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Relationship between serum anti-heat shock protein 27 antibody levels and obesity

Mehrdad Kargari[1,2,3,*], Samira Tavassoli[4,*], Amir Avan[3,5,*], Mahmoud Ebrahimi[6], Mahmoud Reza Azarpazhooh[6], Rasool Asoodeh[7], Mohsen Nematy[3], Seyed Mahdi Hassanian[1,3], Farzad Rahmani[1,3], Elham Mohammadzade[3], Habibollah Esmaeili[3,4], Mohsen Moohebati[6], Gordon A. Ferns[8], Majid Ghayour-Mobarhan[3,5,†], Seyed Mohammad Reza Parizadeh[3,†]

Affiliations:

(1) Department of Clinical Biochemistry, School of Medicine. Mashhad University of Medical Sciences, Mashhad, Iran; (2) Department of Basic Medical Sciences, Neyshabur University of Medical Sciences, Neyshabur, Iran; (3) Metabolic syndrome Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; (4) Department of Biochemistry, Payam Noor University of Mashhad, Mashhad, Iran; (5) Department of Modern Sciences and Technologies, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; (6) Cardiovascular Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; (7) Department of biostatistics and Epidemiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; (8) Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex BN1 9PH, UK.

†Corresponding Author:

Majid Ghayour-Mobarhan, MD, PhD; Metabolic syndrome Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, 99199-91766, Tel:+985118002288, Fax: +985118002287; Email: ghayourm@mums.ac.ir.
Seyed Mohammad Reza Parizadeh, PhD, Metabolic syndrome Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, 99199-91766, Tel:+985118002288, Fax: +985118002287; Email: Parizadehmr@mums.ac.ir.

Running title: Anti-HSP27 antibody levels in obese subjects

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* Equally contributed as first authors

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Abstract

Background: Heat shock protein 27 (HSP27) is an intracellular molecular chaperone that is expressed at high levels following the exposure of cells to environmental stressors such as heat, toxins, and free radicals. High levels of HSP antigens and antibody titers have been reported in several conditions including cardiovascular disease and cancers. We measured serum anti-HSP27 antibody levels in 993 subjects and assessed the associations between serum anti-HSP27 antibody levels and demographic characteristics including coronary risk factors.

Methods: A total of 993 subjects were recruited as part of the Mashhad Stroke and Heart Atherosclerotic Disorders (MASHAD) cohort study. Demographic, clinical, and biochemical parameters and serum anti-HSP27 antibody titers were determined in all the subjects.

Results: Serum anti-HSP27 antibody levels increased with increasing age in men. No significant differences in levels were detected between men and women. Serum anti-HSP27 antibody levels were significantly higher in obese subjects than in nonobese subjects (P=0.046); however, no significant influence of smoking status was observed. Moreover, serum anti-HSP27 antibody titers were positively associated with age, body mass index, waist/hip ratio, the presence of diabetes mellitus, nonsmoking habit, serum triglycerides, cholesterol, and high-sensitivity c-reactive protein.

Conclusion: We have found that serum anti-HSP27 antibody titers are related to several cardiovascular risk factors, necessitating further studies on the value of this emerging marker for risk stratification.

Keywords: Anti-heat Shock Protein 27, Cardiovascular Risk Factors, Determinants

Introduction:

Heat shock protein 27 (HSP27) is an intracellular molecular chaperone that is expressed in response to the exposure of cells to environmental stressors (1) and increases the ability of cells to overcome environmental stress (2). HSPs modify several aspects of cell function, including differentiation, apoptosis, and the repair and refolding of denatured proteins (3). They are associated with cell exposure to a wide variety of environmental stresses including high temperature, viral infection, oxidative stress, ischemia, heavy metals, ultraviolet irradiation, and reactive oxygen species (ROS) (4).

HSPs are highly immunogenic, and it is believed that an autoimmune response to HSPs contributes to rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, lupus erythematosus (5, 6), and diabetes mellitus (1). There are several HSP families of proteins: HSPH (former name HSP110), HSPC (HSP90), HSPA (HSP70), HSPD/E (HSP60/HSP10), CCT (TRiC), DNAJ (HSP40), and HSPB (small HSP or sHSP) including HSP27 (7). HSP27 is a member of the sHSP family of proteins (8), and high serum titers of this protein have been reported in individuals with cardiovascular disease (CVD) (9). HSP27 has been suggested as a marker of inflammation (10), and immune response to HSPs may contribute to the progression of atherosclerosis. Anti-HSP27 antibodies titers were increased during atherogenesis (9, 11). Serum anti-HSP27 antibody titers have been suggested to predict outcomes in cancer and may help to identify individuals at high risk of CVD (1).

There is growing evidence of a relationship between HSPs and inflammatory diseases, including CVD. Most studies have focused on HSP/anti-HSP60, 65, and 70, although recent data support the role of HSP/anti-HSP27 in CVD, which is the leading cause of death worldwide (9, 12, 13).

We have previously demonstrated an association between hypertension and serum anti-HSP27 antibody levels; however, the association with diabetes mellitus and smoking status

was not statistically significant for a sample of 168 patients. Moreover, we previously reported a relationship between anti-HSP27 antibody levels and risk of myocardial infarction (13). The present study aimed to investigate serum anti-HSP27 antibody levels and determine factors affecting it in a large population.

Materials and methods:

Population

A total of 933 subjects, including 456 men and 477 women, of age 35-64 years, were enrolled as part of the Mashhad Stroke and Heart Atherosclerotic Disorders Study, Mashhad University of Medical Sciences (14). Exclusion criteria included subjects with severe hypertension, CVD or history of CVD, cerebrovascular disease, endocrine abnormalities, infectious disease, diabetes mellitus, and pregnant or breastfeeding women (14, 15). The protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences, and all the subjects provided written informed consent to participate in the study.

Anthropometric measurements

Anthropometric parameters including height, body weight, waist circumference, and hip circumference were measured for all participants as described previously (15). Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). In addition, we collected information about the socioeconomic status of our subjects. The subjects were divided into three subgroups: obese (BMI≥30 kg/m^2), overweight (30>BMI≥25 kg/m^2), and normal weight (BMI<25 kg/m^2) (14, 15).

B*iochemical analysis*

Lipid profile, including total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), was evaluated in all the subjects.

Fasting blood glucose (FBG) levels were measured as described before (14-15) using the BT-3000 auto-analyzer machine (Biotechnica, Rome, Italy). High-sensitivity C-reactive protein (hs-CRP) was measured by a PEG-enhanced immunoturbidimetry method using an Alycon analyzer (ABBOTT, Chicago, IL, USA).

Serum anti-HSP27 antibody titer measurements

Anti-HSP27 antibody titers were determined in serum as previously described (16, 17). Briefly, microtiter plates (Nunc Maxisorp, 3Nunc) were coated with 100 ng of recombinant human HSP27 dissolved in 50 μL of carbonate buffer followed by incubation for 18 h at 4 °C. The wells were washed thrice with wash buffer (Phosphate-buffered saline (PBS) containing 0.05% Tween-20). Nonspecific binding was reduced by blocking each well with 2% goat serum in PBS: 250 μL added to each well and incubated for 30 min at 37 °C and 30 min at room temperature. Serum was diluted to 1:100 with 2% goat serum in PBS. After washing, 100 μL of peroxidase-conjugated goat anti-human IgG (Sigma-Aldrich, 4Inc., USA) diluted to 1:500 with 2% goat serum in PBS was added to each well and incubated for 30 min at room temperature. Subsequently, 100 μL of tetramethylbenzidine (TMB) substrate [200 μL of 6 mg⁄mL TMB in Dimethyl Sulfoxide (DMSO) added to 10 mL of 50 mM acetate buffer, pH 4.5, containing 6 μL of hydrogen peroxide (H_2O_2)] was added to each well, and the plate was incubated for 15 min in the dark at room temperature. The reaction was terminated by adding 50 μL of 2 M HCl to each well. Optical density was measured using a Labsystems iEMS Reader Microtiter plate reader. After correction for nonspecific background absorbance (subtracting the absorbance of uncoated wells from the antigen-coated wells for each sample), the results were expressed in optical density units.

Statistical analysis

Data were analyzed using SPSS software (version 11.5). The normality of distribution was assessed using the Kolmogorov-Smirnov test. Descriptive statistics (mean±standard deviation (SD) or median±interquartile range (IQR) were determined for normally or non-normally distributed variables, respectively. For normally and non-normally distributed variables, Student's t-test and Mann-Whitney U test were used, respectively, to compare the clinical characteristics and baseline demographics between the groups. Chi-square test and/or Fisher exact test were used for comparing categorical variables. Pearson and Spearman correlation tests were used to show significant correlation between two normal and two non-normal quantitative variables, respectively. Non-normally distributed data were analyzed by Kruskal–Wallis test (for more than two groups). Spearman correlation was used to investigate the relationship between the anti-HSP antibody titers and atherosclerosis risk factors including age, serum total cholesterol, hs-CRP, triglyceride, HDL-C, LDL-C, cholesterol/HDL-C ratio, BMI, systolic and diastolic blood pressure, FBG, and waist and hip circumferences, height, and waist/hip ratio. We determined the factors that were significantly related to serum HSP27 antibody titers using stepwise multiple regression. A two-sided $P<0.05$ was considered significant.

Results

Effect of gender, age, and smoking status on serum anti-HSP27 antibody levels

As shown in Table 1, age, FBG, waist/hip ratio, LDL-C, serum triglycerides, systolic blood pressure, and presence of other CVD risk factors including diabetes mellitus and hypertension ($P>0.05$) were not significantly different between the groups. However, women had a significantly higher BMI, waist and hip circumferences, HDL-C, and hs-CRP (Table 1). Serum anti-HSP27 antibody levels did not differ between men and women. Similar results were also observed after dividing the participants in to age cohorts: 35-44, 45-54, and 55-65 years (Table 2).

Effect of BMI on serum anti-HSP27 antibody level

Obese subjects had significantly higher BMI, waist/hip ratio, FBG, serum triglyceride, hs-CRP, SBP/DBP, and LDL-C; HDL-C levels were lower in obese than in nonobese subjects ($P<0.05$). Hypertension and dyslipidemia were more common in the obese subjects than in the nonobese subjects (Table 3). Serum anti-HSP27 antibody titer was significantly higher in the obese subjects than in the nonobese subjects ($P=0.046$, Table 5). After adjustment, the differences between the groups remained significant (BMI: $\beta=0.036$, $P=0.037$).

Effect of socioeconomic status on serum anti-HSP27 antibody levels

Subjects were divided into six subgroups of educational attainment: primary school, secondary school, high school, diploma, bachelor or postgraduate, and PhD or MD. Serum anti-HSP27 antibody titers were not significantly different between the groups. Similar results were observed for the effect of employment status on anti-HSP27 antibody levels (Table 4-5).

Effect of atherosclerosis risk factors on anti-HSP27 antibody levels

Associations between anti-HSP27 antibody levels and atherosclerosis risk factors were assessed by general linear model analysis as the dependent variable (anti-HSP27) is quantitative and independent variables are both qualitative and quantitative. These results showed that age, BMI, waist/hip ratio, triglycerides, cholesterol, hs-CRP, hyperlipidemia, and hypertension were positively associated with serum anti-HSP27 antibody titers (Table 6).

Discussion

To the best of our knowledge, this is the first study evaluating the effect of demographic characteristics and socioeconomic status with respect to serum anti-HSP27 antibody titers. We found that obese subjects had significantly higher anti-HSP27 antibody titers than the nonobese group, which is in agreement with our previous report (16). Therefore, our recent data suggest that this biological marker can be used as a novel risk biomarker for CVDs.

There is growing body of data suggesting the important role of this marker in CAD and stroke (12-13, 16-18). In the present study, we also investigated its relationship with age. Our data showed that serum anti-HSP27 antibody titer did not differ with gender, which is consistent with the results of Zilaee et al, who observed no significant relationship between gender and antibody titers to HSP27 and 60 (18). Moreover, Rea et al, in a study of 60 individuals aged between 20 and 96 years, showed that the anti-HSP70 antibody levels increased significantly with age (19). Another study also reported that serum anti-HSP27 antibody levels increased with age, and it was associated with hypertension (20). We found a significant increase in anti-HSP27 antibody levels with respect to age in the male subgroup, which is in agreement with a previous report (20). Moreover, we explored the effect of smoking status on serum anti-HSP27 antibody titers. However, no statistically significant differences were observed between groups, which is consistent with our previous data (21).

There is accumulating evidence showing the association of inflammatory markers such as CRP, TNF-α, and IL-6 with obesity (22). Our previous data revealed the association of body mass and antibody titers to HSP60, 65, and 70 in 170 healthy Caucasians (23). In the current study, we showed for the first time the important role of serum anti-HSP27 antibody titers as a biomarker in obese subjects. Previous studies have shown that serum anti-HSP27 antibody levels may reflect inflammatory status (10, 23). The role of anti-HSP antibody titers has been shown during the process of atherosclerosis (9, 11), and the high levels of anti-HSP27 antibodies in obese subjects suggest that an immune response to HSPs contributes to the progression of atherosclerosis in these subjects.

The effect of socioeconomic status on serum anti-HSP27 antibody titers was studied in our population, although no statistically significant relationship was observed for these parameters. Victora et al (2007) showed no association between serum anti-HSP60 antibody

titers and maternal schooling, skin color, or any other socioeconomic or environmental status (24).

A major strength of the present study is that it was performed in a large number of subjects, and the effect of demographic, biochemical, and socioeconomic status of the population on the anti-HSP27 antibody levels was studied. Moreover, this is the first study showing the effect of obesity, a risk factor for CVD, on the level of serum anti-HSP27 antibody titers.

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Table 1: Comparison of clinical and biochemical characteristics between men and women				
	Total	**men**	**women**	p-value
	933	456	477	-
Age (year)	47.35±7.97	47.37±7.97	47.34±7.98	0.974
Height (m)	1.62±0.09	1.69±0.07	1.56±0.06	0.001
Weight (kg)	73.44±13.14	75.75±13.7	71.24±12.2	0.001
BMI (kg/m^2)	27.92±4.75	26.39±4.32	29.39±4.69	0.001
FBG (mg/dL)	90.16±35.32	90.37±30.92	89.96±39.11	0.081
WC (cm)	95.58±12.25	93.49±11.07	97.56±12.98	0.001
HC (cm)	103.72±9.34	101.06±8.05	106.27±9.78	0.001
WHR	0.92±0.09	0.93±0.1	0.92±0.09	0.635
Serum LDL-C (mg/dL)	116.17±36.7	113.85±36.25	118.39±37.02	0.064
Serum HDL-C (mg/dL)	42.42±9.91	40.05±9.73	44.71±9.55	0.001
Serum TG (mg/dL)	116(84-168)	121 (87-172)	113 (81-162)	0.153
Serum hs-CRP (mg/dL)	1.61(1-3.46)	1.45 (0.89-3)	1.9 (1.11-3.7)	0.001
SBP (mmHg)	121.44±18.06	121.69±16.23	121.21±19.66	0.246
DBP (mmHg)	79.12±11	79.85±10.44	78.42±11.48	0.025
Diabetics (%)	7.45% (69.926)	7.51% (34.453)	7.4% (35.473)	0.771
Smoker (%)	24.44% (228.933)	29.61% (135.456)	19.5%(93.477)	0.001
Hypertensive (%)	22.07% (203.920)	22.27% (100.449)	21.87% (103.471)	0.883
Hyperlipidemic (%)	36.02% (335.930)	32.68% (149.456)	39.24% (186.474)	0.037
Anti-HSP 27 (OD)	0.23(0.1-0.43)	0.24 (0.11-0.43)	0.22 (0.1-0.43)	0.563

BMI, body mass index; FBG, fasting blood sugar; WC, waist circumferences; HC, hip circumferences; WHR, waist/hip ratio; 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.

Diabetes was defined as fasting blood glucose of ≥126 mg/L. Hypertension was defined as SBP of ≥140 mmHg and DBP of ≥90 mmHg. Hyperlipidemia was defined as a total cholesterol ≥5.2 mmol/L (200 mg/dL), triglycerides ≥1.5 mmol/L (150 mg/dL), and HDL-C <0.9 mmol/L (40 mg/dL) (for men) and <1.29 mmol/L (50 mg/dL) (for women).

Values are expressed as mean±SD, and interquartile range or median and Mann–Whitney U test were used for normally and non-normally distributed data, respectively.

Table 2: Comparison of clinical and biochemical characteristics between subgroups for age				
Age (year)	**35-44**	**45-54**	**55-65**	**p-value**
number	393	349	191	-
Height (m)	1.63±0.09	1.62±0.09	1.6±0.1	0.004
Weight (kg)	74.08±14.45	72.82±12.11	73.26±12.1	0.783
BMI (kg/m^2)	27.81±5.05	27.72±4.57	28.53±4.42	0.047
FBG (mg/dl)	85.73±27.18	91.85±35.74	96.17±46.61	0.001
WC (cm)	94.28±12.36	95.65±12.11	98.11±11.93	0.001
HC (cm)	104.2±9.74	103.34±9.12	103.47±8.95	0.53
WHR	0.91±0.11	0.93±0.08	0.95±0.07	0.001
Serum LDL-C (mg/dL)	109.81±36.14	118.83±35.94	124.46±37.15	0.001
Serum HDL-C (mg/dL)	41.84±9.68	43.4±10.54	41.85±9.08	0.118
Serum TG (mg/dL)	112 (77.25-157.75)	119 (84-166)	124 (99.75-186.25)	0.002
Serum hs-CRP (mg/dL)	1.54 (0.9-3.02)	1.62 (1.08-3.3)	2.01 (1.08-5.08)	0.006
SBP (mmHg)	115.11±14.53	123.23±17.21	131.19±20.91	0.001
DBP (mmHg)	76.52±10.76	80.25±10.67	82.39±10.93	0.001
Diabetics (%)	5.13% (20/390)	8.96% (31/346)	9.47% (18/190)	0.003
Smoker (%)	27.99% (110/393)	22.06% (77/349)	21.47% (41/191)	0.097
Hypertensive (%)	14.21% (55/387)	23.77% (82/345)	35.11% (66/188)	0.001
Hyperlipidemic (%)	28.75% (113/393)	38.9% (135/347)	45.79% (87/190)	0.001
Anti-HSP 27 (OD)	0.29±0.26	0.32±0.28	0.33±0.29	0.134

BMI, body mass index; FBG, fasting blood sugar; WC, waist circumferences; HC, hip circumferences; WHR, waist/hip ratio; 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.

Diabetes was defined as fasting blood glucose of ≥126 mg/L. Hypertension was defined as a SBP ≥140 mmHg and a DBP ≥90 mmHg. Hyperlipidemia was defined as a total cholesterol (TC) ≥5.2 mmol/l (200 mg/dl), triglycerides (TG) ≥1.5 mmol/l (150 mg/dl), and HDL-C <0.9 mmol/l (40 mg/dl) (for men), and <1.29 mmol/l (50 mg/dl)(for women).

Values are expressed as mean±SD or median(interquartile range) and One-Way ANOVA and Kruskal Wallis test were used for normal and non-normal distributed data, respectively.

Table 3: Comparison of clinical and biochemical characteristics between subgroups of BMI				
	obese	**overweight**	**normal weight**	**p-value**
number	283	393	256	-
Age (year)	48±8.07	47.16±7.78	46.95±8.15	0.104
Height (m)	1.59±0.09	1.63±0.09	1.65±0.09	0.001
Weight (kg)	85.19±11.92	72.94±8.2	61.23±8.21	0.001
BMI (kg/m^2)	33.49±3.06	27.52±1.39	22.4±1.97	0.001
FBG (mg/dl)	93.14±36.48	89.46±30.3	87.72±40.5	0.006
WC (cm)	106.4±10.53	94.66±8.54	84.98±8.32	0.001
HC (cm)	112.9±8.15	102.51±5.9	95.41±5.01	0.001
WHR	0.94±0.07	0.93±0.11	0.89±0.07	0.001
Serum LDL-C (mg/dL)	120.58±36.79	.115.21±38	.112.73±33	0.013
Serum HDL-C (mg/dL)	41.8±8.96	42.09±10.3	43.67±10.1	0.047
Serum TG (mg/dL)	130(104.25-179.75)	124 (89.25-178)	92.5 (66.75-125)	0.001
Serum hs-CRP (mg/dL)	2.81 (1.47-5.63)	1.51 (0.97-2.79)	1.22 (0.75-2.3)	0.001
SBP (mmHg)	125.52±19.64	121.37±17.0	117.02±16.0	0.001
DBP (mmHg)	81.68±11.15	79.18±10.5	76.19±10.7	0.001
Diabetics (%)	8.9% (25/281)	7.7%	5.1%	0.033
Smokers (%)	24.03% (68.283)	24.17%	25.39%	0.714
Hypertensive (%)	27.86% (78/280)	21.13% (82/388)	17.06%	0.003
Hyperlipidemic (%)	41.13%	39.03%	25.88%	0.001
Anti-HSP27 (OD)	0.27 (0.12-0.48)	0.21 (0.1-0.42)	0.23 (0.1-0.42)	0.046

BMI, body mass index; FBG, fasting blood sugar; WC, waist circumferences; HC, hip circumferences; WHR, waist/hip ratio; 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.

Diabetes was defined as fasting blood glucose of ≥126 mg/L. Hypertension was defined as a SBP ≥140 mmHg and a DBP ≥90 mmHg. Hyperlipidaemia was defined as a total cholesterol (TC) ≥5.2 mmol/l (200 mg/dl), triglycerides (TG) ≥1.5 mmol/l (150 mg/dl), and HDL-C <0.9 mmol/l (40 mg/dl) (for men), and <1.29 mmol/l (50 mg/dl)(for women).

Values are expressed as mean±SD, and interquartile range or median and Mann–Whitney test were used for normality and non-normality distributed data, respectively.

Table 4: Comparison of clinical and biochemical characteristics between subgroups of educational attainment							
	Primary School	**Secondary School**	**High School**	**Diploma**	**Bachelor or Postgraduate**	**PhD or MD**	**p-value**
Number	110	358	362	27	69	6	-
Age (year)	53.07±8.08	47.98±8	45.15±7.08	47.74±8.15	46.73±7.57	47.5±4.32	0.001
Height (m)	1.57±0.09	1.61±0.08	1.64±0.1	1.68±0.07	1.68±0.08	1.68±0.08	0.001
Weight (kg)	70.34±11.7	72.63±12.28	75±13.98	75.41±11.28	73.47±14.37	84.67±14.69	0.041
BMI (kg/m^2)	28.7±4.79	28.2±4.61	27.88±4.9	26.84±4.34	25.99±4.06	30.26±6.37	0.001
FBG (mg/dL)	95.36±54.78	90.5±30.22	88.37±32.29	90.38±29.19	88.55±38.16	113±50.63	0.153
WC (cm)	99.3±12.21	97.14±12.64	93.99±11.71	92.9±10.77	90.51±11.07	101±11.1	0.001
HC (cm)	104.06±9.11	104.46±9.24	103.62±9.9	102.09±6.97	100.4±7.19	107.08±11.67	0.045
WHR	0.95±0.07	0.93±0.08	0.91±0.12	0.91±0.07	0.9±0.07	0.94±0.03	0.001
Serum LDL-C (mg/dL)	123.23±39.14	118.99±37.67	111.92±33.7	118.96±38.76	114.46±39.52	100.16±20.17	0.063
Serum HDL-C (mg/dL)	44.42±9.91	42.98±10.06	41.55±9.92	41.03±8.53	42.22±9.61	37.43±6.67	0.042
Serum TG (mg/dL)	128 (96.75-178)	118 (83-161)	113 (83.75-162)	112 (63-176)	114.5 (74.75-170.75)	192.5 (114-267.25)	0.286
Serum hs-CRP (mg/dL)	1.9 (1.27-4.29)	1.64 (1.06-3.63)	1.51 (0.9-3.21)	1.37 (0.77-5.75)	1.18 (0.68-2.32)	3.92 (1.42-9.05)	0.006
SBP (mmHg)	129.56±21.65	122.63±18.2	118.51±16.16	115.75±16.96	119.79±16.32	133.39±14.85	0.001
DBP (mmHg)	81.97±11.7	79.47±11.42	78.17±10.15	77.48±10.55	78.39±11.18	86.84±10.99	0.013
Diabetics (%)	7.41% (8/108)	8.15% (29/356)	6.44% (23/357)	7.69% (2/26)	7.58% (5/66)	33.33% (2/6)	0.516
Smoker (%)	30% (33/110)	27.65% (99/358)	20.89% (75/359)	22.22% (6/27)	18.18% (12/66)	33.33% (2/6)	0.245
Hypertensive (%)	34.86% (38/109)	24.23% (86/355)	15.86% (56/353)	25.93% (7/27)	19.05% (12/63)	50% (3/6)	0.001
Hyperlipidemic (%)	43.52% (47/108)	38.94% (139/357)	30.64% (110/359)	37.04% (10/27)	34.85% (23/66)	66.67% (4/6)	0.14
Anti-HSP 27 (OD)	0.21 (0.09-0.43)	0.21 (0.1-0.43)	0.26 (0.13-0.44)	0.2 (0.07-0.45)	0.22 (0.08-0.41)	0.23 (0.08-0.59)	0.295

BMI, body mass index; FBG, fasting blood sugar; WC, waist circumferences; HC, hip circumferences; WHR, waist/hip ratio; 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.

Diabetes was defined as fasting blood glucose of ≥126 mg/L. Hypertension was defined as a SBP ≥140 mmHg and a DBP ≥90 mmHg. Hyperlipidaemia was defined as a total cholesterol (TC) ≥5.2 mmol/l (200 mg/dl), triglycerides (TG) ≥1.5 mmol/l (150 mg/dl), and HDL-C <0.9 mmol/l (40 mg/dl) (for men), and <1.29 mmol/l (50 mg/dl)(for women).

Values are expressed as mean±SD, and interquartile range or median. Kruskal–Wallis and Chi-square tests were used.

Table 5: Comparison of clinical and biochemical characteristics between subgroups of employment status				
	Employed	**Unemployed**	**Retired**	**p-value**
Number	414	435	84	-
Age (year)	45.69±7.32	47.53±8.02	54.65±6.46	0.001
Height (m)	1.68±0.08	1.56±0.07	1.66±0.09	0.001
Weight (kg)	76.24±14.12	70.57±11.68	74.57±12.32	0.001
BMI (kg/m^2)	27.07±4.8	28.9±4.67	27.06±3.96	0.001
FBG (mg/dl)	88.68±28.89	91.2±40.93	92.07±32.89	0.411
WC (cm)	94.53±11.69	96.85±12.86	94.09±11.13	0.012
HC (cm)	102.58±9.2	105.25±9.49	101.29±7.95	0.001
WHR	0.92±0.11	0.92±0.9	0.93±0.07	0.39
Serum LDL-C (mg/dL)	115.18±37.61	117.43±35.5	114.55±38.39	0.507
Serum HDL-C (mg/dL)	40.91±10.06	43.98±9.46	41.85±10.31	0.001
Serum TG (mg/dL)	120 (84-170)	113 (82-161.25)	122.5 (94-182)	0.273
Serum hs-CRP (mg/dL)	1.44 (0.89-3.3)	1.84 (1.12-3.59)	1.59 (1.03-3.02)	0.02
SBP (mmHg)	120.44±15.76	121.41±20.11	126.49±16.62	0.011
DBP (mmHg)	79.01±10.4	78.61±11.69	82.33±9.71	0.026
Diabetics (%)	6.81% (28/411)	7.89% (34/431)	8.33% (7/84)	0.96
Smokers (%)	28.74% (119/414)	21.84% (95/435)	16.67% (14/84)	0.014
Hypertensive (%)	20.69% (84/406)	22.27% (96/431)	27.71% (23/83)	0.369
Hyperlipidemic (%)	34.06% (141/414)	38.19% (165/432)	34.52% (29/84)	0.436
Anti-HSP27 (OD)	0.23 (0.1-0.41)	0.23 (0.1-0.44)	0.28 (0.15-0.47)	0.183

BMI, body mass index; FBG, fasting blood sugar; WC, waist circumferences; HC, hip circumferences; WHR, waist/hip ratio; 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.

Diabetes was defined as fasting blood glucose of ≥126 mg/L. Hypertension was defined as a SBP ≥140 mmHg and a DBP ≥90 mmHg. Hyperlipidemia was defined as a total cholesterol (TC) ≥5.2 mmol/l (200 mg/dl), triglycerides (TG) ≥1.5 mmol/l (150 mg/dl), and HDL-C <0.9 mmol/l (40 mg/dl) (for men), and <1.29 mmol/l (50 mg/dl)(for women).

Values are expressed as mean±SD, and interquartile range or median. Kruskal–Wallis and Chi-square tests were used.

Table 6: Association between anti-HSP27 antibody levels and risk factors of atherosclerosis using general linear model analysis				
Parameter Estimates				
Dependent Variable: Anti_HSP27				
Parameter	**β**	**Std. Error**	**t**	**p-value**
Intercept	-1.901	0.974	1.951	0.051
Age	0.001	0.001	0.789	0.430
Height	1.216	0.587	2.072	0.039
Weight	-0.012	0.006	1.948	0.052
BMI	0.037	0.017	2.210	0.027
WHR	0.100	0.103	0.972	0.331
Serum LDL-C	-0.002	0.001	3.988	0.000
Serum HDL-C	-0.002	0.001	1.648	0.100
Serum TG	0.000	0.000	3.620	0.000
Serum total cholesterol	0.002	0.001	2.842	0.005
Serum hs-CRP	0.001	0.001	0.687	0.492
Diabetes	-0.040	0.036	1.123	0.262
Smokers	-0.020	0.032	0.623	0.534
Hypertension	0.006	0.023	0.271	0.786
Hyperlipidemia	0.032	0.031	1.040	0.299

BMI, body mass index; WHR, waist/hip ratio; LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; hs-CRP, high sensitivity c-reactive protein.

Diabetes was defined as fasting blood glucose of ≥126 mg/L. Hypertension was defined as a SBP ≥140 mmHg and a DBP ≥90 mmHg. Hyperlipidemia was defined as a total cholesterol (TC) ≥5.2 mmol/l (200 mg/dl), triglycerides (TG) ≥1.5 mmol/l (150 mg/dl), and HDL-C <0.9 mmol/l (40 mg/dl) (for men), and <1.29 mmol/l (50 mg/dl)(for women).

General linear model analysis was performed as dependent variable (anti-HSP27) is quantitative and independent variables are both qualitative and quantitative.

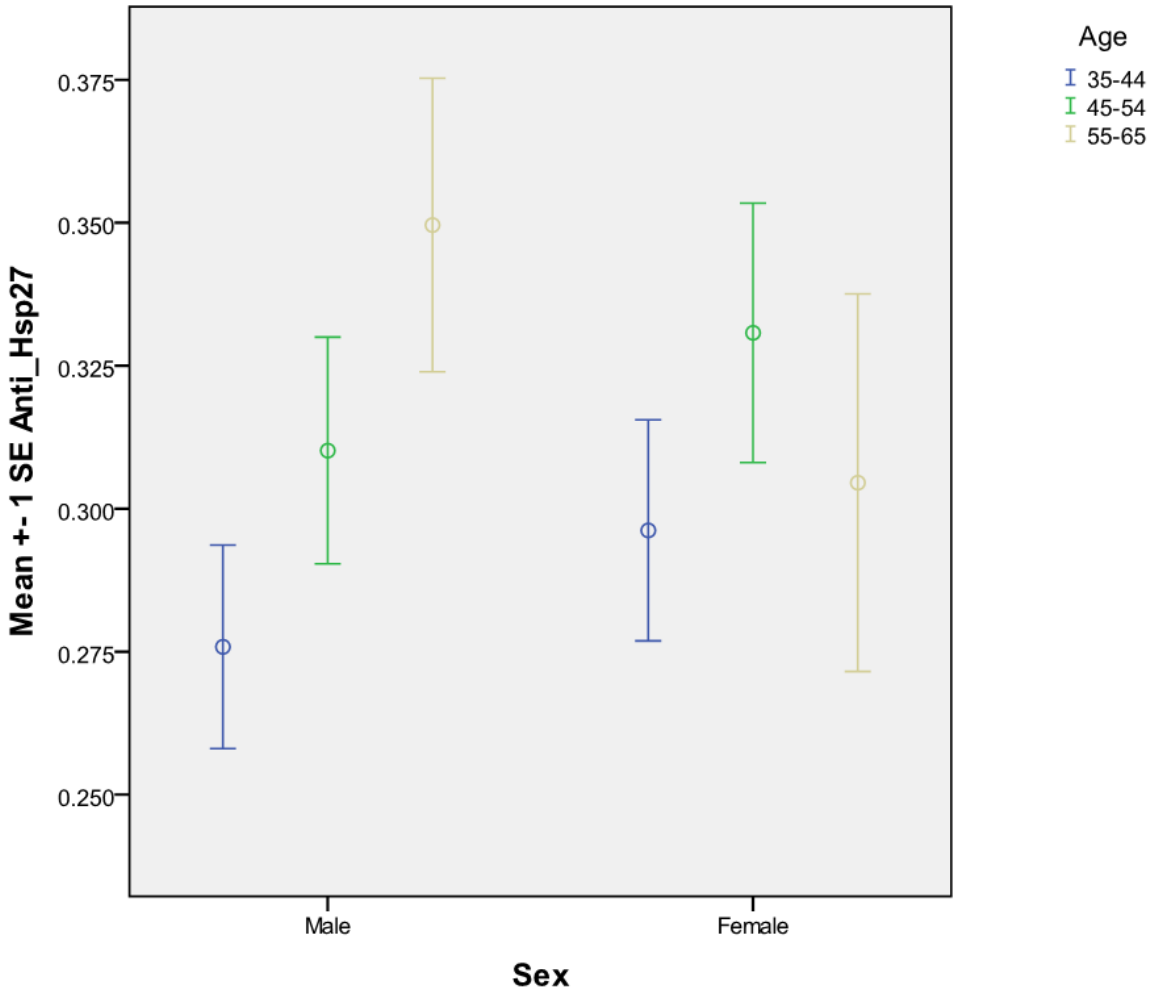

Fig. 1

Highlights

1. Serum anti-HSP27 antibody titers have an important role as biomarker in obese subjects
2. Obese subjects had a significantly higher anti-HSP27 antibody levels than the nonobese group
3. Serum anti-HSP27 antibody titers did not differ with gender
4. Serum anti-HSP27 antibody titers are related to several cardiovascular risk factors