Serum antibody titers against heat shock protein 27 are associated with the severity of coronary artery disease

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Abstract Antibody titers to several heat shock proteins (anti-Hsps) have been reported to be associated with the severity and progression of cardiovascular disease. However, there are little data regarding anti-Hsp27 titers in patients with coronary artery disease (CAD). A total of 400 patients with suspected CAD were recruited. Based on the results of coronary angiography, these patients were classified into CAD⁺ ($n=300$) and CAD⁻ ($n=100$) groups defined as patients with $\geq 50\%$ and $<50\%$ stenosis of any major coronary artery, respectively. Eighty-three healthy subjects were also recruited as the control group. Serum anti-Hsp27 IgG titers were measured using an in-house enzyme-linked immunosorbent assay. CAD⁺ patients had significantly higher anti-Hsp27 titers compared with both CAD⁻ and control groups. Anti-Hsp27 titers were also

higher in the CAD⁻ group compared with the control group. With regard to the number of affected vessels in the CAD⁺ group, patients with three-vessel disease had higher anti-Hsp27 titers compared with both two-vessel disease (2VD) and one-vessel disease (1VD) subgroups. However, there was no significant difference between 1VD and 2VD subgroups. In multiple linear regression analysis, the number of narrowed vessels and smoking were significant independent determinants of serum anti-Hsp27 titers. The present findings indicate that serum anti-Hsp27 titers may be associated with the presence and severity of coronary artery disease.

Keywords Coronary artery disease · Heat shock protein 27 · ELISA · Angiography

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Introduction

The heat shock proteins (Hsps) are families of highly conserved protein chaperones that are expressed by cells in response to a variety of environmental stresses. Hsps have been classified into seven major families on the basis of their molecular mass and include: Hsp10 (HSPE), small Hsps [15–30 kDa, of which Hsp27 (HSPB1) is a member], Hsp40 (DNAJ), Hsp60 (HSPD), Hsp70 (HSPA), Hsp90 (HSPC), and Hsp100; Ghayour-Mobarhan et al. 2009). Among the factors that have been shown to induce Hsps are several known potential risk factors for cardiovascular disease (CVD). These include infection, inflammation, ischemia, oxidized low-density lipoprotein, oxidative stress, hypertension, and toxins (Morimoto 1993). Therefore, there is a potential relationship between serum Hsps, their antibody concentrations, and CVD that has been the subject of several investigations in the past two decades. It has been shown that antibody titers to Hsp 60/65 are associated with the severity and progression of cardiovascular disease (Xu et al. 1999, 2000; Burian et al. 2001; Zhu et al. 2001). Interest has been particularly focused on Hsp60 and Hsp70 (Xu et al. 1999, 2000; Burian et al. 2001; Zhu et al. 2001; Xu 2002; Mehta et al. 2005), with little data on other Hsps.

Hsp27 is a member of the small Hsp family with an apparent molecular mass of approximately 27 Kda. It is expressed at high levels in a variety of normal tissues including the heart (Ciocca et al. 1993; Tallot et al. 2003). In addition to its function as a chaperone, Hsp27 may also be cardio-protective by other mechanisms that include increasing myocardial resistance to oxidative stress and has an effect on apoptosis (Martin et al. 1997; Vander Heide 2002; Ferns et al. 2006). Furthermore, there is evidence indicating the role of Hsp27 phosphorylation in the migration of smooth muscle cells (Hedges et al. 1999).

Several studies have indicated that over-expression of Hsp27 protects cardiac myocyte against ischemic injury (Yamboliev et al. 2000; Vander Heide 2002). Hsp27 has also been shown to promote cell protection and survival during inflammation by regulating the expression of pro- and anti-inflammatory genes (De et al. 2000 and Park et al. 2003). There is a body of evidence on the role of inflammation in the pathophysiology of atherosclerosis and coronary artery disease (CAD; Sarwar et al. 2009; Corrado et al. 2010). Since inflammation and ischemia may induce Hsp27 expression, it seems plausible that myocardial ischemia and heightened inflammatory state in CAD are associated with increased intracellular and plasma concentrations of Hsp27, and this may lead to an auto-immune response. While Hsp27 may be released into the blood stream as a consequence of myocardial necrosis, Hsp27 is also thought to be secreted from the cells via non-classical protein secretion pathways (Thery et al.

1999; Skokos et al. 2003; De et al. 2004; Lancaster and Febbraio 2005).

There have only been two previous reports on the relationship between Hsp27, in patients with acute cardiac chest pain (Ghayour-Mobarhan et al. 2008) and patients with acute coronary syndrome (Bluhm et al. 1998). The present study aimed to investigate whether serum anti-Hsp27 titers were related to the extent of coronary artery disease in patients with angiographically defined CAD and whether concentrations of this antibody were higher in patients with established disease from those without.

Methods

Study population

The study participants were selected from those subjects who underwent coronary angiography in the Ghaem Hospital (Mashhad, Iran). Angiography was indicated principally for stable angina, in patients who were positive for at least one objective test of myocardial ischemia including: exercise stress test, dobutamin stress echocardiography, and thallium SPECT (single photon emission computed tomography). Almost all patients were on statin treatment. Patients who were on oral contraceptives or hormone replacement therapy as well as pregnant women were excluded from the study. None of our subjects had a prior history of coronary angioplasty or coronary artery bypass graft. None of the subjects had overt clinical features of infection or chronic inflammatory disease, and all subjects were negative for HBs antigen, anti-HCV antibody, and anti-HIV antibody.

Coronary angiograms were performed using routine procedures. Analysis of the angiograms was performed offline by a specialist cardiologist. The presence of one or more stenoses $\geq 50\%$ in diameter of at least one major coronary artery (left main, right coronary artery, left anterior descending, circumflex) was considered evidence of significant CAD (CAD⁺; Marroquin et al. 2004). Patients in whom stenoses $< 50\%$ in diameter were identified were considered to have a normal angiogram (CAD⁻).

The CAD⁺ patients were classified according to the number of significantly affected stenotic vessels into: one-vessel ($n=100$), two-vessel ($n=100$), and three-vessel ($n=100$) disease groups. Selected CAD⁺ ($n=300$; 150 females, 150 males; mean age, 58.75 ± 9.98 years) and CAD⁻ ($n=100$; 50 females, 50 males; mean age, 58.50 ± 10.03 years) patients were matched for their age and gender. Eighty-three age- and sex-matched healthy volunteers were also recruited as a normal control group ($n=83$; 34 females, 49 males; mean age, 56.81 ± 8.71 years). These individuals had no personal or family history of

cardiovascular disease or diabetes. The study protocol was approved by the ethics committee of Mashhad University of Medical Sciences (MUMS) and written informed consent was obtained from each participant.

Anthropometric and other measurements

For all patients, anthropometric parameters including weight, height, and body mass index (BMI) were measured. Weight was measured with the subjects 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. BMI was calculated as weight (in kilograms) divided by height squared (in square meters).

Routine biochemical analysis

A full fasted lipid profile was determined for each subject. Serum lipid and fasting blood glucose (FBS) concentrations were measured by enzymatic methods. High-sensitivity C-reactive protein (hs-CRP) was measured by a PEG-enhanced immunoturbidimetry method with an Alcyon® analyzer (ABBOTT, Chicago, IL, USA).

Serum anti-Hsp27 assay

Serum Hsp27 antibody titers were measured using an in-house enzyme-linked immunosorbent assay (ELISA; Ghayour-Mobarhan et al. 2008). Briefly, micro-titer plates were coated with 100 ng per well recombinant human Hsp27 dissolved in 50 µl carbonate buffer pH 9.6 incubated for 18 h at 4°C under humidified conditions. The wells were washed three times in wash buffer phosphate-buffered saline (PBS) containing 0.05% Tween-20. Non-specific binding was reduced by blocking each well with 2% goat serum in PBS, and 250 µl was added to each well and then incubated for 30 min in 37°C and 30 min at room temperature. Wells were washed three times with PBS. Serum was diluted 1:100 with 2% goat serum in PBS, and 100 µl was added to the each well in duplicate and then incubated for 30 min at room temperature. After washing (four times in wash buffer and two times in PBS), 100 µl peroxide conjugated-goat anti-human IgG (Sigma-Aldrich, Poole, UK) diluted 1:500 with 2% goat serum in PBS was added to each wells and incubated for 30 min at room temperature. After washing (four times in wash buffer and two times in PBS), 100 µl

of tetramethylbenzidine (TMB) substrate [100 µl of 6 mg/ml TMB in DMSO was added to 10 ml of 50 mM acetate buffer, pH 4.5, containing 3 µl H₂O₂] was added per well and plate incubated for 15 min in the dark at room temperature.

Statistical analysis

All statistical analyses were performed using the SPSS for Windows™, version 11.5 software package (SPSS Inc., Chicago, IL, USA). Data were expressed as means±SD or median and interquartile range (in the case of anti-Hsp27). Group comparisons were performed using ANOVA or Kruskal–Wallis (in case of non-normally distributed data such as anti-Hsp27) test. Categorical data were compared using Chi-square test. A two-sided *P* value<0.05 was considered statistically significant. Bivariate correlations between different parameters and anti-Hsp27 titers were performed using Spearman's rank correlation. Stepwise multiple linear regression analysis was used to determine which of the conventional risk factors could influence anti-Hsp-27 IgG concentrations (expressed as optical density values). Anti-Hsp27 IgG titers were entered into the model after a square root transformation. The predictor variables classified as dichotomous (1=yes/0=no) including diabetes mellitus, hyperlipidemia, hypertension, and smoking were entered into the initial model. Height, weight, FBS, waist circumference, hip circumference, hs-CRP, high-density lipoprotein (HDL), systolic blood pressure, and number of narrowed vessels (VD) were entered as continuous variables in the same model. Data for age and gender were not included since the patients and healthy subjects were matched for these parameters.

Results

Demographic characteristics

The three subject groups (CAD⁺, CAD[−], and control) were well-matched for age and gender ratio. There was no significant difference in BMI and waist/hip ratio between the groups. However, weight in the CAD⁺ group (*p*<0.05), and height, waist circumference, and hip circumference in both CAD⁺ (*p*<0.01, *p*<0.001 and *p*<0.001, respectively) and CAD[−] (*p*<0.05, *p*<0.001 and *p*<0.001, respectively) groups were significantly higher than those of the control group. With respect to blood pressure, mean systolic blood pressure was significantly higher in CAD⁺ and CAD[−] groups compared with the control group (*p*<0.001). However, no significant difference was observed in diastolic blood pressure between the three groups (*p*>0.05). CAD⁺ patients had significantly higher serum CRP and FBS values compared with CAD[−] (*p*<0.001 and

$p < 0.01$, respectively) and control groups ($p < 0.01$ and $p < 0.001$, respectively). CAD^- patients had also higher FBS than control subjects ($p < 0.05$). Finally, no significant difference in lipid profile parameters (HDL-C, LDL-C, and triglycerides) was observed among the three groups ($p > 0.05$, Table 1) which may be partially because of statin treatment in both groups. In regard to the subgroups of CAD^+ patients with different number of stenosed vessels [one-vessel disease (1VD), two-vessel disease (2VD), and three-vessel disease (3VD)], no significant difference in demographic parameters was observed between different subgroups ($p < 0.05$, Table 2). Demographic characteristics of study subjects are summarized in Tables 1 and 2.

Anti-Hsp27 titers in relation to the CAD

Serum IgG antibody titers to Hsp27 were significantly higher in the CAD^+ group compared to CAD^- and control groups ($p < 0.001$). CAD^- subjects also had significantly higher titers compared with the control group ($p < 0.001$, Fig. 1). With respect to associations with the severity of CAD, serum anti-Hsp27 titers were found to be significantly elevated in CAD^+ patients with 3VD compared with subgroups with 1VD and 2VD ($p < 0.05$). However, no

significant difference was observed between 1VD and 2VD subgroups ($p > 0.05$, Fig. 2).

Correlations between serum IgG anti-Hsp27 titers and CVD risk factors

On bivariate analyses, serum anti-Hsp27 titers were only significantly correlated with serum HDL-C ($p < 0.05$) in the CAD^+ group, and height ($p < 0.05$) in the CAD^- group. However, in the control group, significant correlations were found between serum anti-Hsp27 titers and serum FBS ($p < 0.01$) as well as anthropometric parameters including weight ($p < 0.01$), height ($p < 0.05$), and BMI ($p < 0.05$, Table 3).

Association between serum anti-Hsp27 IgG titers and CVD risk factors

In stepwise multiple linear regression analysis, only the number of VD (β , 0.042; $p < 0.001$) and smoking (β , 0.065; $p < 0.01$) were found to have a significant independent association with serum anti-Hsp27 titers. The regression model yielded the following equation for the prediction of serum IgG anti-Hsp27 titers.

Table 1 Demographic and clinical characteristics of CAD^+ , CAD^- , and control subjects

	CAD^+	CAD^-	Control
Number of subjects	300	100	83
Gender (F/M)	150/150	50/50	34/49
Age	58.75 $\pm$ 9.98	58.50 $\pm$ 10.03	56.81 $\pm$ 8.71
Height	159.03 $\pm$ 10.66 ^b	159.92 $\pm$ 10.69 ^a	162.74 $\pm$ 8.45
Weight	68.53 $\pm$ 12.86 ^a	67.41 $\pm$ 13.91	73.57 $\pm$ 12.40
FBS	129.87 $\pm$ 68.94 ^{c, d}	105.79 $\pm$ 41.27 ^a	91.21 $\pm$ 13.73
BMI	27.27 $\pm$ 6.35	26.32 $\pm$ 5.66	28.03 $\pm$ 4.60
Waist/hip ratio	0.96 $\pm$ 0.10	0.94 $\pm$ 0.08	0.95 $\pm$ 0.11
Waist circumference	90.27 $\pm$ 11.41 ^c	88.07 $\pm$ 14.66 ^c	98.77 $\pm$ 14.17
Hip circumference	94.54 $\pm$ 11.49 ^c	93.88 $\pm$ 13.39 ^c	103.44 $\pm$ 11.94
LDL-C	100.95 $\pm$ 39.56	105.62 $\pm$ 43.81	99.82 $\pm$ 19.54
HDL-C	43.31 $\pm$ 11.66	42.90 $\pm$ 10.92 ^a	50.66 $\pm$ 50.34
TG	126.00 (94.00–173.00)	122.50 (83.00–188.50)	129.00 (94.00–161.00)
hs-CRP	3.33 (1.47–9.24) ^{b, c}	1.31 (0.91–4.37)	1.63 (0.88–3.39)
SBP	144.37 $\pm$ 30.59 ^c	149.06 $\pm$ 26.09 ^c	123.27 $\pm$ 11.24
DBP	77.03 $\pm$ 14.04	75.75 $\pm$ 15.50	78.27 $\pm$ 9.12
Diabetes mellitus (%)	31.2 ^g	17.1	5.9
Smoking or addiction (%)	45.7 ^g	35.0	21.8
Hypertension (%)	51.2 ^f	45.7	30.0
Hyperlipidemia (%)	43.1	26.8	22.2

Values are presented as mean $\pm$ SD. FBS fasting blood sugar, BMI body mass index, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol, TG triglycerides, hs-CRP high-sensitivity C-reactive protein, SBP systolic blood pressure, DBP diastolic blood pressure. Compared with the control group: ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$; compared with the CAD^- group: ^d $p < 0.01$, ^e $p < 0.001$; comparison between all groups (using Chi-square test): ^f $p < 0.05$; ^g $p < 0.001$

Table 2 Demographic and clinical characteristics of CAD⁺ subjects with 1-, 2-, and 3VD

	SVD	2VD	3VD
Number of subjects	100	100	100
Gender (F/M)	50/50	50/50	50/50
Age	58.77±10.25	58.67±9.97	58.82±9.82
Height	161.26±9.80	158.57±10.97	158.09±10.79
Weight	69.73±15.03	67.83±12.38	68.43±11.91
FBS	134.85±75.12	131.72±75.03	123.04±55.04
BMI	26.57±4.95	27.23±6.77	27.68±6.67
Waist/hip ratio	0.94±0.07	0.96±0.11	0.97±0.10
Waist circumference	89.69±12.24	91.05±10.35	89.93±11.85
Hip circumference	95.31±11.73	95.15±10.42	93.54±12.25
LDL-C	97.01±33.99	100.77±41.72	104.56±42.10
HDL-C	46.35±12.27	42.26±11.20	41.63±11.15
TG	114 (83.00–175.50)	129 (88.00–167.00)	129.00 (103.00–187.00)
hs-CRP	3.80 (1.39–16.03)	3.19 (1.03–12.20)	3.33 (1.60–8.41)
SBP	142.61±29.46	143.07±32.62	147.37±29.91
DBP	76.54±12.74	76.82±15.28	77.74±14.23
Diabetes mellitus (%)	34.0	26.4	32.7
Smoking or addiction (%)	46.4	41.7	49.0
Hypertension (%)	50.5	51.1	52.0
Hyperlipidemia (%)	35.1	46.6	48.0

Values are presented as mean±SD. *FBS* fasting blood sugar, *BMI* body mass index, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TG* triglycerides, *hs-CRP* high-sensitivity C-reactive protein, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

Predicted IgG anti Hsp27 titer = $0.510 + 0.042 \times \text{VD} + 0.065 \times \text{smoking}[\text{yes}(\text{present or past habit or addiction}) = 1/\text{no} = 0]$

Discussion

According to the findings of the present study, serum anti-Hsp27 titers were elevated in patients with angiographically

defined CAD compared with healthy control subjects and patients with suspected CAD but with less than 50% coronary stenosis. The present findings are in conformity with our a priori hypothesis. Considering inflammation and ischemia as inducers of Hsp27 expression (Bluhm et al. 1998; Yamboliev et al. 2000; Vander Heide 2002) and Hsp27 as a protein which is expressed at high concentrations in the heart (Ciocca et al. 1993), it may be anticipated that cardiomyocytes in patients with documented CAD contain

Fig. 1 Serum anti-Hsp27 status in CAD⁺, CAD⁻, and control groups. Compared with the control group: *** $p < 0.001$; compared with the CAD⁻ group: ††† $p < 0.001$. Group comparisons were assessed using Kruskal–Wallis test (post hoc analyses were performed by paired samples t test)

Fig. 2 Serum anti-Hsp27 status in CAD⁺ subjects with 1-, 2- and 3VD. Compared with the 1VD group: * $p < 0.05$; compared to the 2VD group: † $p < 0.05$. Group comparisons were assessed using Kruskal–Wallis test (post hoc analyses were performed by paired samples t test)

Table 3 Correlations between serum anti-Hsp27 titers and CVD risk factors

	CAD ⁺		CAD ⁻		Control	
	<i>r</i> values	<i>p</i> values	<i>r</i> values	<i>p</i> values	<i>r</i> values	<i>p</i> values
Age	0.069	0.231	-0.136	0.224	0.203	0.042*
Height	-0.069	0.290	0.241	0.032*	-0.127	0.211
Weight	-0.091	0.160	0.049	0.665	-0.280	0.005*
FBS	-0.002	0.973	0.032	0.784	-0.341	0.002*
BMI	-0.017	0.809	-0.137	0.250	-0.246	0.014*
Waist/hip ratio	0.056	0.403	-0.030	0.798	-0.113	0.268
Waist circumference	-0.003	0.967	0.021	0.856	-0.194	0.056
Hip circumference	-0.047	0.480	0.018	0.873	-0.208	0.040*
LDL	0.002	0.980	-0.015	0.911	0.058	0.627
HDL	-0.157	0.021	-0.082	0.532	-0.095	0.410
TG	0.028	0.684	-0.028	0.829	0.152	0.171
hs-CRP	-0.101	0.201	-0.063	0.657	-0.101	0.525
SBP	0.101	0.093	0.138	0.260	0.141	0.248
DBP	-0.069	0.247	0.212	0.083	-0.026	0.832

Correlations were assessed using Spearman's correlation coefficients

The significant correlations are marked with an asterisk

higher intracellular concentrations of Hsp27 and as a result, increased extracellular release of Hsp27 into blood stream may occur. Moreover, despite lacking any identified signal sequence in Hsps, their secretion from eukaryotic cells is not a rejected hypothesis as non-classical protein secretion pathways might come into play. Notably, one such a mechanism has been identified which is via exosomes (50–100-nm membrane vesicles; Thery et al. 1999; Skokos et al. 2003; De et al. 2004; Lancaster and Febbraio 2005). These increased extracellular concentrations may stimulate the immune response resulting in the rise of anti-Hsp27 titers.

As far as we are aware, serum anti-Hsp27 status has not been investigated specifically in CAD patients. Among related studies are those of Józefowicz-Okonkwo et al. (2009), Kardys et al. (2008), and Park et al. (2006) which are discussed further below: all of these studies have investigated serum Hsp27 antigen concentrations rather than antibody titers. The findings of the first study, which was performed in 62 patients with CAD and 21 healthy controls, implied that plasma Hsp27 concentrations are significantly higher in patients with 2VD or 3VD CAD compared with patients with 1VD CAD or healthy control subjects. In this study, the authors did not observe a significant difference in plasma Hsp27 between patients with 1VD, 2VD, and 3VD, but when 2VD and 3VD subgroups were combined, the difference with the 1VD subgroup reached statistical significance. Furthermore, they did not find any significant correlation between the grade of CAD severity (defined using the Gensini scale) and plasma Hsp27 concentrations. However, in the same study, it was stated that the Gensini scale may not reliably predict the extent of myocardial inflammation and ischemia if a

collateral circulation is present (Józefowicz-Okonkwo et al. 2009). The second study (Kardys et al. 2008) had a prospective, nested design and was performed among initially healthy women. No significant association was reported between baseline plasma Hsp27 concentrations and incident cardiovascular events (including non-fatal MI or ischemic stroke and cardiovascular death) during a period of 5.9 years follow-up. This finding is in contrast with those of other studies reporting higher circulatory Hsp27 or anti-Hsp27 levels in patients with CAD or acute coronary syndrome. The inconsistency between the findings of this latter study and others may be due to its prospective design. It is possible that there is a shorter temporal relationship between high serum levels of Hsp27 antigen and its antibody with overt coronary disease, as the latter are predicated on the presence of inflammation and ischemia. The participants in the latter study were exclusively women, who were aged ≥ 45 years (mean age of 61 years). The results of this study may not be generalizable to the whole population as previous studies have indicated that Hsp27 expression is influenced by estrogen (Ciocca et al. 1993; Porter et al. 1996). The third study also reported increased plasma levels of Hsp27 and Hsp70 in patients with acute coronary artery disease compared with the healthy reference group (Park et al. 2006). The authors concluded that risk factors for CAD may not be related to the increased plasma Hsp27 because the plasma Hsp27 levels of a group of subjects with CAD risk factors but no symptomatic coronary stenosis were not different compared with those of the healthy reference group.

All these previous reports have evaluated the serum concentrations of Hsp27 antigen rather than Hsp27 antibody. There have been numerous previous reports on the

associations between antibody titers against several other Hsps (mainly 60/65 and 70/72) with extent of atherogenesis as well as progression of cardiovascular disease (Xu et al. 1999, 2000; Burian et al. 2001; Zhu et al. 2001; Kocsis et al. 2002; Wysocki et al. 2002). For instance, a prospective study has indicated that antibody titers to Hsp60 are predictive of cardiovascular events (Huittinen et al. 2002). However, compared with the Hsp60 and Hsp70 families, there are few clinical data available for anti-Hsp27 in CVD. Two previous studies have reported elevated anti-Hsp27 titers in patients with acute cardiac chest pain (Shams et al. 2008) and acute coronary syndrome (in the first 12 h following the onset of chest pain; Ghayour-Mobarhan et al. 2008). In addition, serum anti-Hsp27 antigen and antibody titers (IgM and IgG) have been reported to be related to the presence of cardiovascular complications in patients with glucose intolerance. This latter finding is consistent with the present findings as we have found a significant difference in the prevalence of diabetes mellitus among the groups, with the highest rate in the CAD⁺ group (Pengiran Burut et al. 2010).

In the present study, serum anti-Hsp 27 titers in the CAD⁻ group were higher than those in the control subjects but lower than those of CAD⁺ group. These elevated titers in the CAD⁻ group who had <50% coronary artery stenosis—and therefore might have degrees of CAD (which may progress to overt CAD over time)—implies possible association between serum anti-Hsp27 and inflammation and extent of myocardial ischemia. More importantly, the high levels of serum anti-Hsp27 titers in CAD⁺ and CAD⁻ subjects as well as subjects with acute coronary syndrome (Ghayour-Mobarhan et al. 2008) could be attributed to the underlying inflammatory vascular disease (e.g., atherosclerosis). The expression and phosphorylation of Hsp27 appears to be influenced by inflammation, and there is evidence indicating direction of Hsp27 expression and phosphorylation by cytokines (Hastie et al. 1997; Hatakeyama et al. 2002; Njemini et al. 2006). There are also several reports on the role of Hsp27 in regulating the inflammatory response via alterations in the expression of pro- and anti-inflammatory mediators (De et al. 2000 and Park et al. 2003; Sur et al. 2008; Liu et al. 2010). In the current study, we found that serum anti-Hsp27 titers are also related to the degree of CAD: with patients having triple-vessel disease having higher titers than those with single- or double-vessel disease. However, there was no significant difference in anti-Hsp27 titers between the subgroups of CAD⁺ patients with one and two narrowed coronary arteries. As proposed by Józefowicz-Okonwo et al. (2009), this could be due to the relatively small myocardial mass supplied by a single coronary artery, which may not be sufficient to induce significant increase in the intracellular and consequently, extracellular concentration of Hsp27.

Hence, serum Hsp27 antibody titers are increased in patients with CAD, and this may be due to the effects of inflammation and ischemia on Hsp27 expression (Bluhm et al. 1998; Vander Heide 2002; Yamboliev et al. 2000; Ferns et al. 2006). Increased expression of several Hsps, including Hsp27, have been reported in infarcted heart tissue, and they are subsequently released into the circulation for several hours following myocardial ischemia (Knowlton 1995; Vander Heide 2002; Dybdahl et al. 2005), rendering them accessible to the immune system.

In conclusion, the findings of the present study indicate that serum anti-Hsp27 titers are elevated in patients with angiographically defined CAD compared with those without established disease and healthy control subjects. In addition, patients with three-vessel disease had higher anti-Hsp27 titers compared with patients who had one or two affected vessels. Therefore, serum anti-Hsp27 titers may be associated with the presence and severity of coronary artery disease. Future prospective studies are warranted to clarify the prognostic value of these raised serum anti-Hsp27 titers in CAD⁺ patients and their association with future cardiovascular events. In addition, the relationship between anti-Hsp27 titers and other known biomarkers of myocardial ischemia and inflammation remains to be elucidated.

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References

- Bluhm WF, Martin JL, Mestrlil R, Dillmann WH (1998) Specific heat shock proteins protect microtubules during simulated ischemia in cardiac myocytes. *Am J Physiol* 275:H2243–H2249
- Burian K, Kis Z, Virok D, Endresz V, Prohaszka Z, Duba J, Berencsi K, Boda K, Horvath L, Romics L, Fust G, Gonczol E (2001) Independent and joint effects of antibodies to human heat-shock protein 60 and *Chlamydia pneumoniae* infection in the development of coronary atherosclerosis. *Circulation* 103:1503–1508
- Ciocca DR, Oesterreich S, Chamness GC, McGuire WL, Fuqua SA (1993) Biological and clinical implications of heat shock protein 27,000 (Hsp27): a review. *J Natl Cancer Inst* 85:1558–1570
- Corrado E, Rizzo M, Coppola G, Fattouch K, Novo G, Marturana I, Ferrara F, Novo S (2010) An update on the role of markers of inflammation in atherosclerosis. *J Atheroscler Thromb* 17:1–11
- De AK, Kodys KM, Yeh BS, Miller-Graziano C (2000) Exaggerated human monocyte IL-10 concomitant to minimal TNF- α induction by heat-shock protein 27 (Hsp27) suggests Hsp27 is primarily an anti-inflammatory stimulus. *J Immunol* 165:3951–3958
- De GA, Geminard C, Hoekstra D, Vidal M (2004) Exosome secretion: the art of reutilizing nonrecycled proteins? *Traffic* 5:896–903
- Dybdahl B, Slordahl SA, Waage A, Kierulf P, Espevik T, Sundan A (2005) Myocardial ischemia and the inflammatory response: release of heat shock protein 70 after myocardial infarction. *Heart* 91:299–304
- Ferns G, Shams S, Shafi S (2006) Heat shock protein 27: its potential role in vascular disease. *Int J Exp Pathol* 87:253–274

- Ghayour-Mobarhan M, Sahebkar A, Parizadeh SMR et al (2008) Antibody titers to heat shock protein 27 are elevated in patients with acute coronary syndrome. *Int J Exp Pathol* 89:209–215
- Ghayour-Mobarhan M, Rahsepar AA, Tavallaie S, Rahsepar S, Ferns GAA (2009) Chapter 2 the potential role of heat shock proteins in cardiovascular disease. Evidence from in vitro and in vivo studies. *Adv Clin Chem* 48:27–72
- Hastie AT, Everts KB, Zangrilli J, Shaver JR, Pollice MB, Fish JE, Peters SP (1997) HSP27 elevated in mild allergic inflammation protects airway epithelium from H₂SO₄ effects. *Am J Physiol* 273:401–409
- Hatakeyama D, Kozawa O, Niwa M, Matsuno H, Ito H, Kato K, Tatematsu N, Shibata T, Uematsu T (2002) Upregulation by retinoic acid of transforming growth factor-beta-stimulated heat shock protein 27 induction in osteoblasts: involvement of mitogen-activated protein kinases. *Biochim Biophys Acta* 1589:15–30
- Hedges JC, Dechert MA, Yamboliev IA, Martin JL, Hickey E, Weber LA, Gerthoffer WT (1999) A role for p38(MAPK)/HSP27 pathway in smooth muscle cell migration. *J Biol Chem* 274:24211–24219
- Huittinen T, Leinonen M, Tenkanen L, Mänttari M, Virkkunen H, Pitkänen T, Wahlström E, Palosuo T, Manninen V, Saikku P (2002) Autoimmunity to human heat shock protein 60, *Chlamydia pneumoniae* infection, and inflammation in predicting coronary risk. *Arterioscler Thromb Vasc Biol* 22:431–437
- Józefowicz-Okonkwo G, Wierzbowska-Drabik K, Kasielski M, Trzos E, Goraca A, Kasprzak J, Krzemińska-Pakuła M (2009) Is Hsp27 a marker of myocardial ischaemia? *Kardiologia* 67:947–952
- Kardys I, Rifai N, Meilhac O, Michel JB, Martin-Ventura JL, Buring JE, Libby P, Ridker PM (2008) Plasma concentration of heat shock protein 27 and risk of cardiovascular disease: a prospective, nested case-control study. *Clin Chem* 54:139–146
- Knowlton AA (1995) The role of heat shock proteins in the heart. *J Mol Cell Cardiol* 27:121–131
- Kocsis J, Veres A, Vataj A, Duba J, Karadi I, Fust G, Prohaszka Z (2002) Antibodies against the human heat shock protein hsp70 in patients with severe coronary artery disease. *Immunol Invest* 31:219–231
- Lancaster GI, Febbraio MA (2005) Exosome-dependent trafficking of HSP70: a novel secretory pathway for cellular stress proteins. *J Biol Chem* 280:23349–23355
- Liu J, Hong S, Feng Z, Xin Y, Wang Q, Fu J, Zhang C, Li G, Luo L, Yin Z (2010) Regulation of lipopolysaccharide-induced inflammatory response by heat shock protein 27 in THP-1 cells. *Cell Immunol* 264:127–134
- Marroquin O, Kip K, Kelley DE, Johnson BD, Shaw LJ, Bairey Merz CN, Sharaf BL, Pepine CJ, Sopko G, Reis SE (2004) Metabolic syndrome modifies the cardiovascular risk associated with angiographic coronary artery disease in women: a report from the women's ischemia syndrome evaluation. *Circulation* 109:714–721
- Martin JL, Mestral R, Hilal-Dandan R, Brunton LL, Dillmann WH (1997) Small heat shock proteins and protection against ischemic injury in cardiac myocytes. *Circulation* 96:4343–4348
- Mehta TA, Greenman J, Ettelaie C, Venkatasubramanian A, Chetter IC, McCollum PT (2005) Heat shock proteins in vascular disease: a review. *Eur J Vasc Endovasc Surg* 29:395–402
- Morimoto RI (1993) Cells in stress: transcriptional activation of heat shock genes. *Science* 259:1409–1410
- Njemini R, Lambert M, Demanet C, Mets T (2006) The effect of aging and inflammation on heat shock protein 27 in human monocytes and lymphocytes. *Exp Gerontol* 41:312–319
- Park KJ, Gaynor RB, Kwak YT (2003) Heat shock protein 27 association with the I kappa B kinase complex regulates tumor necrosis factor alpha-induced NF-kappa B activation. *J Biol Chem* 278:35272–35278
- Park HK, Park EC, Bae SW, Park MY, Kim SW, Yoo HS, Tudev M, Ko YH, Choi YH, Kim S, Kim DI, Kim YW, Lee BB, Yoon JB, Park JE (2006) Expression of heat shock protein 27 in human atherosclerotic plaques and increased plasma level of heat shock protein 27 in patients with acute coronary syndrome. *Circulation* 114:886–893
- Pengiran Burut DF, Borai A, Livingstone C, Ferns G (2010) Serum heat shock protein 27 antigen and antibody levels appear to be related to the macrovascular complications associated with insulin resistance: a pilot study. *Cell Stress Chaperon*. doi:10.1007/s12192-009-0152-7
- Porter W, Wang F, Wang W, Duan R, Safe S (1996) Role of estrogen receptor/Sp1 complexes in estrogen induced heat shock protein 27 gene expression. *Mol Endocrinol* 10:1371–1378
- Sarwar N, Thompson AJ, Di Angelantonio E (2009) Markers of inflammation and risk of coronary heart disease. *Dis Markers* 26:217–225
- Shams S, Shafi S, Bodman-Smith K, Williams P, Mehta S, Ferns GA (2008) Anti-heat shock protein-27 (hsp-27) antibody levels in patients with chest pain: association with established cardiovascular risk factors. *Clin Chim Acta* 395:42–46
- Skokos D, Botros HG, Demeure C, Morin J, Peronnet R, Birkenmeier G, Boudaly S, Mécheri S (2003) Mast cell-derived exosomes induce phenotypic and functional maturation of dendritic cells and elicit specific immune responses in vivo. *J Immunol* 170:3037–3045
- Sur R, Lyte PA, Southall MD (2008) Hsp27 regulates pro-inflammatory mediator release in keratinocytes by modulating NF-kappa B signaling. *J Invest Dermatol* 128:1116–1122
- Tallot P, Grongnet JF, David JC (2003) Dual perinatal and developmental expression of the small heat shock proteins [FC12]αB-crystallin and Hsp27 in different tissues of the developing piglet. *Biol Neonate* 83:281–288
- Thery C, Regnault A, Garin J, Wolfers J, Zitvogel L, Ricciardi-Castagnoli P, Raposo G, Amigorena S (1999) Molecular characterization of dendritic cell-derived exosomes. Selective accumulation of the heat shock protein hsc73. *J Cell Biol* 147:599–610
- Vander Heide RS (2002) Increased expression of HSP27 protects canine myocytes from simulated ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 282:H935–H941
- Wysocki J, Karawajczyk B, Gorski J, Korzeniowski A, Mackiewicz Z, Kupryszewski G, Glosnicka R (2002) Human heat shock protein 60 (409–424) fragment is recognized by serum antibodies of patients with acute coronary syndromes. *Cardiovasc Pathol* 11:238–243
- Xu Q (2002) Role of heat shock proteins in atherosclerosis. *Arterioscler Thromb Vasc Biol* 22:1547–1559
- Xu Q, Kiechl S, Mayr M, Metzler B, Egger G, Oberhollenzer F, Willeit J, Wick G (1999) Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis: clinical significance determined in a follow-up study. *Circulation* 100:1169–1174
- Xu Q, Schett G, Perschinka H, Mayr M, Egger G, Oberhollenzer F, Willeit J, Kiechl S, Wick G (2000) Serum soluble heat shock protein 60 is elevated in subjects with atherosclerosis in a general population. *Circulation* 102:14–20
- Yamboliev IA, Hedges JC, Mutnick JL, Adam LP, Gerthoffer WT (2000) Evidence for modulation of smooth muscle force by the p38 MAP kinase/HSP27 pathway. *Am J Physiol Heart Circ Physiol* 278:H1899–H1907
- Zhu J, Quyyumi AA, Rott D, Csako G, Wu H, Halcox J, Epstein SE (2001) Antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease: evidence for an autoimmune component of atherogenesis. *Circulation* 103:1071–1075