failure (HF).

Patient classification according to HF	Abroug classification				p-value
	No HF group	Mild	Moderate	Severe	
No HF group	12 (13.8%)	0	0	< 0.001**	
Sub-Clinical HF	9 (10.3%)	20 (23%)	0		
Clinical HF	0	0	46 (52.9%)		
<i>p</i> -value between groups					
mild vs. moderate		mild vs. severe		moderate vs. severe	
< 0.001**		< 0.001**		< 0.001**	

HF: Heart failure

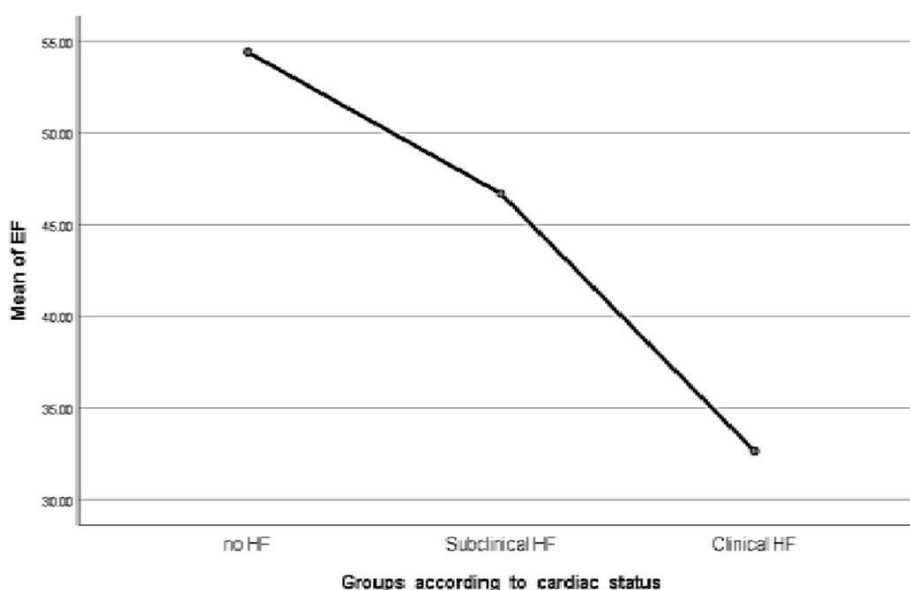


Figure (1): One-way Anova Test with post hoc-Tuckey of EF in the 3 groups (clinical, subclinical and free)

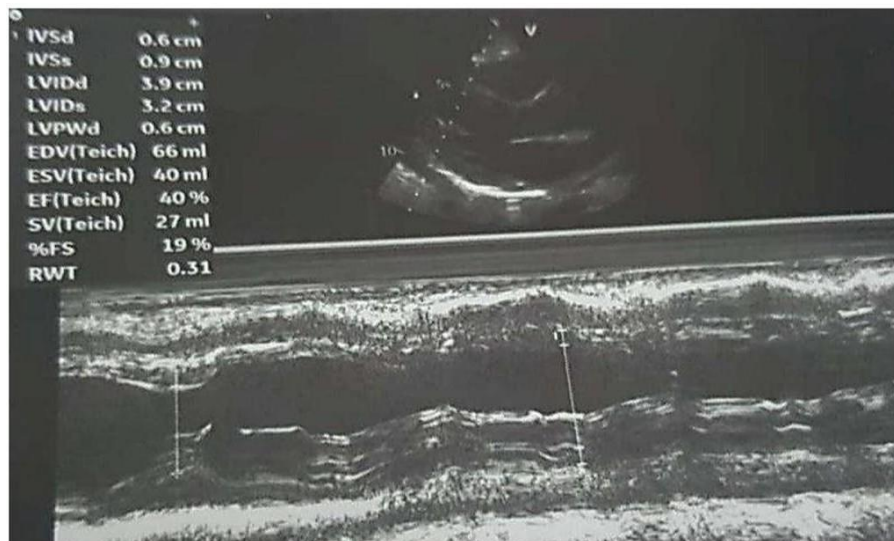


Figure (2) (A): Echocardiography of patient in sub-clinical HF group showing moderate decrease in ejection fraction.



Figure (2) (B): Echocardiography of patient in sub-clinical HF group showing moderate decrease in ejection fraction.

Table (5): Comparison between age and development of heart failure (HF) by using Independent-Samples Kruskal-Wallis Test with pairwise.

		N	Mean RANK	p-value
	No HF group	12	69.83	< 0.001**
	Sub-Clinical HF	29	45.81	
	Clinical HF	46	36.12	
<i>p</i> -value between groups				
Clinical Free group	Clinical Sub-Clinical	Sub-Clinical Free		
< 0.001**	.102	.005*		

HF: Heart failure

Table (6): Comparison between ICU stay and development of heart failure (HF) by using Independent-Samples Kruskal-Wallis Test with pairwise.

		N	Mean	Standard dev.	p-value
	No HF group	12	1.75	.87	< 0.001**
	Sub-Clinical HF	29	3.07	.79	
	Clinical HF	38	4.4	1.4	
<i>p</i> -value between groups					
Clinical- Free group		Clinical Sub-Clinical		Subclinical -Free	
< 0.001**		< 0.001**		0.008*	

HF: Heart failure

Table (7): Simple logistic regression analysis of heart failure (HF) prediction by MR-pro ANP and troponin in patients at admission.

Clini HF	OR	95% CI	p value
ANP 0	1.019	1.002 - 1.037	.028*
Troponin0	1.235E55	.000 - 1.287E+141	.209

Table (8): Simple logistic regression analysis of heart failure (HF) prediction by MR-pro ANP and troponin in patients 6 hrs post admission.

Clini HF	OR	95% CI	p value
ANP 6	1.055	1.001 - 1.113	.045*
Troponin6	22.885	.780 – 671.209	.069

Table (9): Simple logistic regression analysis of heart failure (HF) prediction by MR-pro ANP and troponin in patients at 12 hrs post admission.

Clini HF	OR	95% CI	p value
ANP 12	1.013	1.005-1.022	0.003*
Troponin12	8.354	2.152-32.434	0.002*

Table (10): Multiple logistic regression analysis of heart failure (HF) prediction in patients at 12 hours post admission by MR-pro ANP & troponin.

Clini HF	AOR	95% CI	p value
ANP 12	1.01	1.001-1.02	0.033*
Troponin12	2.486	0.397-15.580	0.3

Table (11): Simple logistic regression analysis of subclinical heart failure (HF) prediction by MR-pro ANP and troponin in patients at admission.

Clini HF	OR	95% CI	p value
ANP 0	1.028	0.996-1.060	0.09
Troponin0	2.201E+53	0-2.366E+134	0.197

Table (12): Simple logistic regression analysis of subclinical heart failure (HF) prediction by MR-pro ANP and troponin in patients at 6 hrs post admission.

Clini HF	OR	95% CI	p value
ANP 6	1.038	1.006-1.070	0.019*
Troponin6	27.850	0.517-1499.4	0.1

Table (13): Simple logistic regression analysis of subclinical heart failure (HF) prediction by MR-pro ANP and troponin in patients at 12 hrs post admission.

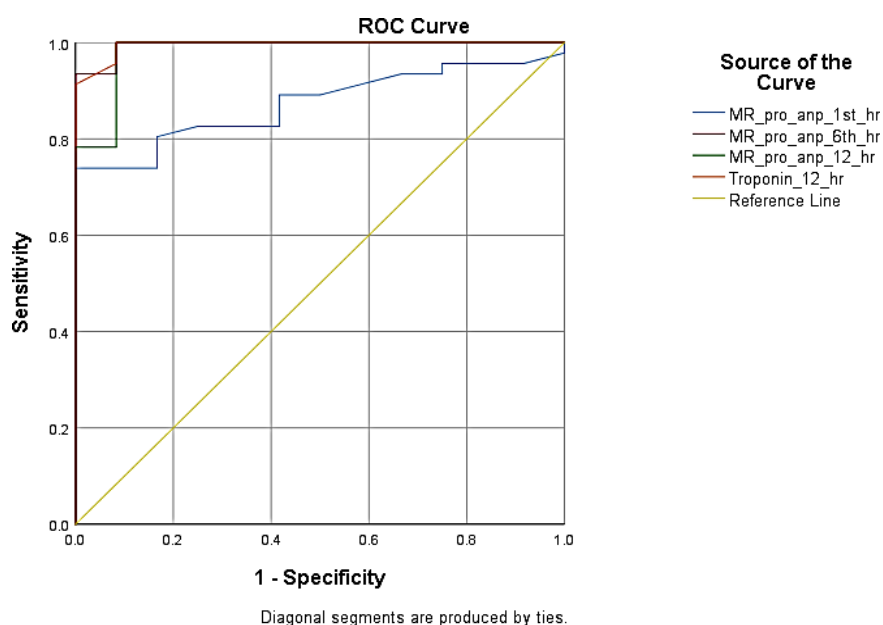
Clini HF	OR	95% CI	p value
ANP 12	1.014	1.005-1.023	0.002*
Troponin12	179.552	6.3-5140.3	0.002*

Table (14): Multiple logistic regression of subclinical heart failure (HF) in patients at 12 hrs post admission by MR-pro ANP & troponin.

Clini HF	AOR	95% CI	p value
ANP 12	1.011	1-1.021	0.043*
Troponin12	7.287	0.112-474.3	0.35

Table (15): Values of Receiver Operator Characteristic Curve (ROC) analysis of clinical heart failure (HF) in study patients.

	Cut-off value	AUC	P value	Sensitivity	Specificity	PPV	NPP	Accuracy
MR pro ANP 0	120	0.873	<0.0001	73.91%	100%	100	50	79.3
MR pro ANP 6	135	0.995	<0.0001	93.48%	100%	100	80	94.83
MR pro ANP 12	140	0.982	<0.0001	100%	91.67	97.9	100	98.28
Troponin 12	0.1	0.947	<0.0001	91.3%	91.67	97.7	73.3	91.38

**Figure (3):** ROC curve analysis of MR pro ANP & Troponin in predicting clinical HF at admission, 6th hrs & 12 hrs post admission.**Table (16):** Values of Receiver Operator Characteristic Curve (ROC) analysis of subclinical heart failure (HF) in study patients.

	Cut-off value	AUC	P value	Sensitivity	Specificity	PPV	NPP	Accuracy
MR pro ANP 6	55	0.966	<0.0001	89.66 %	91.67 %	96.3	78.6	90.24
MR pro ANP 12	81	0.940	<0.0001	100 %	91.67 %	96.7	100	97.56
Troponin 12	0.1	0.855	<0.0001	89.66	91.67	96.3	78.6	90.24

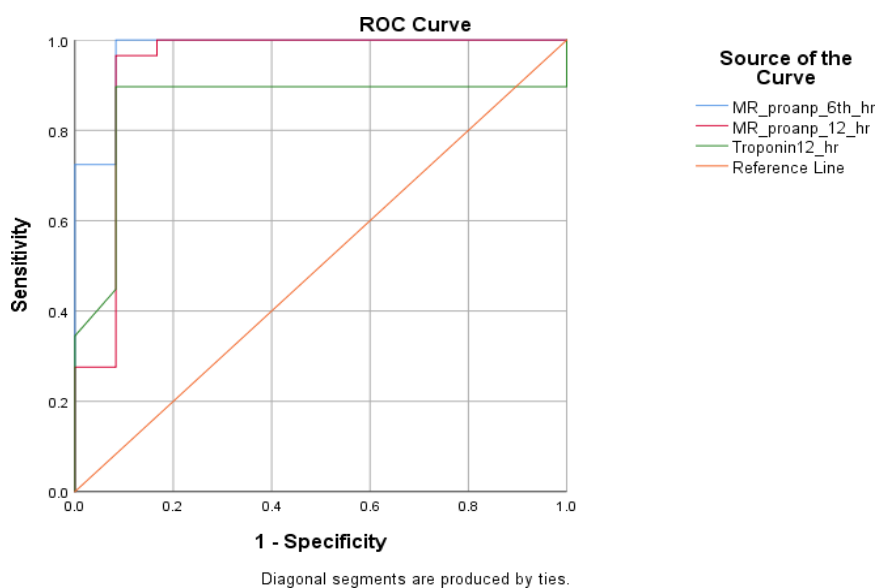


Figure (4): ROC curve analysis of MR pro ANP & Troponin in predicting subclinical HF at 6th hours & 12 hours post admission.

DISCUSSION

Scorpion sting is still considered a bothering health issue that endangers many victims' lives in developing communities, especially children. For example, Sofer and Gueron (2008) in Saudi Arabia revealed that 70.6 % of scorpion sting patients were younger than 18 years old and Dehesa-Davilla and Possani (2004) in Morocco showed that 36% of patients were younger than 15 years old.

The current study was conducted on 87 children with their ages ranged between 5- 17 yrs. The reason behind selecting this young age span is the greater possibility of cardiac-related high morbidity and mortality because of their small body weight and unstable hemodynamics (Khattabi et al., 2001; Mohamed et al., 2016).

Multiple mechanisms of venom-related cardiotoxic effects are postulated, including: **First**, the venom-induced stimulation of Na and K voltage-gated ion channels leading to increased catecholamine release and development of heart failure (Abroug et al., 2015; Bahloul et al., 2013). **Second**, it includes releasing chemical mediators leading to coronary vasospasm, platelet aggregation, and thrombosis (Yang et al., 2009). **Third**,

venom-induced direct myocarditis by reduction of Na-K-ATPase leading to heart failure **Isbister and Bawaskar (2014)**. **Fourth**, the venom acts as an antigen that causes an anaphylactic reaction with subsequent vasodilatation and decreases cardiac blood supply (**Nugent et al., 2004**). **Fifth**, it assumes the angiotensin-converting enzyme (ACE) inhibition causing pulmonary edema (**Isbister and Bawaskar (2014)**).

Using Abroug classification, most patients were categorized to the severe group (52.9%). These findings were concordant with data published by **Ahmed et al. 2018** who reported that severe manifestation was predominant in the studied individuals (78%); these manifestations are usually caused by the broad content of scorpion venom being consisted of hemolytic toxin, cardio toxin, neurotoxin, nephrotoxin, phosphodiesterases, hyaluronidases, glycosaminoglycans, phospholipases, histamine, acetylcholine, and cytokine releasers (**Ahmed et al., 2018; Neale 2000; and Geoffrey et al., 2003**).

The time spent in the Intensive care unit (ICU) by patients manifested clinically by HF was significantly longer than that spent by those who were clinically free.

This is considered logical because of the extra care needed by those patients until their hemodynamics become normalized, requiring more time to stay in the ICU (**Dehesa-Davila 2009; Chippaux and Goyffon 2008; and Wirtz and Azad 2010**).

Hausfater et al., 2017 revealed that atrial natriuretic polypeptide (ANP) is usually secreted from the left ventricle of patients with left ventricular dysfunction in response to mechanical wall stretch thus; it can be used to detect the severity of cardiac affection. However, because of its short half-life, ANP's clinical application is limited; however, its precursor NT-proANP is more stable in plasma and has a longer half-life, but it is inadequate for immunoassay detection due to its development various sub fragments. Recently, **Francis et al., 2016** reported that a mid-regional sequence of pro-atrial-type natriuretic peptide (MR-proANP), which is more stable, was successfully used as a biomarker of the prognosis of acute HF.

Troponin I was an excellent diagnostic marker for myocardial infarction (MI), but its level also increased in some cases of heart failure, i.e., when the causes of heart failure were MI, cytotoxicity, apoptosis, or inflammation (**Thygesen et al., 2007**).

In our study, the echocardiography identified 46 patients with EF < 45% who showed clinical signs of HF, 29 patients with subclinical HF who were clinically free but having EF ≥ 45% and less than 55 % (**Shah et al., 2012; Meki et al., 2003**).

Coinciding with results published by (**shah et al., 2012 and Maisel et al.,2010**), current results showed that at the time when MR-pro ANP predicted clinical heart failure in the 1st-hour post scorpion sting with a cutoff point of 120 pmol/L, sensitivity 73.9 % and specificity 100 %, troponin was only able to predict clinical heart failure after 12 hours from the sting.

Subclinical heart failure was reported in 29 children where EF has fallen below the lower limit of normal value, but no clinical manifestations of heart failure were

recorded. Furthermore, as before, MR-pro ANP was earlier and better than troponin in predicting subclinical heart failure (**Maisel et al.,2010**).

In agreement with these results, **Mohamed et al. (2016)** reported that Troponin I was a late predictor of heart failure.

This study's result is that the MR-pro ANP predicts heart failure primarily even in the absence of myocardial damage, while troponin detects the cardiac cell damage that subsequently results in heart failure. Therefore, substantial damage in cardiac myocytes might be imperative to permit troponin I to predict heart failure.

CONCLUSION

Mid regional pro atrial natriuretic peptide is a good predictor of clinical and subclinical heart failure induced by scorpion stings.

RECOMMENDATION

Further studies for early diagnosis of complications of scorpion sting.

FUNDING

No funding has been received for this study.

CONFLICT OF INTEREST DISCLOSURE

The authors declare that there is no conflict of interest.

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الملخص العربي

منتصف طليعة البروتين الأذيني المدر للصدويوم كعامل متنبئ بإصابة القلب بعد لدغة العقرب بين الأطفال

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يمثل سم العقرب مشكلة صحية مهمة خاصة عند الأطفال فهو يسبب مجموعة متنوعة من المضاعفات على أجهزة الجسم المختلفة ؛ ومع ذلك ، فإن أخطرها هو التأثير السام على القلب. لذا تهدف هذه الدراسة إلى استخدام منتصف طليعة البروتين الأذيني المدر للصدويوم كعامل متنبئ بإصابة مرضى لدغ العقرب بفشل في عضلة القلب. أجريت الدراسة على 87 مريضاً (تتراوح أعمارهم بين 5- 17 عام) تم حجزهم بمركز السموم بمستشفى المنيا الجامعي بادعاء لدغ عقرب (خلال الساعة الأولى من اللدغة) خلال الفترة من 1 يونيو 2018 إلى 31 مايو 2020و تم سحب عينات الدم لعمل التحليل الكيميائي لمادة التروبونين ومنتصف طليعة البروتين الأذيني المدر للصدويوم كما تم عمل ايكو على القلب. ووضحت النتائج نجاح مادة منتصف طليعة البروتين الأذيني المدر للصدويوم في تشخيص فشل القلب الإكلينيكي في خلال الساعة الأولى من اللدغ (حساسية 73.91% وخصوصية 100%) وتشخيص فشل القلب تحت الإكلينيكي بعد 6 ساعات (حساسية 89.66% وخصوصية 91.6%) مما يدل على تفوق منتصف طليعة البروتين الأذيني المدر للصدويوم على مادة التروبونين الذي يشخص فشل القلب بعد اثني عشر ساعة من اللدغ. الخلاصة: تم التوصل الى أن منتصف طليعة البروتين الأذيني المدر للصدويوم من أفضل العلامات البيولوجية في تشخيص اصابة القلب في الساعة الأولى في المرضى المصابون بلدغ عقرب.