Original Article

The role of human heart-type Fatty Acid Binding Protein in the early detection of myocardial injury in Acute Coronary Syndrome

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Abstract

Objectives: To evaluate the use of H-FABP as a novel marker in early detection of cardiac damage (MI) in patients presenting with symptoms of the acute coronary syndrome (ACS).

Methods: This cross-sectional study included 250 subjects; 125 patients of ACS presenting within four hours of the onset of chest pain and/dyspnea and 125 age and sex-matched healthy controls. An initial blood sample was taken from patients at presentation. Blood samples of healthy control subjects were also taken. The blood samples of both groups were centrifuged and stored at -200 C for H-FABP analysis. All the patients and control subjects were thoroughly examined and detailed history was taken. The diagnostic test was troponin-T.H-FABP concentrations of all samples were measured by ELISA-kit. The results were analyzed statistically. A p-value≤0.05 was considered statistically significant.

Results: In 125 patients of ACS, H-FABP showed a sensitivity of 85% and specificity of 83.3% for acute MI diagnosis at a cut-off level of 16 ng/ml. Positive predictive values and negative predictive values were 96.8% and 48.4% respectively. The accuracy was 84.4%and the area under the ROC curve was 0.842.

Conclusion: Evaluation of heart-type fatty acid-binding Protein (H-FABP) within four hours of onset ACS symptoms may be a valuable tool in the diagnosis of AMI.

Keywords: Heart-type fatty acid-binding protein, Acute coronary syndrome, Myocardial infarction.

Introduction

Cardiovascular disease (CVD) has emerged as a serious health issue and according to recent statistics, it almost contributes to one in every three deaths globally and this number will surely rise in both under-developed and developed countries.[1] The inhabitants of Indo-Asian origin have a huge burden of coronary artery disease (CAD) which is the leading cause of death in the Indo-Pakistan subcontinent.[2] The incidence of ischemic heart disease (IHD) is increasing in Pakistan and one in every four individuals with age ≥ 40 years may be having underlying coronary artery disease in urban areas of Pakistan.[3] Inflammation is increased in case of risk factors and their reduction, elimination or treatment may result in slowing inflammation and decreasing the threat of MI.[4]

ACS refers to a group of clinical diseases as a result of acute myocardial ischemia and includes unstable angina (UA), non-ST-segment elevation myocardial infarction (NSTEMI), and ST-segment elevation myocardial infarction (STEMI).[5] The annual incidence of NSTEMI has increased compared to STEMI as it is difficult to diagnose and hence to manage effectively.[6] Also, it has been seen that a 12-lead ECG has low sensitivity and specificity for the diagnosis of coronary artery ischemia and has shown a range of accuracy from 58.5% to 62% in identifying ACS. [7] In such clinical situations cardiac biomarkers play a critical role for diagnosis, risk assessment and disease management Currently, cardiac troponins (T or I) are the preferred biomarkers to establish the diagnosis of acute MI.[8]

But a major limitation of this current protocol is that troponin levels do not raise to a detectable level until six hours after the start of symptoms.[9] It has also been observed that patients with NSTEMI at high risk will benefit from earlier revascularization if it is done within four hours of the onset of occluding coronary thrombus but unfortunately, troponins do not confirm NSTEMI due to their low sensitivity within initial four hours.[10] There has been a great interest in seeking alternative or additional markers which could detect cardiac damage earlier.[11] Heart-type fatty acid-binding protein (H-FABP) may have the potential to fulfill the requirement.[12] After the damage to cardiomyocytes, it is quickly released in the blood from injured myocardium, detectable within 20 minutes of myocardial injury, reaches to the maximum level in three-four hours and comes to basal level within twenty-four hours due to rapid renal excretion.[13]

H-FABP is a low molecular weight membrane-bound protein present in high concentration in heart tissues and carries out transportation of insoluble long-chain fatty acids (LCFA) from the blood into the cardiomyocytes for energy. Due to cardiac cell injury, it appears in circulation even earlier than troponin.[14] Due to its rapid release and early detection in the blood there has been a great interest in its utility as an early indicator of MI.[15] It been found that H-FABP can detect early cardiac damage within three hours of symptoms onset and can rule out non-ischemic acute chest pain.[16]

The major issue of H-FABP availability to use it in routine testing is that its assay has not been standardized and the validity of effectiveness of this assay has not been established so far.[17]

Materials and Methods

This analytical cross-sectional study was carried out at the emergency department (ED) of Punjab Institute of Cardiology (PIC) Lahore from November 2014 to January 2015. The study was approved by the ethical committee of the Postgraduate Medical Institute Lahore and was conducted after approval of the head of institute PIC Lahore.

The sample size was calculated using the formula $n=Z^2_{1-\alpha/2} \times S_N \times (1-S_N)/ L^2 \times$prevalence.

The calculated sample size was 492 (246 patients and 246 healthy control subjects) but due to limited resources, the sample size was reduced and we finally included 250 subjects of both sexes. Among 250 subjects, 125 were patients showing symptoms of ACS presenting to the ED with the final diagnosis of ACS established by the expert panel of doctors and 125 were healthy age and sex-matched blood donors as controls.

The inclusion criteria for patients were Age ≥ 40 years with typical chest pain and/or dyspnea suggestive of ischemic origin within 4hours of onset and had the final diagnosis of ACS. The exclusion criteria were patients who had undergone an intervention or received fibrinolytic therapy at an early stage and those with serum creatinine level ≥1.3 mg/dl

The control group had no history or clinical findings of cardiac or other systemic diseases. Informed written consent was obtained from all the participants. The values of cardiac biomarkers (Troponin-T, CK-MB, and H-FABP), creatinine and other routine investigations were also documented in the Performa.

At a presentation in ED, three ml of the blood was drawn from each patient and stored for H-FABP estimation as per the recommendation of the manufacturer. Blood for routine investigation was also taken. Another blood sample was drawn from each patient at eight hours after the presentation in ED for the estimation of the Troponin T level. General physical and systemic examination and complete history was taken from all participants. Blood samples for H-FABP and routine investigations were also obtained from the control group.

Heart-type fatty acid-binding protein was measured by using human heart-type fatty acid-binding protein (H-FABP) ELISA kit manufactured by Glory Biosciences USA for the quantitative determination of H-FABP with a detection range of 0.4-22ng/ml.

The serum samples were stored at -20^{0}C until the quantitative determination of H-FABP. Samples were analyzed in batches with three levels of controls in each batch and before analysis, samples were thawed, centrifuged and the supernatant was used for analysis. Repeated freeze-thaw cycles were avoided. The samples were analyzed according to the manufacturer's instructions.

Data were analyzed statistically by SPSS software, version 18. Quantitative variables were presented as mean and standard deviation SD. Qualitative variables were presented as frequency and percentages. For the comparison of quantitative variables between cases and controls independent samples t-test was applied while for the comparison of qualitative variables chi-square test was applied. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for H-FABP using Trop-T as the gold standard. ROC curve was also drawn and AUC was calculated. A p-value ≤ *0.05* was considered significant.

Results

Two hundred and fifty subjects were included in the study. One hundred and twenty-five patients of ACS were based on clinical features (±ECG changes) and cardiac troponin T. One hundred and twenty-five healthy subjects who served as a control group were also enrolled in the study.

The average age of cases was 54.72 years (SD=7.472) while the average age of controls was 53 years (SD=7.381). The difference in the age of cases and controls was insignificant (p=0.068).

Among cases, there were 99 (79.2%) males and 26 (20.8%) females. Among controls, there were 87 (69.6%) males and 38(30.4%) females. There was an insignificant difference in gender between cases and controls (p=0.082). Both groups were similar with respect to age and gender distribution.

Among cases, the average time duration of symptoms was 2.584 hours (SD=0.839) with a minimum duration of 1 hour and a maximum duration of 4 hours. Among cases, the final diagnosis was STEMI in 73(58.4%), NSTEMI in 34(27.2%) and UA in 18(14.4%) cases based on Trop-T. Median H-FABP level in cases was 25.68ng/ml (IQR: 17.78-35.78) with a minimum level of 5.62ng/ml and a maximum level of 100.25ng/ml. Median H-FABP level in controls was 5.38 ng/ml (IQR: 3.78-6.42) with a minimum level of 1.56ng/ml and a maximum level of 19.83 ng/ml. H-FABP level was significantly higher in cases as compared to controls (p ≤ 0.001). Mean H-FABP comparison between cases and control is shown (Table-1).

Using a cut-off value 16ng/ml for H-FABP,91 out of 125 patients of ACS were correctly diagnosed to have acute MI. The sensitivity and specificity of H-FABP in diagnosing AMI were 85% and 83.3% respectively at this cut-off value (Table-2). The positive predictive value (PPV) and negative predictive value (NPV) were 96.8% and 48.4% respectively. The accuracy of H-FABP in ACS diagnosis was 84.4% (Table-3).ROC curve was drawn and calculated AUC was 0.842 (p ≤ 0.001, 95% CI: 0.735 – 0.949) (Figure-1).

Table-1: Comparison of mean H-FABP level among cases and controls

	N	*H – FABP Level (ng/ml)*		*P-value*
		Mean	*Std. Deviation*	
Cases	125	28.76	15.77	0.0001
Controls	125	6.73	4.68	

Table-2: Sensitivity & Specificity at the different cut off values of H-FABP

H-FABP level (ng/ml)	*Sensitivity*	*Specificity*
7.44 ng/ml	100%	11.1%
10.89 ng/ml	96.3%	22.2%
14.78 ng/ml	89.7%	50%
16 ng/ml	85%	83.3%
18.94 ng/ml	81.3%	83.3%
21. ng/ml	70.1%	83.3%

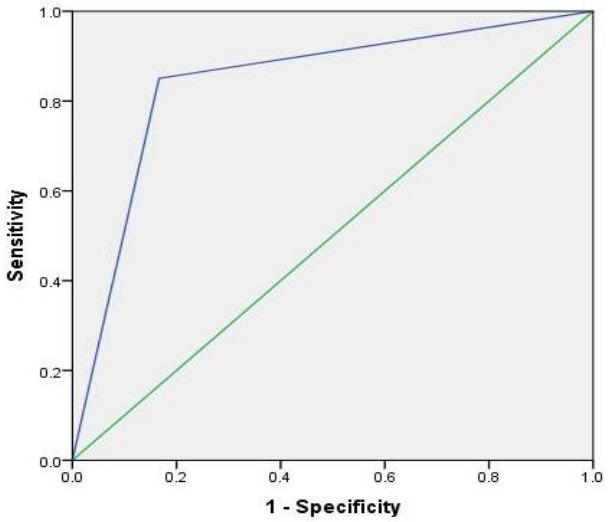

Figure-1: ROC curve of H-FABP for the cut-off value 16ng/ml

Table-3: H-FABP & Trop-T using 16ng/ml as cut-off value area under the curve (AUC)

H-FABP	*Trop-T*		*Total*	*Area*	*P-value*
	Positive	*Negative*			
Positive	91	3	94	.795	0.0001
Negative	16	15	31		
Total	107	18	125		

The test result variables: H–FABP Level (ng/ml) has at least one tie between the positive actual state group and the negative actual state group.
Sensitivity = 91/107 = 85%
Specificity = 15/18 = 83.3%
Positive predictive value (PPV) = 96.8%
Negative Predictive value (NPV) = 48.4%
Accuracy = 106/125 = 84.4%

Discussion

Acute coronary syndrome (ACS) is a chief cause of morbidity and mortality worldwide. It is strongly believed that identification and management of AMI at early hours greatly influences the AMI related morbidity and mortality and hence it's related expenditure.[18] It has also been found that the serum levels of cardiac troponins and CK-MB are not sufficient to establish the diagnosis of ACS (NSTEMI-ACS) in the patients seeking urgent medical aid in the casualty department within three hours of the onset of chest pain. So, for decision making, biomarkers facilitating identification of ACS at the initial point of time will gain particular significance.[19] H-FABP is one of the novel biomarkers, which has the potential to re-enforcing cardiac troponins in the early confirmation of AMI.[20] At presentation, the combined assessment of H-FABP and high-sensitivity troponins (hs-cTn) has been proposed as an early rule-out strategy for MI for early discharge from ED.[21] So far, it has not given consistent results as a diagnostic tool for MI, though many studies have given excellent results. The main reason may be the lack of standardization of its assay as different methods are being used for its detection in the serum and different researchers have used different assays and cut-off values for diagnosis of MI. Recently, Vupputuri *et al* reported the sensitivity of 89.7% and specificity 68% for H-FABP in early six hours for AMI detection.[22] Banuet al have found a sensitivity of 25% and AUC of 0.61 in 36 cases diagnosed with AMI(both STEMI and NSTEMI) presenting within 48 hours of the onset of chest pain of which majority presented within four hours. The diagnostic cut-off was 7ng/m.[23] Ramaiah *et al* reported a sensitivity of 77%, specificity 91% area under ROC curve equal to 0.83± 0.05 in ACS patients presenting within four hours of symptoms onset by a qualitative H-FABP immune-test with a diagnostic cut-off value of 10ng/L.[24]

The other issue concerned with the fact that some studies have not given convincing results may be due to the kinetics of H-FABP i.e. early release and rapid clearance from plasma, hence having its prime diagnostic role in early six hours after which it starts declining. It might be the reason for poor results in studies in which the patients were included with a larger duration of symptoms. The variation in performance of H-FABP in ACS diagnosis may also be due to several other factors like age group, the gender ethnic variations or sample size as this biomarker has not been evaluated at a very large scale for generalization. Also, the age-specific cut-off values have not been established so far. Our study was aimed to evaluate the effectiveness of H-FABP in the early diagnosis of acute ischemic myocardial damage (AMI) in patients with ACS. We have compared H-FABP levels with controls to show that H-FABP levels are higher in AMI cases. We also established cut-off value for H-FABP and the best cut-off level of H-FABP was 16ng/ml with sensitivity and specificity of 85% and 83.3% respectively. Our results are consistent with

many studies where the same method (ELISA) was used to analyze H-FABP.

Our results are consistent with those of Gururajan *et al*. They conducted a case-control study on 485 subjects including 99 healthy controls and found 87% sensitivity and 93% specificity of H-FABP to diagnose AMI in patients presenting within four hours of chest pain by quantitative ELISA technique keeping a diagnostic cut-off level of17.7ng/ml.[25] The results of our study are also in complete agreement with those of Elmadbouh *et al* who found a diagnostic sensitivity of H-FABP 85% and specificity equal to 83.3% within three hours of the onset of chest pain by quantitative ELISA technique with a diagnostic cut-off of 21.85ng/ml.[16] Our study is further potentiated by Cubranic *et al* who evaluated the performance of H-FABP and glycogen phosphorylase isozyme BB in AMI diagnosis within three hours after onset of chest pain and found that H-FABP had a sensitivity 99%, specificity 99% by ELISA technique at a cut-off value of 5ng/ml.[26]

Similar work was done by Figiel *et al*.[27] They found that H-FABP was the most sensitive and accurate biomarker among H-FABP, glycogen phosphorylase BB (GP-BB) and cTnT in the subgroup presenting in the initial three hours of chest pain onset. H-FABP gave 79%sensitivity and 100% PPV and an accuracy of 80% in AMI diagnosis at the 7ng /ml cut-off level. Earlier, Pasuoglu *et al* also assessed 66 patients with ACS and found that H-FABP had 100% sensitivity and 97% specificity and an area of 1.0 under the ROC curve in patients presenting within three hours of symptoms onset. H-FABP was measured by ELISA with cut-off 19ng/ml for AMI diagnosis.[28]

In our country, a large number of people come to ED with chest pain as the main complaint out of which a small fraction of cases are with true ACS diagnosis. In our study H-FABP at cut-off level, 16ng/ml has 85% sensitivity which makes it a better tool to rule out the disease at an early phase. The specificity of H-FABP is also significant and a more specific test gives a minimum number of false-positive results. In our study the AUC was equal to 0.842 which confirms that the methodology used to analyze H-FABP is good and it can discriminate among ACS and non-ACS cases efficiently. The positive predictive value which helps in confirming ACS was significantly raised which shows H-FABP as a useful early diagnostic tool for the ischemic type of chest pain in an emergency clinical setting.

Conclusion

In conclusion, the measurement of H-FABP is a valuable tool in the early diagnosis of patients with chest pain (4hrs). H-FABP is a promising biomarker for the early detection of ischemic myocardial damage (infarction) in patients presenting with the acute coronary syndrome and seems to be a preferred biomarker in the differential diagnosis of NSTE-ACS. More studies are needed to establish the exact role of this biomarker in diagnosing ACS at the early phase of ischemia.

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