Heart-Type, Fatty-Acid Binding Protein Can Be a Diagnostic Marker in Acute Coronary Syndromes

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Objectives: Chest pain is one of the most common complaints among patients admitted to emergency departments. Cardiac troponins, CK-MB and myoglobin, which are used routinely in the diagnosis of acute coronary syndrome (ACS), are not elevated in the initial hours of ACS—precluding their usefulness in the early diagnosis. The aim of this study is to determine the efficacy of H-FABP compared to myoglobin and CK-MB in the early diagnosis of ACS.

Methods: Sixty-seven patients with ACS were enrolled in the study. An initial blood sample was obtained for CK-MB, cTnT, myoglobin and H-FABP. At the fourth, eighth, and 12th hours, repeat ECGs and cardiac enzyme samples were obtained. H-FABP test was repeated at the fourth hour.

Results: H-FABP has sensitivity equal to that of CK-MB and superior to that of myoglobin (97.6%, 96.7%, 85.4%, respectively) on the first hour. This trend extends to the fourth hour of myocardial injury as well. H-FABP was more specific than CK-MB, myoglobin and troponin T at the first hour (38.5%, 34.6%, 34.6%, 23.1%, respectively), whereas its specificity at the fourth hour was equal to those of CK-MB and troponin T and exceeded that of myoglobin.

Conclusions: It can be suggested that in patients with an initial diagnosis of ACS and within 20 hours from symptom onset, H-FABP levels may be measured. For this purpose, point-of-care H-FABP test may be utilized, which has the advantage of bedside testing and rapid test results.

Key words: H-FABP ■ acute coronary syndrome ■ cardiac enzymes ■ early diagnosis ■ chest pain

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INTRODUCTION

Chest pain is one of the most common complaints among patients presenting to emergency departments. It may be the initial and sole complaint of acute coronary syndrome (ACS). Diagnosis of ACS is based on an assessment of risk factors, careful and rapid assessment of ECG, and measurement of cardiac enzymes. Cardiac troponins, CK-MB and myoglobin, which are routinely used in the diagnosis of ACS, are not elevated in the initial hours, precluding their usefulness in the early diagnosis.[1]

Fatty-acid binding proteins (FABPs) are members of cytosolic protein family. The name FABP originates from their ability to adhere fatty acids noncovalently in a high-affinity manner. FABP is relatively tissue specific; and liver, heart and intestinal FABPs are named L-FABP, H-FABP and I-FABP, respectively. They are most abundantly found in heart and liver tissue. H-FABP is an equivalent protein to albumin, the principle extracellular fatty-acid transporter, in regard to its function to transport fatty acids intracellularly.[2] H-FABP distribution in the heart is 0.57 mg/g, whereas myoglobin's distribution is 2.7 mg/g. Skeletal tissue contains FABP in an amount of 0.04–0.14 mg/g and myoglobin 2.2–6.7 mg/g.[3-5] This difference helps one to differentiate myocardial and skeletal muscle injury. The reasons for using H-FABP in early diagnosis of ACS are high myocardial content, presence in mainly cytosole (unclear), low molecular weight, relative tissue specificity, and early (within two hours) appearance in plasma and urine after AMI onset.

The aim of this study is to determine the efficacy of H-FABP compared to myoglobin and CK-MB in the early diagnosis of ACS, and to assess its cardiosensitivity and specificity.

METHODS

Subjects

This prospective study was conducted in 67 patients admitted with a chief complaint of chest pain to the

Hacettepe University, Faculty of Medicine, Adult Emergency Department between July 2004 and March 2005. Inclusion criteria were as follows: patients presenting within one hour of symptom onset and having typical chest pain. Patients were excluded if they presented after one hour of symptom onset, had renal insufficiency or any renal disease impairing renal clearance, were <18 years of age or had atypical chest pain. Patients who underwent percutaneous transluminal coronary angioplasty or coronary artery bypass grafting within 30 days; had prior AMI within 30 days; had chronic muscle disease, pulmonary thromboembolism or pericarditis were also excluded. The protocol of this study was approved by the local ethics committee, and written informed consent was obtained from every subject participating in this study.

All patients underwent a comprehensive inquiry regarding the degree of angina pectoris, risk factors and past history. A complete blood count, biochemical profile and an initial 12-lead ECG were obtained. Subjects underwent serial ECG and cardiac enzyme follow-up every four hours. All subjects were managed medically in conformity with ACC/AHA ST elevation myocardial infarction (STEMI), non-STEMI (NSTEMI) and unstable angina pectoris (USAP) guidelines.[6] All patients received treatment with p.o. aspirin (300 mg), IV unfractionated heparin (UFH) (bolus of 60 U/kg body weight up to a maximum of 5,000 U followed by continuous infusion of 7 U/kg per hour titrated to an activated partial thromboplastin time of 60–70 seconds) or 100 IU/kg subcutaneously enoxaparin 1 mg/kg every 12 hours as low-molecular-weight heparin, and clopidogrel (loading dose of 300 mg followed by 75 mg daily). USAP/NSTEMI patients with high-risk factors underwent coronary angiography in 24 hours. Patients with low-risk factors initially received medical therapy and then underwent coronary angiography in five days. Fibrinolytic therapy was administered to STEMI patients, and they underwent coronary angiography in five days.

Table 1. Number and percentage of the patients with high myoglobin, CK-MB levels and positive H-FABP at first and fourth hours

	Myoglobin	CK-MB	H-FABP
First hour [n(%)]	7 (10%)	10 (15%)	11 (16%)
Fourth hour [n(%)]	22 (32%)	23 (34%)	24 (34%)

Table 2. Specificity of H-FABP at first and fourth hours

Specificity	Myoglobin	CK-MB	Troponin T	H-FABP
First hour	34.6%	34.6%	23.1%	38.5%*
Fourth hour	73.1%	88.5%	88.5%	88.5%**

* p<0.05 for H-FABP versus myoglobin, CK-MB and troponin T at first hour; ** p<0.05 for H-FABP versus myoglobin at fourth hour; p=NS for H-FABP versus CK-MB and troponin T at fourth hour

Biochemical and Laboratory Analysis

Levels of conventional cardiac markers—namely, myoglobin, CK-MB and troponin T—were measured by in vitro quantitative electrochemiluminescence immunoassay (ECLIA), sandwich test-specific antibody system and Myoglobin STAT (Short Turn-Around Time), Troponin STAT and CK-MB STAT kits. Normal reference levels for myoglobin, CK-MB and troponin T were accepted as 0–72 ng/ ml, 0.0–5.0 ng/ml and 0.0–0.1 ng/ml, respectively. At the fourth, eighth and 12th hours, repeat ECGs and cardiac enzyme samples were obtained. At the initial blood sampling, a 0.5–1.0 cc blood sample was left in the syringe and 3–4 drops (60–100 µl) were used in the bedside point-of-care CardioDetect (Berlin, Germany) apparatus for H-FABP testing. H-FABP test was repeated at the fourth hour. The test uses two different monoclonal antibodies specific to H-FABP: monoclonal anti-hh-FABP antibodies (2.0 µg) and monoclonal anti-hh-FABP antibodies (5.0 µl) binding to colloidal gold-labeled substance (OD540=12). The subject's blood sample draws out the gold-labeled H-FABP antibody from its matrix. This antibody forms an intermediary compound that appears as a purple line. A blood sample free of H-FABP does not produce this compound and so the purple line does not appear. Gold-labeled antibodies are responsible for the purple line present on the control portion of the panel. A single purple line on the indicator demonstrates a negative test result, whereas a double purple line demonstrates a positive test result for H-FABP. It can detect serum H-FABP level to a sensitivity level of 7 ng/ml. An H-FABP level of >7ng/ml in a patient presenting with chest pain within two hours of symptom onset was considered positive for an AMI. A negative test result was a level of <7 ng/ml. The diagnostic window period for the test is first 20 minutes to 24 hours after symptom onset. This period may decrease to 16 hours if medical intervention occurs.

Statistical Analysis

Values are expressed as mean ± standard deviation. Preliminary data exploration by Kolmogorove Smirnov test showed no significant deviation from normality. Categoric variables are reported in percentages. Means were compared by ANOVA method. For all statistics, a two-sided p value <0.05 was considered statistically significant. SPSS for Windows® version 10.0 statistical package was used. The eighth-hour cTnT level was accepted as gold standard for diagnosis of ACS. Cardiospecificity and sensitivity levels for initial, fourth-hour and eighth-hour CK-MB and myoglobin, and fourth-hour H-FABP levels were calculated according to the reference cardiosensitivity and specificity levels for eighth-hour cTNT level by using the Pearson Chi-squared test.

RESULTS

Sixty-seven subjects were enrolled in the study. Fifty-one were male (76%) and the mean age was 57 ±

11 (max 85, min 29, median 54). The final diagnoses of subjects were as follows: 42 (63%) had USAP, seven (10%) had NSTEMI, 18 (27%) had STEMI. Sixty-three patients had a complaint of chest pain only, two patients had both chest and back pain, one patient had chest pain together with dyspnea, and one patient suffered from nausea and vomiting. Diabetes mellitus was present in 15 patients (22%), and history of hypertension was present in 34 (49%). Thirty patients (45%) gave a history of smoking. The mean time period between the symptom onset and admission was 51 ± 12 (mean ± standard deviation) minutes (min 15 minutes, max 60 minutes). Among all patients, 33 (49%) had a normal admission ECG, 18 (27%) had an acute ST elevation, six (9%) had ST depression, five (7.5%) had pathologic Q waves, and five (7.5%) had any type of dysrhythmia.

The number and percentage of the patients with high myoglobin, CK-MB levels and positive H-FABP at first and fourth hours are shown in Table 1. Of the 42 USAP subjects, only one (2%) had a positive H-FABP both on admission and at fourth hour, while 41 (97.6%) of them developed a negative result at both time points. In the subgroup of NSTEMI patients, H-FABP was negative on admission turning to positive at the fourth hour in three (43%) patients, was positive both on admission and at the fourth hour in two (28.5%) patients, and negative both on admission and at the fourth hour in two (28.5%) patients. Ten (56%) out of 18 STEMI patients had an H-FABP negative at the first hour and positive at the fourth hour, and eight (44%) had a positive H-FABP both at the first and fourth hours. Using a cTnT level >0.1 at the eighth hour as gold standard for diagnosis of AMI, cardiosensitivity and cardiospecificity values for CK-MB, myoglobin and H-FABP at the first and fourth hours were calculated and the following results were obtained (Tables 2 and 3).

DISCUSSION

Our data demonstrate that at the first hour of myocardial injury, H-FABP has sensitivity equal to that of CK-MB and superior to that of myoglobin. This trend extends to the fourth hour of myocardial injury as well. With regard of specificity, H-FABP was more specific than CK-MB, myoglobin and troponin T at the first hour, whereas its specificity at the fourth hour was equal to those of CK-MB and troponin T, and exceeded that of myoglobin. H-FABP is released from injured myocardium and may be a useful marker in early diagnosis of acute myocardial infarction.[7] H-FABP is released within two hours of symptom onset, reaches its peak concentration within 4–6 hours and returns to its normal basal levels by 20 hours.[8] A few hours after acute myocardial infarction, H-FABP increases 18-fold, while cTnT increases two-fold and myoglobin eight-fold. H-FABP and cTnT increase to a peak plasma concentration 30–40 times the normal levels, while myoglobin increases to a peak plasma concentration 15 times the normal plasma concentration. This comparison suggests that H-FABP is more sensitive than myoglobin and cTnT in the detection of myocardial cell necrosis.[9,10] Hence, within 30–210 minutes after symptom onset, sensitivity of H-FABP in diagnosis of acute myocardial infarction is >80%.[11]

The same parameter for other cardiac markers within 0–6 hours after symptom onset has been reported to be around 64%.[12] Seino et al. reported that in acute myocardial infarction the sensitivity of H-FABP within two hours after symptom onset was 89%, and this number was greater than those of cTnT (22%) and myoglobin (38%).[13] They also reported that the area under roc curve for H-FABP in patients presenting within two hours after symptom onset was greater than that of myoglobin (0.72 vs. 0.61, p=0.01), and they suggested that bedside whole blood rapid panel H-FABP test may be used in early assessment of patients with acute chest pain. Similarly, in another study reported by Senio et al., in patients within three hours of symptom onset, sensitivity levels for H-FABP and cTnT were 100% and 50%, specificity levels were 63% and 96.3%, and negative predictive values were 100% and 86%, respectively.[14] 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).[15-17] Both myoglobin and H-FABP are low-molecular-weight proteins released into the bloodstream in early periods of ACS.

A rapid test kit of H-FABP is essential in early diagnosis of ACS. Recently, a rapid and sensitive monoclonal antibody-based enzyme-immunosensor test and a rapid microparticle-augmented turbidimetric FABP immunoassay test were developed.[18] The former one is an easy-to-use choice in emergency departments. We used a whole blood rapid panel point-of-care test that measures blood H-FABP level qualitatively (giving a positive or negative result) with a sensitivity of 7 ng/ml. This method utilizes two different monoclonal antibodies specific to FABP in cardiac tissue and gives a result within 10–15 minutes. In our study, when a positive test result was obtained in patients presenting within the first hour after symptom onset, the H-FABP concentration was considered as >7 ng/ml, favoring an ACS. In the case of two negative consecutive test results, the H-

Table 3. Sensitivity of H-FABP at first and fourth hours

Sensitivity	Myoglobin	CK-MB	Troponin T	H-FABP
First hour	85.4%	97.6%	100%	97.6%*
Fourth hour	90.2%	97.6%	100%	97.6%**

* p<0.05 for H-FABP versus myoglobin at first hour; ** p<0.05 for H-FABP versus myoglobin at fourth hour; p=NS for H-FABP versus CK-MB and troponin T at first and fourth hours

FABP concentration was considered <7 ng/ml, making the diagnosis of an ACS unlikely.[19-26]

Study Limitations

There are some limitations in the use of H-FABP measurement. It has been reported that skeletal muscle FABP and H-FABP are similar in structure, and this may cause acute elevations in H-FABP in patients with myocardial injury if skeletal muscle injury coincides.[4] In this case, applying H-FABP alone may be misleading. Combined measurement of H-FABP and myoglobin may facilitate discrimination between myocardial and skeletal muscle damage since by means of the ratio of these two proteins in plasma it is possible to determine the exact localization of damage. In myocardial damage, the myoglobin/H-FABP ratio is 4–5 units, whereas it is 20–70 units in skeletal muscle injury.[5,10] It therefore may be logical to determine the myoglobin/H-FABP ratio in plasma to favor specificity. Another problem is that cardiac or noncardiac surgery as well as renal failure may cause elevations in H-FABP plasma concentrations.[27] Kleine et al. showed that in severe renal insufficiency, H-FABP plasma levels may remain high as long as 25 hours after myocardial infarction.[11] Hence, we excluded from the study patients with severe renal impairment to increase specificity.

CONCLUSION

Point-of-care assays of H-FABP in patients presenting within 20 hours of symptom onset may lead to an earlier diagnosis of ACS. Its use in ACS appears feasible, as it has the advantage of bedside testing and rapid test results.

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