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RESEARCH ARTICLE

The significance of H-FABP and BNP levels in diagnosis of acute myocardial infarction.

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Abstract

Early interventions aimed at restoring coronary blood flow in patients with acute myocardial infarction (AMI) reduces myocardial damage and improves patient outcome. Yet, a timely diagnosis can be a diagnostic challenge for the clinician. We aimed to compare the potential diagnostic efficacy of Brain-type natriuretic peptide (BNP) and heart-type fatty acid binding protein (H-FABP) with other cardiac-markers to examine their usefulness in early confirmatory markers of acute myocardial infarction. Eighty subjects were recruited in this study, including 40 AMI patients, 40 patients suffering from non-cardiac chest pain as well as 10 healthy subjects. Receiver Operator Characteristic Curve (ROC) analysis showed H-FABP to be a good discriminator between patients with AMI and patients without AMI. Both H-FABP and BNP were found to be more sensitive than troponin (89%, 79.5% *vs.*61.3%), in the same line, H-FABP and BNP showed higher specificity when compared to troponin (89.9%, 82.4%, *vs.* 61.5%) in diagnosing of AMI.

In conclusion; H-FABP is a promising discriminator biomarker for the early detection of patients with acute myocardial infarction admitted within 3-6 hours from the onset of chest pain either alone or in combination with troponin.

Introduction

Biomarkers are helpful tests for making a diagnosis. They are usually the golden standard for their respective diseases. Cardiology, especially, uses biomarkers extensively. Because a great margin of cardiologic emergencies present with a risk of mortality; swift diagnosis and treatment is essential. Biomarkers are helpful in the manner that they shorten diagnosis time (**Yalcinkaya and Oztas 2015**). Acute myocardial infarction (AMI) is one of the most frequent cardiological emergencies. When it is taken into account that AMI presents with vital risk and that the outcome is dependent on swift diagnosis and treatment speed; it is obvious that it is of utmost importance to have a biomarker that defines the diagnosis with certainty (**Sandroni et al 2004**).

Diagnosis of an AMI in the past, during the early 1990s, utilized the World Health Organization (WHO) criteria defining MI as the presence of two out of three characteristics: symptoms of acute ischemia (chest pain), ST segment elevation on electrocardiography (ECG), and increase in traditional enzyme activities in serum, total creatine kinase (CK), creatine kinase MB (CK-MB), Aspartate transaminase (AST) and lactate dehydrogenase (LDH) (**Madias**, **1995**).

Inappropriately long patient time delay is the main cause for undesirable pre-hospitalization delay. General awareness of basic symptomatology and the importance of time factor for further curse of the disease may substantially influence the duration of AMI prehospitalization phase. Therefore, prompt diagnosis of all patients with AMI is an elusive goal (**Bredero et al 2008**).

Heart-type fatty acid binding protein (H-FABP) is a low molecular weight (14.5 kDa) protein, which contains 132 amino acid residues. Fatty acid-binding proteins bind the long-chained fatty acids reversibly and non-covalently, facilitating the intracellular cytoplasmic transport of the fatty acids, and are highly expressed in tissues with active fatty acid metabolism such as the heart muscle (**Valle et al 2008**).

Brain natriuretic peptide (BNP) is produced from heart muscle cells, mainly in the left ventricular myocardium but also in the atrial myocardium, as a pro-hormone and released into the cardiovascular system in response to ventricular dilation and pressure overload. Regulation of BNP is at the level of gene expression; there is no storage of BNP in cardiomyocytes. BNP exhibits several physiologic functions including vasodilation, promotion of natriuresis and diuresis, inhibition of the sympathetic nervous system and several hormone systems such as the renin-angiotensin aldosterone system, as well as inhibitory and beneficial effects on the physiological mechanisms associated with the cardiovascular system (**Roberts et al 2015**).

The study was designed to investigate the performance of HFABP and BNP in detecting AMI. Also, to compare the diagnostic efficiency of the targeted parameters with the routine benchmarks in the early phase of MI onset.

Subjects and Methods:-

Subjects:-

This study was conducted on 80 patients with manifestations suggestive of acute myocardial infarction admitted to the cardiology clinics; cardiovascular hospital, Ain shams university during the period from December, 2014 to July, 2015.The protocol was conducted in accordance with guidelines approved by local research ethics committee that confirms to the ethical guidelines of the 1975 Declaration of Helsinki. All subjects in this study were matched in regard to sex and age and an informed written consent was obtained before enrollment.
Patients with liver and kidney disorders, brain ischemia and tumor were excluded from the study.

Protocol of the study:-

An expert panel consisting of two cardiologists established the final diagnosis in each patient. The patients were assorted into two groups each included 40 subjects. In all patients, a venous blood sample was collected between 3 and 6 h after onset of complaints, for measurement of cardiac biomarkers. Also, we obtained a twelve-lead ECG in every patient. The patients with ST segment elevation on ECG and elevated cardiac biomarker levels were taken into ***Group 1*** (the patients with AMI). The patients with non-specific ECG changes and negative cardiac biomarker levels were taken into ***Group 2*** non cardiac chest pain (NCCP).

Biochemical analyses:-

Routine biochemical parameters; AST, LDH, Total CK, and CK MB were measured using Beckman Coulter kits, with an Olympus AU400 auto-analyzer. The serum levels of troponin were evaluated according to instruction included in ELISA kits (Abcam, USA). The serum concentrations of BNP and H-FABP were determined using a recently developed ELISA kits, Oxis Research Inc, USA.

Data presentation and statistical analysis:-

Statistical evaluations were performed using SPSS software 17 (PASW Statistics for Windows, Version 17.0. Chicago: SPSS Inc.). Data are expressed as mean ±S.D. Significance among groups were analyzed using one way analysis of variance (ANOVA). Operating characteristic (ROC) curves were plotted for each marker to examine its performance as a marker for AMI. With the use of each cardiac marker cutoff point, sensitivities and specificities were calculated.

Results and Discussion:-

Acute myocardial infarction (AMI) is the main cause of mortality and disability in the modern world. Early and correct diagnosis is of great importance to enable immediate and intensified treatment which consequently reduces mortality (**Chen et al., 2004**). If treatment of AMI is done within 1 h (the golden hour) effectively, mortality can be reduced from 9% to 3%, if delay of 3–4 h, mortality could be five times higher (**Alhashemi, 2006**). However, the golden moment for optimal outcome of coronary revascularization is within 4 h after the onset of occlusive coronary thrombosis so early diagnosis is important for cardiac etiology in patients presenting with acute chest pain (**Gravning and Kjekshus, 2008**). Unfortunately, at least one-fifth of AMI is clinically unrecognized because of the absence of chest discomfort or atypical ECG changes (**Li et al., 2010; Figiel et al., 2008**). Early diagnosis of AMI

has great clinical significance, because the duration of symptoms has a strong impact on the hospital management strategy (**Bruins et al., 2008**).

The baseline demographic and clinical characteristics of the subjects in the study groups are summarized in **Table** 1.

Table 1: The demographic and clinical characteristics of the study groups:

Characteristics	Healthy subjects (25)	NCCP (40)	AMI (40)
Gender:			
Male	15 (60%)	22 (55%)	25 (52.5%)
Female	10 (40%)	18 (45%)	15 (37.5%)
Age (years):	50.6±13.2	51.9±11.8	54.7±8.3
Cigarette smoking:			
Non smoker	12(48%)	18(45%)	15(37.5%)
Past smoker	4(16%)	10(25%)	5(12.5%)
Current smoker	9(36%)	12(30%)	20(50%)
Family history:			
Yes	18(72%)	20(50%)	22(55%)
No	7(28%)	20(50%)	18(45%)
Hypertension:			
Present	0(0%)	10(25%)	22(55%)
Absent	25(100%)	30(70%)	18(45%)
Systolic blood pressure (mmHg)	119±8.3	129±5.1	145.8±12.5
Diastolic blood pressure (mmHg)	77±4.2	89±2.3	95.1±10.8
Hypertension medication:			
ACEI		2(20%)	5(22.7%)
β Blocker		0(0%)	9(40.9%)
Ca channel blocker		5(50%)	2(9.1%)
Diuretics		1(10%)	1(4.6%)
Combination		2(20%)	5(22.7%)
Rhythmicity:			
Present			32(80%)
Absent			8(20%)
Rhythmicity medication:			
Yes			25(78.1%)
No			7(21.9%)

In our study, the sensitivity of tested cardiac markers; AST, LDH, CK, CK-MB and troponin; at hospital admission within 4:6 hours after the onset of chest pain were low compared with previous reports (**Ruzgar et al 2006, Sayre et al 1998 and Zarich et al., 2001**). This difference might be due to differences not only in cutoff level, but also in the characteristics of study subjects that make the sensitivities of these markers were inevitably low.

H-FABP is a small cytosolic protein (14 to 15 kDa) that is present in abundance in both skeletal and cardiac muscle. Because of its small size, H-FABP is released quickly into the circulation when membrane integrity is compromised in response to cardiac ischemia (**McCann et al 2008**). Following myocardial injury, H-FABP is released into the blood stream by damaged myocytes and is rapidly cleared from the blood by renal filtration (**Schaap et al., 1999**).

In the present study, H-FABP levels appeared to be significantly higher in AMI patients than those in NCCP patients and healthy subjects. Our results are in line with those of **Hayashida et al (2000)** who have demonstrated plasma H-FABP levels may be an early and sensitive biochemical marker for the diagnosis of myocardial injury in AMI patients.

BNP is a cardiac hormone, released from the cardiac ventricles in response to increased myocardial stretch or wall tension (**McCullough et al., 2003**). When ventricular myocytes secrete pro-BNP_{108}, furin, corin or other currently

unknown proteases are thought to cleave pro-BNP_{108} to amino terminal pro-$BNP_{1–76}$ (NT-pro-BNP_{-76}) and pro-$BNP_{77–108}$ (BNP-32) (**Daniels and Maisel, 2007**)

Our results showed that BNP levels were significantly higher in AMI patients when compared to those in NCCP patients and normal reference control. Suggesting that processing of this peptide is compromised as the disease progresses. The increased BNP levels in AMI may be explained in part by the increased production and secretion of BNP from the ventricle.

Indeed, **Morita et al. (1993)** and **Richards et al. (1999)** by studying patients in AMI found out also significantly higher BNP plasma levels in comparison with healthy controls.

BNP and HFABP levels were found to be significantly higher in MI patients than those in non-cardiac chest pain (NCCP) patients and those in healthy subjects. Furthermore, the plasma levels of BNP, HFABP and other biochemical parameters in the different study groups are summarized in **Table 2**.

Table 2: The levels of different biochemical parameters of the study groups (mean ± SD):

Biomarker	Healthy subjects	NCCP	AMI	*P value*
T.C. (mg%)	149.4 ± 22.1 (114 – 179)	146.7 ± 39.1 (106 - 218)	224 ± 49.3 (133 - 364)	0.754 ϒ 0.05 Ϋ*
LDL- C (mg%)	98.6 ± 29.4 (71 - 138)	127.3 ± 19.6 (114.8 - 183.4)	154.1 ± 20.9 (125 - 214)	0.151 ϒ 0.01 Ϋ*
HDL-C (mg%)	41.1 ± 8.6 (35 - 56)	47.8 ± 10.3 (39 - 64)	37.7 ± 9.1 (15 - 49)	0.699 ϒ 0.05 Ϋ*
TG (mg%)	139.8 ± 59.7 (99 - 258)	191.7 ± 55.3 (89 - 264)	264.7 ± 77.1 (126 - 396)	0.446 ϒ 0.05 Ϋ*
AST (IU/L)	25.80 ± 7.65 (17.58 - 34.02)	29.3 ± 6.0 (20.1 - 43.1)	228.60 ± 35.33 (180.2 - 276.9)	0.704 ϒ 0.001 Ϋ*
CK (IU/L)	57.82 ± 14.18 (47.16 – 20.74)	64.30 ±19.16 (50.43 - 78.17)	1902.3 ± 91.21 (1476.3 - 2329.2)	0.526 ϒ 0.001 Ϋ*
CK-MB (IU/L)	16.90 ± 4.76 (14.67 - 19.13)	17.4 ± 6.1 (15.3 - 44.2)	258.45 ± 72.73 (177.61- 339.29)	0.151 ϒ 0.001 Ϋ*
LDH (IU/L)	335.0 ± 29.47 (290.5 - 400.5)	383.15 ± 32.85 (230.65 - 535.65)	1382.5 ± 178.1 (971.5 - 1793.18)	0.145 ϒ 0.001 Ϋ*
Troponin (ng/ml)	0.49 ± 0.21 (0.21 - 0.89)	0.45 ± 0.14 (0.15 - 0.75)	41.95 ± 12.54 (36.08 - 47.82)	0.466 ϒ 0.001 Ϋ*
BNP (pg/ml)	85.72 ± 25.71 (56.5 - 96.9)	87.50 ± 29.39 (64.47 - 106.53)	339.20 ± 91.69 (139.82- 818.58)	0.381 ϒ 0.001 Ϋ*
H-FABP (pg/ml)	5.73 ± 2.45 (2.44 - 11.91)	5.94 ± 1.72 (5.13 - 6.74)	103.10 ± 34.22 (82.87 - 123.33)	0.479 ϒ 0.001 Ϋ*

NCCP, non-cardiac chest pain; AMI, acute myocardial infarction; TC, total cholesterol; HDL-C, LDL-C, low-density lipoprotein cholesterol; high-density lipoprotein cholesterol; TG, triglycerides; AST, aspartate transaminase; CK, creatine kinase LDH, lactate dehydrogenase; ϒ healthy subjects *vs.* NCCP; Ϋ NCCP *vs.* AMI and ***** indicates a statistically significant difference.

In patients suffering from myocardial infarction, a positive correlation was found for HFABP level compared with LDH level. Furthermore, there was a significant positive correlation between LDH and troponin levels in AMI patients. Also, AST level was positively correlated with CKMB level in the same group.

Prior reports have shown >80% sensitivity of H-FABP within the period of 30–210 min for diagnosis of AMI. One study by **Glatz et al (1992)** has further demonstrated increased area under the ROC for H-FABP (0.945) than other cardiac markers in patients admitted after 3–6 h after symptom onset. Our results were in complete agreement with the above studies demonstrating an area under the curve of 0.986 with 89% sensitivity and 89.9% specificity. These results indicate the fact that H-FABP is well suited for early detection of AMI.

ROC analysis was done to demonstrate BNP and HFABP levels as an individual risk determinant in patients with AMI. Receiver operating characteristic curve analysis showed that HFABP is superior good test to differentiate between patients with non-cardiac chest pain, AMI participants and healthy subjects, with an area under the curve of only 0.986 (**Figure** 1). From the ROC analysis, the optimum cut-off value above which BNP and HFABP can be considered positive was found to be 217.7 pg/ml and 85.3 pg/ml respectively.

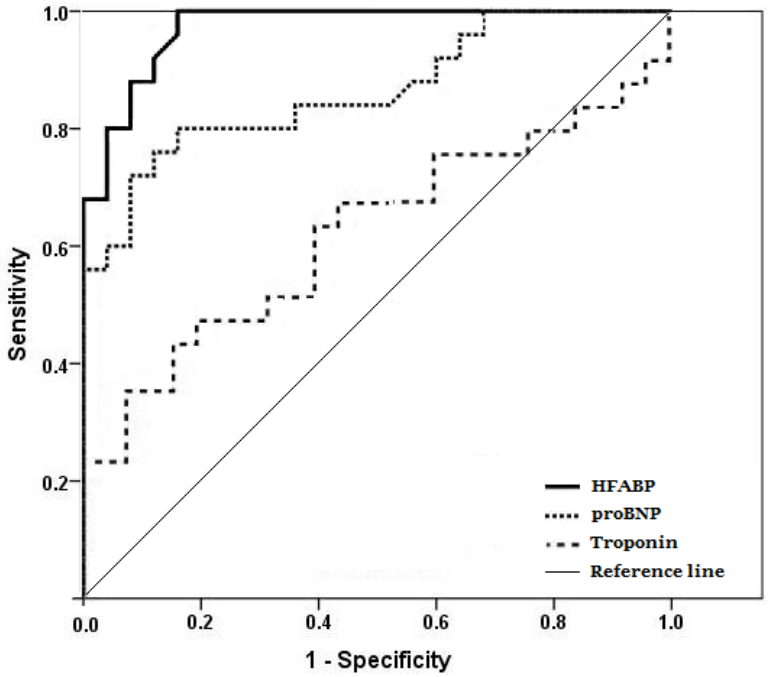

Figure 1: Receiver operating characteristic (ROC) curves display of H-FABP, BNP and troponin for AMI patients.

The current report also emphasises the fact that increased H-FABP levels seemed to be an important tool for differentiating patients with AMI from non-cardiac chest pain. As suggested by **Wu (1998)**, the optimal set of biochemical markers for assessment of AMI may be H-FABP together with troponin. Fortunately, the combination of troponin with H-FABP statistically improves their sensitivities in patients admitted within 3-6 hours from the onset.

The sensitivity, specificity and accuracy of AMI diagnosis in patients participated in the study by HFABP level were significantly higher than that by BNP and troponin. The comparative analysis of sensitivity, specificity, area under the curve, positive predictive value (PPV), negative predictive value (NPV) and accuracy of troponin, BNP and HFABP are shown in **table 3**.

Table 3: cut-off values, sensitivity, specificity, positive predictive value, negative predictive value and area under the curve of Troponin, BNP and H-FABP for predicting AMI.

Parameter	Cut-off value	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
Troponin	39.9 ng/ml	0.639	61.3	61.5	62.5	66.3	63.2
BNP	217.7 pg/ml	0.845	79.5	82.4	85.4	87.1	84.6
H-FABP	85.3 pg/ml	0.986	89.0	89.9	90.5	95.7	92.4
HFABP and Troponin		0.992	92.5	90.7	93.4	97.1	95.5

In conclusion, to the best of our knowledge, the H-FABP rapid test does provide additional diagnostic certainty in suspected AMI patients in primary care when added to general patient and symptom characteristics. The need

remains for more alternative biomarkers for the detection of myocardial infarction. It is possible that H-FABP could fill that gap.

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