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Changes in Plasma Level of Heat Shock Protein 27 After Acute Coronary Syndrome

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Abstract

We assessed the association between serum heat shock protein 27 (Hsp-27)concentrations in patients with acute coronary syndrome (ACS) and compared them with healthy participants. Patients with ACS (n = 75) were recruited and their biochemical parameters were compared with 75 healthy participants. Heat shock protein 27 concentrations were measured from blood samples taken on admission and 12 hours after the onset of chest pain. In the patient group, Hsp-27 concentrations (31.62 [20.12-38.51] ng/mL) in the first blood samples were significantly ($P < .001$) higher than in control samples (20.12 [16.67-28.17] ng/mL). In patients, serum Hsp-27 levels on admission were significantly ($P < .001$) higher than for the samples collected 12 hours after the onset of chest pain (25.87 [15.52-31.62]); the latter did not differ significantly from samples of healthy controls. In conclusion, serum Hsp-27 concentrations are elevated in the early hours following ACS, but fall to levels near to those in healthy individuals after about 12 hours from the onset of chest pain.

Keywords

heat shock protein 27, acute coronary syndrome, coronary artery disease

Introduction

Acute coronary syndrome (ACS) encompasses a spectrum of coronary artery diseases (CADs), ranging from unstable angina to non-ST-elevation and ST-elevation myocardial infarction (MI). Acute coronary syndrome is often the first presentation of CAD and also is the principal cause of mortality and morbidity in many parts of the world.[1] The major cause of CAD has been attributed to atherosclerosis which is a multifactorial and chronic disease. Over the last few decades, some of the risk factors for progression of atherosclerosis have been identified, such as hypertension and dyslipidemia. But several emerging risk factors for cardiovascular disease have been reported including oxidative stress, inflammation, and autoimmunity.[2]

Heat shock proteins (Hsps) are highly conserved families of proteins found in the cells of all organisms. Several Hsps are known to function as molecular chaperones. However, in addition to their roles as molecular chaperones, there have other putative roles especially in cardiovascular tissue.[3-5] Heat shock proteins may stimulate autoimmune responses, causing the production of antibody against them. This notion has been proposed by Wick et al[6] who hypothesized that an immune response to Hsps, either endogenously derived from cells involved in atherogenesis, or exogenously, from microorganisms, may lead to endothelial injury and subsequent atherosclerosis.

While most research has focused on Hsps-60 and -70 and there have been a number of cross-sectional and cohort studies investigating the relationship between antigen and antibody concentrations in CAD (reviewed by Ghayour-Mobarhan et al[7]), there has been recent interest and investigations of the possible role of the smaller Hsps, such as Hsp-27, in atherogenesis.

Heat shock protein 27 is a 27 kDa chaperone with an ability to interact with a large number of different proteins and is

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found at high levels in several human cell types including heart and even tumor cells.[8,9] Heat shock protein 27 is induced during the stress response and its expression is correlated with increased survival in cells exposed to cytotoxic stimuli.[10]

Lower levels of Hsp-27 also have been related with the occurrence of cardiovascular disease.[11,12] In addition to Hsp-27 antigen, the role of expressed antibodies against Hsp-27 in the pathogenesis of atherosclerosis has been studied and strong correlation has been found. Shams et al[13] found higher antibody concentrations to Hsp-27 in patients with chest pain compared to healthy participants. We[14] have also reported that in patients with ACS, Hsp-27 antibody titers are high during the first 12 hours following the event, then fall to near normal levels after about 12 hours. In the present study, we aimed to study the association between Hsp-27 concentrations in patients with ACS and compare it with normal healthy participants.

Materials and Methods

Participants

A total of 75 patients (48 males and 27 females, mean age 58.5 $\pm$ 7.6) with ACS were recruited. The patients with chest pain attended the Accident and Emergency Centre within the preceding 12 hours and were subsequently admitted to the Coronary Care Unit (CCU) of Ghaem Hospital, Mashhad, Iran. Two blood samples were taken from each patient for analysis; the first sample on admission and the second sample about 12 hours later. The presence of MI or unstable angina was determined by a cardiologist, according to the World Health Organization (WHO) criteria using echocardiography (ECG) and serum troponin I positivity. Seventy-five age- and sex-matched healthy participants (48 males and 27 females, mean age 59.3 $\pm$ 13.3) without a history of CAD, who were referred to the Mashhad Central Laboratory for a routine checkup of serum biochemical parameters, were recruited as the control group. Hypercholesterolemia was diagnosed by a serum total cholesterol concentration $\geq$200 mg/dL or treatment with lipid-lowering drugs, diabetes by a fasting glycemic level $\geq$126 mg/dL on $\geq$2 occasions or treatment with antidiabetic drugs, and hypertension by blood pressures $\geq$140/90 mm Hg on $\geq$2 occasions or if patients were on treatment with antihypertensive medication.[15] Patients were considered smokers if they smoked $\geq$1 cigarette/d at the time of admission or in the preceding 12 months.

Each participant gave written consent to participate in the study, which had been given approval previously by the Mashhad University of Medical Sciences' research ethics committee.

Anthropometric and Other Measurements

For all participants, anthropometric parameters including weight, height, and waist circumference were measured using a standard protocol. Waist circumference was measured midway between the lower rib margin and the iliac crest, without any pressure to body surface. Participants were asked to breathe normally and to breathe out gently at the time of the measurement. The hip circumference measurement was taken at the point yielding the maximum circumference over the buttocks. Weight was measured with the participants dressed in light clothing after an overnight fasting using a standard scale. Blood pressure was measured twice while the patients were seated and rested, using a standard mercury sphygmomanometer. The systolic blood pressure was defined as the appearance of the first sound (Korotkoff phase 1), and the diastolic blood pressure was defined as the disappearance of the sound (Korotkoff phase 5) during deflating of the cuff. Body mass index was calculated as weight (in kg) divided by height squared (in meter).

Blood Sampling

Venous blood samples were collected from each patient on hospital admission and approximately 12 hours later, and for the control group 1 blood sample was taken on the day of laboratory sampling after a 12-hour fasting. Following venipuncture of an antecubital vein, blood samples were collected into Vacutainer tubes and centrifuged at 10 000*g* for 15 minutes at 4°C. After separation, aliquots of serum were frozen at −80°C until the day of analysis. A full 12-hour fasted lipid profile comprising total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) was determined for each patient. Serum lipid and fasting serum glucose concentrations were measured by routine enzymatic methods.

Determination of Serum Hsp27 Antigen

Serum Hsp-27 antigen concentrations were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) developed in-house. One hundred microliters of a 2.5-μg/mL solution of monoclonal Hsp-27 antibody (SPA-800, Stressgen Bioreagents, Canada) in phosphate-buffered saline (PBS) was used to coat a 96-well microtiter plate. After an overnight incubation, the plate was washed 3 times with 0.05% Tween-20 in PBS and then blocked with 4% goat serum for 1.5 hours. Fifty microliters of standards, 60, 30, 15, 7.5, 3.75, 1.875, and 0.937 ng/mL of recombinant Hsp-27 (SPP-715, Stressgen Bioreagents), and 1:2 diluted samples were then added into the duplicate wells. After 30 minutes and washing, 50 μL of 1:6000 dilution of rabbit anti-human Hsp-27 polyclonal antibody (SPA-803, Stressgen Bioreagents) was added. After 30 minutes of incubation and 3 cycles of washing, 50 μL of 1:6000 dilution of goat anti-rabbit horseradish peroxidase-conjugated antibody (A 0545, Sigma-Aldrich, UK) was then added and incubated for 30 minutes. After a final wash of 4 cycles, 50 μL of the substrate solution tetramethylbenzidine dihydrochloride (TMB) was added. The reaction was stopped after 20 minutes with 2 mol/L HCl and the absorbance was read at 450 nm. The sensitivity of the assay was 0.94 ng/mL, and the inter- and intra-assay coefficients of variation were 3.7% and 5.8%, respectively.

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Statistical Analysis

All data were analyzed using SPSS16. Values were expressed as mean ± SD for normally distributed data and in the case of nonnormally distributed data (ie, triglyceride and Hsp levels) as median and interquartile range. Normally distributed data were analyzed using Student *t* test and for nonnormally distributed data nonparametric Mann-Whitney test was used (for 2 groups). For comparison between 2 related samples, the Wilcoxon signed ranks test was used. Chi-square test was used to compare the qualitative factors such as hypertension between control and patients. A 2-sided $P < .05$ was considered significant. To analyze the relationship between Hsp-27 concentrations and individual ACS factors, Spearman correlation was used due to the nonnormal distribution of Hsp-27 concentrations.

Results

Demographic Data

There was a high frequency of hypertension (36%), diabetes mellitus (42.7%), and hyperlipidemia (36%) in the patient group, and this was significantly higher than that of the control group, as may be expected ($P < .05$). Fasting serum glucose, waist-hip ratio, systolic, and diastolic blood pressure were also significantly higher in patients compared with the controls. Significantly lower levels of total and LDL-C and higher levels of HDL-C in the patients could be attributed to consumption of statins in 53 patients (70.66%) versus 21 controls (28%). The mean age did not differ significantly ($P > .05$). The demographic data for the patients and controls are presented in Table 1.

Table 1. Comparison of Clinical and Biochemical Characteristics of Patients and Controls[a]

Group	Controls	Patients
N	75	75
Age (years)	58.5 ± 7.6	59.3 ± 13.3
Female (%)	27 (36%)	27 (36%)
Smoker (%)	23 (30.66)	29 (38.7)
Hypertensive (%)	13 (17.3%)	27 (36%)[b]
Hyperlipidemic (%)	50 (66.6%)	27 (36%)[c]
Diabetic (%)	2 (2.7%)	32 (42.7%)[c]
BMI (kg/m^2)	27.20 ± 6.16	26.71 ± 4.20
Waist: hip ratio	0.94 ± 0.10	1.05 ± 0.16[c]
Fasting blood sugar (mg/dL)	86.3 ± 18.6	152.1 ± 0.6[c]
Total cholesterol (mg/dL)	194 ± 43	176 ± 105[b]
Triglycerides (mg/dL)	109 (79-151)	97.5 (68-157)
HDL-C (mg/dL)	43 ± 8	51 ± 8[c]
LDL-C (mg/dL)	122 ± 36	104.54 ± 37[b]
Systolic blood pressure (mm Hg)	94.8 ± 51.7	132.7 ± 29.0[c]
Diastolic blood pressure (mm Hg)	61.3 ± 33.0	84.0 ± 20.0[c]

Abbreviations: BMI, body mass index; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SEM, standard error of the mean.

[a] Values are expressed as mean ± SEM, or median and interquartile range. Between-group comparisons were assayed using independent samples *t* test for normally distributed data and Mann-Whitney for nonparametric data.

[b] $P < .05$.

[c] $P < .01$.

Hsp-27 Concentrations in Patients and Controls

In the patient group, Hsp-27 concentrations in the first blood samples were (interquartile range: 31.62 [20.12-38.51] ng/mL) were significantly higher than those (interquartile range: 20.12 [16.67-28.17] ng/mL) in samples taken from the control group ($P < .001$). Moreover, in the patient group, Hsp-27 levels on admission were statistically higher compared with the samples collected after 12 hours (interquartile range: 25.87 [15.52-31.62] ng/mL; $P < .001$). No difference was observed between samples of control group and the second 12-hour samples taken from patient group ($P > .05$; Figure 1).

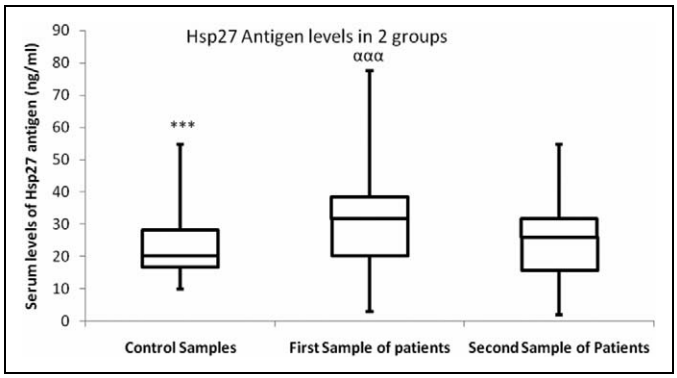

Figure 1. Comparison of heat shock protein 27 (Hsp-27) antigen levels between controls and first and second samples of the patients. ***Significant difference between controls and first samples of the patients ($P < .001$). $^{\alpha\alpha\alpha}$Significant difference between first and second samples of the patients ($P < .001$).

Correlation Between CAD Risk Factors and Hsp-27 Concentrations

In the control group, bivariate analysis between serum Hsp-27 concentrations and individual coronary risk factors indicated that serum Hsp-27 concentrations were significantly related to systolic blood pressure ($r = 0.239$), LDL-C ($r = -0.249$), and serum triglycerides ($r = -0.239$; $P < .05$). In the patient group, Hsp-27 concentrations did not associate with any of the conventional risk factors on bivariate analysis ($P > .05$).

Discussion

We showed that in the first 12-hour postcardiac event, patients with ACS had significantly higher plasma level of Hsp-27 compared with healthy participants. After 12 hours post cardiac event, Hsp-27 titers decreased almost to the level in the control group. In addition to the above results, we found that Hsp-27 was not correlated with any CAD risk factors.

Several studies have reported overexpression of Hsp-27 in cardiac myocytes following ischemia-reperfusion, and suggested the cardioprotective role of Hsp-27.[16,17] In patients with atherosclerosis lesions, decreased concentrations of Hsp-27 in

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serum have been reported, and it has been suggested that Hsp-27 may be a potential biomarker of atherosclerosis.[11] Consistent with the latter study, Park et al,[12] reported that in the atherosclerotic lesion, Hsp-27 expression is increased in the normal-appearing vessel adjacent to atherosclerotic plaque, whereas levels in the plaque itself were significantly decreased. Moreover, it is reported that in humans with cardiac allograft rejection, increased expression of Hsp-27 may be important for cardiac self-protection.[18]

There have been a few studies that report changes in Hsps or their antibody levels in patients with ACS.[19-21] In accordance with our study, it has been previously reported that there is a significant increase in serum Hsp-27 and -70 antigen levels in patients with ACS compared with healthy participants.[12] In another study by Dybdahl et al,[22] increased levels of serum Hsp-70 were reported following MI. Heat shock protein 27 levels remained elevated for about 24 hours. In another study, we[23] found that anti-Hsp-27 antibody levels decreased after the placement of bare metal and drug eluting stents, and that this may be associated with a more favorable outcome.[24] Moreover, the time course of Hsp60 release has also been studied by Schett et al[25] in an animal study. They found that after induction of MI in a rat heart, serum Hsp-60 concentrations begin to increase after 6 hours, reaching a peak level at 24 hours, and decreasing thereafter. Interestingly, the changes in Hsp-60 titers were accompanied by a reduction in serum anti-Hsp-65 levels. The following result for Hsp-60 is similar to ours for Hsp-27,[14] showing the increase in plasma levels of Hsp-27 in the first 12 hours following cardiac event and decreasing to its basal levels thereafter.

In addition, in our previous study, we showed the same pattern for serum levels of anti-Hsp-27. Moreover, Shams et al[26] found higher antibody concentrations against Hsp-27 in patients with chest pain compared to healthy controls.

The fall in both Hsp-27 and anti-Hsp-27 antibody levels in the present and last study,[14] after about 12 hours post event could be related to the formation of immune complexes between anti-Hsp-27 antibody and Hsp-27 antigen that may be released from the necrotic myocardial tissue. These complexes are then probably cleared via Fc-receptor-mediated uptake by cells of the reticuloendothelial system as has been previously suggested for Hsp-65 following MI.[25]

Conclusions

We found that Hsp-27 levels rise rapidly after the onset of cardiac event and fall after 12 hours to basal levels. This may be caused by an increase in systemic levels of inflammation and oxidative stress which is followed by the release of this protein from atherosclerotic plaque and necrotic tissue.

Study Limitations

Sequential blood sampling and measurement of Hsp-27 at different time points following the onset of cardiac event would give a better understanding of the pattern of changes in serum Hsp-27. Another limitation of the study is that we were not able to determine the exact time of the cardiac event. Finally, it remains to be clarified that whether the elevated serum Hsp-27 levels are only observed as a result of acute cardiac event or they precede the onset of that event and are of prognostic importance. Other limitation is that the groups were not matched for the presence of diabetes, hypertension, differences in drug treatment, and so on; factors that may themselves cause an elevation in Hsp-27.

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Declaration of Conflicting Interests

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