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The value of heat shock protein (HSP) 60 on in-hospital and short-term prognosis in patients with acute ST segment elevation myocardial infarction

Akut ST elevasyonlu miyokard infaktüsünde ısı şok proteini 60'ın hastane içi ve kısa dönem prognostik değeri

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Abstract

Aim: HSP60 has previously been shown to be an independent risk factor for congestive heart failure. Recently, increased HSP60 values have been shown to be associated with increased mortality in patients hospitalized with acute heart failure. The purpose of our study was to determine whether HSP 60 is an independent predictor of in-hospital or short-term mortality in patients with acute ST segment elevation myocardial infarction (STEMI) and to assess whether it may be used as an early predictor of in-hospital heart failure or cardiogenic shock development in the same population.

Methods: A total of 221 patients over 18 years of age presenting within 12 hours after the onset of symptoms with STEMI between 2011 and 2013 were enrolled to our study. Our study was designed as a prospective cohort. Several clinical conditions, such as clinical heart failure, development of cardiogenic shock and death during hospitalization were recorded together with the data pertaining to the length of hospital stay, MI localization, revascularization strategy and risk factors associated with coronary heart disease. Patients were called 1 month after discharge to find out if anyone had died.

Results: HSP 60 values were significantly higher in patients who died during hospitalization (P=0.004) or within 1 month after discharge (P=0.020). These results were independent from other factors associated with mortality following STEMI. Significant association was found between HSP60 value and in-hospital development of cardiogenic shock (P=0.03) or heart failure (P=0.03).

Conclusion: HSP60 was found to be independently associated with increased mortality in patients admitted for STEMI and therefore may be used as a promising predictor of mortality.

Keywords: Myocardial infarction, HSP60, Prognosis, Inflammation, Atherosclerosis

Öz

Amaç: HSP60'ın daha önce konjestif kalp yetmezliği için bağımsız bir risk faktörü olduğu gösterilmiştir. Son zamanlarda artan HSP60 değerinin mortalite ile ilişkili olduğu gösterilmiştir. Çalışmanın amacı HSP 60'ın akut ST segment yükselmeli miyokard enfarktüslü (STEMI) hastalarda bağımsız bir hastane içi veya kısa süreli mortalite belirleyicisi olup olmadığını belirlemek ve aynı popülasyonda kardiyojenik şok gelişimi ve hastanede kalp yetmezliğinin erken bir belirleyicisi olarak kullanılıp kullanılmayacağını değerlendirmektir.

Yöntemler: Çalışmamıza 2011-2013 yılları arasında STEMI semptomlarının başlamasından sonraki 12 saat içinde başvuran 18 yaş üstü 221 hasta alındı. Çalışmamız prospektif kohort olarak dizayn edildi. Klinik kalp yetmezliği, kardiyojenik şok gelişimi ve hastanede yatış sırasındaki ölüm gibi çeşitli klinik durumlar, hastanede kalış süresi, MI lokalizasyonu, revaskülarizasyon stratejisi ve koroner kalp hastalığı ile ilişkili risk faktörleri ile ilgili verilerle birlikte kaydedildi. Hastaların taburcu olduktan 1 ay sonra mortalite açısından telefonla aranarak bilgi alındı.

Bulgular: Hastanede yatış sırasında ölen hastalarda (P=0,004) veya taburcu olduktan sonraki 1 ay içinde HSP 60 değerleri anlamlı olarak daha yüksekti (P=0,020). Bu sonuçlar STEMI sonrası mortalite ile ilişkili diğer faktörlerden bağımsızdı. HSP 60 değeri ile hastanede kardiyojenik şok (P=0,03) veya kalp yetmezliği (P=0,03) arasında anlamlı bir ilişki izlendi.

Sonuç: HSP60'ın STEMI ile başvuran hastalarda artmış mortalite ile bağımsız olarak ilişkili olduğu ve bu nedenle umut verici bir mortalite belirleyicisi olarak kullanılabileceği düşünülebilir.

Anahtar kelimeler: Miyokard enfarktüsü, HSP60, Prognoz, İnflamasyon, Ateroskleroz

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Introduction

Atherosclerosis is a structural disease of medium-sized and large arteries, which commences as an endothelial dysfunction in the intima and media layers in its early phases, then transforms into true atherosclerotic plaque formations in its further phases. It begins to affect the arteries during the early stages of bodily development, and follows a continuous and uninterrupted pattern through a lifespan [1]. Among the most commonly affected arteries are coronary arteries, aorta, ilio-femoral arteries and carotid arteries. It may also affect, albeit less frequently, the intracranial arteries.

Development of atherosclerosis is multifactorial. Inflammation has been known to play role in every stage of atherosclerosis, as well as plaque rupture and thrombus formation [2]. Inception of the disease relies upon the interaction of the immune mechanisms with metabolic risk factors. Once initiated, the disease shows progression in various rates. Moreover, disrupted endothelial structure contributes further to the progression of the disease.

Cells begin to synthesize some proteins known as heat shock proteins upon exposure to sudden increase in temperature, anoxia, reactive oxygen species and changing glucose levels. The heat shock proteins (HSP) are subgrouped according to their molecular weights (HSP 10, 40, 60, 70, 90, 110, small HSP). HSPs are secreted from cytosol, mitochondria, endoplasmic reticulum and cell nucleus [3-6]. Moreover, importance of the HSPs relies on their ability to interact with other proteins, thereby modifying both their structure and function. HSPs exert strong cytoprotective effect and behave as chaperons for other cellular proteins. They also resist aggregation of the proteins during their folding, disassembly and exposure to stress, like high temperature. Many HSPs are synthesized and secreted into the cytosol in response to stress.

HSP 60 is found in high amounts in the vicinity of atherosclerotic areas, despite their absence in non-atherosclerotic vessel segments within the same vascular lumen. Vascular endothelial cells do not secrete HSP60 under physiological circumstances. Upon encountering any stress induced by classical atherosclerotic risk factors, however, they begin to secrete HSP60 and some other adhesion molecules simultaneously, thus triggering both cellular and humoral immunity which further causes intimal layer of the vessel to be infiltrated by mononuclear cells. This reversible process may convert into an irreversible one, should the atherosclerotic risk factors still persist [7]. Plasma HSP 60 level was found to be elevated in patients with increased carotid intima-media thickness, independently of such other atherosclerotic risk factors as gender and age [8]. HSP 60 and HSP 70 plasma levels were reported to be significantly elevated in patients with borderline hypertension, which was considered to be associated with premature atherosclerosis [9]. Major cardiovascular events were observed in greater frequency in patients with higher plasma level of HSP 65, a member of HSP 60 family. The afore-mentioned findings led the physicians to extrapolate that plasma HSP 60 level could have a prognostic value on the morbidity and mortality related to atherosclerosis.

In the light of the afore-mentioned premises, we intended to inquire the in-hospital and 30-day prognostic importance of HSP 60 in patients with acute ST-segment elevation myocardial infarction (STEMI).

Materials and methods

Patient selection

Patients older than 18 years of age who were admitted to the emergency department with acute STEMI within 12 hour of the onset of symptoms between 2011 and 2013 were included in the study. Our study was designed as a prospective cohort. After excluding those with any co-existing active infection or inflammation, autoimmune connective tissue disorder and malignancy, which are among the clinical states that may have been implicated in higher baseline HSP60 levels measured on admission, a total of 221 patients were considered to be appropriate for study enrollment. All the patients included in the study were interrogated with regards to age, gender, risk factors for atherosclerosis, any previous history of ischemic diseases, myocardial infarction (MI) or percutaneous coronary intervention, and medications used. Informed consent was obtained from every participants and the local ethics committee (Selçuk University Ethics Committee) approved our study. (Approval Number:2011-50, Date:31.05.2011)

Biochemical analysis

10 ml of blood sample was collected into plain tubes from the patients admitted to the emergency department with acute STEMI within 12 hours from onset of the symptoms. Respective supernatants obtained from the blood samples through centrifugation at 3000 ppm for 5 minutes were transferred to Eppendorf tubes and were frozen at -80°C until the time of assay. The analyses for HSP 60 were performed through Enzyme-Linked Immunosorbent Assay (ELISA) method using commercial kit in Rayto-2100C Microplate Reader (India) and expressed in ng/mL.

Medications and devices used

Patients considered appropriate for percutaneous coronary intervention received 300 mg acetylsalicylic acid and 600 mg loading dose of clopidogrel. The other medications were used in compliance with the relevant guideline of STEMI management [10].Right femoral artery was selected as the main site of entry for the angiographic procedure. In patients deemed to have high thrombus burden, a loading of 25 μg/kg tirofiban hydrochloride as glycoprotein IIb-IIIa antagonist was administered intracoronarily in 3 minutes, followed by a constant intravenous infusion at a rate of 0.15μg/kg/min for 18 hours. The patients in whom fibrinolytic therapy was performed received 300 mg acetylsalicylic acid and 300 mg loading dose of clopidogrel. However, 75 mg oral clopidogrel was administered without any loading dose in patients older than 75 years of age. Successful reperfusion of myocardium was decided to be achieved if the patient was rendered asymptomatic and there appeared to be 70% or more resolution in relevant ST segments in the ECG strip compared to baseline ECG.

All patients received high dose of statin (atorvastatin 40-80 mg and rosuvastatin 20-40 mg, preferably), beta-blocker treatment (except those with manifest cardiac decompensation and/or with any contraindication for beta blocker use),

angiotensin-converting enzyme (ACE) inhibitor or angiotensin-II receptor blocker (ARB), and maintenance doses of 100 mg acetylsalicylic acid and 75 mg clopidogrel. Primary percutaneous coronary interventions were performed by experienced interventional cardiologists (more than 75 PCI cases/year), using standard methods in Toshiba Infinix DFP-8000D interventional angiography system. Echocardiographic evaluations were conducted using Toshiba Aplio XV echocardiography device.

End points

Primary end point: Analysis of predictive value of HSP 60 in in-hospital and 30-day mortality after acute STEMI.

Secondary end point: Analysis of predictive value of HSP 60 in in-hospital development of cardiogenic shock and heart failure, and length of hospital stay.

In-Hospital and short-term follow-up of the patients

Data regarding in-hospital end-points of the patients was obtained either through interview with the patients or through interrogation of their hospital records. In order to gather data regarding first-month follow-up, on the other hand, the patients were summoned to out-patient clinic via phone call. For those unable to attend to out-patient clinic visit, relevant data was obtained via direct phone call either with the patients themselves or one of his/her relatives. Furthermore, hospital records of the patients, if any, who presented to the hospital within one month after hospital discharge were also utilized in this regard.

Statistical analysis

SPSS (Statistical Package for Social Sciences) for Windows 15.0 software was utilized in the statistical analysis of the study findings. Numbers, percentage, mean (SD), median, minimum (min), maximum (max) and 25-75 percentiles were used for the descriptive statistics. Z test and Kruskal-Wallis tests were used for the comparison of quantitative data. Chi-square test, on the other hand, was used for the comparison of qualitative data. The parameters found to be significant were further assessed using logistic-regression analysis so as to determine the predictors of mortality. In this regard, such variables as age, length of hospital stay, HSP 60 value and development of cardiogenic shock were involved in the logistic regression model as independent predictor, as they provided significant contribution to the model in the univariate analysis (P<0.05). The other variables lacking any significant contribution (P>0.05), on the other hand, were not included in the logistic regression model. Furthermore, cut-off value of HSP60 level was determined by using ROC curve analysis. P-values <0.05 was regarded as statistically significant.

Results

Clinical and demographic characteristics of the patients

The ages of all 221 patients ranged between 25 and 89 years. Mean age was found to be 59.8(4.6) years. Of all patients, 176 (80%) were male, while 45 (20%) were female. The mean ages of the males and the females were 57.69 and 68.57 years, respectively. Respective incidences of hypertension, diabetes mellitus, hyperlipidemia and history of smoking were 40.5%, 18.6%, 13.2% and 70.9%. 85% of the patients was admitted with Killip class 1. GpIIb/IIIa antagonistic agent (tirofiban) was used in 33.2% of the patients.

There was no relationship between HSP60 value and such personal demographic characteristics as gender, hypertension, hyperlipidemia, diabetes mellitus and smoking (Table 1).

Primary end-point was observed in 30 (13.6%) out of all 221 patients included in the study, most of which occurring as in-hospital mortality (21 patients) (Table 2).HSP60 value was measured to be greater in patients in whom in-hospital or one-month mortality occurred (for one-Month mortality P=0.020 and for In-Hospital mortality P=0.004).

Logistic regression analysis revealed that mortality was associated with age, length of hospitalization, development of cardiogenic shock and HSP60 value (P<0.05).Accordingly, age, development of cardiogenic shock and greater HSP60 value were found to be positively correlated with mortality, while length of hospital stay being found to be negatively correlated with mortality. Furthermore, on the basis of odd ratios, every increase of 1 year of age was related to 1.16 time increased risk of death (Table 3).

HSP60 value was significantly increased in patients developing cardiogenic shock and in those with in-hospital heart failure (Table 4).

Table 1: The relationship between HSP60 value and risk factors of coronary arterial disease

Risk factors of coronary arterial disease		(n)	HSP60 Median (IQR 25-75)	Statistical analysis Z	*P*-value
Gender	Female	176	6.075 (4.455-8.665)	1.811	0.070
	Male	45	7.110 (4.820-11.710)		
Hypertension	No	131	6.360 (4.510-9.360)	0.124	0.901
	Yes	90	6.450 (4.660-9.080)		
Hyperlipidemia	No	192	6.450 (4.510-9.360)	0.159	0.874
	Yes	29	6.230 (4.890-8.800)		
Diabetes Mellitus	No	179	6.540 (4.660-9.360)	-1.193	0.233
	Yes	42	5.770 (4.220-8.420)		
Smoking	No	65	6.590 (4.660-10.370)	-0.412	0.680
	Yes	156	6.185 (4.530-9.165)		

Table 2: Relationship between mortality and HSP60 value

Mortality		(n)	Median HSP60 (ng/mL) (IQR 25-75)	Statistical analysis Z	*P*-value
In-Hospital Mortality	No	200	6.230 (4.510-8.690)	2.853	0.004
	Yes	21	8.720 (5.750-20.820)		
One-Month Mortality	No	185	6.140 (4.510-8.580)	2.325	0.020
	Yes	9	21.880 (6.590-45.070)		

Table 3: Depiction of the results from the logistic regression analysis

Variables	B	Wald	*P*-value	*Odds*	95 % Confidence Interval for Odds Minimum	Maximum
Age	0.149	23.116	<0.001	1.161	1.093	1.234
Length of Hospital Stay	-0.457	5.479	0.019	0.633	0.432	0.928
HSP60 value	0.055	8.750	0.003	1.056	1.019	1.095
Development of Cardiogenic Shock	4.865	12.712	<0.001	129.719	8.943	1881.658
Constant	-11.549	23.352	<0.001	0.000		

Table 4: Relationship between HSP60 value and in-hospital cardiogenic shock or in-hospital heart failure

		(n)	Median HSP60 value (ng/mL) (IQR 25-75)	Statistical analysis *Z*	*P*-value
In-Hospital Cardiogenic Shock	No	211	6.270 (4.510-9.250)	1.637	0.021
	Yes	10	7.650 (5.750-22.100)		
In-Hospital Heart Failure	No	131	6.450 (4.330-9.080)	1.101	0.033
	Yes	90	6.405 (4.890-10.370)		

Determination of HSP60 cut-off point

ROC curve analysis was utilized in an attempt to define any potential cut-off point of HSP60 in the determination of mortality, which yielded a cut-off point >7.325 ng/mLvalue with sensitivity of 67.7% and specificity of 66.8% (AUC: 0.70; *P*<0.001) (Figure 1). In addition, this cut-off was analyzed through Chi-Square test to evaluate further its accuracy which showed that HSP60 in 127 out of 191 patients in whom mortality did not occur was lower than the cut-off value. The HSP60 value in 20 out of 30 patients in whom mortality occurred, on the other hand, can be seen to be greater than the cut-off value. Chi-square test also yielded a significant relationship between HSP60 cut-off value and mortality (*P*<0.001). The accuracy of predicting patients in whom mortality did not occur was 97.4% and that of the patients in whom mortality occurred was 56.7%. Overall accuracy of prediction was 91.8%.

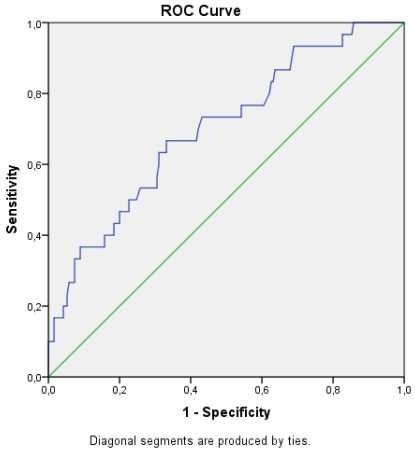

Figure 1: ROC curve analysis for HSP60 values in the determination of mortality (a cut-off point >7.325 ng/mL value with sensitivity of 67.7% and specificity of 66.8%, AUC: 0.70; *P*<0.001)

Discussion

HSP60 is a nuclear-encoded protein which is predominantly found in the mitochondria. In addition to the widely accepted definition that it is an intracellular molecule, HSP60 has also been known to be secreted from cells and can be present in the peripheral blood of healthy individuals [11,12]. Extra cellular HSPs are one of the most powerful ways of sending a "danger signal'' to the immune system and thereby triggering an immune response [13,14]. HSPs function in cell cycle control, cell proliferation, development, organization of the cyto-architecture, and regulation of cell death and survival [15,16]. It is not surprising therefore that HSPs possess a pivotal role in the development of atherosclerosis [17].

Seroepidemiologic studies also point out to the role of circulating HSP60s in the development and progression of atherosclerosis [8,9]. Atherosclerosis has been accepted as an inflammatory process and previous studies reported a significant relationship between atherosclerosis and the inflammation markers (such as CRP) and infections with *Chlamydia pneumoniae*, *cytomegalovirus (CMV)* and *Helicobacter pylori* [18]. Minor relationship was reported between the inflammation markers of CRP and HSP60 in a study by Xiao et al [19].

Compatible with the findings by Xiao et al. [12] study by Zhang et al. reported no correlation between CRP and HSP60. The findings from these studies might translate into the possibility that CRP and HSP60 may exert their atherosclerosis-triggering effect through distinct mechanisms. Previous studies reported that high levels of HSP60 could stimulate the expression and release of respective pro-inflammatory mediators and vascular adhesion molecules from the myeloid cells and the endothelial cells. Accordingly, these secreted molecules, together with the immune responses they initiated, were reported to have been implicated directly in the pathogenesis of atherosclerosis [20,21].

A prospective study in 2008 by Zhang et al. [14] which compared the risk of coronary arterial(CAD) disease development between individuals with very high level of HSP 60 (> 1000 ng/ml) and those with low level of HSP60 reported a positive and strong correlation between high levels of HSP60 and risk of CAD development, independently of such traditional risk factors of CAD development as age, gender, smoking status, body-mass index, hypertension, diabetes mellitus and positive family history of premature coronary arterial disease AMI was also shown to induce the secretion of HSP60.

It was reported in previous studies that human HSP60 (hHSP60) and HSP60 secreted from *C.pneumoniae* were co-localized in the atherosclerotic plaques and both play a pivotal role chronic bacterial and viral infection of the plaques [22]. Many other studies conducted on cardiovascular diseases reported the presence of antibodies against HSP60 and such pathogens as *C.pneumoniae*, *H. pylori* and *CMV* along with microbial/human HSP60 [23].

Biasucci et al. [24] suggested *C.pneumoniae*-HSP60 antibodies as specific markers in acute coronary syndromes. Burian et al. [25] reported that high level of antibodies against hHSP60 and *C.pneumoniae* were independent risk factors for CAD. Considering such a relationship between CAD development and anti-HSP60 antibody level, it would be rational to assume that high levels of HSP60 together with high levels of anti-HSP60 antibody might reflect a over-activated immunity and exacerbated atherosclerotic process [18,26]. Addition of HSP60 and anti-HSP60 levels to such well-known CAD risk factors as smoking, hypertension and diabetes mellitus act as a booster in the development of CAD. In a prospective study, HSP60 level was reported to show a transient increase in blood circulation following STEMI, the reason for which was proposed to be cardiomyocyte necrosis and accompanying endothelial dysfunction [27]. In this regard, Schett et al. [28] also reported abundant HSP60 secretion into circulation as a result of experimentally-induced myocardial ischemia and necrosis in rat models. Shear-stress related to STEMI is also likely to be another contributor of high levels of HSP60 [29].

In a study conducted on 826 patients and investigating the relationship between HSP60 level and carotid atherosclerosis, serum HSP60 level was found to be elevated in patients with increased carotid intima-media thickness, independently of other traditional atherosclerosis risk factors [10]. Similarly, in a study by Pockley et al. [11] comparing HSP60 and HSP70 levels between healthy individuals those with borderline hypertension, HSP60 and HSP70 levels were found to be significantly increased in the individuals with borderline hypertension and this was considered to have a possible relationship with early atherosclerosis.

In another study including 79 patients with documented history of CAD, incidence of major cardiovascular event development was increased in patients with high level of HSP65, a member of HSP60 family. All these findings direct clinicians to infer that increased serum HSP60 level may have a prognostic importance on morbidity and mortality in patients with atherosclerosis.

In a recent prospective study conducted by Bonanad et al. [30] patients presenting with acute heart failure were assigned into 3 groups according to the HSP60 levels on admission and followed-up for 7 months. The incidence of death and re-admission to the hospital for acute cardiac decompensation was statistically higher in the group of high HSP60 level. Therefore, HSP60 level was suggested to have a prognostic significance on patients with heart failure

Our study showed that HSP60 level had a prognostic significance in the prediction of mortality. Especially the cut-off value in our study was found to possess a high accuracy in the prediction of mortality. We believe that patients at high risk of in-hospital mortality may be successfully predicted by measuring HSP60 level on admission so that these high-risk patients can be closely followed-up to abate further the mortality rates. As for the secondary end point of our study, we also found a significant relationship between admission HSP60 level and in-hospital development of heart failure or cardiogenic shock. To the best of our knowledge, our study is the first in this regard.

Limitations

Our study should be assessed together with some limitations. First, our study population is relatively small and studies conducted on larger groups are warranted to verify our results. Secondly, although our in-hospital and 30-day mortality rates (9.5% and 13.6%, respectively) seems higher than expected, we consider that this relatively greater mortality rates could be attributable to the fact that our hospital operates as a tertiary center and hence receive a great number of patients in diverse clinical conditions.

Conclusion

Our study showed that HSP60 possess a prognostic significance in the prediction of in-hospital and 30-day mortality in patients presenting with acute STEMI within 12 hours of onset symptom onset. However, the fact that our study recruited a relatively small group of patients may hinder true interpretation of our findings. Hence, future studies including larger groups of patients are needed to confirm our results.

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