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SERUM NT- PRO-ATRIAL NATRIURETIC PEPTIDE AND PENTRAXIN 3 AS COMPARATIVE TO ROUTINE CARDIAC FUNCTIONS TESTS IN DIABETIC PATIENTS WITH MYOCARDIAL INFARCTION

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ABSTRACT

The study conducted as a cross-sectional to include 62 Saudi diabetic patients admitted to intensive-care unit (ICU) with silent cardiac symptoms; 39 of them had MI and 23 had other cardiac disease. The control group involved 40 healthy patients. Blood samples were taken from participants at the moment they were hospitalized in ICU to determine CK-MB, homocysteine, troponin I (cTnI), N-terminal pro-atrial natriuretic peptide (NT-proANP) and pentraxin (PTX3) using ELISA methods. NT-proANP levels showed a significant high increase in the MI patient compared to the control and non-MI patients. Such raised levels were also found in the results of CK-MB, cTnI and homocysteine. While PTX3 levels in non-MI patients showed insignificant elevation comparing to the control. Not like the routine cardiac markers, both serum NT-proANP and PTX3 levels were insignificantly changed in MI male and MI female patients. In conclusion, all patients admitted to ICU showed significant increase in the routine markers, whereas NT-proANP and PTX3 were independent risk factors in the onset of MI.

Key words: NT-proANP, PTX 3, myocardial infarction, CK-MB, troponin I, homocysteine, diabetes mellitus.

INTRODUCTION

Despite recent advances in the management of high blood pressure, diabetes mellitus, hyperlipidemia, but still the death due to heart disease is the most common (Schumacher *et al.*, 2018). Myocardial infarction (MI) usually develops to sudden or gradual anterior chest problems (Saleh and Ambtose, 2018). There is a substantial interest in the use of newer biomarkers to identify persons who are at risk for the development of cardiac disease. Measurements of several biomarkers simultaneously will enhance risk stratification in Coronary Artery Disease (CAD) (Härstedt *et al.*, 2018). Diagnosis of MI is done by the patient's history of illness and physical examination, in addition to laboratory cardiac biomarkers (CK-MB, troponins, us-C reactive protein and homocysteine) and electrocardiogram (ECG) findings. Reliance upon clinical impression alone leads to diagnostic uncertainty because the signs and symptoms of MI are relatively nonspecific (Nordenskjöld *et al.*, 2019). Furthermore, some people have a heart attack without having any symptoms (a silent MI). A silent MI may appear among all patients however it is very prevalent among diabetic patients. Key symptoms such as shortness of breath are nonspecific in patients with comorbidities such as reactive airway disease (Delma, 2018). Likewise, routine laboratory tests, ECG, and radiographs cannot be relied upon to always guide an accurate and appropriate diagnosis. Despite these challenges, diagnostic capabilities in heart failure have improved in recent years with recognition of the role that many factors play in the disease. Now, some newer tests are also being considered to be valuable in the diagnosis of the vascular events (Härstedt *et al.*, 2018).

NT-pro ANP (N-terminal pro-atrial natriuretic peptide) means N-terminal polypeptide of ANP (atrial natriuretic peptide) precursor. In cardiomyocytes, corin converts pro-ANP to active ANP, a hormone that regulates blood pressure by promoting natriuresis, diuresis, and vasodilatation. ANP also suppresses rennin and endothelin release. Plasma ANP very rapidly disappears owing to binding with its receptor and so-called clearance receptor, making its exact measurement difficult. On the other hand, NT-pro ANP, because it does not bind clearance receptor, has a long half-life, and serves as an excellent marker of ANP secretion (Gangnus and Burckhardt, 2019).

Numerous studies have recently examined the role of pentraxin 3 (PTX3) in clinical situations (Fornai *et al.*, 2016). It is expressed predominantly in atherosclerotic lesions. PTX3 synthesis is activated by cytokines and endotoxins in endothelial cells, myeloid cells, macrophages and also in dendritic cells. Once synthesized, PTX3 is predominantly organized into covalent octamers through disulfide bonds (Sahin *et al.*, 2017). Regardless that PTX3 is specifically confined in lactoferrin positive-specific granules, it has been translocated to the surface of late apoptotic neutrophils upon activation, in which it stores in blebs and then released swiftly. PTX3 later binds with the high-affinity C1q to begin the pathway of complement activation and help microbe identification by macrophages.

The PTX3 is found to be elevated in patients with acute MI. Plasma PTX3 levels were found to peak at 7 hours after AMI, and to return to normal levels after 3 days (Wirestam *et al*., 2017). Moreover, PTX3 mRNA reaches peak levels after 24 hours and returns to normal levels 5 days later (Fornai *et al*., 2016).

The aim of our study was to determine whether the introduction of combined serum NT-pro ANP together with PTX3 has an impact on patients presenting with suspected acute MI to the emergency department as a protocol for emergency diagnosis for acute MI comparing to cTnI, Homocysteine and CK-MB

MATERIALS AND METHODS

This study was a cross-sectional study conducted in Saudi Arabia, between June 2018 and April 2019. The study included 62 Saudi patients, 42 males and 20 females in mean age of 50.89±14.33 years. All the patients of either sex were admitted in Intensive Cardiac-Care Unit (ICCU) wards in different hospitals in Qassim, Jeddah, and Riyadh. The patients were diabetic with no previous history of malignancy, infection, inflammatory disease. The control group consisted of 40 healthy volunteers (25 males and 15 females) whose mean ages were matched (49.19 ± 5.67 years). All subjects were nonsmokers, received no drugs and had no metabolic disease and had no recent history of surgery or trauma. To avoid other confounding factors, we excluded overweight patients and controls and/or with a history of folic acid or vitamin B complex supply, a history of folic acid or vitamin B complex deficiency, and renal insufficiency.

Informed consent was obtained from all patients and controls, and then pre-prepared questionnaire was used to collect the subjects' data including their past medical history, smoking status, family and clinical histories, and the prescribed drugs. The blood samples were collected from participants at the moment they were hospitalized in ICCU with cardiac disease, regardless of the type of that disease. 10 mL venous blood samples were taken in serum separator tube patients in the first 3 hours after chest pain, while blood samples were collected after an overnight (for 10 hours) fasting from the controls group. Samples were left to clot, centrifuged for 5 min at 15000 RPM and serum was separated and stored at - 40°C till the time of analysis. Then serum was used to determine creatine kinase-MB (CK-MB), total homocysteine (Hyc), troponin I (cTnI), NT-pro ANP and PTX3 using commercially available ELISA kits from Fine Biotech Company; Wuhan, China.

During the next 24 hours, and as a result of the medical follow-up of patients and after determining the type of cardiac disease, the patients were divided into 2 groups, MI group (39 patients; 31 males and 8 females) and non-MI group (23 patients; 11 males and 12 females). Data analysis statistically was performed using SPSS version 22 (SPSS Inc., Chicago, IL, USA). The level of significance was determined using repeated measures ANOVA followed by Tukey's post-test. $P<0.05$ was considered significant. All values are expressed as mean±SD.

RESULTS

Table 1 showed the demographic data of all participants of healthy persons and ICU admitted patients. The levels of NT-pro ANP and PTX3 in the control, myocardial infarction and admitted non-MI patients were shown in Table 2. Comparing to the control group, all parameters in the MI and non-MI groups showed significant elevations except for PTX 3 in non-MI patients (Table 2). Table 3 included a comparison between MI and non-MI patients, in which, all parameters in MI group showed highly significant increase levels. Table 4 classified the MI group into two sub-groups according to the gender of the patients, males and females. NT-pro ANP and PTX3 levels showed insignificant variation between male and female MI patients; while CK-MB, Hyc and cTnI levels showed significant changes.

Table 1. Demographic characteristics of the participants (n = 50 in each group).

	Control	Patients	P value
Number	40	62	
Age	49.19 ± 5.67	50.89 ± 14.33	0.5979
BMI	23.8 ± 7.09	24 ± 6.22	0.8937
Males (%)	25 (62.5%)	42 (67.74%)	-
Females (%)	15 (37.5%)	20 (32.26%)	-
Married (%)	40 (100%)	62 (100%)	-
Blood Pressure (mmHg)	129/76	132/84	0.553
Duration of diabetes (years)	-	6.35 ± 2.99	-
Signs and symptoms	-	Not specified	
Duration of MI onset	-	- 19.4%: 2 days - 40.3%: 3 days - 40.3%: > 3 days	-

Table 2. Clinical cardiac tests in controls, MI and non-MI groups (mean ± SD).

	Control	MI	Non-MI	P value
Number of cases	40	39	23	
NT-pro ANP (pmol/L)	34.72 ± 9.92	272.82 ± 83.755*	92.77 ± 23.19*	0.001
PTX3 (ng/mL)	1.27 ± 0.681	3.95 ± 0.667*	1.39 ± 0.912	0.001
CK-MB (U/L)	6.14 ± 2.42	14.52 ± 3.17*	10.51 ± 3.89*	0.001
Hyc (µmol/L)	9.11 ± 2.32	19.46 ± 3.81*	10.15 ± 6.17*	0.001
Troponin I (ng/ml)	0.327 ± 0.193	8.631 ± 1.427*	2.029 ± 1.085*	0.001

*significant change when compared to control (p. value≤0.001)

Table 3. Clinical cardiac tests (mean ± SD) in all admitted subjects between MI and non-MI patients.

	MI	Non-MI	P value
Number of cases	39	23	
NT-pro ANP (pmol/L)	272.82 ± 83.755	92.77 ± 23.19	0.000
PTX3 (ng/mL)	3.95 ± 0.667	1.39 ± 0.912	0.000
CK-MB (U/L)	14.52 ± 3.17	10.51 ± 3.89	0.000
Hyc (µmol/L)	19.46 ± 3.81	10.15 ± 6.17	0.000
Troponin I (µg/L)	8.631 ± 1.427	2.029 ± 1.085	0.000

mean ± SD

Table 4. Clinical cardiac tests (mean ± SD) in MI all patients between males and females.

	MI males	MI females	P value
Number of cases	31	8	NA
NT-pro ANP (pmol/L)	273.22 ± 90.652	271.24 ± 53.462	0.953
PTX3 (ng/mL)	3.978 ± 0.709	3.84 ± 0.524	0.611
CK-MB (U/L)	15.55 ± 2.602	10.51 ± 1.576	0.000
Hyc (µmol/L)	21.04 ± 2.97	17.88 ± 3.32	0.012
Troponin I (µg/L)	9.88 ± 2.842	5.63 ± 1.091	0.000

mean ± SD

DISCUSSION

Biomarkers are measurable and quantifiable biological parameters that can have an important impact on clinical situations. Ideal biomarkers are those that are associated with disease clinical endpoints in observational studies and clinical trials, and in some cases, they may even be used as surrogate endpoints (Härstedt *et al*., 2018; Miao *et al*., 2014). Confounding factors that would change the results of tests such as obesity, hypertension, smoking, and age difference between control and patients were excluded in this study.

In this study, the NT-proANP levels showed a significant high increase in the blood of the MI patient compared to the control (p=0.001) and to non-MI patients (p=0.000). Such significant increased levels (p=0.000) were also found in the results of CK-MB, cTnI and homocysteine. While the elevation in PTX 3 levels in non-MI patients compared to control subjects were insignificant (p>0.05).

This significant increase in NT-proANP for non-MI patients compared to control in the current study may be caused by several reasons other than heart disease. Bala *et al*. (2018) have shown that the rate of daily insulin doses correlated with most of the inflammatory factors except PTX 3. The same study showed that the factors leading to the increase in PTSX are very limited and the most obvious is MI. On the other hand, many studies correlated NT-proANP with MI. These studies showed that the rate of serum NT- proANP for patients with myocardial infarction increased by about ten folds the normal rate, while the rates do not exceed four folds in non-infarct heart patients (Nakagawa *et al*., 2019; Roberts *et al*., 2015). Moreover, Taylor *et al*. (2018) reported that dyspnoea and/or chest pain increases NT-pro ANP; on the same subject, Büttner *et al.* (2018) reported that CK-MB, NT-proANP, and C reactive protein –as cardiac markers- showed similar significances in variations when compared control healthy

persons with patients diagnosed as cardiovascular or pulmonary diseases; but NT-proANP showed highly significant changes in MI.

The present study illustrated that both of serum NT-proANP and PTX 3 levels were insignificantly changed in MI male and MI female patients (p value>0.05); and this contrasted with what appeared in other cardiac markers which emphasized significant rises (p=0.000) in CK-MB, Hyc, as well as cTnI through MI male comparing to MI female patients. On top of that, it has affirmed that NT-pro ANP levels in different cardiac diseased depend on many confounding factors that could interfere with the creation of the result in a predictable or unexpected manner. These factors have an effect on the prescription of medication; which should be clarified when writing the results of the pathological laboratory for heart patients. The most important factors are sex, age and BMI (Hidayet *et al*., 2018).

Abdelsalam (2015) reported that us-CRP and homocysteine levels were significantly increased in all subjects admitted to ICCU with all types of cardiac problems. However, these levels are gradually reduced within two to three days after the onset of angina and may return to the highest normal rate. If measured at that time, fail to interpret or case misunderstand may occur. Wirestam *et al*. (2017) found that acute coronary artery diseases (CAD) and chronic ischemia are both lead to increased serum Hcy and CK-MB concentration as well as most of the cardiac markers, but only PTX 3 in that study was found to be insignificantly changed in chronic cases. In the same vein, Pérez-López *et al.* (2010) and Devaraj *et al.* (2009) illustrated that hs-CRP, cTnI, and CK-MP were affected by age and gender in one way or another and in varying degrees; while Sahin *et al*., (2017) and Banaszak *et al*., (2019) reported that the plasma NT-proANP and PTX 3 may be independent risk factors in acute MI patients.

CONCLUSION:

The routine cardiac markers showed significant elevations in all patients admitted to ICCU whether the patient admitted with MI or other heart diseases. Also, these markers showed variations in the results of MI patients depending on the gender of the patient. While the results of NT-proANP and PTX 3 were not affected by interfering factors as well as they give clear readings in MI comparing to control persons and non-MI patients.

Conflict of interest

No conflict of interest

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