INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

N-TERMINAL PRO BNP AND SOME OTHER BIOCHEMICAL AS INDICATORS OF CARDIAC DAMAGE IN PATIENTS WITH ACUTE CORONARY SYNDROME

Wafa M Merza[1], Basil N Saeed[2], Ahsan K Abbas[1] and Mohamad M Alanee[3]

[1]Department of Biochemistry, College of Medicine, University of Baghdad.
[2]Department of Medicine, College of Medicine, University of Baghdad.
[3]Department of biology, College of Medicine, University of Baghdad.

ABSTRACT

Background: Acute coronary syndrome (ACS) refers to any group of symptoms attributed to obstruction of the coronary arteries. ACS include Unstable angina , Extensive MI, Anterior MI, Antero septal MI and Inferior MI. **Objective:** To evaluate the diagnostic value of a single measurement of plasma BNP in patients with ACS admitted to cardiology care unite within 24hour. **Methods:** Clinical, echocardiographic, and laboratory characteristics, including N Terminal pro brain natriuretic peptide (NT-PRO BNP), troponin I (TnI) and C-reactive protein (CRP) were measured within 24 hours of hospitalization for 70 patients, The measurement were done by enzyme linked immunosorbent assay .Twenty healthy subjects were considered as control group. The present study was conducted at the Department of Bio Chemistry, College of Medicine, University of Baghdad and Baghdad Teaching Hospital during the period from April 2012 to May 2014. **Results:** The patients with **Acute coronary syndrome (A.C.S)** were found to have significantly higher mean (± SEM) value of serum NT-PRO BNP ($p<0.001$) compared with mean (± SEM) value of serum control groups. , mean (± SEM) value of troponin I were Non significantly higher ($p<0.01$) compared with mean (± SEM) value of serum control groups,and significantly higher serum CRP ($p<0.001$) compared with mean (± SEM) value of serum control groups.A significant positive correlation ($r = + 0.77$; $p_{=0.0144}$) was observed between serum NT-PRO BNP concentrations and serum TnI concentrations and Non significant inverse relationship ($r = -0.19$; $p=0.388$) was noted between serum NT-PRO BNP concentrations and the serum CRP concentrations in patients with ACS.There were significant differences in mean(± SEM) value of NT-PRO BNP ($P\leq0.05$) among 5 types of **A.C.S** , mean(± SEM) value of NT-PRO BNP in Inferior MI > Unstable angina > Extensive MI > Anterior MI > Anteroseptal MI. **Conclusions:** A single measurement of BNP on admission can predict earyl and degree of ischemi in types of Acute coronary syndrome.

Keywords: B-type natriuretic peptide, ACS, Diagnosis.

1. INTRODUCTION

Acute coronary syndrome (ACS) refers to any group of symptoms attributed to obstruction of the coronary arteries. The most common symptom prompting diagnosis of ACS is chest pain, often radiating of the left arm or angle of the jaw, pressure-like in character, and associated with nausea and sweating. These types are named according to the appearance of the electrocardiogram (ECG/EKG) as non-ST segment elevation myocardial infarction (NSTEMI) and ST segment elevation myocardial infarction (STEMI). There can be some variation as to which forms of myocardial infarction (MI) are classified under acute coronary syndrome[1].The availability of serum cardiac markers with markedly enhanced sensitivity for myocardial damage enables clinicians to diagnose MI in

approximately an additional one third of patients who would not have fulfilled criteria for MI in the past.[2] Inflammation characterizes all phases of atherothrombosis and provides a critical pathophysiologic link between plaque formation and acute rupture, which lead to occlusion and infarction.[3] The expression of cytokines such as interleukin-6, which can travel from local sites of inflammation to the liver and trigger a change in the program of hepatic protein synthesis, is characteristic of the acute-phase response. The acute-phase reactant CRP, a simple downstream marker of inflammation, has now emerged as a major cardiovascular risk marker.[4]

There is evidence that the release pattern of troponin complexes and degradation into various troponin fragments may affect the results of various commercial assays, especially for cTnI, and may be useful in the future to gain insight into pathophysiologic events (e.g., ischemia, reperfusion).[5]

B-type natriuretic peptide (BNP) and its precursor, N-terminal pro-BNP (NT-proBNP), are secreted by human atrial and ventricular myocardium. Given the larger mass of ventricular rather than atrial myocardium, the total amount of mRNA for BNP is higher in the ventricles than in the atria. Natriuretic peptides are released early after STEMI, peaking at about 16 hours.[6]

2. Subjects and Methods

2.1 Patients Selection

The present study was conducted at the Department of PhysiologicalChemistry, College of Medicine, University of Baghdad and BaghdadTeaching Hospital- during the period from April 2013 to May 2014. Thediagnosis in every patient was done by a specialist in cardiology based on clinical presentation and history of ischemic heart disease, which was confirmed by ECG and cardiac Troponin .70 patient with ACS and 20 healthy persons as Controls group (10 females and 10 males).

2.2 Blood Collection

From each patient (during 24h from admition) and control, five ml of venous blood were aspirated from a suitable vein. Samples were collected between (8-9 A.M.) after 10 hours fasting. Blood samples were divided into two parts, three ml transferred to a plain tube to measure the levels of (glucose and lipid profile). The remaining of blood transferred to another sterile plain tubes for storage to measure the levels of (N-PRO BNP, CRP, and Troponin I) by a kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology. The non heparinized blood in the plain tubes were left to clot and then centrifuged by cold centrifuge at 4000 rpm for 5 minutes many times (1-5) to separate the serum and dispensed into tightly closed Eppendorf tubes 1.0 ml and stored at -20 C° until assayed.

Electrocardiography

ECGs were performed upon hospital admission and were repeated soon after medication was started. ECGs were analyzed blind to the results of coronary angiography. Each tracing was evaluated by two cardiologists for the presence or absence of acute ischemic changes based on the presence or absence of (1) ST-segment depression $ 0.5 mm and (2) T wave changes. Patients with acute ischemic changes were wave changes. Patients with acute ischemic changes were categorized according to the location of the acute ischemic region into groups with anterior (V1, V2, V3, V4), inferior DII, DIII, aVF), or lateral wall (V5, V6, DI, aVL) involvement.

2.3 Statistical Analysis

All data are described as rates and frequencies or means with standard deviations, as appropriate. Differences in the distribution of selected characteristics between patient groups were examined using the chi-square test and Fisher's exact test for categorical variables. The analysis was performed using the Student's *t*-test for normally distributed continuous variables and the Mann-Whitney and Kruskal-Wallis tests for nonparametric variables. Pearson correlation coefficients were used to study the correlations.

3. Results

3.2 Biochemical markers in Acute Coronary Syndrome(A.C.S.) patients and control

serum levels of NT-PRO Brain Natriuretic Peptide (NT-PRO BNP), troponin I and C-reactive protein (CRP) were compared between the patients groups and controls groups using analysis of variance t-test of significant as in table 1 . The patients with A.C.S. were found to have significantly higher mean (± SEM) value of serum NT-PRO BNP ($p<0.001$) compared with mean (± SEM) value of serum control groups, mean (± SEM) value of troponin I was Non significantly higher ($p<0.01$) compared with mean (± SEM) value of serum control groups and mean (± SEM) value of serum CRP ($p<0.001$) was significantly higher than mean (± SEM) value of serum control groups.

Table 1: Comparison between patients & control according to biochemical markers

Group	No.	Mean ± SE		
		NT-PRO BNP	TnI: Troponin I	CRP: C-reactive protein
Patients	70	203.95 ± 21.42	8.10 ± 0.87	12.73 ± 0.97
Control	20	107.79 ± 4.23	7.74 ± 0.48	0.316 ± 0.143
T-test value P-value	--	34.782 ** 0.0144	1.973 NS 0.372	3.019 *** 0.00252
** (P≤0.01), *** (P≤0.001), NS: Non-significant(p≥0.05)9.				

Results expressed as Mean (± SEM).

Table 2: Distribution of TnI& CRP according to NT-PRO BNP quintiles

NT-PROBNP quintiles	No.	Mean ± SE	
		TnI: Troponin I	CRP: C-reactive protein
1 (≤110)	25	5.42 ± 0.32	11.32 ± 1.47
2 (110-400)	36	6.14 ± 0.24	15.05 ± 1.39
3 (≥400)	9	23.37 ± 3.94	7.36 ± 2.28
LSD value P-value	----	2.984 **** 0.0001	5.406 * 0.0211
* (P≤0.05),**** (P≤0.0001).			

3.3. Relation between NT-PRO Brain Natruretic Peptide (NT-PRO BNP) and Troponin I(TnI) and C-Reactive protein(CRP)

In the present study, a significant positive correlation ($r_{=}$ + 0.77 ; $p_{=0.0144}$) was observed between serum NT-PRO BNP concentrations and serum TnI concentrations in patients with ACS (Figure 1) .

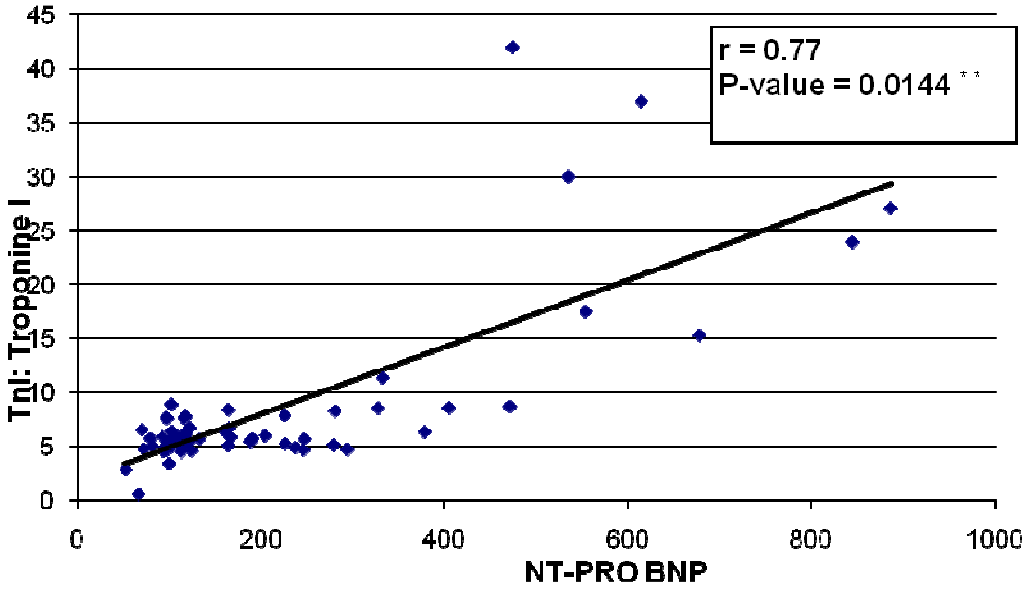

Fig. 1: correlation between serum NT-PRO BNP(ng/l) concentrations and serum Troponin (ng/l) concentrations in patients with ACS

Non significant inverse relationship (r = -0.19; p=0.388) was noted between serum NT-PRO BNP concentrations and the serum CRP concentrations in patients with ACS (Figure 2).

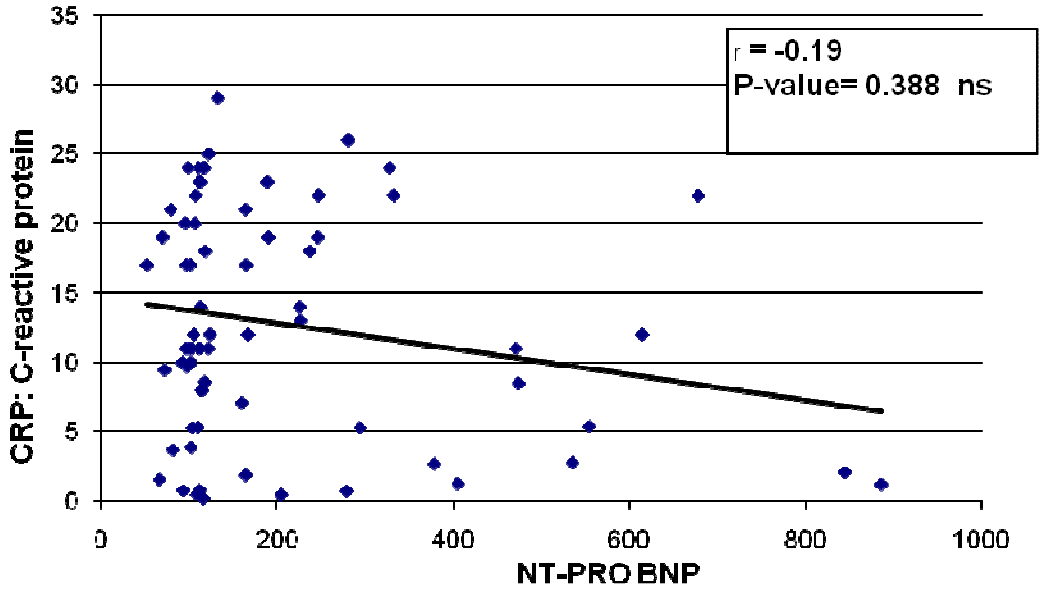

Fig. 2: Relationship between serum NT-PRO BNP (ng/l) concentrations and the serum C-reactive protein: CRP (mg/l) concentrations in patients with ACS

Study patients divided into 5 groups according to type of A.C.S, table (3)

1. Extensive MI: this group include 26 (37.14%) patients, Male Frequency was 18(69.23%) higher than Female Frequency 8(30.77%) .
2. Anteroseptal MI: this group include patients10 (14.29%), Male Frequency was 9(90%) higher than Female Frequency 1(10%) .
3. Anterior MI: this group include 2(2.86%) patients, Male Frequency was 1(50%) equal to Female Frequency 1(50%) .
4. Inferior MI: this group include18 (25.71%) patients, Male Frequency was 15(83.33%) higher than Female Frequency 3(16.67%).
5. Unstable angina: this group include14 (20.00%) patients, Male Frequency was 9(64.29%) higher than Female Frequency 5(35.71%).

Table 3: Distribution of study sample according to type of Acute Coronary Syndrome (A.C.S.) and gender (No. & %)

Type	Total No. (%)	Male		Female	
		No.	%	No.	%
1: Extensive MI	26 (37.14%)	18	69.23	8	30.77
2: Anteroseptal MI	10 (14.29%)	9	90.00	1	10.00
3: Anterior MI	2 (2.86%)	1	50.00	1	50.00
4: Inferior MI	18 (25.71%)	15	83.33	3	16.67
5: Unstable angina	14 (20.00%)	9	64.29	5	35.71
Total	70	52	74.29	18	25.71

Table(4)shows Distribution of biochemical markers according to types of Acute coronary syndrome. There were significant differences in mean(± SEM) value of NT-PRO BNP (P≤0.05) among 5 groups, mean(± SEM) value of NT-PRO BNP in Inferior MI > Unstable angina > Extensive MI > Anterior MI > Anteroseptal MI .

There were no significant differences in mean(± SEM) value of Troponine I (P≥0.05) among 5 groups, mean (± SEM) v alue of Troponine I in Inferior MI > Unstable angina > Extensive MI > Anterior MI > Anteroseptal MI .

There were significant differences in mean(± SEM) value of C-reactive Protien (P≤0.05) among 5 groups, mean (± SEM) value of C-reactive Protien in Extensive MI > Anterior MI > Anteroseptal MI > Inferior MI > Unstable angina.

Table 4: Distribution of Biochemical markers according to types of Acute coronary syndrome

Parameters	types of ACS					P-value
	1ExtensiveMI	2 Anteroseptal MI	3 Anterior MI	4 Inferior MI	5 Unstable angina	
NT-PRO BNP	199.67 ± 15.47	142.41 ± 19.06	195.37 ± 22.53	277.63 ± 26.42	229.08 ± 22.61	0.0255 *
TnI: Troponin I	8.12 ± 0.48	5.39 ± 0.71	6.31 ± 0.59	8.60 ± 0.62	9.85 ± 1.04	0.146 NS
CRP: C-reactive Protein	15.56 ± 0.84	13.45 ± 0.72	14.66 ± 0.92	11.97 ± 0.73	7.12 ± 0.12	0.0478 *

DISCUSSION

Cardiac biomarkers are an integral component in the evaluation and risk-stratification of patients with cardiac diseases in general and acute coronary syndromes (ACS) in particular.

The present study result showedthe mean (± SEM) of CRP was significantly higher ($p<0.001$) compared with mean (± SEM)value of serum control groups .C-Reactive Protein binds to a large number of autologous and extrinsic ligands, including native and modified plasma lipoproteins, phospholipids, and apoptotic cells, which are present in the atherosclerotic lesions. When bound to ligands, CRP activates the classic pathway of complement, a major player in the immune and inflammatory response, and reacts with Fcγ receptors on phagocytic cells. Both CRP and complement are known to co localize in human atherosclerotic lesions, which suggests that CRP, by activating the complement, may be an active participant in atherosclerosis development.[7]

Michael H.et al[8] reportead that the predictive value of hs-C-reactive protein decreased gradually with further adjustment indicating that it was the sum of established CV risk factors rather than markers of subclinical CV damage which reduced the prognostic effect of hs-C-reactive protein to an insignificant level. This is in agreement with the findings by Danesh et al.,[9] who demonstrated that hs-C-reactive protein was a moderate predictor of coronary heart disease, and by Kistorp et al.,[10] who showed that hs-Creactive protein was not as strongly associated to CV outcome as Nt-proBNP and UACR.

The mean (± SEM) value of troponin I was Non significantly higher ($p<0.01$) compared with mean (± SEM) value of serum control groups.An increased circulating cardiac troponin concentration indicates myocardial injury and aids in the diagnosis of acute myocardial infarction (MI)[11]. Fred S.et al[12] study's attempted to evaluate the independent diagnostic information contributed by a spectrum of biomarkers representing inflammation, plaque destabilization, plaque rupture, myocardial necrosis, and myocardial dysfunction for a heterogeneous group of low - to high – risk patients who presented with symptoms suggestive of ACS. In their study, cTnI measured with a newer-generation assay was the most effective diagnostic biomarker for detecting MI. No other combination of biomarkers added any diagnostic sensitivity.

cTnI the biomarkers were measured only in a single sample obtained at the time of enrollment. Serial measurements of the multiple biomarkers over time would potentially be of great interest for better understanding the differences in biomarker kinetics, as well as for future elucidation of effective therapeutic strategies.

The present study result showed mean (± SEM) value of NT-PRO BNP was significantly higher ($p<0.001$) compared with mean (± SEM)value of serum control groups . B-type natriuretic peptide (BNP) is a counter-regulatory peptide hormone predominantly synthesized in the ventricular myocardium. BNP is released into the circulation in response to ventricular dilatation and pressure overload, and reflects ventricular wall stress and tissue hypoxia rather than cell injury perse[13,14].

Brügger-Andersen et al[15] reported that The main findings of thier study in a group of unselected patients admitted to the ED with chest pain and potential ACS, indicate that BNP is an important and independent prognostic biomarker for both short- and long-term mortality.

SeonGyuet al[16] reported that NT-proBNP is a natriuretic hormone released primarily from the heart ventricles. NT-proBNP is released from myocardial cells in response to volume expansion in HF. Therefore, NT-proBNP has been used for diagnosis of symptomatic or asymptomatic HF. However, NT-proBNP is also thought to be released from ischemic myocardial cells due to an unknown mechanism.

In the first hours of acute myocardial infarction, BNP is released as a result of ischemia and necrosis of myocardial cells. Afterwards, BNP rises as a result of systolic and diastolic dysfunction and increased wall stress of the left ventricle[17-19].

A significant positive correlation ($r = + 0.77$; $p_{=0.0144}$) was observed between serum NT-PRO BNP concentrations and serum TnI concentrations in patients with ACS, the cause may be due to increase serum NT-PRO BNP concentrations indicate present of heart muscle ischemia ,prolonged ischemia

lead to muscle necrosis and releasing of Troponin I .there was no studies express the cause of this result .

B. Vergèsa et al[20] found Nt-proBNP was negatively associated with with peak plasma troponin level (P=0.0002).

Non significant inverse relationship (r = -0.19; p=0.388) was noted between serum NT-PRO BNP concentrations and the serum CRP concentrations in patients with ACS, the possible reason for this result may be that NT-PRO BNP act as thrombolytic and vasodilator which has been reported previously that lead to decrease inflammatory response to harmful events and decrease C-Reactive protein. There was no previous studies to be rely on for comparison.

There were significant differences in mean(± SEM) value of NT-PRO BNP (P≤0.05) among 5 groups, mean(± SEM) value of NT-PRO BNP in Inferior MI > Unstable angina > Extensive MI > Anterior MI > Anteroseptal MI .

Roge´rio Bicudo Ramos et al [21] in there study included 170 patients with non-ST-elevation myocardial infarction showed that the BNP level was the only variable able to locate ischemic myocardium in the LV anterior wall in patients with NSTEMI, independent of multiple other characteristics including heart disease risk factors and history of previous cardiovascular disease.

Maria Dorobantu MD, PhD, FESC, FACC;et al [22] In their study BNP AND LV FUNCTION IN STEMI PATIENTS they have demonstrated that the early BNP measurement provides important information regarding systolic LV dysfunction in STEMI with anterior location patients undergoing revascularization.

Thus they've minimalised the factors that could bias their data. Their study provides support for the use of BNP as a screening tool for systolic LV dysfunction in anterior AMI patients at a threshold of 90pg/ml. This threshold is very similar to that established for the diagnostic of chronic heart failure (100 pg/ml) in a recent prospective study.

CONCLUSIONS

A single measurement of BNP on admission can predict early and degree of ischemi in types of Acute coronary syndrome.This may explain one of the underlying mechanisms accounting for the independent ability of the baseline BNP levels to predict events during index admission hospitalization and long-term follow-up.

REFERENCES

1. Mercedes Torres and Siamak Moayedi. Evaluation of the Acutely Dyspneic Elderly Patient. Clinics in Geriatric Medicine. 2007;23(2):307-325.
2. Ravkilde J, Horder M and Gerhardt W. Diagnostic performance and prognostic value of serum troponin T in suspected acute myocardial infarction. Scand J Clin Lab Invest. 1993;53:677.
3. Libby P, Ridker PM and Hansson GK. Inflammation in atherosclerosis: From pathophysiology to practice. J Am Coll Cardiol. 2009;54:2129.
4. Ridker PM. C-reactive protein: Eighty years from discovery to emergence as a major risk marker for cardiovascular disease. Clin Chem. 2009;55:209.
5. Jaffe AS and Van Eyk JE: Degradation of cardiac troponins: implications for clinical practice. In Morrow DA (ed): Cardiovascular Biomarkers: Pathophysiology and Disease Management. Totowa, NJ, Humana Press. 2006;161-174.
6. Kosiborod M, Inzucchi SE and Krumholz HM. Glucose normalization and outcomes in patients with acute myocardial infarction. Arch Intern Med. 2009;169:438.
7. Ward CW and Lawrence MC. Ligand-induced activation of the insulin receptor: a multi-step process involving structural changes in both the ligand and the receptor. Bioassays. 2009;31 (4):422–434.
8. Michael H Olsen, Tine W Hansen, Marina K Christensen and Finn Gustafsson. N-terminal pro-brain natriuretic peptide, but not high sensitivity C-reactive protein, improves cardiovascular risk prediction in the general population. European Heart Journal. 2007;28: 1374–1381.
9. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, Lowe GD, Pepys MB and Gudnason V. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med. 2004;350:1387-1397.
10. Kistorp C, Raymond I, Pedersen F, Gustafsson F, Faber J and Hildebrandt P. N-terminal pro-brain natriuretic peptide, C-reactive protein, and urinary albumin levels as predictors of mortality and cardiovascular events in older adults. Jama. 2005;293:1609–1616.
11. Thygesen K, Alpert JS and White HD. On behalf of the Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. Eur Heart J. 2007;28:2525–38.

12. Assessment of the Multiple-Biomarker Approach for Diagnosis of Myocardial Infarction in Patients Presenting with Symptoms Suggestive of Acute Coronary Syndrome Clinical Chemistry. 2009;55(1):93–100.
13. Maeda K, Tsutamoto T, Wada A, Hisanaga T and Kinoshita M. Plasma brain natriuretic peptide as a biochemical marker of high left ventricular end-diastolic pressure in patients with symptomatic left ventricular dysfunction. Am Heart J. 1998;135:825-32.
14. Nakagawa O, Ogawa Y, Itoh H, Suga S, Komatsu Y, Kishimoto I, Nishino K, Yoshimasa T and Nakao K. Rapid transcriptional activation and early mRNA turnover of brain natriuretic peptide in cardiocyte hypertrophy. Evidence for brain natriuretic peptide as an emergency cardiac hormone against ventricular overload. J Clin Invest. 1995;96:1280-7.
15. B-type natriuretic peptide is a long-term predictor of all-cause mortality, whereas high-sensitive C-reactive protein predicts recurrent short-term troponin T positive cardiac events in chest pain patients: a prognostic study. Trygve Brügger-Andersen, VolkerPönitz, Harry Staines, David Pritchard, Heidi Grundt and Dennis WT Nilsen.
16. SeonGyu Choi MD, Myung Ho Jeong MD, Youngkeun Ahn MD, JeongGwan Cho MD and Jung Chaee Kang MD.Relationship Between Obesity and N-Terminal Brain Natriuretic Peptide Level as a Prognostic Value After Acute Myocardial Infarction. DOI 10.4070/kcj.2010.40.11.558.
17. Watanabe I, Tani S and Washio T. Relationship between the plasma levels of brain natriuretic peptide and left ventricular ejection fraction in asymptomatic patients with previous myocardial infarction. Int Heart J. 2005;46:1007–14.
18. Talwar S, Squire IB and Downie PF. Profile of plasma N-terminal proBNP following acute myocardial infarction; correlation with left ventricular systolic dysfunction. Eur Heart J. 2000;21:1514-21.
19. Štambuk K, Bulj N, Trbušić M, Krčmar T and Lukinac L. B-type Natriuretic Peptide as Predictor of Heart Failure in Patients with Acute ST Elevation Myocardial Infarction, Single-vessel Disease, and Complete Revascularization: Follow-up Study. New Croat Med J. 2009;50(5):449–54.
20. B. Vergèsa et al, Vergès B, Zeller M, Desgres J, Dentan G, Laurent Y and Janin-Manificat L. High plasma N-terminal pro-brain natriuretic peptide level found in diabetic patients after myocardial infarction is associated with an increased risk of in-hospital mortality and cardiogenic shock. Eur Heart J. 2005;26:1734-41.
21. Roge´rioBicudo Ramos, Ce´ lia M Strunz, SolangeDesire´eAvakian, Jose Antonio Ramires and Antonio de Padua Mansur. B-type natriuretic peptide as a predictor of anterior wall location in patients with non-ST-elevation myocardial infarction. Clinics. 2011;66(3):437-441.
22. Maria Dorobantu MD, PhD, FESC, FACC; Ana-Gabriela Fruntelata MD, PhD, FESC, CBNC; AlexandruScafa-Udriste MD, PhD; Oana-FlorentinaTautu MD.B-Type Natriuretic Peptide (BNP) and Left Ventricular (LV) Function in Patients with ST-Segment Elevation Myocardial Infarction.Journal of Clinical Medicine. 2010;5(4).