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A cross-sectional study of the association between heat shock protein 27 antibody titers, pro-oxidant–antioxidant balance and metabolic syndrome in patients with angiographically-defined coronary artery disease ☆

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ABSTRACT

Objective: To investigate the association between serum antibody titers to Hsp27 (anti-Hsp27) and pro-oxidant–antioxidant balance (PAB) in patients with angiographically-defined coronary artery disease (CAD) with or without the metabolic syndrome (MS).
Design: Subjects ($n=243$) were classified into MS+ ($n=161$) and MS− ($n=82$) subgroups, based on the AHA/NHBLI criteria.
Results: Serum anti-Hsp27 titers were found to be significantly higher in the MS+ vs. MS− group. However, no significant difference was observed in serum PAB values. When assessed for individual components of MS, increased serum anti-Hsp27 was found to be higher in subgroups with elevated triglycerides, elevated blood pressure and reduced high-density lipoprotein cholesterol (HDL-C). Subgroups of patients with elevated triglycerides had higher PAB values. HDL-C was the only significant predictor of anti-Hsp27 in the population as a whole.
Conclusion: The evidence from this investigation indicates the presence of elevated anti-Hsp27 in patients with concurrent CAD and MS compared to those with CAD alone.

Introduction

Metabolic syndrome (MS) is a term that has been used to describe the clustering of several cardiometabolic disorders including abdominal obesity, hypertension, dyslipidemia and hyperglycemia. As the criteria for MS are also important cardiovascular risk factors, their concurrence in MS is associated with significant elevation of cardiovascular disease (CVD) risk [1,2].

Owing to the increasing prevalence of the characteristic features of the MS, in particular obesity, the syndrome is now regarded as a significant public health problem. Health statistics in the USA have shown a prevalence of 27% among adults [3]. Furthermore, the prevalence of MS is also high in populations of developing countries ranging from 13.3% in China to a strikingly high prevalence of about 30% in Iran [4]. As a consequence there is predicted to be a dramatic increase in the prevalence of CVD and diabetes.

Being present in all organisms, heat shock proteins (HSPs) represent a ubiquitous and highly conserved class of proteins. The primary function of most Hsps is chaperonin activity, which involves correction of protein mis-foldings and preventing the generation of unwanted protein aggregates [5,6]. Hsps have been suggested to be implicated in the pathophysiology of CVD [7–9]. Several common characteristics of CVD risk and MS such as hypertension, diabetes, hyperlipidemia and oxidative stress have been reported to induce the expression of Hsps

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[8–10]. However, data regarding the levels of Hsps or their corresponding antibodies in MS are scarce. Hsp27 is a member of small Hsp family which possesses cardio-protective properties [11]. Serum levels of antibody titers against this protein (anti-Hsp27) have been reported to be higher in patients with CVD including established coronary artery disease (CAD) and acute coronary syndrome (ACS) [12–14]. The present study aimed to evaluate the association of antibody titers to Hsp27 (anti-Hsp27) with MS and its individual components among Iranian subjects with angiographically defined CAD. In addition, the level of pro-oxidant–antioxidant balance (PAB) was also assessed in relation with MS as a secondary goal.

Methods

Study population

The study population consisted of 243 patients (126 females, 117 males; mean age: 58.46 ± 9.87) with CAD who were selected from subjects undergoing coronary angiography in the Ghaem Hospital (Mashhad, Iran). Angiography was principally indicated for stable angina, in patients who were positive for at least one objective test of myocardial ischemia including: exercise stress test, dobutamin stress echocardiography, or Thallium SPECT (single photon emission computed tomography). Patients who were on oral contraceptives or hormone replacement therapy as well as pregnant women were excluded from the study. None of the subjects had overt clinical features of infection, chronic inflammatory disease, or a prior history of coronary angioplasty or coronary artery bypass graft (CABG). All subjects were negative for HBs antigen, anti-HCV antibody and anti-HIV antibody.

Coronary angiography was performed using routine procedures. Analysis of the angiograms was performed offline by a Specialist Cardiologist. The presence of one or more stenoses ≥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. Patients in whom stenoses of <50% in diameter were identified and were considered to have a normal angiogram (CAD−).

Based on the presence of MS, patients were classified into: MS+ ($n = 161$; 107 females, 54 males; mean age: 59.11 ± 9.46 years) and MS− ($n = 82$; 19 females, 63 males; mean age: 57.45 ± 10.58 years) groups. The study protocol was approved by the Mashhad University of Medical Sciences (MUMS) Ethics Committee and written informed consent was obtained from each participant.

Definition of metabolic syndrome

The American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) guideline was used to categorize subjects into MS+ and MS− subgroups [15]. Metabolic syndrome was defined as the co-occurrence of at least 3 of the following 5 metabolic abnormalities: 1) elevated serum fasting glucose (≥100 mg/dL) or use of medication for hyperglycemia; 2) elevated serum triglycerides (≥150 mg/dL); 3) reduced serum HDL-C (<40 mg/dL in males and <50 mg/dL in females); 4) elevated systolic (≥130 mm Hg) or diastolic (≥85 mm Hg) blood pressure or use of medication for hypertension; and 5) elevated waist circumference (≥102 cm in males and ≥88 cm in females).

Routine anthropometric and biochemical analyses

Anthropometric parameters including weight, height and body mass index (BMI), together with systolic and diastolic blood pressures, fasted lipid profile [comprising low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C) and triglycerides] and fasting blood glucose (FBS) were measured. Lipid profile parameters and 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). Blood pressure was measured using a 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 (kg) divided by height squared (m^2).

Serum anti-Hsp27 assay

Serum Hsp27 antibody titers were measured using an in-house ELISA assay [13]. Briefly, micro-titer plates were coated with 100 ng per well recombinant human Hsp27 dissolved in 50 μL carbonate buffer (pH 9.6) and incubated for 18 h at 4 °C under humidified conditions. The wells were washed three times in washing buffer PBS (phosphate buffer saline) containing 0.05% Tween-20. Non-specific binding was reduced by blocking each well with 2% goat serum in PBS and 250 μL added to each well and 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 then added to each well in duplicate and incubated for 30 min at room temperature. After washing (four times in washing 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 well and incubated for 30 min at room temperature. After washing (four times in washing buffer and two times in PBS), 100 μL of tetramethylbenzidine (TMB) substrate [100 μL of 6 mg/mL TMB in dimethyl sulfoxide (DMSO)] was added to 10 mL of 50 mM acetate buffer, pH 4.5. Finally, 3 μL H_2O_2 was added per well and plate was incubated for 15 min in the dark at room temperature.

Pro-oxidant–antioxidant balance (PAB) assay

A modified PAB assay was applied based on a previously described method [16]. The standard solutions were prepared by mixing varying proportions (0–100%) of 250 μM hydrogen peroxide with 3 mM uric acid (in 10 mM NaOH). TMB powder (60 mg) was dissolved in 10 mL DMSO. For preparation of the TMB cation, 400 μL of the TMB/DMSO solution was added to 20 mL of acetate buffer [0.05 M buffer, pH 4.5], and then 70 μL of fresh chloramine T (100 mM) solution was added to this 20 mL. The solution was mixed well and incubated for 2 h at room temperature in a dark place. Then 25 U of peroxidase enzyme solution was added to 20 mL of TMB cation solution, dispensed in 1 mL and stored at −20 °C. In order to prepare the TMB solution 200 μL of TMB/DMSO was added to 10 mL of acetate buffer [0.05 M buffer, pH 5.8] and the working solution was prepared by mixing 1 mL TMB cation with 10 mL of TMB solution. This working solution was incubated for 2 min at room temperature in a dark place and immediately used. Ten microliters of each sample, standard or blank (distilled water) were mixed with 200 μL of working solution in each well of a 96 well plate, which was then incubated in a dark place at 37 °C for 12 min. At the end of the incubation time, 100 μL of 2 N HCl was added to each well, and the optical density (OD) was measured in an ELISA reader at 450 nm with a reference wavelength of 620 or 570 nm. A standard curve was provided from the values relative to the standard samples. The values of the PAB are expressed in arbitrary (HK) unit, which is the percentage of hydrogen peroxide in the standard solution. The values of the unknown samples were then calculated based on the values obtained from the above standard curve.

Statistical analysis

All statistical analyses were performed using the SPSS for Windows™, version 11.5 software package (SPSS Inc., Chicago, Illinois, USA). Data

Table 1
Demographic characteristics of study groups.

		MS+	MS−	*p*-value
Age (years)		59.11 ± 9.46	57.45 ± 10.58	0.28
Female (%)		66.5	23.2	<0.001
Smokers (%)		45.3	48.8	0.76
Weight (kg)		70.21 ± 13.35	66.67 ± 12.57	0.06
Height (m)		157.33 ± 11.28	163.30 ± 8.77	0.001
BMI (kg/m^2)		28.66 ± 6.87	24.95 ± 4.02	0.001
Waist (cm)		94.46 ± 10.96	83.37 ± 10.30	0.001
Hip (cm)		97.86 ± 10.83	89.27 ± 11.09	0.001
Waist/hip ratio		0.96 ± 0.09	0.93 ± 0.09	0.002
SBP (mm Hg)		151.87 ± 30.00	129.83 ± 25.39	0.001
DBP (mm Hg)		78.71 ± 14.23	73.35 ± 12.18	0.005
FBS (mmol/L)		8.15 ± 4.32	5.56 ± 1.51	0.001
LDL-C (mmol/L)		2.67 ± 1.03	2.49 ± 0.97	0.38
HDL-C (mmol/L)		1.08 ± 0.30	1.19 ± 0.29	0.009
Triglycerides (mmol/L)		1.86 ± 0.92	1.29 ± 0.72	0.001
hs-CRP (mg/L)		15. 48 ± 25.30	12.69 ± 23.64	0.23
CAD severity	SVD (%)	32.9	32.9	1.00
	2VD (%)	32.9	29.3	0.69
	3VD (%)	34.2	27.8	0.91

Values are expressed as mean ± SD. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein; SVD: single vessel disease.

were expressed as mean ± SD or mean ± SEM (in figures). Group comparisons were performed using independent samples *t*-test or Mann–Whitney *U* test in case of normally and non-normally distributed data, respectively. Categorical data were compared using Chi-square test. A two-sided *p*-value of <0.05 was considered statistically significant. Bivariate correlations of different parameters with anti-Hsp27 titers and PAB values were performed using Pearson's (for normally distributed data) or Spearman's (for non-normally distributed data) rank correlation coefficients. Stepwise multiple linear regression analysis was used to identify the independent parameters that are related to anti-Hsp27 titers or PAB values.

Results

Demographic characteristics

MS+ and MS− were comparable in their age distribution ($p>0.05$), but significantly different with respect to gender ($p<0.001$). BMI ($p<0.001$), waist/hip ratio ($p=0.002$), FBS ($p<0.001$), systolic ($p=0.001$) and diastolic pressure ($p=0.005$) were significantly higher in the MS+ compared to MS− group as would be expected. As for the lipid profile, the MS+ group had significantly higher serum levels of triglycerides ($p<0.001$) and lower HDL-C ($p=0.009$) compared to the MS− group as would be expected from the definition of MS. Severity of CAD (based on the number of stenotic coronary vessels), was not significantly different between the groups ($p>0.05$). Demographic characteristics of the study population are summarized in Table 1. The prevalence of individual MS components in MS+ and MS− groups (total and segregated for each gender) is illustrated in Fig. 1.

Bivariate analysis

Bivariate correlations between serum anti-Hsp27 titers and different parameters were assessed in the total study population as well as MS+ and MS− subgroups, separately. In the total population serum anti-Hsp27 was negatively correlated with HDL-C ($p=0.01$), and positively correlated with systolic blood pressure ($p<0.001$). A borderline significant positive correlation with age was also observed ($p=0.07$). In the MS+ group, negative correlation with HDL-C ($p=0.01$) and borderline positive correlations with systolic blood pressure ($p=0.06$) were found. In the MS− group, serum anti-Hsp27 was positively associated with age ($p=0.01$) and systolic blood pressure ($p=0.01$) (Table 2).

With respect to PAB values, a borderline negative correlation with HDL-C ($p=0.05$) was observed in the total population. In the MS+ group, there was a negative correlation with LDL-C ($p=0.01$). In the MS− group, positive correlation with diastolic blood pressure ($p=0.02$) and borderline negative correlations with HDL-C ($p=0.06$) and LDL-C ($p=0.06$) were found (Table 3).

Multivariate analysis

Stepwise multiple regression analysis was used to assess the impact of predictor variables on serum levels of anti-Hsp27 and PAB. Anti-Hsp27 titers were entered into the model after a square root transformation. Predictor variables included in the analysis were those which differed significantly between the MS+ and MS− groups: height, weight, BMI, waist, hip, waist/hip ratio, FBS, triglycerides, LDL-C, HDL-C, systolic and diastolic blood pressures. Among the

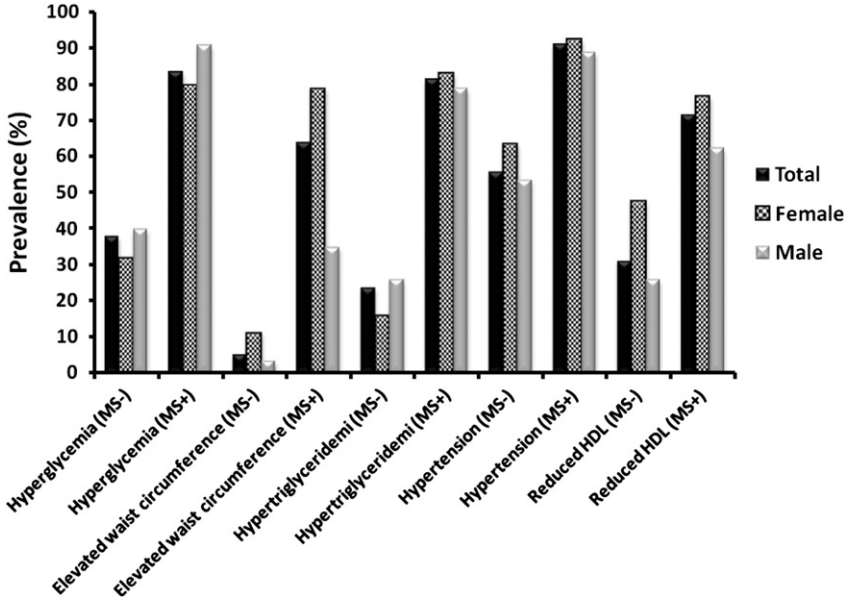

Fig. 1. Prevalence of individual MS components in study groups.

Table 2
Bivariate correlations between anti-Hsp27 titers and different parameters.

	Total		MS+		MS−	
	r	*p*	*r*	*p*	*r*	*p*
Age (years)	0.115	0.07	0.28	0.72	0.27	0.01
Weight (kg)	−0.035	0.62	−0.28	0.75	−0.117	0.33
Height (m)	−0.068	0.33	−0.007	0.93	−0.118	0.33
BMI (kg/m^2)	0.044	0.50	−0.026	0.74	−0.018	0.87
Waist circumference (cm)	0.080	0.22	0.027	0.74	−0.023	0.84
Hip circumference (cm)	0.011	0.86	−0.020	0.81	−0.150	0.19
Waist/hip ratio	0.073	0.27	0.048	0.56	0.058	0.61
SBP (mm Hg)	0.226	0.001	0.147	0.06	0.290	0.01
DBP (mm Hg)	0.004	0.94	−0.071	0.37	0.111	0.33
FBS (mmol/L)	0.037	0.57	0.063	0.43	−0.106	0.34
LDL-C (mmol/L)	0.049	0.48	0.031	0.72	0.077	0.49
HDL-C (mmol/L)	−0.169	0.01	−0.215	0.01	−0.085	0.45
Triglycerides (mmol/L)	0.085	0.21	0.075	0.38	0.042	0.70
Serum PAB (HK)	0.093	0.14	0.133	0.09	0.01	0.92
Serum hs-CRP (mg/L)	−0.125	0.11	−0.151	0.13	−0.050	0.70

MS: metabolic syndrome; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; PAB: pro-oxidant–antioxidant balance; hs-CRP: high-sensitivity C-reactive protein.

aforementioned variables, only HDL-C had significant influence on serum anti-Hsp27 titers ($\beta = -0.002$, 95% CI: −0.004 to −0.001, $p = 0.007$). With respect to PAB values, results of regression analysis did not demonstrate any significant predictor.

Serum anti-Hsp27 in relation with metabolic syndrome and its individual components

Comparison of serum anti-Hsp27 titers between MS+ and MS− groups revealed a significant elevation of titers in the former group (115.63 ± 2.89 and 116.75 ± 4.65 for MS+ and MS− groups, respectively; $p = 0.04$). However, this difference did not remain significant after gender segregation ($p > 0.05$ in males and $p = 0.07$ in females) (Fig. 2a). When assessed for individual components of MS, serum anti-Hsp27 titers were found to be higher in subgroups with elevated triglycerides vs. normal triglycerides ($p = 0.01$), reduced HDL-C vs. normal HDL-C ($p = 0.02$) and elevated blood pressure vs. normal blood pressure ($p = 0.01$). Among females, serum anti-Hsp27 titers were only found to be higher in subjects with elevated triglycerides vs. normal triglycerides ($p = 0.001$). In males, serum anti-Hsp27 titers were not found to be different between subgroups with and without individual MS features, apart from a significant elevation in subjects with reduced HDL-C vs. normal HDL-C ($p = 0.03$) (Table 4).

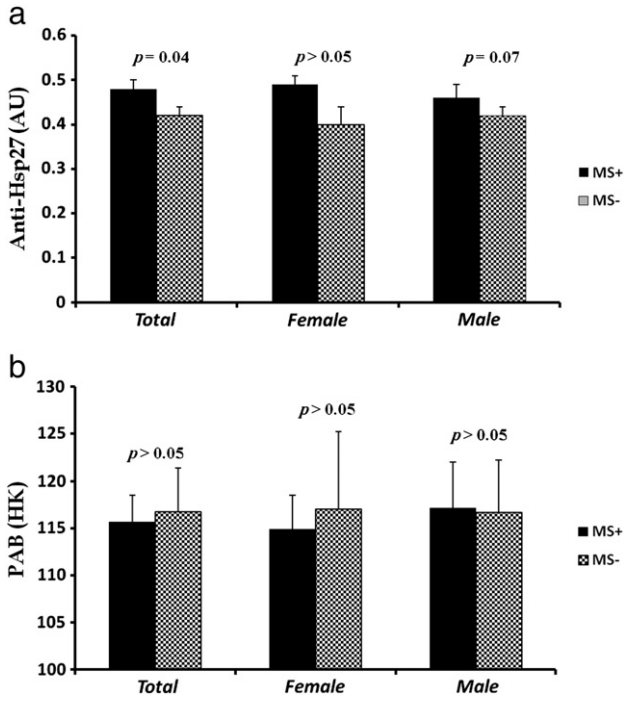

Fig. 2. a. Comparison of anti-Hsp27 titers in MS+ and MS− groups. Values are mean ± SEM. b. Comparison of PAB values in MS+ and MS− groups. Values are mean ± SEM.

Serum PAB in relation with metabolic syndrome and its individual components

Serum PAB values were not found to be significantly different between MS+ and MS− groups ($p > 0.05$). A similar finding was also observed after separate analysis of each gender ($p > 0.05$) (Fig. 2b). With respect to the individual MS components in total population, serum PAB values were significantly higher in subgroups with elevated triglycerides vs. normal triglycerides ($p = 0.03$). After separate analysis in females and males, increased PAB values were only observed in hypertriglyceridemic vs. normotriglyceridemic subjects ($p = 0.054$ and 0.03 in females and males, respectively) (Table 5).

Table 3
Bivariate correlations between PAB values and different parameters.

	Total		MS+		MS−	
	r	*p*	*r*	*p*	*r*	*p*
Age (years)	−0.058	0.37	−0.110	0.16	0.026	0.82
Weight (kg)	−0.008	0.91	−0.038	0.66	−0.073	0.54
Height (m)	0.049	0.48	0.083	0.34	−0.51	0.67
BMI (kg/m^2)	−0.006	0.93	−0.017	0.82	0.029	0.79
Waist circumference (cm)	0.020	0.76	−0.003	0.97	−0.035	0.76
Hip circumference (cm)	−0.029	0.66	−0.102	0.22	0.100	0.38
Waist/hip ratio	0.061	0.36	0.042	0.61	0.065	0.57
SBP (mm Hg)	0.001	0.99	−0.040	0. 62	0.059	0.60
DBP (mm Hg)	0.053	0.41	−0.057	0.47	0.252	0.02
FBS (mmol/L)	0.039	0.55	0.102	0.20	−0.058	0.60
LDL-C (mmol/L)	−0.052	0.45	−0.210	0.01	0.205	0.06
HDL-C (mmol/L)	−0.134	0.05	−0.112	0.20	−0.208	0.06
Triglycerides (mmol/L)	0.080	0.24	0.115	0.18	0.083	0.46
Serum Anti Hsp27 (AU)	0.093	0.14	0.133	0.09	0.010	0.92
Serum hs-CRP (mg/L)	−0.005	0.94	−0.066	0.51	0.137	0.28

MS: metabolic syndrome; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; PAB: pro-oxidant–antioxidant balance; hs-CRP: high-sensitivity C-reactive protein.

Table 4
Anti-Hsp27 titers in subgroups with and without MS components.

		Total	Male	Female
Hypertriglyceridemia	No	0.41 ± 0.17	0.44 ± 0.18	0.36 ± 0.14
	Yes	0.48 ± 0.21	0.45 ± 0.19	0.52 ± 0.21
	p	0.01	0.87	<0.001
Reduced HDL-C	No	0.40 ± 0.16	0.39 ± 0.16	0.43 ± 0.14
	Yes	0.46 ± 0.20	0.47 ± 0.19	0.46 ± 0.20
	p	0.02	0.03	0.58
Elevated waist circumference	No	0.43 ± 0.18	0.45 ± 0.18	0.48 ± 0.21
	Yes	0.49 ± 0.21	0.42 ± 0.20	0.50 ± 0.21
	p	0.11	0.42	0.61
Hypertension	No	0.40 ± 0.21	0.41 ± 0.19	0.49 ± 0.26
	Yes	0.47 ± 0.19	0.46 ± 0.17	0.49 ± 0.20
	p	0.01	0.10	0.97
Hyperglycemia	No	0.44 ± 0.19	0.43 ± 0.15	0.46 ± 0.22
	Yes	0.46 ± 0.20	0.44 ± 0.19	0.48 ± 0.20
	p	0.77	0.70	0.93

MS: metabolic syndrome; HDL-C: high-density lipoprotein cholesterol.

Table 5
PAB values in subgroups with and without MS components.

		Total	Male	Female
Hypertriglyceridemia	No	109.59 ± 40.20	111.28 ± 42.77	106.62 ± 35.39
	Yes	120.54 ± 36.74	125.72 ± 36.72	119.37 ± 37.10
	p	0.03	0.03	0.054
Reduced HDL-C	No	114.14 ± 39.34	119.43 ± 41.54	104.33 ± 31.75
	Yes	113.71 ± 38.15	115.04 ± 3763	112.75 ± 38.20
	p	0.93	0.57	0.28
Elevated waist circumference	No	119.64 ± 41.62	126.43 ± 43.15	125.85 ± 36.74
	Yes	112.56 ± 37.94	113.17 ± 37.51	115.33 ± 38.62
	p	0.17	0.14	0.15
Hypertension	No	121.48 ± 40.11	128.79 ± 44.09	126.18 ± 20.87
	Yes	114.41 ± 37.96	122.13 ± 42.22	116.33 ± 37.94
	p	0.25	0.45	0.27
Hyperglycemia	No	117.14 ± 41.26	125.63 ± 42.85	116.53 ± 40.53
	Yes	115.25 ± 37.76	120.51 ± 41.86	117.49 ± 36.86
	p	0.72	0.72	0.93

PAB: pro-oxidant–antioxidant balance; MS: metabolic syndrome; HDL-C: high-density lipoprotein cholesterol.

Discussion

In the current study, significantly higher anti-Hsp27 titers were found in patients with MS. These elevated titers could be attributed to the impact of different MS-related risk factors which affect the expression, release and consequently immune response to Hsp27. In previous investigations, several common characteristics of CVD and MS such as hypertension, diabetes, hyperlipidemia and smoking have been found to induce the expression of Hsps which is most probably due to the promotion of oxidative stress [8–10,17]. As for the Hsp27, increased expression in association with hypertension [18] and diabetes [19] has been mentioned. There have also been several reports on the potential cardioprotective effects of Hsp27. Martin-Ventura and colleagues have reported an inverse correlation between tissue levels of Hsp27 in atherosclerotic plaques compared to non-plaque areas of carotid artery, which suggests a cardioprotective function for Hsp27 [20]. Robinson et al. have reported decreased levels of phosphorylated-Hsp27 in coronary arteries of patients with ischemic heart disease compared to non-diseased vascular specimens [21]. However in a prospective survey, Kardys et al. could not find a significant association between baseline plasma Hsp27 antigen and future CVD during a ~6 year follow-up [22]. The latter may be for one of several reasons: for example Hsp27 upregulation and release might be a relatively late consequence of acute coronary events and CVD, rather than their cause. Therefore elevated titers may not always be expected to precede CVD; or there may be a shorter temporal relationship between increasing levels and cardiac events. Furthermore, the participants of the latter study were women with mean age of approximately 61 years. Such a population may not be representative of a general population as the impact of estrogen on Hsp27 expression has been previously demonstrated [23,24]. All of the aforementioned studies have dealt with Hsp27 and not its antibody titers. It is worth noting that Hsp27 and anti-Hsp27 may have quite different functions in the cardiovascular system. Having in mind the cardioprotective function of Hsp27, an important issue which deserves further attention is the autoimmune response to Hsp27, reflected in raised anti-Hsp27 titers, that has the potential to further increase cardiovascular risk. Such a relationship between anti-Hsp titers and progression of cardiovascular disease has been shown for Hsp classes 60 [25,26]. However, investigations regarding serum anti-Hsp titers have been far less, and in relation to metabolic syndrome, data are scant.

Raised antibody titers to Hsps have been reported to be associated with the presence and progression of vascular disease [7,8]. Elevated anti-Hsp60 titers have been documented in patients with CAD [27] and peripheral vascular disease [28], and found to correlate with the severity of CAD [26,29]. High levels of anti-Hsp65 have also been reported to correlate with carotid atherosclerosis [30] and borderline hypertension [31], and predict the development of myocardial infarction, stroke, or cardiovascular mortality of patients [32,33]. However, findings have not been consistent [34] and the clinical significance of such elevated anti-Hsp titers is yet to be clarified.

In comparison to Hsps 60, 65 and 70, there have been fewer studies investigating antibody titers to Hsp27 in cardiovascular disorders. There have been reports on the elevated anti-Hsp27 titers in patients with dyslipidemia [35], CAD [14], acute cardiac chest pain [12] and acute coronary syndrome (in the first 12 h following the onset of chest pain) [13]. Furthermore, a recent study has shown that a plausible association exists between serum anti-Hsp27 titers and presence of cardiovascular complications in patients with glucose intolerance [36]. However, serum anti-Hsp27 has not been investigated in CAD patients with MS. The observed elevation of anti-Hsp27 titers in the MS+ compared to the MS− group could not be attributed to the advancement of CAD in the former group as the two groups were comparable regarding the severity of CAD (evaluated based on the number of stenotic vessels in angiography).

A significant finding to emerge from this study was the role of HDL-C as the negative predictor of serum anti-Hsp27 titers. Owing to the well-known paraoxonase activity and antioxidant functions of HDL [37], there is a plausible mechanistic basis for this inverse association. Higher HDL could mitigate oxidative stress, thereby decreasing Hsp27 expression, release and immune response.

Another evaluated measure in the present study was PAB status. The PAB assay that was applied here is a recently described rapid and inexpensive method for the assessment of oxidative status. Using this method, heightened oxidative stress has been reported in patients with type 2 diabetes [38], CAD [16], exfoliative glaucoma [39], stroke [40] and acute coronary syndrome [41]. Moreover, PAB values obtained by this method have been reported to be decreased following percutaneous coronary intervention (PCI) [42] and simvastatin therapy [43].

Upon comparing PAB values between MS+ vs. MS− groups, no significant difference was observed, even when each gender was looked at separately. There are two possible explanations for this lack of significance. First, although subjects in the MS− group did not fulfill criteria to be categorized as having MS, the prevalence of MS features such as hyperglycemia, hypertension and reduced HDL-C, together with smoking was high in this group. All of these disorders could be linked with oxidative stress and hence contribute to the heightened PAB state in the MS− group [44–47]. Second, all patients in both MS+ and MS− groups had established CAD, a condition which is closely associated with an augmented oxidative stress status [48]. Therefore, it is likely that PAB values in both groups had already been increased as a consequence of CAD, as previously reported.

In summary, the most obvious finding to emerge from this large cohort is the presence of elevated anti-Hsp27 titers in patients with documented CAD and MS compared to those with only CAD. However, current results do not support any elevation in PAB values in the MS group beyond what is caused by CAD. In order to confirm this observation, further research is needed to provide more definitive evidence using other biomarkers of oxidative stress. Features of MS, such as glucose intolerance, in patients with established CAD could be associated with increased risk of vascular complications and more susceptibility to future cardio- and cerebrovascular endpoints. Therefore, the elevated anti-Hsp27 titers in subjects with MS might be of prognostic value for the prediction of future cardiovascular events. To clarify this issue, prospective studies need to be undertaken. Finally, it is recommended that further work be undertaken in the following areas: investigation of the causative relationship between raised anti-Hsp27 titers and CVD; comparison of anti-Hsp27 status between non-CAD individuals with and without MS; exploring the association between anti-Hsp27 titers and different echocardiographic and angiographic findings of CAD patients; and determination of anti-Hsp27 level in different subcategories of CVD.

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