

Human Heart-Type Fatty Acid Binding Protein as a Biomarker in Patients with Acute Chest Pain

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Abstract: Human Heart-Type Fatty Acid Binding Protein (H-FABP), a small (15kDa) cytoplasmic tissue specific protein, is mainly expressed by myocytes. **Aim:** The aim of this work is to compare between (H-FABP) as a sensitive biomarker than other traditional biomarkers for early differential diagnosis of acute chest pain. **Methods:** This study was carried out on 60 subjects and classified into four groups; Control group, acute myocardial infarction (AMI), unstable angina (UA) and Non cardiac chest pain (NCCP). We assessed serum levels of LDH, Troponin-I, CK-MB, H-FABP and Myoglobin. **Results:** We found that Serum H-FABP was significantly higher in AMI and UA in comparison with control and NCCP groups ($P < 0.05$). Serum H-FABP differentiated between AMI group & UA group at cut off 22.35 ng/ml with sensitivity 85%, specificity 70 %, and over all accuracy 81.2%. Serum H-FABP differentiated between AMI group cases of 0 – 3 hours, as it has the highest sensitivity of the studied cardiac markers. **Conclusion:** This study showed that Measurement of serum H-FABP can be used in early differential diagnosis of acute chest pain patients.

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Keywords: Heart Type-Fatty Acid Binding Protein (H-FABP), acute chest pain, Myocardial infarction, cardiac Biomarkers.

1. Introduction

Chest pain is the leading symptom that initiates the diagnostic and therapeutic cascade in patients with suspected acute coronary syndromes [1]. Rapid triaging of patients presenting with chest pain is needed to facilitate early initiation of appropriate treatment in patients with acute myocardial infarction [2]. Cardiac marker tests help physicians to diagnose acute coronary syndromes. They are myoglobin, Creatine kinase-MB (CK-MB), troponin T (cTnT), troponin I (cTnI) and others. CK-MB, cTnT, and cTnI may also be used to identify and manage high-risk patients. Till now the ideal marker is not present [3]. H-FABP, a small (15kDa) cytoplasmic tissue specific protein, is mainly expressed by myocytes. H-FABP can be transported from severely damaged cardiomyocytes to the blood more rapidly than other markers such as cTn-I and CK-MB. Recent research suggests that human heart-type fatty acid binding protein (H-FABP), might have potential as an early cardiac marker. It appears in plasma 1-3 h. after cardiac damage, and may be the earliest available plasma marker of acute myocardial injury and returns to normal values in 12-24 hours. It may have better diagnostic accuracy than other cardiac markers in the early stages after symptom onset unclear [4]. The aim of this work was to compare between (H-FABP) as a sensitive biomarker than other traditional biomarkers (CK-MB, Troponin-I, LDH and myoglobin) for early

differential diagnosis of acute chest pain.

2. Materials and methods

This study was carried out on 60 subjects: 51 patients and 9 healthy subjects served as controls. The patients were selected from Intensive Care Unit, Cardiology Department, Al- Azhar University Hospital. The subjects were divided into 4 groups: Group1; included 21 AMI patients: 13 males & 8 females. The diagnosis was based on The Joint European Society of Cardiology/American College of Cardiology Committee criteria of AMI (2010) [5]. Group2; included 19 UA patients (13 males, 6 females). Patients were classified as UA if they experienced new onset angina, angina that came on with less exertion or angina that increased in severity, duration or frequency relative to their baselines [6]. Group3; included 11 NCCP patients (6 males, 5 females) NCCP patients included chest wall diseases & trauma, gallstones, gastritis, peptic ulcer, pulmonary embolism, pleurisy, psychic, asthma, mitral valve prolapse and reflux oesophagitis [6]. Group4; included 9 age and gender matched healthy subjects served as controls (6 males, 3 females). They were selected on the basis of healthy condition.

Without cardiovascular diseases, lung and kidney diseases, with normal blood pressure and electrocardiogram, normal serum lipids and normal blood glucose [6].

2.1. Exclusion criteria: Patients with one of the following criteria are excluded; arrival to the hospital over 6 h after onset of chest pain, patients who underwent percutaneous transluminal coronary angioplasty or coronary artery bypass grafting within 30 days, patients who had prior AMI within 30 days, patients having chronic muscle disease, patients receiving direct current (DC) shock, patients with renal insufficiency or any renal disease impairing renal clearance and patients have recent surgery (cardiac & non-cardiac). All studied subjects were submitted to the following: Full history taking, General clinical examination, ECG examination. Blood samples were obtained from the patients at the time of admission to the hospital within 4 hours after the onset of symptoms. 10 ml venous blood sample was withdrawn by sterile vein puncture and clean dry syringes from the cubital vein of every investigated subject into plain tube. Blood was allowed to clot and centrifuged for 3 minutes at 4000 rpm. The clear supernatant serum was separated from the clot and liquated under complete

aseptic condition. The serum obtained was kept frozen at - 20°C till analysis of LDH (Spectrophotometric method), Troponin-I (ELFA method), CK-MB, H-FABP and Myoglobin (ELISA method).

2.2. Statistical analysis: Data were expressed as Mean \pm SD. Data comparisons were performed by using student t-test. The levels of significance were accepted with $P < 0.05$. P. value considered insignificant if it > 0.05 , significant if it ≤ 0.05 , highly significant if it ≤ 0.01 and the results were presented in Tables as mean \pm SD. The statistical analysis were done using SPSS version 20.0. program.

3. Results

3.1. The study shows that there is statistically significance difference between group 1 and 4 as regard to cardiac markers (LDH, troponin I, CK-MB, myoglobin, and H-FABP level) with high mean among acute MI cases (p-value < 0.05) as presented in table 1, figure 1 and figure 2:

Table 1: Comparisons of cardiac markers between group (1) and group (4).

Variables	Group 1		Group 4		p-value	Sig.
	Mean	SD	Mean	SD		
LDH	258.1	72.5	184.7	85.5	0.02	S
CK MB (ng/ml)	8.04	4.9	1.2	0.94	< 0.001	HS
Troponin I ($\mu\text{g/l}$)	3.29	2	0.024	0.02	< 0.001	HS
Myoglobin (ng/ml)	274.4	76	22.9	13.6	< 0.001	HS
H-FABP (ng/ml)	56.3	27.9	17.5	5.9	< 0.001	HS

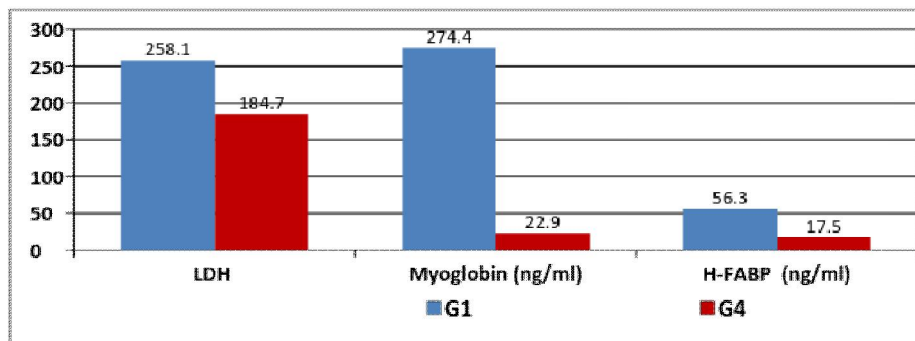


Figure 1: Comparisons of cardiac markers LDH, myoglobin and H FABP between group (1) and group (4).

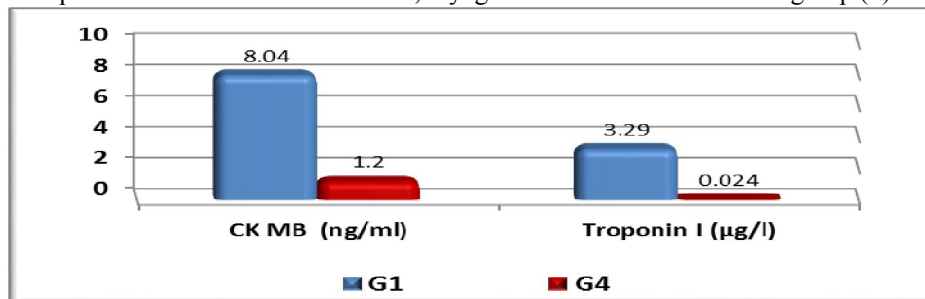


Figure 2: Comparisons of cardiac markers troponin and CKMB between group (1) and group (4).

3.2. As regard to comparison between group 1 and group 2 that there is statistically significance difference in all cardiac markers (LDH, troponin I,

CK-MB, myoglobin, and H-FABP level) with high mean among acute MI cases (p-value <0.05) as presented in table 2, figure 3:

Table 2: Comparisons of cardiac markers between group (1) and group (2).

Variables	Group 1		Group 2		p-value	Sig.
	Mean	SD	Mean	SD		
LDH	258.1	72.5	191.5	77.3	0.008	HS
CK MB (ng/ml)	8.04	4.9	2.8	0.59	<0.001	HS
Troponin I (µg/l)	3.29	2	0.052	0.02	<0.001	HS
Myoglobin(ng/ml)	274.4	76	40.2	19.4	<0.001	HS
H-FABP (ng/ml)	56.3	27.9	23.4	7.5	<0.001	HS

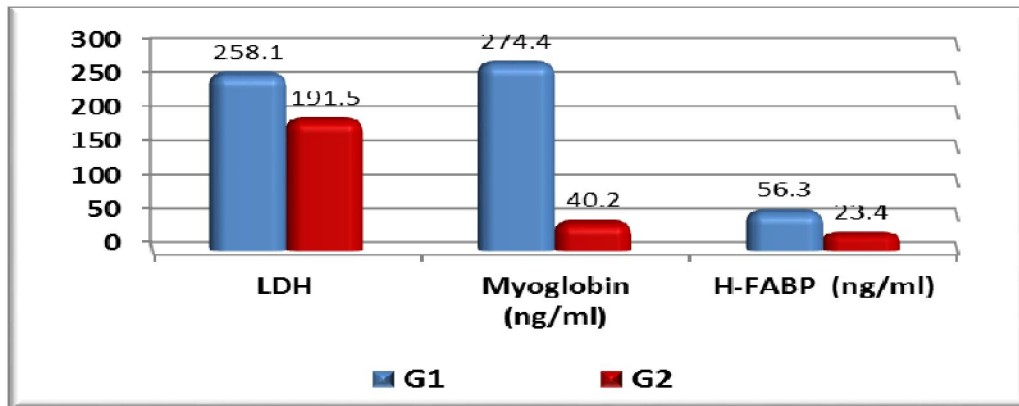


Figure 3: Comparisons of cardiac markers LDH, myoglobin and H FABP between group (1) and group (2).

3.3. There is statistically significance difference between group 1 and 3 as regard to cardiac markers (LDH, troponin I, CK-MB, myoglobin, and H-FABP

level) with high mean among acute MI cases (p-value <0.05) as shown in Table 3, figure 4 and figure 5:

Table 3: Comparisons of cardiac markers between group (1) and group (3)

Variables	Group 1		Group 3		p-value	Sig.
	Mean	SD	Mean	SD		
LDH	258.1	72.5	165.5	67.9	0.001	HS
CK MB (ng/ml)	8.04	4.9	1.98	0.92	<0.001	HS
Troponin I(µg/l)	3.29	2	0.025	0.009	<0.001	HS
Myoglobin(ng/ml)	274.4	76	28.9	19.1	<0.001	HS
H-FABP (ng/ml)	56.3	27.9	20.4	6.7	0.001	HS

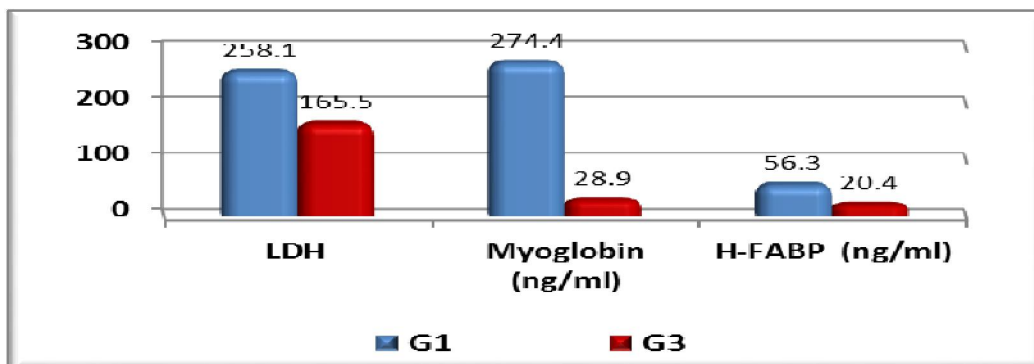


Figure 4: Comparisons of cardiac markers LDH, myoglobin and H FABP between group (1) and group (3).

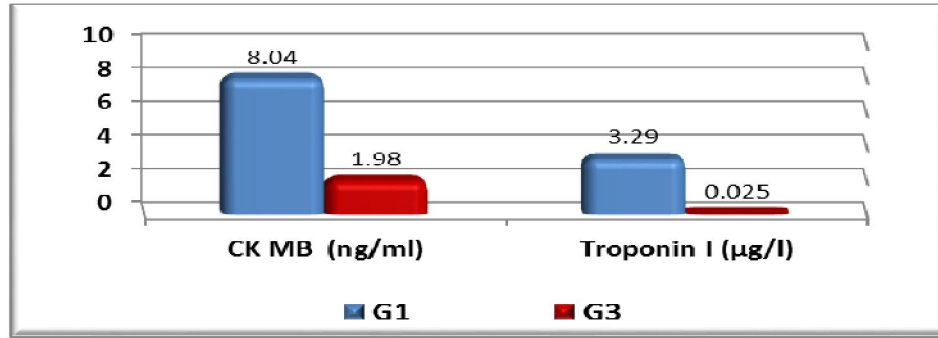


Figure 5: Comparisons of cardiac markers CKMB and troponin between group (1) and group (3).

3.4. There is statistically significant difference between cases groups as regard to cardiac markers (LDH, troponin I, CK-MB, myoglobin, and H-FABP

level) with high mean among acute MI cases (p-value <0.05) as presented in table 4:

Table 4: Comparisons of cardiac markers between different patients groups.

Variables	G1		G2		G3		p-value	Sig.
	Mean	SD	Mean	SD	Mean	SD		
LDH	258.1	72.5	191.5	77.3	165.5	67.9	0.004	HS
CK MB (ng/ml)	8.04	4.9	2.8	0.59	1.98	0.92	<0.001	HS
Troponin I(µg/l)	3.29	2	0.052	0.02	0.025	0.009	<0.001	HS
Myoglobin(ng/ml)	274.4	76	40.2	19.4	28.9	19.1	<0.001	HS
H-FABP (ng/ml)	56.3	27.9	23.4	7.5	20.4	6.7	<0.001	HS

3.5. The diagnostic ability of each marker to distinguish between AMI and non-AMI groups was assessed by receiver operating characteristic curve (ROC) analysis figure 6 and table 5, both demonstrate that the H FABP and troponin had the highest

sensitivity (86%) and (84%) respectively than myoglobin and CKMB. While the troponin shows the highest specificity (90%) and H FABP show specificity 70%.

Table 5: Sensitivity and specificity of different cardiac markers in diagnosis of cardiac cause of chest pain.

Source of the Curve	Sensitivity	Specificity	Accuracy	Cut off point
CK MB (ng/ml)	80%	55%	77.8%	2.45
Troponin I(µg/l)	84%	90%	93.4%	0.037
Myoglobin(ng/ml)	75%	85%	86.6%	40.1
H-FABP (ng/ml)	86%	70%	81.2%	22.35

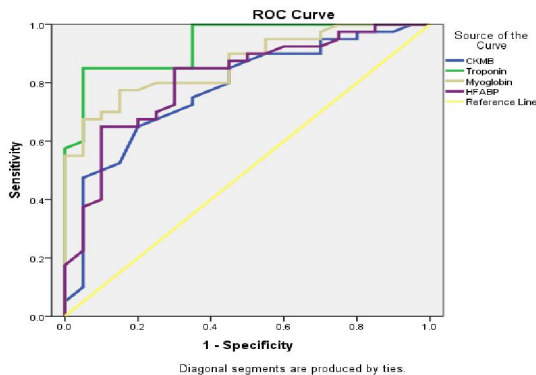


Figure 6: Diagnostic value of sensitivity and specificity of ROC curves of different cardiac markers in chest pain

4. Discussion

Management or excluding of patients with acute chest pain due to acute coronary syndrome (ACS) is a common and difficult challenge for emergency physicians [7, 8]. Therefore, rapid triaging of patients presenting with chest pain is needed to facilitate early initiation of appropriate treatment in patients with acute MI. Missing an ACS due to non-specific symptoms and unclear ECG does, may lead to excess morbidity and mortality that could have been prevented with optimal treatment. H-FABP is released rapidly from the myocardium into the blood stream after ischemic injury. Since H-FABP is a smaller molecule than myoglobin, cTnI, and CK-MB, it peaks earlier than these other molecules when there is cardiomyocyte damage [9]. In an attempt to

investigate whether studied cardiac markers can differentiate between AMI and UA groups, this study found a cutoff value for serum H-FABP of 22.3ng/ml gives a sensitivity of 86% and a specificity of 80% for differentiation between AMI and UA groups. Myoglobin & H-FABP have been relied on particularly for their negative predictive value. Recent data suggest that with modern contemporary sensitive assays and sensitive cutoffs for cTnI, cTnI can assume this role [10]. This is confirmed by this study. *Newby et al., (2006)*[11] reported that, the cardio-specificity of CK-MB is not 100%. False-positive elevations occur in a number of clinical settings, including trauma, heavy exertion, and myopathy. In this study, for differentiation between UA group & NCCP group, the reported cutoff values of the studied cardiac markers demonstrate that cTn-I has the highest specificity, PPV, NPV and accuracy while has the same sensitivity as H-FABP. It was found that H-FABP perform better than or similar to myoglobin [12]. *Alansari and Croal, (2004)*[13] suggested that H-FABP and myoglobin provide little clinical value, compared to cTnI, when measured at presentation in patients presenting with chest pain (3-12 h). For differentiation between AMI cases of 0 – 3 hours onset and non-AMI cases of 0-4 hours onset, the reported cutoff values of the studied cardiac markers demonstrate that H-FABP has the same sensitivity as cTnI & CK-MB but has higher sensitivity than myoglobin while cTnI has the highest specificity, PPV, NPV and accuracy. This study showed that the cut off value of H-FABP for the diagnosis of AMI from cases of acute chest pain within 3 hours of onset was 22.35 ng/ml. At this cut off value the sensitivity of H-FABP is equal to that of cTnI and CK-MB and superior to that of myoglobin similar to that reported by *Cavus et al.,(2006)* [14]. The present study showed that within the 0-3 hours of acute chest pain H- FABP has specificity more than CK-MB. This comes in line with that reported by *Cavus et al, (2006)* [14]. Whereas the specificity of myoglobin was superior to that of H-FABP similar to that reported by *Pasaoglu et al.,(2007)*[9]. In another study, in patients within 3 hours of symptom onset, sensitivity level for H-FABP was 100%, specificity level was 63%, and negative predictive value was 100% [15]. The multicenter EUROCARDI trial revealed a more pronounced superiority of H-FABP to myoglobin in patients admitting to hospital early after symptom onset (0-3 hours) [16]. This study showed that within 3 hours of acute chest pain the areas under the ROC curve for H-FABP and myoglobin in the AMI group was highly significant than in the non-AMI groups similar to that reported by *Pasaoglu et al., (2007)*[9]. The current study found that within the 0-3 hours of acute chest pain the specificity of cTnI within 3 hours was superior to that of H-FABP. These results

agree with that reported by *Seino et al, (2003)* [17]. Another study reported that patients with non-AMI myocardial damage associated with unstable angina showed positive H-FABP test results so affected the specificity [15]. This study showed that within the 0-3 hours of acute chest pain the specificity of cTnI is 90% close to the percentage 87.6% that reported by *McCord et al.,(2001)*[18]. The AUC of cTnI was 93.4% close to the percentage 86% that reported by *McCord et al., (2001)* [18]. Positive predictive value (PPV) of cTnI is 81.8% close to the percentage 78.6% that reported by *Melanson et al., (2004)* [19]. This study showed that within the 0-3 hours of acute chest pain the sensitivity of myoglobin was 75.% close to the percentage 69.7% that reported by *Melanson et al., (2004)* [19], and the percentage 70.8% (at admission) that reported by *McCord et al.,(2001)* [18]. Negative predictive value (NPV) (for exclusion of AMI) of myoglobin was 83.3% similar to the percentage 83% that reported by *Montague & Kircher., (1995)* [20]. AUC of myoglobin was 86.6% similar to the percentage 82% that reported by *McCord et al.,(2001)* [18], PPV of cTnI is 81.8% close to the percentage 78.6% that reported by *Melanson et al.,(2004)* [19] i.e cTnI had a higher PPV than either CK-MB or myoglobin similar to that reported by *Melanson et al., (2004)* [19]. Skeletal muscle injury can reduce the specificity of CK-MB [21]. The present study observed that the cut off value of H-FABP for the diagnosis of AMI from cases of acute chest pain within 0-3 hours of onset was 22.35 ng/ml. At this cut off value the sensitivity was 86% close to the percentage 88.8% that reported by (*Willemsen et al., 2015*)[22]. The specificity was 70% lower than the percentage 86.4% as that reported by (*Willemsen et al., 2015*) [22]. The present study cannot exactly explain why there were more than a few patients with non-cardiovascular etiology who showed positive test results, but it may be related to contamination by skeletal muscle damage or diminished renal clearance similar to that reported by *Seino et al.,(2004)*[15] & *Seisuta et al.,(2002)*[23], also may be due to the presence of cases of traumatic chest wall cases with elevated H-FABP level). Sensitivity of H-FABP is higher than CK-MB similar to that reported by *Ruzgar et al., (2006)* [24]. It was concluded by *Carroll et al., (2012)* [25] that the combining H-FABP and cardiac-specific troponin (cTn) significantly improves the diagnostic sensitivity assays. Also a systematic review by quality specifications of these assays have recently been produced on four clinical studies (total of 1,598 patients) on combinations of quantitatively assessed H-FABP and cTn versus cTn alone at presentation revealed that the addition of H-FABP to cTn increased sensitivity from 42–75 % to 76–97 % but decreased specificity from 95–100 % to 65–93 % [26].

5. Conclusion

At 0 – 3 hours of acute chest pain, for differentiation between AMI cases and non-AMI cases, the reported cutoff values of the studied cardiac markers demonstrate that H-FABP has the same sensitivity as cTn-I but has higher sensitivity than myoglobin & CK- MB while cTn-I has the highest specificity, PPV, NPV and accuracy. It is preferred to use the combination of a highly sensitive marker that appears early e.g. H-FABP and more specific marker as cTnI to facilitate true rapid exclusion of AMI (i.e. eliminate false-positives) and enable discharge of patients who do not require prolonged observation.

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