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# A Case/Control Study of Evaluating the Serum Levels of New Risk Factors for Cardiovascular Diseases in Iranian patients

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## ABSTRACT

Early diagnosis is the cornerstone of cardiovascular diseases management. In this study the predictive value of N-Terminal Probrain Natriuretic Peptide (NT-proBNP), High-Sensitivity Cardiac Troponin T (hs-cTnT), Myeloperoxidase (MPO) and Pregnancy-Associated Plasma Protein A (PAPP-A) were investigated in Iranian Acute Myocardial Infarction (AMI) patients, Unstable Angina (UA) patients, Coronary Artery Disease (CAD) patients and normal subjects. The present study was performed on 35 AMI patients, 35 UA patients, 35 CAD patients and 53 healthy individuals. NT-proBNP, hs-cTnT, MPO and PAPP-A were quantitated by ELISA kits. The cardiovascular patients had significantly higher levels of NT-proBNP, hs-cTnT, MPO and PAPP-A ( $p < 0.05$ ) as compared with normal individuals. The obtained results indicated that the serum levels of NT-proBNP, hs-cTnT, MPO and PAPP-A significantly increased in cardiovascular patients and would be strongly predictive in these diseases.

**Key words:** Cardiovascular diseases, High-Sensitivity Cardiac Troponin T, Myeloperoxidase, N-terminal probrain natriuretic peptide, Pregnancy-Associated Plasma Protein.

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## 1. INTRODUCTION

Cardiovascular diseases are considered as one of the main leading causes of death in human societies. Deal with their complications, CAD imposed a huge cost to the health system of various countries including Iran every year. Statistics indicated that cardiovascular diseases were the first cause of death in people over 35 years in Iran. The mortality arising from cardiovascular diseases was increased from 26.6% in 1981 to 47.3% in 1995 (1). Several major risk factors have been known for this disease. They included fibrinogen, cardiac troponin I, C-reactive protein (CRP), high levels of homocysteine, hypertension, smoking, hyperlipidemia, diabetes and obesity. Other new studies also continued to identify the new factors (2-4). According to the recent studies, N-Terminal Probrain Natriuretic Peptide (NT-proBNP), High-Sensitivity Cardiac Troponin T (hs-cTnT), Myeloperoxidase (MPO) and Pregnancy-Associated Plasma Protein A (PAPP-A) have been proposed as risk

factors (1, 4, 5). The NT-proBNP is a neurohormone synthesized in atrioventricular parts of the heart muscles. It generates in the heart myocytes and releases in response to injury, ischemia, stress or inflammation (6). Hs-cTnT is a heterogenic protein that plays an important role in regulation of contractions in the skeletal and heart muscles. This protein contains three subunits, termed troponin I (TnI), troponin T (TnT) and troponin C (TnC). TnT and TnI were observed as various forms in the skeletal and heart muscles. Recently, TnT measurement with high sensitivity or hs-cTnT has been considered as a prognostic marker for the diagnosis of heart diseases including myocardial infarction (7). MPO is an enzyme released from neutrophils in response to acute inflammatory reactions arising from heart damage. More recently, there was a special attention to this enzyme in clinical trials related to cardiovascular damages (8). PAPP-A is a protein that released from unstable atherosclerotic plaques in the coronary artery wall. Recently, it is considered as a

substantial laboratory marker in prognosis of heart muscle damage (9). Due to an increasing prevalence of cardiovascular diseases in our population without a known major risk factor in many cases of people suffering from these diseases, especially younger individuals (<50 years), we decided to investigate new risk factors in patients with cardiovascular diseases as well as normal healthy subjects. The purpose of this study was to determine exactly four factors including NT-proBNP, hs-cTnT, MPO and PAPP-A in patients with acute coronary diseases, Unstable Angina (UA) and acute myocardial infarction (AMI), and coronary artery diseases (CAD). The results obtained from this study could determine whether these laboratory markers were effective in the diagnosis of these diseases.

## 2. MATERIALS AND METHODS

The present study was performed as a case/control study on 105 cardiovascular patients referred to Ekbatan hospital in Hamadan City include 35 AMI patients, 35 UA patients and 35 CAD patients. The presence of disease was proved by angiography and confirmed by cardiologist in each patient. 53 healthy individuals were studied as control group. After 14 hours of fasting, 10ml blood was taken from all the studied patients as well as the control individuals. The blood samples were taken from hospitalized patients almost 6-8 hours after hospitalization. The plasma was isolated from blood including EDTA solution with concentration 0.47 mol/L by centrifugation at 2000×g for 15 min. After removal of plasma, four risk factors for cardiovascular diseases include NT-proBNP, hs-cTnT, MPO and PAPP-A were measured. NT-proBNP, MPO, hs-cTnT and PAPP-A were evaluated by ELISA kit (Usan Life Science, Dignosis, Roche Diagnostics and Demeditec companies, respectively). Tests for blood glucose, total cholesterol, HDL cholesterol, triglyceride and uric acid were measured by enzymatic method (by pars azmon enzyme test kit). LDL cholesterol levels were calculated by Friedewald formula. All of these experiments were carried out in duplicate. In order to obtain accurate results the tests were repeated if needed.

The sample size was determined by using formula:

$$n = \frac{(\sigma_1^2 + \sigma_2^2)(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2}{d^2} \quad (1)$$

It was estimated according to the information obtained by Khan et al [11] for each of the patients and healthy subjects. Statical parameters include ( $\alpha = 0.05$ ,  $\beta = 0.1$ ,  $\sigma_1 = 140.64$ ,  $\sigma_2 = 3.25$ ,  $\mu_1 = 40$ ,  $\mu_2 = 85$ ,  $d = \mu_1 - \mu_2 = 45$ ).

After putting the values in the formula, the number of samples for test (including cardiovascular disease) and control groups were obtained 105 and 53 individuals, respectively. The individuals of both groups were matched in terms of demographic characteristics such as age and gender. None of them affected by other diseases such as diabetes, gout, metabolic disorders or chronic diseases. Furthermore, those taking enhancing drugs (such as B-complex, vitamin B6, vitamin B12 and folic acid) or drugs nicotinic acid, allopurinol, codeine, dizopiramid, Probuco, estrogen and neomycin were excluded from the study. Descriptive statistical methods including charts, tables and statistical indications were used for analysis of data. Independent t-test or Mann-Whitney test were used to compare two groups. Logistic regression was used to investigate the effects of risk factors on cardiovascular diseases. Data analysis was performed using SPSS software. Estimated P-value less than 0.05 was considered as significant.

## 3. RESULTS AND DISCUSSION

This study was performed on 158 patients including CAD, UA and AMI patients and healthy subjects. The studied population was evaluated in terms of age, gender, the level of NT-proBNP, hs-cTnT, MPO, PAPP-A, fasting blood glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and uric acid. The obtained data was shown in Table 1.

**Table 1. Clinical and laboratory characteristics of four groups: CAD (n=35), UA (n=35), AMI (n=35) and normal (n=53)**

Variables	CAD (n=35)	UA (n=35)	AMI (n=35)	Normal (n=53)
Age	65±10	79±9	63±12	45±15
Sex (male/female)	15.20	17.18	11.24	23.30
NT-proBNP (pmol/l)	40±35	4430±	135±70	35±25
hs-cTnT (ng/l)	150±80	107±50	220±70	15±7
MPO (Unit/ml)	155±75	118±57	211±99	63±19
PAPP-A (µg/ml)	2.9±0.7	1.1±0.3	10.5±5.5	0.5±0.1
fasting blood glucose (mmol/l)	90±45	97±40	110±50	85±35
Total cholesterol (mmol/l)	197±22.5	174±17.5	215±20.7	158±10.7
HDL cholesterol (mmol/l)	38±11.1	45±9.2	38±9.7	48±10.5
LDL cholesterol (mmol/l)	129±14	111±13	134±9	100±15
Triglycerides (mg/dl)	173±49.5	130±41	186±77	115±25
Uric acid (mg/dl)	7.9±3.7	6.1±2.5	8.5±5.5	5.5±2.2

Data were expressed as mean ± SD.

CAD=Coronary artery disease

UA=Unstable angina

AMI=Acute myocardial infarction

NT-proBNP= N-Terminal Probrain Natriuretic

hs-cTnT= High-Sensitivity Cardiac Troponin T

MPO= Myeloperoxidase

PAPP-A= Pregnancy-Associated Plasma Protein A

The results shown that NT-proBNP levels increased to three times in AMI patients as compared with CAD and UA patients and the normal healthy individuals ( $p > 0.001$ ). In addition, levels of hs-cTnT in AMI cases was reported 1.5, 2 and 15 times more than CAD patients, UA patients and healthy subjects, respectively ( $p > 0.001$ ). MPO level in all three groups of CAD, UA and AMI patients was higher than normal healthy subjects. However, the most level of MPO was observed in AMI ( $p > 0.001$ ). Finally, the most levels of PAPP-A was in AMI cases among the studied population. So that the level of PAPP-A in AMI patients was 3, 10 and 20 times higher than CAD patients, UA patients and healthy subjects, respectively ( $p > 0.001$ ). Regression analysis was performed to determine the correlation between the groups, CAD, UA, AMI patients

and healthy individuals with four measured factors including NT-proBNP, hs-cTnT, MPO and PAPP-A. The results shown that there is a clear correlation between these groups with four factors NT -proBNP ( $\chi^2=21$ ;  $p < 0.001$ ), hs-cTnT ( $\chi^2=25$ ;  $p < 0.001$ ), MPO ( $\chi^2=18$ ;  $p < 0.001$ ) and PAPP-A ( $\chi^2=25$ ;  $p < 0.001$ ). Hs-cTnT was correlated with NT-proBNP ( $r=0.44$ ,  $p < 0.001$ ), MPO ( $r=0.32$ ,  $p < 0.01$ ) and PAPP-A ( $r=0.59$ ,  $p < 0.001$ ). On the other hand, hs-cTnT was correlated with both factors MPO and PAPP-A ( $r=0.42$ ,  $p < 0.001$ ). MPO was also correlated with PAPP-A ( $r=0.52$ ,  $p < 0.001$ ). Table 2 shown the results obtained from comparison of NT-proBNP, hs-cTnT, MPO and PAPP-A levels between four groups including CAD, UA, AMI patients and normal healthy individuals by  $\chi^2$  test.

**Table 2. The Comparison of NT-proBNP, hs-cTnT, MPO and PAPP-A levels between groups CAD, UA, AMI patients and healthy individuals**

Factor	Healthy individuals	CAD	$\chi^2$	P value	UA	$\chi^2$	P value	AMI	$\chi^2$	P value
NT-proBNP	6.94	8.17	4.2	0.04	26.24	52	0.001	34.66	16	<0.001
hs-cTnT	6.94	10.15	17	<0.001	46.4	89	0.01	62.38	32	<0.001
MPO	6.94	12.13	21	<0.001	49.1	100	<0.001	68.32	62	<0.001
PAPP-A	6.94	16.9	83	<0.001	49.1	100	<0.001	89.11	111	<0.001

The ratio between positive and negative results was shown with  $\chi^2$  amounts estimated by Yates continuity test. P-value was obtained from comparison with normal healthy group.

In this project, the values of NT-proBNP, hs-cTnT, MPO and PAPP-A were measured in CAD, UA and AMI patients in comparison to normal healthy individuals. This research was performed according to evaluation of four relatively new laboratory markers for cardiovascular diseases including NT-proBNP, hs-cTnT, MPO and PAPP-A. NT-proBNP that are released as a part of BNP (Brain natriuretic peptide) hormone in heart failures. The results of this study shown that this factors in AMI patients was 4 times higher than healthy controls. hs-cTnT is known as an important laboratory biomarker of cardiac damage. This marker increased 15, 10 and 7 times in AMI, CAD and UA patients, respectively as compared with healthy individuals. The MPO, an enzyme released by leukocytes during inflammation, in groups of AMI, CAD and UA patients were reported 3, 2.5 and 2 times more than healthy individuals, respectively. In addition, PAPP-A, a measurable indicator of heart damage, was obtained in AMI, CAD and UA patients almost 20, 6 and 2 times more than control group, respectively. In a previous article all the laboratory parameters measured in this project including NT-proBNP, hs-cTnT, MPO and PAPP-A have been introduced as biomarkers of Coronary artery diseases (10). In research performed On 398 cardiovascular patients, four experimental factors including MPO, PAPP-A, CRP and MRP (Myeloid-related protein) were measured. The concentration of MPO, CRP and MRP in AMI patients was reported significantly more than healthy individuals. Their results indicated that measurement of inflammatory factors and platelet markers could be useful in identifying early stages of heart disease (11). The findings of another study, has introduced two factors NT-proBNP and hs-cTnT as suitable markers for identifying patients with cardiovascular disease, especially AMI patients. They have also proposed the simultaneous use of both factors (12). The results of different studies also shown that PAPP-A could be used as laboratory factor for the diagnosis of CAD disease. They also reported that increase of PAPP-A

concentration will increase mortality in CAD patients. The results of the present study indicated that the amount of this factor also shown a significant increase in CAD group as compared with normal healthy individuals and UA group (9). Also previously two experimental factors NT-proBNP and hs-cTnT were introduced as biomarkers of Acute Coronary Syndromes. These two factors were measured on 115 patients and it was reported that hs-cTnT measurement could be a relatively suitable prognosis for the detection of cardiovascular disease, especially in people under age 65 years. These two factors should be evaluated in people over the age range 65-70 years (13). In another study on patients with cardiovascular disease in the Netherlands it was reported that hs-cTnT factor shown the assay values more than other factors including NT-proBNP, Myoglobin, GDF15 (Growth-differentiation factor 15) and PIGF (Placenta-derived growth factor) in the diagnosis of disease (14). The results of studies carried out by Mayr et al showed that NT-proBNP levels were significantly increased in AMI patients and it had a positive correlation with increased mortality (15). According to the other findings MPO levels increased significantly in AMI patients as compared with UA groupe and healthy individuals (16) and these observations were consistent with the results of the present study. The study conducted on 379 patients with CAD in India shown that the amount of PAPP-A was significantly increased in those patients as compared with other measured factors including CRP and Leptin (17). These observations shown a completely direct correlation with the results obtained from the present study. The results also shown that the NT-proBNP, hs-cTnT, MPO and PAPP-A increased in CAD,UA and AMI patients as compared with healthy subjects. However, this increase in AMI patients was significantly more than others. In AMI patients, myocardial ischemia caused vascular endothelial damage and rapid speed of prostaglandins biosynthesis through cyclooxygenase-dependent pathway in these cells that results in increased

platelet activation, their aggregation and adhesion as well as increasing the plasma fibrinogen concentration. These reactions lead to release toxic aldehydes such as MDA. On the other hand, endothelial injury leads to myocardial necrosis and increasing troponin I concentration as its index. These two latter processes including endothelial injury and myocardial necrosis stimulated inflammation reactions and increased laboratory parameters including NT-proBNP, hs-cTnT, MPO and PAPP-A in these patients. The results shown that the amount of four risk factors including NT-proBNP, hs-cTnT, MPO and PAPP-A in cases with cardiovascular disease was higher than normal healthy individuals and this increase in AMI patients was significantly more than others. Therefore, the simultaneous measurement of four parameters including NT-proBNP, hs-cTnT, MPO and PAPP-A was very suitable for detection of cardiovascular diseases.

#### 4. CONCLUSION

The present study suggested that the four mentioned factors could be evaluated along with other routine laboratory tests for cases with cardiovascular disease. It could provide early diagnosis of patients that followed by successful treatment strategies and reduced the mortality rate of these patients.

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#### AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

#### CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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