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Biochemical analysis of the natriuretic peptides BNP and NT-proBNP in patients with cardiovascular disease

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Abstract : Objectives: The study was designed to assess the serum concentrations of BNP and NT-proBNP in patients with ACS and HF and compare the results that will obtain with healthy control. In addition, we chose almost equal number of males and females to control the effect of age and gender on the levels of BNP and NT-proBNP.
Design and methods: The study was comprised of (70) patients, (35) of them withACS and other (35) with HF. The study also included (22) subjects were taken as control group. The sera obtained from the blood of patients and healthy control subjects were used to measure the concentrations of natriuretic peptides (BNP and NT-proBNP) by ELISA method.
Results: Both the ACS and HF patients had significantly higher mean levels of BNP and NT-proBNP than control group, also HF patients had higher mean level of both parameters than ACS patients, in all comparison $p<0.001$. The receiver operating characteristics (ROC) curve showed that both BNP and NT-proBNP were valid in prediction of ACS and HF with high sensitivity and high specificity, the differences in validity rates between both parameters were statistically nonsignificant, ($P>0.05$). There was a statistically significant positive correlation between the level of BNP and NT-proBNP with the age, in addition, its mean levels was higher in female than male patients with ACS and HF.
Conclusion: the two parameters are good predictors, highly sensitive and highly specific and accurate in prediction of ACS and HF with relatively high accuracy in NT-proBNP than BNP. Both parameters levels are elevated with progressing age of patients, in addition, its mean levels are higher in women than men patients.
Key words : Acute coronary syndrome, heart failure, B-type natriuretic peptide, and N-terminal pro-B-type natriuretic peptide.

Introduction

Acute coronary syndrome (ACS) is a group of signs and symptoms due to reduced blood flow in the coronary arteries such that part of the heart muscle is unable to function properly or dies [1]. Three problems can cause it: STEMI, NSTEMI, or UA[2]. These kinds are named according to the outcomes of the electrocardiogram (ECG)[3]. The main symptom supporting occurrence of ACS is chest pain, predominantly extending to the left arm or angle of the jaw, pressure-like in character, and associated with sweating, breathlessness and vomiting [2].Heart failure (HF) means failing of the heart to fill with (diastolic HF) or to eject (systolic HF) blood, or both [4]. Frequently, the diagnosis is performed clinically on the basis of the presence of dyspnea, fatigue, signs of fluid overload, such as pulmonary crepitation, peripheral edema, and jugular distension[5]. Nearly 10- 20% of patients with ACS have concomitant HF, and up to 10% of ACS patients develop HF during hospitalization[6].

When the heart fails, a considerable changes take place to the heart and peripheral vascular system in response to the hemodynamic change associated with HF which included ventricular dilatation, myocyte hypertrophy, altered myosin gene expression, altered sarcoplasmic Ca ion-ATPase density, increased NP secretion, salt and water retention, peripheral vasoconstriction, and sympathetic stimulation[7].Brain natriuretic peptide (BNP) is cardiac neurohormone synthesized and released from the cardiac ventricular cells owing to increased wall tension such as volume or pressure overload. BNP has special systemic effects: vasodilatation, increase the excretion of water and sodium, inhibition of SNS and of RAAS[8]. The precursor of circulating BNP (active peptide) and NT-proBNP (inactive peptide) is a 134 amino acid pre prohormone, which yields a 108 amino acid prohormone molecule[9], a precursor molecule stored in cardiomyocyte[10]. The prohormone is released during homodynamic stress from the left and right cardiac ventricle in response to ventricular volume expansion and pressure overload. Modern data suggest that LV diastolic wall stress and wall stiffness may be the predominate triggers of BNP release[11]. Upon release, pro BNP 108 is cleaved in the circulation by way of proteolytic enzyme into two polypeptides: NT- proBNP, 76 amino acids in length, and BNP, 32 amino acids in length[12]. The inactive NT-proBNP is more stable in vitro and in vivo, with a longer plasma half-life than active BNP[13, 14]. BNP binds specifically to the NPR-A to make use of its effect in the regulation of intravascular blood volume and vascular tone. Existing proof suggests that BNP acts not only as a circulating hormone, but additionally as an autocrine and/or paracrine factor in order to exert its cardioprotective position[15].

Methods

This study was achieved at the laboratories of Biochemistry Department, Medicine College / Babylon University. The collection of samples was conducted during the period from 1st of December 2015 until 30th of April 2016. The study was comprised of (70) patients, (35) of them withACS in the age group ranging from 40-75 years (this group comprised of males 48.6 % and females 51.4%) and other (35) with HF in the age group ranging from 48-79 years (this group comprised of males 48.6% and females 51.4%). The study additionally included (22) apparently healthy persons have been taken as a control group of the age ranging from 40 - 79 years (this group comprised of men (50%) and women (50%). The age and sex of this group had been matched to age and sex of patient groups, where statistical p.value (> 0.05).

Determination of serum BNP and NT-ProBNP

The sera obtained from the blood of patients and control subjects were used to measure the concentrations of BNP and NT-proBNP. Human NT-ProBNP kit was based on standard sandwich ELISA. The kits were provided from Elabscience®/.

Statistical Analysis

SPSS- version 23 was used to perform statistical analysis. Data have been expressed as (mean ± SD) and percentages. Analysis of variance (ANOVA) and post-hoc (LSD) tests have been used to compare the mean BNP and NT-proBNP among the three studied groups. Level of significance (P.value) = 0.05 used to be regarded significant.

Results

As it shown in figures (1 and 2), the mean BNP and NT-proBNP level was significantly higher in HF group patients than ACS patients and controls.

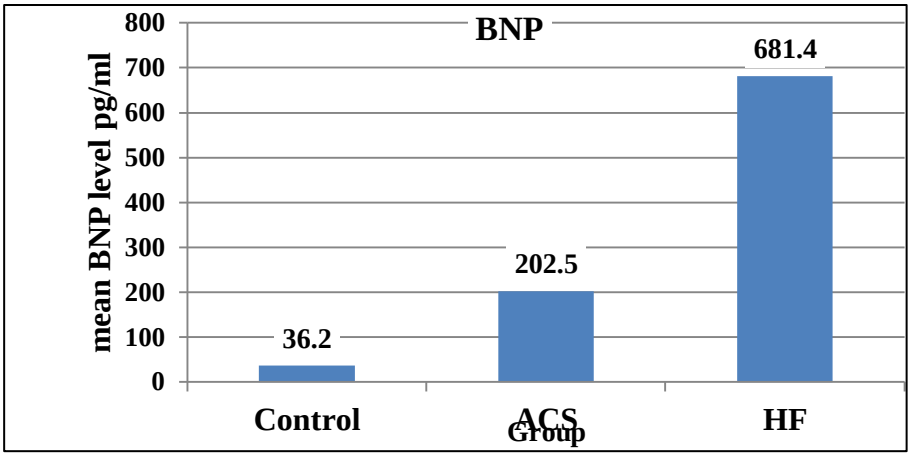

Figure (1): Comparison of mean BNP level among the studied groups

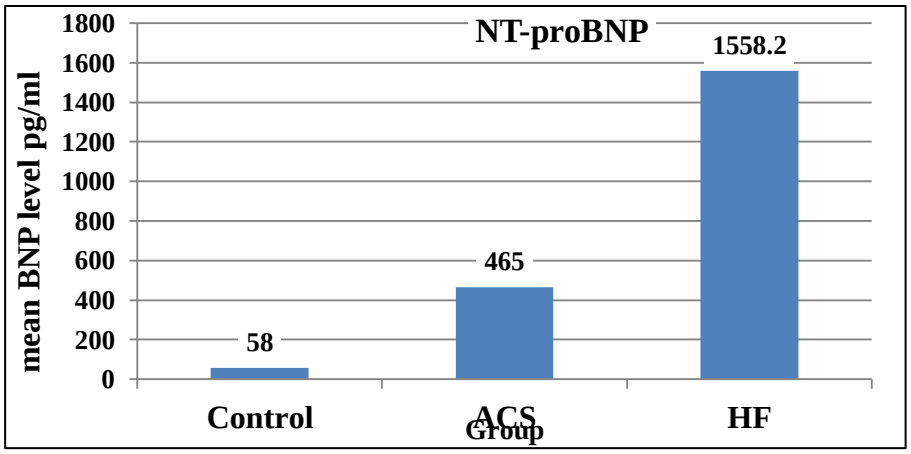

Figure (2): Comparison of mean NT-proBNP level among the studied groups.

On the other hand, the mean BNP and NT-proBNP level of ACS group was also significantly higher than that of control group, in all comparison. The differences were statistically significant, (P <0.001) as shown in table (1).

Table (1): Comparison of BNP and NT-proBNP levels among the studied groups

Post hoc analysis (LSD) for multiple comparisons between groups	
Comparison	**P. value**
BNP	
HF vs. ACS	< **0.001** Significant
HF vs. Control	< **0.001** Significant
ACS vs. Control	< **0.001** Significant
NT-proBNP	
HF vs. ACS	< **0.001** Significant
HF vs. Control	< **0.001** Significant
ACS vs. Control	< **0.001** Significant

According to the values of BNP and NT-proBNP and using the receiver operating characteristics (ROC) curve to assess the validity of BNP and NT-proBNP in prediction of ACS and HF, it had been significantly found that both parameters were valid in prediction of ACS and HF with relatively higher validity in NT-proBNP than BNP. Figure (3) indicated that both tests can predict ACS and they were excellent, accurate

and valid predictors, additionally, the validity tests of BNP and NT-proBNP in prediction of ACS; sensitivity, specificity, accuracy, positive predictive value and negative predictive value are shown in table (2), where both tests were highly sensitive and specific with high accuracy rate with relatively higher sensitivity rate in BNP and higher specificity in NT-proBNP, however, the differences in validity rates between both parameters were statistically insignificant, (P > 0.05). Similar trend was reported in validity of these two parameters in prediction of HF, it had been significantly found that both tests are good predictors, highly sensitive and highly specific and accurate in prediction of HF, figure (4), and the validity rates are shown in table (3).

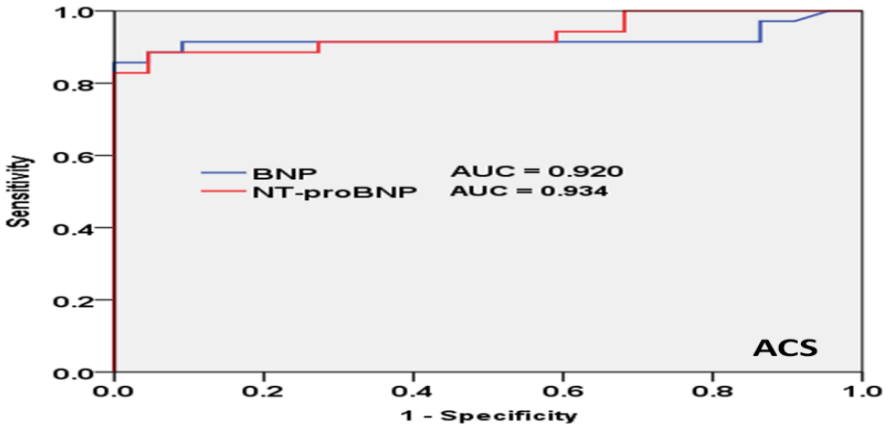

Figure (3): Receiver operating characteristic (ROC) curve for the validity of BNP and NT-proBNP in prediction of ACS.(AUC: area under the curve, ACS: acute coronary syndrome).

Table (2): Tests of Validity of BNP and NT-proBNP in prediction of ACS

	BNP	NT-proBNP
Sensitivity	90.7%	87.8%
Specificity	86.8%	91.6%
Accuracy	88.8%	89.7%
PPV	87.3%	91.3%
NPV	90.3%	88.2%
*PPV: Positive predictive value, NPV: Negative predictive value		

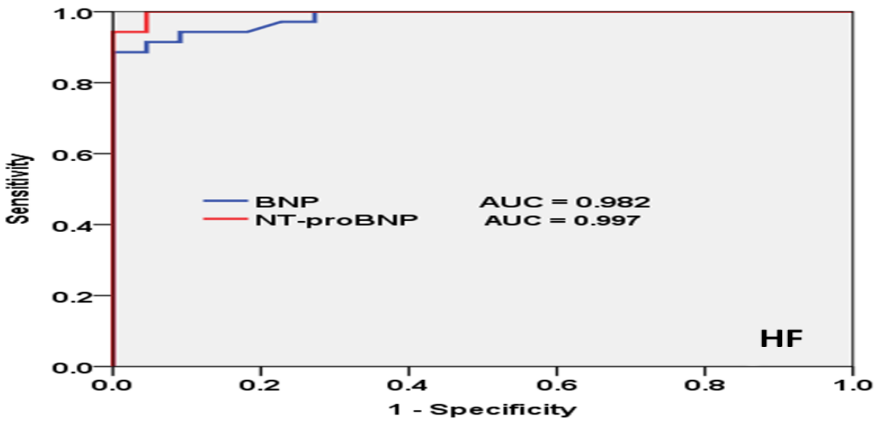

Figure (4): Receiver operating characteristic (ROC) curve for the validity of BNP and NT-proBNP in prediction of HF. (AUC: area under the curve, HF: heart failure).

Table (3): Tests of Validity of BNP and NT-proBNP in prediction of Heart failure

	BNP	NT-proBNP
Sensitivity	91.2%	94.3%
Specificity	95.1%	90.9%
Accuracy	93.2%	92.6%
PPV	94.9%	91.2%
NPV	91.5%	94.1%
*PPV: Positive predictive value, NPV: Negative predictive value		

Relationship of BNP and NT-proBNP with the age

There was a statistically significant direct correlation between the level of BNP and NT-proBNP with the age, as it shown in figure (5), the BNP levels increased directly with the advancing age in patients with ACS, (r = 0.31, P< 0.05). Similar correlation and trend were found between the age and NT-proBNP level, (r=0.56, P< 0.001), figure (6). Regarding the correlation of the two parameters with the age of patients in the HF group, it had been significantly found that both parameters, BNP and NT-proBNP levels were increased with advancing age, (r =0.63, P<0.001) and (r = 0.64, P< 0.001), for the correlation of age with BNP and NT-proBNP levels, respectively, figures (7 and 8).

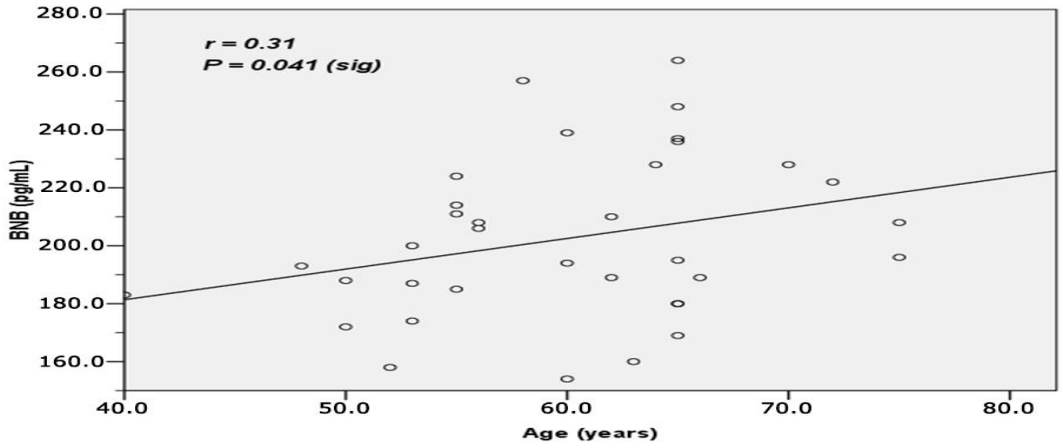

Figure (5): Correlation between BNP level and age in ACS group

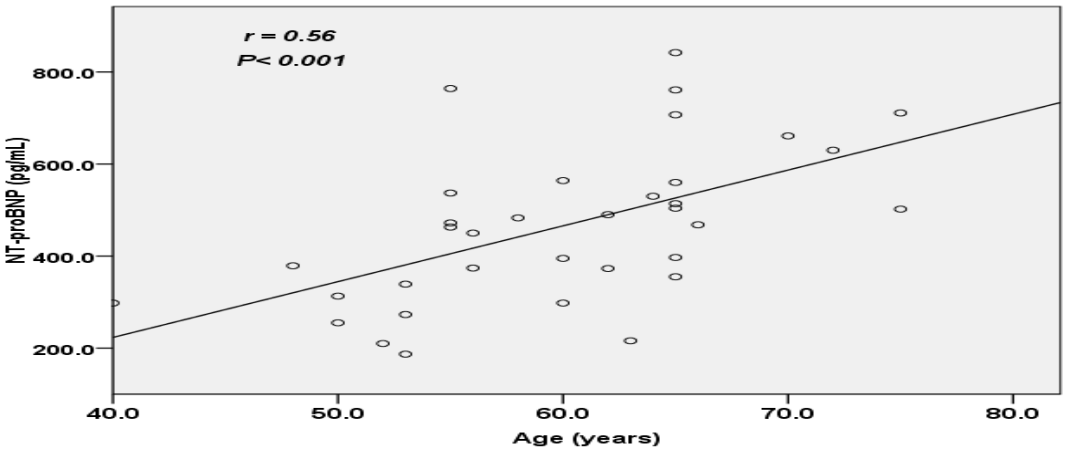

Figure (6): Correlation between NT-proBNP level and age in ACS group.

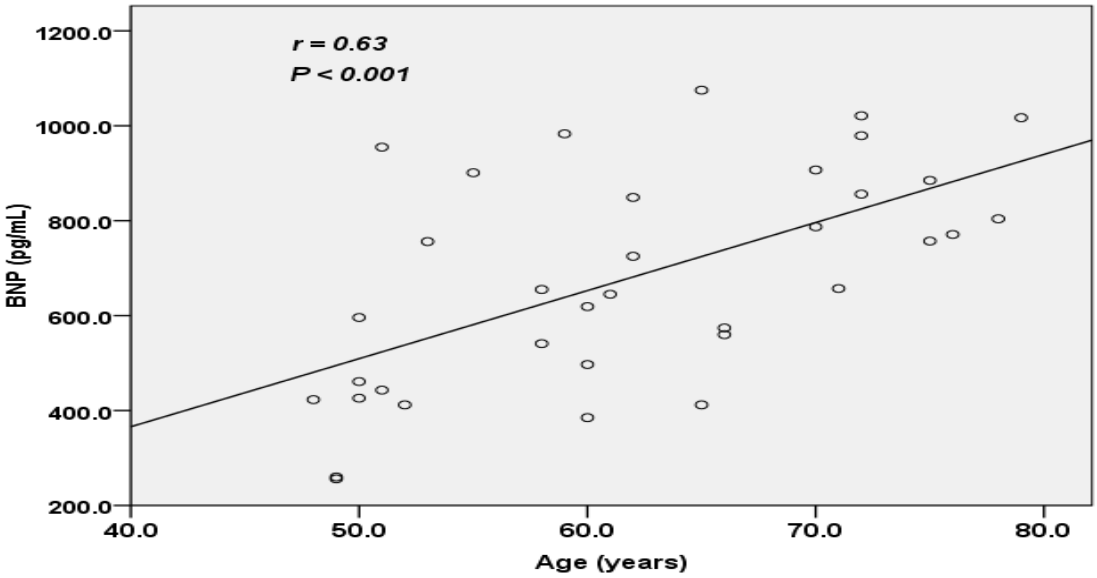

Figure (7): Correlation between BNP level and age in HF group.

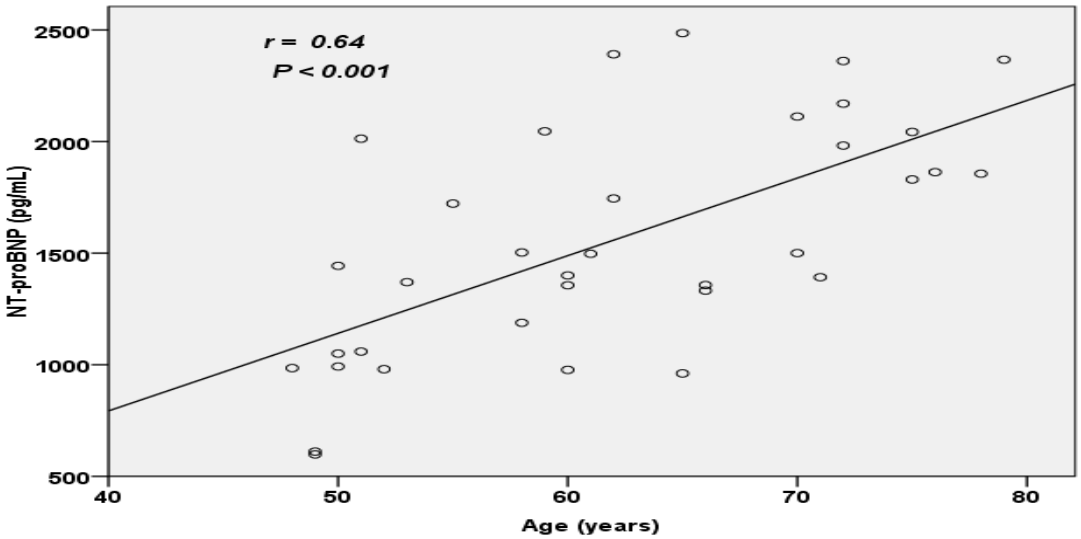

Figure (8): Correlation between NT-proBNP level and age in HF group.

Relationship of BNP and NT-proBNP with the gender:

As it shown in table (4), the mean level of BNP and NT-proBNP was significantly higher in female than male patients with ACS, (P< 0.05), while the difference in BNP level between both genders was statistically insignificant in HF group (p=0.11) and control (p=0.92). The mean level of NT-proBNP was significantly higher in female than male patients with HF (p=0.045), while insignificant in control (p=0.90).

Table (4): Association of BNP and NT-proBNP levels with gender

		Group		
		HF	**ACS**	**Control**
BNP	Male	616.6±195.7	191.1 ± 23.3	36.3± 6.1
	Female	742.7±249.3	212.3±28.8	35.9 ± 6
P. value male vs. female		0.11 ns	0.038 sig	0.92 ns
NT-proBNP	Male	1379.2±424.2	386.2±146.2	59 ± 9.7
	Female	1727.3±550.6	539.6±156.4	56.4±10.6
P. value male vs. female		0.045 sig	0.026 sig	0.90 ns

Discussion

BNP is a counter neurohormone mainly synthesized in myocardial cells. BNP is released into the blood stream due to ventricular dilatation and pressure overload, thus it reflects ventricular wall stress and tissue hypoxia rather than cell damage as such [16]. It is a well-known marker of reduced LV function and HF, and it delivers prognostic information beyond and above LVEF in ACS patients[17]. This marker of neurohormonal activation shows a vital role through the spectrum of ACS, including patients with STEMI and NSTEMI. Moreover, elevated NPs in reality label patients about ACS who are at standing threaten of extermination and HF and add information to that provided by the troponin[18]. BNP secretion is moved by myocardial stretch but is also related to structural abnormalities including fibrosis, myocardial and peripheral ischemia[19].NT-proBNP is released by cardiac myocytes in response to wall stress (myocyte injury) in conditions associated with volume overload as in HF and ischemia owing to coronary disease[20]. HF is the activation of NE has been conjectural to edict a pathophysiological work in the conduct of LV dysfunction and development of HF. In patients with HF, serum ranges of NT-proBNP are elevated due to the fact of cardiac hormonal system activation prompted by way of increased wall stretch due to raised volume expression and pressure overload. The ventricular wall stretch was attributed to reduced ventricular systolic and diastolic function[21]. Nali*et al* 2007[22] reported that NT-proBNP is synthesized in ventricular tissue in response to volume expansion and pressure overload and showed that serum NT-proBNP level was drastically multiplied in mild, average and severe HF than healthy controls. Lewandowsky*et al* 2007[23] found that serum NT-proBNP levels were raised in mild, moderate and severe IHF compared to healthy controls .Sedlak*et al* 2008 [24] found that serum NT-proBNP levels amplify appreciably with each growing stage of disease, there is a wider vary of NT-proBNP levels in NYHA classes III and IV. Arnhof-Hermann *et al* 2005[25] found significant relationship between serum NT-proBNP and cardiac function in severity stages of IHF. The authors also reported that severe IHF group had significantly higher serum NT-proBNP level than mild and moderate groups ($p<0.0001$). kallistratos *et al* 2008 [26] showed that serum NT-proBNP levels also correlated strongly with severity of IHF (mild, moderate and sever). Pfister*et al* 2004[27] demonstrated that NT-proBNP levels correlate well with clinical severity of CHF expressed by the stages of severity of HF and are directly related to filling pressure and LV function. Also Hammerer-Lercher*et al* 2004[28] and Richards et al 2004[29] showed a statistical relationship between NT-proBNP concentration and severity of IHF. Luchner*et al* 2005[30] demonstrate that NT-proBNP is secreted during NE stimulation of the heart. Because of their close correlation with the severity of symptoms, they have been developed as markers of HF[30]. Friese *et al*2007[31]recorded the serum NT-proBNP levels were significantly increased in systolic and diastolic IHF in comparison with healthy controls ($p<0.001$). Sahu*et al* 2010[32] showed that the serum of NT-proBNP levels have been notably elevated in patients with CVD than healthy controls ($p<0.05$). Kragelund *et al* 2006[33] suggested that the main stimulus for NT-proBNP is believed to be local or complete impairment of LV systolic or diastolic function leading to extended LV wall stretch. However, emerging evidence suggests that extra mechanisms, along with MI, might also make contributions to the release of NPs. Seino et al 2004[34] reported the relationship of LV ischemic area with serum NT-proBNP level in patients with ischemia, they only found significant correlation with systolic IHF (LVEF<50%), ($p<0.05$). These

findings indicate the origin for the elevation of serum NT-proBNP levels in IHF especially after MI. Sabtine*et al* 2002[35] suggested that myocardial ischemia may increase regional wall stretches which stimulate NT-proBNP secretion. Mueller *et al* 2004[36] reported that the increase in NT-proBNP in patients with AMI have a higher discriminative value for early cardiac dysfunction, suggesting that it can also additionally be a greater sensitive marker of decreased lv function. Homocysteine may consider as an independent risk factor for ischemic stroke and myocardial infarction[37]. DD genotype and D allele of ACE gene can be considered as an independent risk factors for ischemic stroke[38].Many studies were showed that ROS are a known to cause oxidative nucleobase modifications in DNA which may lead to carcinogenesis[39].

Serum NT-proBNP level prepares precious predictive facts on short- and long-term mortality in AMI patients[40]. Hall *et al* 2004[41] showed that NT-proBNP syntheses are increased in the infarcted and non-infarcted tissue. It has been suggested that the amount of increase in NT-proBNP after MI reflects not only size of the necrosis but also the extent of ischemia [42]. Krishnaswamy *et al* 2001[43]showed that serum of NT-proBNP concentration used to be considerably increased in sufferers with systolic IHF and diastolic IHF when compared to the healthy individuals. However, some studies showed serum NPs to be normal unless systolic dysfunction develops[44]. Uusimaa *et al* 1999 [45]reported that serum NT-proBNP levels are markedly increased in sufferers with AMI at the hospital set. Sumide *et al* 1995[46]reported the magnitude of the elevation of serum NT-proBNP is related to the size of the infarction, the severity of global LV dysfunction or both. Nielson *et al* 2000[47] considered the probability of finding LV systolic dysfunction based on the combined use of diastolic blood pressure and NT-proBNP concentration without echocardiography. This protocol used to be accurate adequate to note patients who ought to be referred to echocardiography[48]. Costello *et al* 2006[49] reported that NT-proBNP correlated well with ventricular size and systolic function. They also validated a connection between NT-proBNP and left atrial size, which would be expected to be multiplied in sufferers with LV dysfunction. Following ACS, The BNP and NT-proBNP are related with the measurement of LV dysfunction however nonetheless supply prognostic statistics independently of LVEF[50].McKie*et al* 2011[51] reported that the risk of mortality and cardiovascular morbidity develops progressively in those with elevated NT-proBNP values. This key observation strongly submits that mild alterations in myocardial function identified by minimal NT-proBNP elevation. Burnett *et al* 1986[52] suggested that measurement of plasma BNP degree in early section of MI may additionally be beneficial as a non-invasive approach for identification of individuals at greater hazard of problems and mortality after MI. In the first hours of AMI, BNP is released because of ischemia and necrosis of myocardial cells. After wards, BNP rises due to the fact of reduced systolic and diastolic function and increased wall stress of the left ventricle[53]. Numerous evidences supporting that variation in plasma BNP level during AMI makes as a prognostic factor. Moreover, a new study has shown that plasma BNP level, measured in ACS, independently might predict mortality rate, HF and degree of expansion of MI[54]. Plasma NT-proBNP displays the measurement of the myocardial necrosis and the extent of ischemic insult. Indeed, plasma NT-proBNP is increased in sufferers with ACS, even in the absence of necrosis [55].TrygveBrugger-Andersen et al[56] reported that the main findings of their study in a group of unselected patients admitted to the ED with chest pain and potential ACS indicate that BNP is a vital and impartial predictive biomarker for mortality. However, few research have examined the predictive value of NPs throughout the spectrum of ACS in blood samples received directly on admission before introducing any variety of therapy[57]. There might also be several attainable explanations for the apparently more strong prognostic value of the NPs, as they share multiple different pathophysiological signals within an individual, including ventricular dysfunction [58], accelerated intra cardiac filling pressures [56], and valvular disease [60]. The finding of a quickly rise in the NPs obtained immediately after offering to the ED with an ACS might be favorably influenced by immediately or early interventions [61]. The accurate mechanisms of NPs rise in coronary disease are not wholly understood. Ischemia may create an independent stimulus for BNP release towards a transient reduction of systolic function and compliance, reflecting not only the impairment in LV function, but also the severity of the ischemic harm[62]. Otherwise, BNP secretion may be owing to the increased local wall stretch that takes place during ischemic attacks even in the absence of pump dysfunction inducing the neurohormonal activation[63]. All together, these records should explain the mechanisms relating BNP to an adverse outcome in CAD; it represents a marker of coronary disease severity and is associated to the existence of plaques diffusion and narrowing. For the above stated reasons BNP need to be measured as an indicator of regional ischemia and as a predictor for adverse activities in sufferers with chest pain[64]. Manola *et al* 2009[65] studied the BNP value in predicting LV systolic dysfunction in sufferers with STEMI and ordinary LV systolic characteristic at admission and successful reperfusion. BNP levels had been measured at three exclusive time points (admission, 1 day, and 7 days), they concluded that BNP can be used as a predictor of decreased systolic

function in sufferers with STEMI who underwent successful reperfusion and had regular ejection fraction at admission. Elevated BNP after AMI identifies sufferers at danger of adverse LV remodeling, persistent LV dysfunction and CHF[66]. Gardner et al 2003[67] suggest NT-proBNP as a new gold standard in predicting mortality in patients with advanced HF, more powerful than LVEF echocardiographic parameters. These increasing data make reasonable to use NT-proBNP as a reference parameter in clinical studies of HF patients [68].

BNP is a newly biochemical marker in patients with CAD. BNP is capable to predict systolic dysfunction, adding new prognostic facts to present classical markers. Oppositely, it is not known if there is a relation between the extent of BNP levels and the severity of CAD[69,70].

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