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Original Article

Internal Medicine Section

A Study on the Role of Heart Type Fatty Acid Binding Protein in the Diagnosis of Acute Myocardial Infarction

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ABSTRACT

Introduction: Heart type Fatty Acid Binding Protein (H-FABP) has been proposed as an early cardiac biomarker for the diagnosis of acute myocardial Infarction (AMI) using animal models and clinical samples.

Aim: The study aimed to evaluate the role of H-FABP in early detection of AMI by comparing its sensitivity, specificity and predictive value with Creatinine Kinase-MB (CK-MB) and Cardiac Troponin I (cTnI).

Materials and Methods: This is a cross-sectional descriptive study of 50 patients admitted with the diagnosis of AMI at a tertiary care hospital in South India. The study group was categorised in to those coming to the hospital within four hours of symptom onset and those coming in between 4 to 12 hours. H-FABP was compared with those of troponin T and myoglobin tests.

Results: Among patients presenting within four hours of symptom onset, the sensitivity of H-FABP was 60% and was significantly higher than that of cardiac Troponin I (cTnI, 18.8%) and Creatinine Kinase (CK)-MB (12.5%). But specificity was only 23.53% and was less than that of cTnI (66.67%) and CK-MB (100%). In patients presenting during 4 to 12 hours of symptom onset, the sensitivity of H-FABP was 86.96% which was comparable to that of cTnI (90.9%) and CK-MB (77.3%). The specificity was 60% in the 4-12 hours group which was comparable to that of cTnI (50%) and CK-MB (50%).

Conclusion: The H-FABP is a sensitive biomarker for the diagnosis of AMI in the initial hours after symptom onset when the standard biomarkers may not be elevated, but it is less specific. During 4-12 hours of symptom onset it is as sensitive and specific as standard cardiac biomarkers troponin and CK-MB. Due to these factors H-FABP can be considered as a promising cardiac biomarker which can be used along with troponins and CK-MB at present.

Keywords: Cardiac biomarkers, Cardiac Troponin I (cTnI), Creatinine kinase, Sensitivity, Specificity

INTRODUCTION

Coronary heart disease which was earlier more common in developed countries is now common in developing countries including India [1]. Asian Indians have much higher incidence of Coronary Artery Disease (CAD) as compared to all other ethnic groups. CAD among Asian Indians has been found to be more severe, diffuse and associated with serious complications and increasing mortality at a younger age and its incidence has dramatically increased in recent years [2]. Heart type Fatty Acid Binding Protein (H-FABP) has been proposed as an early cardiac biomarker for the diagnosis of Acute Myocardial Infarction (AMI) using animal models [3,4] and clinical samples [5,6]. H-FABP is proved to be a sensitive early biomarker in Acute Coronary Syndrome (ACS) [7]. Rapid detection of H-FABP by immuno-testing has shown good sensitivity and negative predictive value compared to conventional methods in patients with AMI [8]. Some promising results were found in previous studies from India on H-FABP in patients with Acute Coronary Syndrome (ACS) [9-12]. Hence in this study the role of H-FABP in the diagnosis of AMI, particularly in the initial hours after symptom onset, is studied and compared with the established biomarkers Creatinine Kinase-MB (CK-MB) and troponin. The study was done with the following objectives: 1) To study the role of heart type fatty acid binding protein in the diagnosis of acute myocardial infarction; 2). To study the sensitivity, specificity and predictive value of H-FABP in the diagnosis of acute myocardial infarction and to compare with the CK-MB and cardiac Troponin I (cTnI).

MATERIALS AND METHODS

This study was carried out at in a tertiary medical college hospital in South India during the period between November 2009 and October 2010. This study was Ethically approved by the Ethical committee of the institution.

This is a cross-sectional descriptive study involving 50 patients admitted to the Intensive Cardiac Care Unit (ICCU) with the diagnosis of acute myocadial infarction. AMI was defined as: typical anginal pain at rest lasting more than 20 minutes; previously diagnosed angina that has become more frequent, longer in duration, or more easily provoked. Typical electrocardiographic changes defined as: ≥ 1 mm ST-segment elevation in at least two anatomically contiguous limb leads, ≥ 1 mm ST-segment elevation in a precordial lead V4 through V6, ≥ 2 mm ST-segment elevation in V1 through V3, ST-segment depression by more than 0.05 mV in two or more contiguous leads, marked symmetrical T-wave inversion by more than 0.2 mV in the precordial leads, new bundle branch block, and/or sustained ventricular tachycardia [13]. Elevation of conventional cardiac enzymes included Troponin-I, and/or CK-MB. Presence of at least two positive factors (history, ECG changes as mentioned above and cardiac markers) constituted the diagnosis of ACS.

The patients were categorised in to those coming to the hospital within four hours of symptom onset and those coming in between 4 to 12 hours. Patients with cerebrovascular accident, known renal disease and those coming after 12 hours of symptom onset were excluded from the study. Applying these criteria, 50 eligible patients were selected with random sampling and included in the study after informed consent.

A detailed history was taken and clinical examination was done in all the patients with particular reference to the cardiovascular system. History included age, gender, family history of hypertension, diabetes, Ischemic Heart Disease (father/mother/siblings), history of smoking and tobacco use. Body Mass Index (BMI) was calculated using the formula BMI=Weight (kg)/(Height in metre) ^{2} and were categorised as normal: 8.5 - 22.9 kg / m^2, overweight: 23- 24.9 kg / m^2 and obese: ≥ 25 kg / m^2. Blood sugar was estimated by Trinder's (Glucose oxidase) method and read at 505/670 nm. Total

cholesterol and triglycerides were measured using Trinder's method. Total cholesterol <200mg/dl was taken as normal and triglycerides <150mg/dl was taken as normal.

Standard 12 lead ECG was taken for all the patients. CK-MB was estimated using immuno-inhibition method. Values <25 IU/L was considered as normal. Two dimensional echocardiography was done for all the patients included in this study. Treatment given, complications and the course in the hospital were recorded.

Estimation of Troponin I and H-FABP

Estimation was done using Cardio-detect qualitative immunological rapid test combi kit. The test field for both troponin I and H-FABP was filled with 3 to 4 drops of whole blood, serum or plasma. The result is read after 15 minutes. If both the test (T) and control (C) lines were seen then it was taken as positive result. If only control (C) is seen then it is taken as negative result. If no line is seen then it was taken as invalid result.

The Test Principle

H-FABP: The test contains two different monoclonal antibodies specific for H-FABP, of which one is gold-labelled. The sample liquid releases the gold-labelled anti-FABP antibody from its matrix. This antibody forms an intermediary complex with the FABP present in the sample. This complex spreads across the test strip up to the position marked by 'T' where a second antibody is located. The intermediary complex and the second antibody form a sandwich complex showing up as a red line. A sample without H-FABP does not form a sandwich complex and therefore, forms no red line [14].

cTnI: If the sample contains cTnI, it interacts with anti cTnI antibodies and particles coated with the bio-tynilised anti cTnI antibodies. This complex passes over the test strip up to the position marked with 'T' where a line is coated with Streptavidin. The complex reacts with the Streptavidin showing up as a red line. A sample without cTnI forms no red line [14].

Evaluation of the Test

For H-FABP: If two lines seen (at 'C' and 'T') - Positive (H-FABP>7ng/ml), if only one line at 'C' - Negative (H-FABP<7ng/ml), no line or one line at 'T' only- Invalid test. For cTnI: If two lines seen (at 'C' and 'T') - Positive (cTnI>1ng/ml), if only one line at 'C' - Negative (cTnI<1ng/ml), no line or one line at 'T' only- Invalid test.

STATISTICAL ANALYSIS

Data was entered in Microsoft excel spread sheet and analysed statistically using SPSS software version 17. Results were considered significant if the p-value was below 0.05. Chi – square test and Pearson's correlation test was done for statistical analysis.

RESULTS

A total of 50 patients were selected for the study. They were divided into two groups those presenting within four hours after the symptom onset and those presenting during 4-12 hours. Out of 50 patients, 22 presented within four hours (44%) and 28 patients presented during 4-12 hours (56%). There was no statistical difference between males and females with regard to time window of presentation to hospital after symptom onset.

Thirty nine patients were males (78%) and 11 were females (22%). Six patients were aged <40 years (12%), 15 were in 40-50 years age group (30%), 17 in 50-60 years age group (34%) and 12 were aged >60 years (24%). The mean age group among H-FABP positive patients was 51.92 ± 9.437 years and 55.92 ± 9.931 among H-FABP negative patients. H-FABP was positive among 20 alcoholics (90.9%) and 18 non-alcoholics (64.3%) [Table/Fig-1].

Thirty two males and six females were positive for H-FABP (82.05% v/s 54.55%). There was statistically significant correlation between ECG changes and echocardiographic findings and the group which received thrombolysis with H-FABP positivity (p-value 0.000) [Table/Fig-2].

In this study, among patients presenting within four hours of symptom onset, the sensitivity of H-FABP was 60% and was significantly higher than that of cTnI (18.8%) and CK-MB (12.5%). But specificity was only 23.53% in the initial four hours which was less than that of cTnI (66.67%) and CK-MB (100%). In patients presenting during 4 to 12 hours of symptom onset, the sensitivity of H-FABP was 86.96% which was comparable to that of cTnI (90.9%) and CK-MB (77.3%). The specificity was 60% in the 4-12 hours group which was comparable to that of cTnI (50%) and CK-MB (50%). Overall diagnostic accuracy compared to troponin was 60% and compared to CK-MB was 56% [Table/Fig-3,4].

Patient characteristics		H-FABP				p-value*
		Positive		Negative		
		N	%	N	%	
Gender	Male	32	64	7	14	0.06
	Female	6	12	5	10	
Age group (in years)	<40	5	10	1	2	0.3
	40 - 50	11	22	4	8	
	50 - 60	15	30	2	4	
	>60	7	14	5	10	
Body Mass Index	Normal	23	46	10	20	0.15
	Increased	15	30	2	4	
Presence of hypertension	Yes	17	34	5	10	0.85
	No	21	42	7	14	
Presence of diabetes mellitus	Yes	9	18	6	12	0.08
	No	29	58	6	12	
History of smoking	Smoker	30	60	6	12	0.05
	Non-smoker	8	16	6	12	
History of alcohol consumption	Yes	20	40	2	4	0.03
	No	18	36	10	20	
Outcome	Improved	35	70	12	24	0.32
	Expired	3	6	0	0	

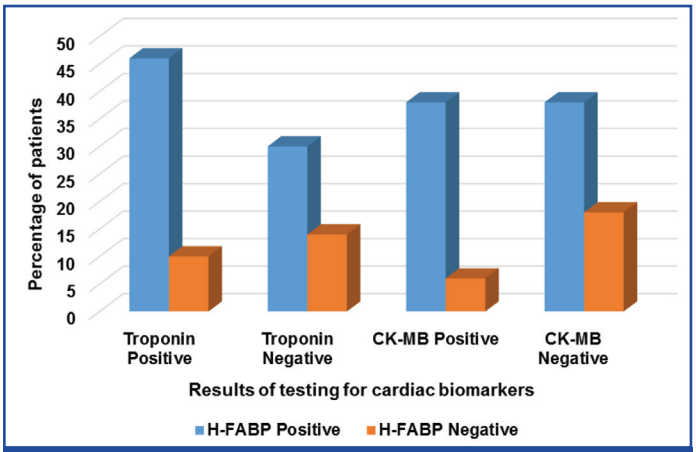
[Table/Fig-1]: Distribution of H-FABP with respect to study characteristics (N=50). *Statistical significance was considered at $p \leq 0.05$.

Variables		H-FABP				p-value*
		Positive		Negative		
		N	%	N	%	
Killip class	I	17	34	6	12	0.58
	II	9	18	6	12	
	III	2	4	0	0	
	IV	10	20	0	0	
Triglyceride levels	Normal	7	14	4	8	0.28
	Increased	31	62	8	16	
Cholesterol levels	Normal	3	6	2	4	0.38
	Increased	35	70	10	20	
Electrocardiograph findings	ST segment elevation	26	52	3	6	<0.001
	Other changes	12	24	2	4	
	No changes	0	0	7	14	
Echocardiograph findings	RWMA	32	64	3	6	<0.001
	No RWMA	6	12	9	18	
Thrombolysis	Done	25	50	3	6	0.013
	Not done	13	26	9	18	

[Table/Fig-2]: Distribution of H-FABP among patients with respect to their Killip class, laboratory parameters and thrombolysis (N=50).
*Statistical significance was considered at $p \leq 0.05$. RWMA – Regional wall motion abnormality.

H-FABP		Troponin		CK-MB	
		Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)
0 - 4 hrs	Positive	3 (6)	13 (26)	2 (4)	14 (28)
	Negative	2 (4)	4 (8)	0 (0)	6 (12)
4 – 12 hrs	Positive	20 (40)	2 (4)	17 (34)	5 (10)
	Negative	3 (6)	3 (6)	3 (6)	3 (6)
Overall	Positive	23 (46)*	15 (30)*	19 (38)†	19 (38)†
	Negative	5 (10)*	7 (14)*	3 (6)†	9 (18)†

[Table/Fig-3]: Comparison of H-FABP distribution with Troponin and CK-MB. *Sensitivity = 82.14%, Specificity= 31.82%, Positive Predictive Value=60.53%, Negative Predictive Value=58.33%, Diagnostic Accuracy= 60%. †Sensitivity = 86.36%, Specificity= 32.14%, Positive Predictive Value=50%, Negative Predictive Value=75%, Diagnostic Accuracy= 56%.



[Table/Fig-4]: Comparison of overall H-FABP distribution with Troponin and CK-MB (N=50)

DISCUSSION

Early diagnosis and treatment is very important in preserving the myocardium and in limiting the ischaemic damage. Studies have shown that the serum levels of gold standard cardiac biomarkers start to rise relatively late (3-4 hours in case of cardiac troponins and 4-6 hours in case of CK-MB) and myoglobin which can be detected early is nonspecific to myocardium. Studies also have shown that non-diagnostic ECGs are recorded in approximately half of the patients presenting to emergency department with chest pain who ultimately are shown to have AMI. Hence an early biomarker is essential for the accurate diagnosis of AMI. Heart Type Fatty Acid Binding Protein (H-FABP) has shown promise in this regard in various studies.

The present study aimed at evaluating the role of H-FABP in the diagnosis of AMI and also to compare it with the standard biomarkers troponin and CK-MB. It also aimed at finding out the distribution of H-FABP with regard to variables like age and sex of the patient, BMI, lipid levels, smoking, and alcoholism and in those with systemic hypertension and diabetes mellitus. It also aimed at correlating the H-FABP positivity with ECG and echocardiographic findings.

This observation is supported by some of the following studies. According to a study by Okamoto et al., the overall sensitivity within 12 hours of symptom onset was 92.9% for H-FABP, 88.6% for myoglobin and 18.6% for CK-MB [9]. The overall specificity was 67.3% for H-FABP, 57.1% for myoglobin and 98.0% for CK-MB. A study from Chennai showed H-FABP to be a good discriminator between patients with and without IHD [15]. It also showed that troponin levels rise more than six hours after symptom onset, H-FABP is usually positive within first four hours. At the optimum cut-off value (17.7 ng/ml), the sensitivity and specificity were found to be 87% and 93% respectively.

In another study, among patients presenting within four hours of symptom onset, sensitivity of H-FABP was higher than cTnT (73% v/s 53%). Specificity of H-FABP was 71% [16]. Combined use of H-FABP and cTnT significantly improved the sensitivities of both to 85%. In a study by Umut Cavus et al., H-FABP had sensitivity equal to that of CK-MB and superior to that of myoglobin (97.6% v/s 96.7% v/s 85.4%) in initial 4 hours [17].

In a study by, Ecollan P et al., a positive H-FABP using cardio-detect assay had a significantly better sensitivity than cTnI, myoglobin and CK-MB (87.3% v/s 21.8%, 64.2% and 41.5% respectively) [18]. A study by Ruzgar et al., showed sensitivity of 38% with troponin, 76% with CK-MB and 95% with H-FABP in patients admitted within 6 hours of chest pain onset [19]. In another study conducted by Mad P et al., in 280 patients presenting to the hospital with a median time of three hours of symptom onset, H-FABP had a sensitivity of 69% and specificity of 74% and AMI was diagnosed significantly earlier than by troponin [20].

In this study, H-FABP was found to be more sensitive but less specific compared to cardiac troponin I and CK-MB during the initial four hours of symptom onset [Table/Fig 4]. During the 4-12 hours of symptom onset, H-FABP showed similar sensitivity and specificity compared to cardiac troponin I and CK-MB. It did not show any significant difference between males and females, different age groups, diabetics and non- diabetics, hypertensives and normotensives, smokers and non-smokers and between those having hyperlipidaemia and normolipidaemia.

LIMITATION

The main limitation of the study was the small sample size. The sample size calculation and the required numbers are not accomplished because of feasibility constraints due to the cost of the kits and this may affect the analysis of study results. Each patient was tested only once and serial evaluation of the biomarkers was not done. Also, quantitative assay for troponin and H-FABP was not performed. Also, cardiac Troponin T and myoglobin were not measured.

CONCLUSION

The heart type fatty acid binding protein is a sensitive biomarker for the diagnosis of acute myocardial infarction in the 4-12 hours of symptom onset when the standard biomarkers may not be elevated, but it is less specific. The H-FABP assay is not influenced by the age, sex, BP, glycaemic status, BMI and lipid levels of the patient. Due to these factors H-FABP can be considered as a promising cardiac biomarker which can be used along with troponins and CK-MB at present.

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