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IMPORTANCE OF APOPITOSIS MARKERS AND OXIDIZED LOW DENSITY LIPOPROTEIN FOR DIAGNOSIS AND PROGNOSIS DETERMINATION OF ACUTE MYOCARDIAL INFARCTION

Akut Miyokart İnfarktüsü Teşhisi Ve Prognozunu Belirlemede Apopitoz Belirteçleri Ve Okside Düşük Dansiteli Lipoproteinin Önemi

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ABSTRACT

To determine the importance of markers such as Ox-LDL, CK-18, HSP70, H-FABP in the diagnosis and prognosis of AMI. Additionally, to determine the correlation between them, and to analyze if there are advantages of these biomarkers to well-known cardiac parameters such as CK-MB, Troponin I.Study was performed on blood taken from 40 male individuals who provide the properties of: between the ages of 45-65, admitted to the emergency room within 6 hours of symptoms, entrance troponin values were high, and MI for the first time. Venous blood samples of first application, 24th hour and 30th day were collected. To determine the values of parameters in healthy volunteers, samples were collected for one time from 30 healthy males who are age and sex matched with our patients. Groups were: group I (blood samples at entrance), group II (24-hour blood values), grup III (30th day values), and grup IV (control). HSP70, CK-18, H-FABP Ox-LDL levels were measured with ELISA, and CK-MB and troponinI parameters measured with chemiluminescence technique.When the results were analyzed: HSP70, H-FABP and Ox-LDL for the entrance blood values; HSP70 and H-FABP for the 24th hour blood samples and CK-18, Ox-LDL and HSP70 for the 30th day blood samples were significantly higher than control group. Additionally, a positive correlation was found between HSP70 and cTnI.H-FABP has a diagnostic accuracy for early detection of AMI, Ox-LDL is an important risk factor for AMI, early detection of MI diagnosis is possible with the detection of Ox-LDL in blood, antioxidant therapy may be necessary in post-MI, in diagnosis of MI, HSP70 increases the diagnostic accuracy with cTnI and carries prognostic value, level of CK-18 is detected, as a result of ischemia-reperfusion releasing in the microvascular endothelial tissue whereas, CK-18 isn't sufficient to demonstrate diagnostic accuracy of early detection of AMI.

Keywords: FABP3 protein, acute coronary syndrome, M30 cytokeratin-18 peptide, HSP70, creatine kinase, troponin I, oxidized low density lipoprotein

ÖZET

Akut miyokard infarktüsü teşhisi ve prognozunu belirlemede CK-18, HSP70, H-FABP ve Ox-LDL gibi biyokimyasal belirteçlerin önemini, aralarındaki korelasyonu ve CK-MB, TROPONİN I gibi bilinen kardiak parametrelere karşı üstünlükleri olup olmadığını belirlemek amaçlanmıştır. Çalışma; semptomlar başladıktan 6 saat içinde acile başvuran ve giriş troponin değerleri 0,04 ng/ml den yüksek, ilk defa MI geçiren 45-65 yaş arası 40 erkek bireyden alınan kanlar üzerinde gerçekleştirilmiştir. Bu hastaların ilk başvuruda, 24. saat ve 30.gün sonrasında venöz kan örnekleri toplanmıştır. Bu parametrelerin sağlıklı bireylerdeki referans aralığının belirlenebilmesi ayrıca parametreler arasında karşılaştırma ve korelasyonların değerlendirilebilmesi amacıyla, hasta grubumuzla benzer yaş ve cinsiyet dağılımı gösteren 30 sağlıklı gönüllü erkek bireyden de bir defaya mahsus kan örnekleri alınmıştır. Gruplar; grup I (Girişteki kan değerleri), grup II (24. saat kan değerleri), grup III (30. gün kan değerleri) ve grup IV (Kontrol grubu kan değerleri) şeklinde düzenlenmiştir. HSP70, CK-18, H-FABP ve Ox-LDL düzeyleri ELİSA tekniği ile çalışan ticari kitlerle, CK-MB ve TroponinI parametreleri ise kemilüminesans esaslara göre ölçülmüştür.Hastaların girişteki kan değerlerine bakıldığında: HSP70, H-FABP ve Ox-LDL'nin; 24. saatteki değerler için HSP70 ve H-FABP nin ve 30. gün kan değerleri için ise CK-18, Ox-LDL ve HSP70'in kontrol grubundaki değerlere oranla önemli düzeyde yüksek ve istatistiksel olarak anlamlı olduğu görülmüştür. HSP70 ve cTnI arasında pozitif korelasyon olduğu saptanmıştır.Bulgularımız; H-FABP' nin AMI'nün erken teşhisi için tanısal doğruluğa sahip olduğunu, Ox-LDL' nin AMI için önemli bir risk faktörü olduğunu, MI erken teşhisinde periferal kanda tepitiyle tanıyı kuvvetlendirebileceğini, HSP70' in MI teşhisinde cTnI ile beraber tanısal doğruluğu artırmakta olduğunu ve prognostik değer taşıdığını, CK-18'in iskemi reperfüzyon sonucu mikrovasküler endotelial dokudan salgılanarak kan serum seviyelerinin tespit edilebilir olduğunu fakat AMI erken teşhisinde tanısal doğruluğu göstermede yeterli olamayacağını göstermiştir.

Anahtar Kelimeler: FABP3 proteini, akut koroner sendrom, HSP70, kreatin kinaz, troponin I, okside düşük dansiteli lipoprotein, m30 sitokeratin-18 peptit

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INTRODUCTION:

Acute myocardial infarction (AMI) is an important cause of mortality and morbidity at present despite all recent developments in medical and surgical treatments (1).

General cause of necrosis in case of myocardial infarction (MI) which appears by necrosis of the myocardium as a result of ischemia is an atherom plaque which was ruptured or caused endothelial function disorder on an atherosclerotic formation or a thrombosis located on the plaque causing total obstruction in the coronary arteries (2).

Different studies suggest that about 1/3 to 1/4 of the patients die within the first hour following onset of the symptoms before arriving to the hospital. Rate of mortality decreases to 15% for the cases who could arrive to the hospital and to 5% for the cases who had invasive intervention in ER (2).

Diagnosis of AMI where early intervention is lifesaving is based on a good medical history, evaluation of ECG and measurement of biochemical cardiac parameters (3).

Cardiac troponine I and T (cTnI and cTnT), Creatine Kinase Myocardial Band (CK-MB) are routinely used for diagnosis of AMI. However, such markers are not sufficient for early diagnosis of MI because they do not increase in the blood within first hours. Troponine values and CK-MB simultaneously exceed the upper normal limit (within 3 to 8 hours). Myoglobin is a rapidly increasing market following myocardial injury (1 to 4 hours). However, myoglobin is not a specific protein for myocardium. Therefore, a negative test may be useful to exclude the diagnosis and a positive test is not much valuable for diagnosis (4).

Cytokeratine (CK) proteins belong to an intermediary filament family and are secreted from apopytotic or proliferating cells; an important characteristics of these protein is low solubility. Therefore, they should be destructed to be able to get into circulation (5). Half-life of CK particles in the circulating blood is about 10 to 15 hours. Release procedure of soluble CK particles to the circulation is not understood well; however, the paths predicted are as follows:

-During proteolytic destruction of CKs in dying cells

-At abnormal mitosis

-From proliferating cells

-At apopitosis and/or neo-vascularization.

Total CK-18 is produced abundantly in the proliferating cells and released to the circulation by disintegration of the membrane during necrosis. Broken CK-18 only appears as a result of breaking down of total CK-18 at aspartate 238 and aspartate 396 points through caspases in the apoptotic cells (6). M30 monoclonal antibody especially recognizes the broken fragment of CK-18 aspartate 396 (M30 antigen). M30 antigen may get into the serum through release from apoptotic cells and levels of M30 antigen may be easily measured via ELISA method. Broken CKs may thereby be used as an apoptotic marker (7, 8). CKs are not specific to any organ; they are useful to evaluate the symptomatic patients during the treatment and after the recovery (9). High titration of serum antibodies against CK-18 molecule in the endothelial cells of cardiac micro-circulation were shown in the patients with ischemic cardiac disease (10, 11). Adlbrecht et al. reported highly increased M30 antigen level in the coronary occlusion area in the patients who had myocardial infarction (12).

HSPs increase the resistance of the cell against stress. They are essential for protein stability and folding of denaturated proteins. They were first discovered in the cells exposed to high temperature and stimulation of these proteins was detected in case of exposure to physical stress, oxidative stress, some chemicals and many conditions causing protein damage (13). HSP70 is an important protein which enables and preserves three dimensional structure of the proteins and exists universally among the species. Synthesis of HSP70 increases in various stress conditions and contributes to overcome with unfolded or denaturated proteins in increased concentrations in the cells (14, 15). HSP70 prevents activation of caspase-3 and SAPK (stres-activated protein kinase)/JNK (jun n-terminal kinase) during heat shock or seramide-stimulated apoptosis (16).

H-FABP abounds in cytoplasms of cardiomyocytes and plays an important role for binding the fat acids and Acyl-CoAs and transmitting to mitochondria (17, 18). Beyond the heart, existence in red muscle cells, renal cortex, testicles and brain was also reported (18, 19). High concentration of H-FABP in the mitochondria, low moleculer weight, relatively tissue-specific characteristics, evelation in the blood during early period (within 2 hours) following mitochondrial injury are basic indicators that H-FAPB may be used as an determinant for cardiac injury (20).

It is reported that H-FABP also exists in the skeletal muscle such as myoblobine; however, myoglobine content of the skeletal muscle is about 2-fold of H-FABP (21).

Exposure to different modifications that may affect structures and functions of the lipoproteins was expressed. Oxidized LDL (Ox-LDL) is the most examined among modified proteins. The LDL existing in atherosclerotic lesions of human is chemically similar to the modified Ox-LDL in terms of intake and degradation by macrophages. Such finding shows that LDL modification my be associated with atherosclerosis (22).

Modified LDLs are early cause for vascular diseases. LDL conglomerates accumulated in the vascular tissues under pathological conditions rapidly transform into Ox-LDL by reactive oxygen radicals including superoxide, hydrogen peroxide, hydroxide radicals in a modified pattern (23).

The oxidative modification causes a series of modifications on LDL. Products of oxidation are very toxic and cause abnormal functioning of the artery (24). Oxidized lipoproteins are removed by reticuloendothelial system including liver through circulation (25).

Activated platelets secrete reactive oxygen types and cause formation of Ox-LDL from LDL. Oxidation is faster in an acidic pH in particular and Ox-LDL intake by macrophages increases (26).

LDL and intermediately oxidized LDL are recognized through normal LDL receptor pattern and taken into the cell. The lipids taken into the cells by these lipoproteins do not accumulate in macrophages. Recognition characteristics of normal LDL receptors decreases by over oxidation of LDL. In such case, the Ox-LDL is taken by scavencer receptors which exist in macrophages and are not affected by intracellular cholesterol concentration and such intake causes lipid accumulation (27).

Some internal and external factors affect oxidation of LDL. Composition of fat acids, antioxidant content of LDL, phospholipase A2 activity, particle size and density of LDL may be regarded as intrinsic factors. Extrinsic factors include concentration of some metals in the plasme and extracellular fluid or concentration of the proteins binding the aforesaid metals, concentration of the antioxidants in the plasma and extracellular fluid, HDL concentration and existence period of LDL in the intima (26).

Although mechanism of onset for atherosclerosis is not completely known, the commonly accepted hypothesis is response to injury (28, 29). Smoking, increased level of Ox-LDL, hypertension and degenerative modifications may be counted as the factors causing injury (30).

Subendothelial accumulation of foam cells primarily originating from the monocytes/macrophages through uptake of low density lipoprotein play a significant role for onset of atherosclerosis. Increase in intracellular cholesterol levels causes a decrease in number of LDL receptors (down-regulation) whereas macrophages chemically tend to intake modified LDLs and accumulate through scavenger receptors which are non-reactive to intracellular cholesterol levels (31).

Since exact mechanism of monocyte migration was not justified, it is accepted that specific chemoattractants such as MCP-1 induced by oxidized-LDL are secreted by endothelial cells, smooth muscle cells and macrophages (31, 32). Activation of endothelial cells by oxidized-LDL causes secretion of granulocyte/monocyte-colony stimulating factor (GM-CSF) which stimulates proliferation and differentiation of macrophages as well as chemoattractant factors and adhesion molecules. M-CSF induces scravenger receptor expression located on the macrophage surface as a result of increase in oxidized-LDL intake and foam cell formation (33).

In the present study, we planned to analyze some parameters which may serve as new biochemical markers for early diagnosis of AMI. We aimed to determine importance of some markers such as Cytokera-

tine 18 (CK-18), Heat Shock Protein 70 (HSP70), Heart Type Fatty Acid Binding Lipoprotein (H-FABP) and Oxidized Low Density Lipoprotein (Ox-LDL), the correlation between these markers and any possible superiority to CK-MB and Troponin I parameters.

MATERIAL AND METHOD:

The present study was performed by approval of Ethical Review Board for Clinical Researches of Necmettin Erbakan University, Meram Faculty of Medicine by approval number of 057 in 2010.

Groups were created by the blood samples collected from male patients between 45 and 65 years of age without any chronic disease such as chronic kidney failure, diabetes mellitus and any previous ischemic cardiac disease who have not received any hormone replacement therapy, are not smoker, had MI first with a troponin level higher than 0.04 ng/ml at referral. Bloo samples collected at referral to ER, for follow-up at 24th hour and in day 30 for control were grouped and included into the study. The blood samples collected for further examination at referral within first 6 hours following a chest pain were accepted as entry blood samples. Secondary blood samples were collected at 24th hour during hospitalization of the patients in intensive care unit of cardiology department. Furthermore, blood samples of day 30 were created from blood samples collected during control visits of the patients in the cardiology polyclinic.

Thirty healthy (in addition to the characteristics above, individuals with CK-MB and troponin I levels not higher than normal reference values) and volunteer males with similar ages with our patients were informed about the study and their blood samples were collected following their consent to create the healthy control group. The CK-MB and Troponin values routinely obtained from the patients referring to ER due to chest pain were used as comparative parameters with the parameters searched in the present study. The values within normal reference limits were enrolled as the volunteer control group. All patients who had AMI received the medications required for their treatments (aspirin, unfractionated heparin, IN nitroglycerine, beta blocker, ACE inhibitor, rtPA).

HSP70, CK-18, H-FABP and Ox-LDL parameters were measured from serum samples by ELISA method and CK-MB and Troponin I parameters were measured by chemiluminescence method.

1-CK-MB and Troponin I parameters were measured by Beckman Coulter DXI 800 device through Access CK-MB and Accu TnI kits.

2- CK-18 parameter was measured by ELISA method through M30-Apoptosense Peviva brand kits

(catalogue no:10010).

3- Ox-LDL parameter was measured by ELISA method through Immune Diagnostic brand kits

(catalogue no:K7810KO2)

4- H-FABP parameter was measured by ELISA method through Hycult brand kits (catalogue no:HK401).

5- HSP70 parameter was measured by ELISA method through Enzo brand kits (catalogue no:ADI-EKS-715).

Our data were evaluated by SPSS 13.0 program. A distribution analysis was performed to determine whether the data was parametric before determination of the statistical test. Mass distributions of the data were detected as not applicable to any distribution; therefore, use of non-parametric tests was planned. Kruskal-Wallis one-way analysis of variance was used to compare the differences within the groups. Mann-Whitney U test was used for binary comparisons. A p value below 0.05 was accepted as significant as a result of the statistical analysis.

FINDINGS

Mean ± standard deviation values of the findings for CK-18, HSP70, H-FABP, Ox-LDL parameters of the present study were presented in Table 1. The groups of the aforesaid parameters were as follows; group 1 (blood values at referral), group II (blood values at 24th hour), group III (blood values in day 30) and group IV (blood values of the control group).

Parameters	Group I	Group II	Group III	Group IV
N	40	40	40	30
CK-18 (U/L)	93.1 ±6.79	92.68±5.73	113.35±51.31	93.96±11.15
HSP70 (pg/ml)	0.73±0.57	0.40±0.44	0.48±0.63	0.03±0.06
H-FABP (pg/ml)	51.33±43.20	10.41±12.73	8.75±10.75	4.4±4.42
Ox-LDL (ng/ml)	810.63±660.05	370.45±540.58	440.22±550.09	120.94±60.58
cTnI (µgram/L)	18.07±24.53			
Ox-LDL (ng/ml)	72.82±67.13			

Table 1: Mean ± standard deviation values of CK-18, HSAP70, H-FABP, Ox-LDL parameters of the groups and cTnI and CK-MB parameters of Group 1

Table 1 indicates that mean and standard deviation values at 1st and 24th hour for CK-18 were very close to those detected in the control group whereas values in day 30 were very different from control values. Each of HSP-70 values at 1st and 24th hour and in day 30 differ from control values. Entry values of H-FABP were very different from control values whereas values at 24th hour and day 30 were closer to the control values when compared with the entry values. For Ox-LDL, all values detected at referral, 24th hour and in day 30 were quite different from the values of the control group.

Kruskal-Wallis test was performed for parameters of CK-18, HSP 70, H-FABP and Ox-LDL; and significant differences were detected between blood values at entry, 24th hour and day 30. For instance, a significant difference was detected for CK-18 values between blood analysis values at the entry, 24th hour and in day 30 (p=0.000>0.05); however, no information was gathered for how groups have changed. Therefore, the difference between group pairs formed for each parameter was evaluated by Mann-Whitney U test and presented in Table 2.

Accordingly, in Table 2: The difference between group I and II for HSP70, H-FABP and Ox-LDL parameters indicates a statistically significant difference. Groups I and III were different for all parameters. When group I and IV were compared, all three parameters other than CK-18, indicates a statistically significant difference. Groups II and III were different for CK-18 parameter. Groups II and IV were different for HSP70 and H-FABP parameters. Groups III and IV were different for all three parameters other than H-FABP.

Parameter		Group I-II	Group I-III	Group I-IV	Group II-III	Group II-IV	Group III-IV
CK-18	Asymp. Sig.	0.718 (p>0.05)	0.00 (p<0.001	0.626 (p>0.05)	0.000 (p<0.001)	0.43 (p>0.05)	0.001 (p=0.001)
HSP70	Asymp. Sig.	0.002 (p<0.05)	0.003 (p<0.05)	0.000 (p<0.001)	0.969 (p>0.05)	0.000 (p<0.001)	0.000 (p<0.001)
H-FABP	Asymp. Sig.	0.000 (p<0.001)	0.000 (p<0.001)	0.000 (p<0.001)	0.063 (p>0.05)	0.004 (p<0.05)	0.278 (p>0.05)
Ox- LDL	Asymp. Sig.	0.000 (p<0.001)	0.000 (p<0.001)	0.000 (p<0.001)	0.157 (p>0.05)	0.192 (p>0.05)	0.002 (p<0.05)

Table 2: Evaluation of the difference between group pairs formed for each parameter by Mann-Whitney U test

A ROC curve analysis was performed to confirm sensitivity and specificity of CK-18, HSP70, H-FABP, Ox-LDL, CK-MB and cTnI of our patient group within first 6 hours. As seen in Table 4, the area under the curve (AUC) for Ox-LDL, HSP70 and H-FABP of the patients referred within first 6 hours following onset of the symptoms were 0.98, 0.96 and 0.91 respectively. Such value for CK-18 was quite low with 0.48. Accordingly: Ox-LDL had the highest value and was followed by HSP70 and H-FABP. All these values are statistically significant for all parameters (p<0.05).

The end values of the control group were ignored to determine the cut-off values for HSP70, H-FABP and Ox-LDL parameters. The cut-off values for HSP70, H-FABP and Ox-LDL were 0.11 ng/ml, 10.99 ng/ml and 220.51 ng/ml, respectively. The sensitivity and specificity values presented in Table 3 were obtained for MI patients.

	Sensitivity (%)	Specificity (%)
Ox- LDL	97.5	86.7
HSP70	95	93
HFABP	77.5	76

Table 3: Sensitivity and Specificity values

DISCUSSION AND CONCLUSION

ACS is a major cause for mortality in adults and life saving results may be obtained in case of early intervention (34).

Hypertension (HT), hyperlipidemia, smoking, DM and extracardiac vascular diseases are major risk factors with a prognostic significance for those with suspicion of ACS. Absence of history of any disease and lack of classical risk factors were considered while creating the patient group of the present study. The purpose for this is to avoid influence of the parameters analyzed analyzed parameters by extra cardiac factors.

The best biochemical marker known to determine the severity of cardiac injury and ischemia is cTnI. Higher levels of cTnI indicates necrotic sites on the myocardial tissue. The apopytosis paths are activated as a result of ischemic damage. Since the apopytosis markers were considered to be aveluated better in the present study, the patients with higher TroponinI levels were included into the study.

The normal plasma and serum levels for the analyzed parameters are dependent to the method and test (37). Normal ranges of CK-18, HSP70, H-FABP and Ox-LDL were determined as 83-120 U/L, 0.01-0,1 ng/ml, 1.2-10.2 ng/ml, 50-210 ng/ml, respectively in the present study. Sensitivity and specificity values of these parameters were calculated for AMI according to the aforesaid values; furthermore, such values were compared with each other as well as sensitivity and specificity values of cTnI and CK-MB which are ordinary cardiac markers. Normal range for TroponinI was detected between 0.01 and 0.04 ng/ml and the cut-off value known was 0.5 ng/ml. Normal range for CK-MB was determined between 0.3 and 6.3 ng/ml and the cut-off value was 8.6 ng/ml. The principal for sensitivity and specificity analysis is dependent to the cut-off values detected (38). The end values of the control group was ignored while determining cut-off values for HSP70, H-FABP and Ox-LDL parameters; and cut-off values for HSP70, H-FABP and Ox-LDL were determined as 0.11 ng/ml, 10.99 ng/ml and 220.51 ng/ml, respectively. Accordingly, sensitivity and specificity values for HSP70, H-FABP and Ox-LDL were 95%, 77.5 and 97.5%, respectively and 93%, 76% and 86.7%, respectively.

A ROC curve analysis was performed to compare the diagnostic accuracy between the parameters. The area under the curve (AUC) for Ox-LDL and HSP70 of the patients referred within first 6 hours following onset of the symptoms were 0.98 and 0.96. The AUC for H-FABP was 0.91, which had a higher level. Such value for CK-18 was quite low with 0.48. Accordingly: Ox-LDL had the highest value and was followed by HSP70 and H-FABP. All these values are statistically significant for all parameters ($p<0.05$). Within the frame of these results, it may be suggested that Ox-LDL, HSP70 and H-FABP have a diagnostic accuracy for diagnosis of myocardial injury within 6 hours. Same suggestions cannot be made for CK-18 according to the results of the statistical analysis.

Lukasz Figiell et al. obtained a higher sensitivity for H-FABP by 84% than cTnT by 50% in their study performed with 52 patients with a suspicion of AMI (39). Decrease in sensitivity was observed as onset period of the symptoms passed. Sensitivity and specificity values calculated in the present study for H-FABP were 77.5%, 76%, respectively; and for cTnI were 100%, 100%, respectively. The area under the curve for CK-MB and Troponin I in the patient group was 1 for both parameters. The reason for that is considered as selection of the patients with excessively elevated troponin and CK-MB values at referral to ER. From the view of patient profile, majority of the patients deferred or were referred from another centers. The outcomes of the present study were based on the levels within 3 to 6 hours following onset of the symptoms. Besides, both parameters reach up to peak levels within 3 to 8 hours (40). However, if blood level of these parameters would have been analyzed within 1 to 2 hours following the symptoms, early detection of H-FABP would have been more likely than other cardiac markers. Luaksz Figiell et al. performed H-FABP measurements through a bedside test; such measurement was performed through Elisa method in the present study. More sensitivity on reliability of our method seems advantageous; however, longer analysis period is disadvantageous. H-FABP increases by 18-fold after several hours following AMI whereas myoglobin increases by 8-fold and cTnT increases by 2-fold. H-FABP and cTnT presented an increase at peak level by 30 to 40-fold; and myoglobin had an increase by 15-fold than normal at peak level. Such comparisons indicate that H-FABP is more sensitive than myoglobin and cTnT for determination of myocardial cell necrosis earlier (41, 42).

Murat Orak et al. analyzed H-FABP from blood samples of the patient who have referred to ER because of chest pain, compared the results with routine cardiac markers and found sensitivity of H-FABP superior to CK-MB and Troponin I by a rate of 98% (43).

Jun Liao et al. showed that blood level of H-FABP increased earlier than blood level of cTnI for the patients who have referred ER because of chest pain and were diagnosed with MI (44).

Use of novel techniques (point of care) that enable measurement of H-FABP within a shorter period would be more appropriate. The Point-of-Care (POC) based on cromotographic immunoassay method provides a result within 15 minutes and eliminates the disadvantage of analysis period. Hafidh A Alhadi and Keith A Fox (45) performed a study on 101 patients with ACS; sensitivity of H-FABP level in serum samples collected within the first hour following onset of the symptoms was found 79.9% which was >62% higher than other cardiac markers. The sensitivity rate of 98% in second hour is advantageous than other cardiac markers for early diagnosis of the patients with acute MI.

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Reference	Number of the patients	Patients with AMI (%)	Onset of The symptoms (hour)	Area under ROC curve H-FABP
Ishii et al. 1997 (22)	165	60	3-12 (mean 3.5)	0.898
Glatz et al.1997 (46)	312	54	1.5-8 (mean3.3)	0.901
Okamato et al. 2000 (47)	189	74	0-12 (mean 4.0)	0.921
Ghani et al. 2000 (48)	460	220	3-7 (mean 5.0)	0.80

Table 4: The studies addressing diagnostic performance of H-FABP in AMI

We found the area under the curve in ROC curves 0.921 for H-FABP for the patients who referred within 0 and 12 hours (3 to 4 hours in average) following onset of the patients; such outcome was consistent with majority of the studies mentioned above. In these patient groups, the H-FABP measurements performed at 24th hour were above the levels detected in the control group. Such values were quite lower when compared with the values at referral. There was not any significant difference in comparison of the blood samples collected from the patients and from the control group in day 30.

The main limiting factor of the present study was to what extent the H-FABP value (10.99 ng/ml) that we based on would correspond to exact cut-off value. We believe that such limitation would be prevailing for other studies and this would be resolved by determination of exact cut-off value with larger patient series.

Many researchers revealed that Ox-LDL is a very important risk factor for AMI and our findings complied with this suggestion. The Ox-LDL levels were statistically and significantly higher in the patients who had AMI when compared with the control group (49, 50).

Levels of Ox-LDL at referral and day 30 were found statistically and significantly higher than the control group of the present study. Sensitivity and specificity of Ox-LDL were quite higher in the patient group who referred to ER within 0 and 6 hours following onset of the symptoms by 97.5% and 86.7%, respectively. Although analysis levels of the patients were higher at 24th hour, such elevation was not statistically significant.

We may suggest that the cause for decrease in Ox-LDL levels at 24th hour was efficiency of medical or surgical treatment performed for reperfusion. The Ox-LDL levels in serum samples of this patient group during control visit in day 30 were observed as re-elevated and such re-elevation was statistically significant. These levels detected in day 30 were very lower than the levels at referral during heart attack. We believe that the aforesaid elevation in blood analysis would be caused by return of the patients to their old life habits, noncompliance to timing and dosage of the medical treatment, sedentary lifestyle, bad nutritional habits. Furthermore, lipid-lowering drugs (i.e. statins) are added into medical therapy regimens of the patient after MI; elevated levels of Ox-LDL despite these drugs show that other lipid parameters are important risk factors. Accordingly, we consider that antioxidant therapy should be added into lipid-lowering therapy in the patients with coronary artery disease.

Frederikson et al (51) showed higher Ox-LDL/cholesterol ratio in the cases who had acute myocardial infarction

Shimada et al. (49) found higher Ox-LDL levels in cardiac events such as cardiac death, non-fatal myocardial infarction or coronary artery bypass graft.

We consider that Ox-LDL has a diagnostic qualification for diagnosis of AMI; however AMI is an urgent condition. The present study performed through Elisa method provides results after a longer period; to conclude the diagnosis with test methods providing results within a shorter period are essential for these patients.

AMI is one of the conditions with most oxidative stress. We searched whether serum level of HSP70 would reveal any diagnostic significance for the patients with AMI.

When blood sample analyses of the patients who have referred to ER within 0 to 6 hours from onset of the chest pain at referral, 24th hour and 30th day were reviewed, all HSP70 levels were observed significantly higher than the control group. Levels at referral were about two-fold higher than the levels at 24th hour and 30th day. Sensitivity and specificity of HSP70 were quite higher in the patient group who referred to ER within 0 and 6 hours following onset of the symptoms by 95% and 93%, respectively. Higher blood serum levels at 24th hour and 30th day indicates that HSP70 would not be sufficient solely to diagnose AMI.

HSP70 levels rapidly decreased in patients who had AMI after 1 to 7 days whereas anti-HSP70 antibody levels increased during the aforesaid period. These findings suggest that higher HSP70 levels or lower anti-HSP70 antibody levels were independently associated with higher risk of AMI. Such risk increases more in combination of higher HSP70 levels and lower anti-HSP70 antibody levels (52).

B Dybdahl et al. analyzed serum HSP70 levels from blood samples collected from the patients who had AMI at referral, 6th hour and in the next morning and detected HSP70 concentrations 686, 868 and 607 pg/ml, respectively (53). All the concentrations above were significantly higher than the reference values created by the control group. Such study concluded that HSP70 might be secreted rapidly to the circulation following AMI, used as a marker for myocardial injury and have a role in inflammatory response following AMI.

Levels of HSP70 in the studies were consistent with findings of the present study; however, serum levels of HSP70 rapidly decreases during following hours and days, but could not reach up to normal reference range for many of the patients even in day 30. We believe that further studies are required to determine the duration for decrease within reference range.

Existence of CK-18 in cardiac microvascular endothelial cells as well as vascular smooth muscle cells was reported and CK-18 is considered as a potential biomarker for cardiovascular diseases (16).

Bialik et al. (54) showed in a study performed both on animals and humand that apoptosis appeared in about 5 to 30% of the cells in the infarction site during acute coronary syndrome.

Mattey et al. (11) performed a study to determine whether the antibody levels increased against CK-18 in the patients with rheumathodi arthritis (RA) was associated with ischemic heart disease (IHD) and reported that the IgG type antibody levels against CK18 were higher in RA patients with IHD when compared with those without IHD.

The outcomes of the present study showed that the CK-18 levels measured in the patients with AMI at referral and 24th hour were similar to those detected in the control group without any statistical significance. Serum CK-18 levels of the patient group in day 30 were higher than the control group with a statistical significance.

Senturk et al. conducted a study on 74 patients with ACS and analyzed CK-18 levels at referral to ER, 24th hour and 48th hour following onset of the symptoms; and found CK-18 levels at referral and 48th hour similar with the control group whereas levels at 24th hour were statistically and significantly higher than the control group. They concluded that increase in M30 level at 24th hour indicates an early apoptosis on endothelial and/or smooth muscle tissue following AMI. Levels detected at referral of the study above were similar with the present study; difference of the present study was absence of any difference between levels at 24th hour and levels of the control group (55). The patient group profile created by Senrutk et al. differed from our patient group with some risk factors that may affect the apoptosis (33 smokers, 20 patients with systemic diseases such as diabetes, 44 patients with hypertension).

Adlbrecht et al. (12) performed a study on 110 patients depending on the suggestion that systemic inflammation and apoptosis plays a major role in the patients with acute coronary syndrome including myocardial infarction. Coronary artery blood samples of the patients who had MI were collected through thromboctemy devices (X-sizer). CK-18 was measured through ELISA method in the MI group and compared with cTnT and CK which are accepted markers. The area under the curve in ROC curve analysis for CK-18, cTnT and CK was found 0.925, 0.62, 0.858, respectively. Levels of CK-18 of the blood samples collected from the coronary obstruction sites of the cases who had AMI were found significantly higher than levels of the blood samples collected from peripheral vessels. The CK-18 was suggested as a superior marker for myocarial injury when compared with cTnT and CK.

The value found for CK-18 was very low by 0.48 in ROC curves of the patients who referred wthin 0 to 6 hours following onset of the symptoms. Adlbrecht et al. collected blood samples from the coronary obstruction sites of the patients and found the levels higher than peripheral blood samples.

In the present study, CK-18 levels were higher and statistically significant in day 30. We could not detect any study for CK-18 within the period above. Endothelial dysfunction, microvascular obstruction caused by the plateles, edema and oxidative injury following ischemia-reperfusion cause microvascular dycfunction. We believe that apoptosis developed in microvascular endothelial tissue during such period and caused an increase in CK-18 levels.

Consequently;

a) it was observed that H-FABP concentration could be detected during acute phase following onset of the symptoms in the cases who had AMI, had a diagnostic accuracy for early diagnosis of AMI and served as a good alternative to conventional cardiac markers. Use of novel techniques (point of care) that enable measurement of H-FABP within a shorter period would be more appropriate.

b) it was determined that Ox-LDL is a significant risk factor for AMI and early diagnosis of MI may be achieved by detection of Ox-LDL in the peripheral blood sample.

c) it was concluded that antioxidant therapy may be necessary in addition to lipid lowering therapy after MI.

d) determination of blood levels of HSP70 increases the diagnostic accuracy with acknowledged cardiac markers for diagnosis of MI; however such marker is not sufficient solely.

e) it was deduced that CK-18 is secreted from microvascular endotheli-

al tissue and blood serum levels are detectable after ischemia-reperfusion; however it is not sufficient to indicate the diagnostic accuracy for early diagnosis of AMI.

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Conflict of Interest
The author declares that he has no conflict of interest