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Association between indices of body mass and antibody titers to heat-shock protein-27 in healthy subjects

Shima Tavallaie [a], Amir Ali Rahsepar [a,b,c], Hamid Abdi [a], Mohsen Moohebati [a,b], Fatemeh Moodi [a,b], Hossein Pourghadamyari [a], Habibollah Esmaily [d], Fatemeh Khorashadizadeh [d], Majid Ghayour-Mobarhan [a,b,*], Gordon A.A. Ferns [e]

[a] Cardiovascular Research Center, Faculty of Medicine, Mashhad University of Medical Science (MUMS), Mashhad, Iran
[b] Biochemistry and Nutrition Research Center, Faculty of Medicine, MUMS, Mashhad, Iran
[c] Young Researchers Club, Mashhad Branch, Islamic Azad University, Mashhad, Iran
[d] Department of Biostatistics and Health sciences research center, school of Health, MUMS, Post Code: 9138813944, Mashhad, Iran
[e] Institute for Science & Technology in Medicine, Thornburrow Drive, University of Keele, Stoke on Trent, Staffordshire ST4 7QB, UK

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ABSTRACT

Objectives: We have assessed the relationship between indices of adiposity and antibody titers to Hsp-27 in healthy subjects.

Design: Two-hundred and fifty subjects were studied, including 50 normal-weight subjects (body-mass-index (BMI) 25 kg/m^2), 100 overweight subjects (BMI 25 to 30 kg/m^2) ($n=100$) and 100 obese subjects (BMI ≥30 kg/m^2).

Results: Anti-Hsp27-antibody levels in obese subjects were [0.34 (0.20–0.39) absorbency unit], being significantly higher than overweight and normal-weight groups ($P<0.05$). Anti-Hsp27-antibody levels in overweight subjects [0.29 (0.15–0.34) absorbency unit] were statistically higher than controls [0.18 (0.10–0.23) absorbency unit] ($P<0.05$).

Conclusion: High anti-Hsp-27-antibody levels in obese-subjects without established coronary disease may be related to a heightened state of immunoactivation associated with obesity.

Introduction

Atherosclerosis is a chronic multi-factorial process that underlies the pathophysiology of cardiovascular disease (CVD). One of the modifiable risk factors for CVD is obesity, in which abdominal or visceral obesity particularly acts as an independent and modifiable risk factor for vascular events such as cardio/cerebrovascular events.

The Hsps are highly conserved families of proteins found in the cells of all organisms and several of them are known to function as molecular chaperones. The Hsps may be divided into seven major families according to their molecular weights. HSP expression is increased in response to several environmental stresses [1]. It has been suggested that an immune response to Hsps, either endogenously derived from cells involved in atherogenesis, or exogenously, from micro-organisms, may lead to complement-mediated endothelial injury and subsequent atherosclerosis. Since then many other animal or human studies have shown the strong and positive relationship between CVD and presence of high anti-Hsp antibodies levels in blood stream of their subjects (reviewed by Ghayour-Mobarhan et al. [1]). Whilst most of the past studies have focused on Hsp-65 and -70, there has been recent interest and investigations of the possible role of the smaller Hsps, such as Hsp27, in CVD [1].

Martin et al. [2] reported Hsp-27 was able to protect cardiac myocytes from the effect of ischemia and that decreasing the level of endogenous Hsp27 resulted in an enhancement of the damaging effects of a subsequent ischemic stimulus. These findings suggest that Hsp27 may also be protective in myocardial cells. It has also been proposed that Hsp27 may be a putative auto-antigen involved during atherogenesis [3]. It is reported that high antibody titers against Hsp27 are associated with cardiovascular events [4]. Thus, we aimed to evaluate the hypotheses that anti-Hsp27 could be affected by obesity and to assess the association between its levels and indices of obesity, as a risk factor for CVD, and anti-Hsp27 antibody levels in patients without overt clinically CVD.

Materials and methods

Two-hundred and fifty subjects including normal-weight ($n=50$), overweight ($n=100$) and obese subjects ($n=100$) were

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; FBS, fasting blood Sugar; HDL-C, high density lipoprotein-cholesterol; HSP, heat shock protein; LDL-C, low density lipoprotein-cholesterol.

* Corresponding author at: Mashhad University of Medical Science, Mashhad, Iran. Fax: +98 511 8827040.
E-mail address: ghayourm@mums.ac.ir (M. Ghayour-Mobarhan).

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recruited from either those who came for routine biochemical check-up or referred to the nutrition clinic, Ghaem Hospital, Mashhad, Iran. In this study overweight was defined as a BMI of 25 to <30, and a BMI of ≥30 was defined as obesity. They had neither received any other weight control measures nor had any medical and/or drug history within the last 3 months before their participation in the study. Participants were provided with information about the study by verbal explanation and written information sheets. Those who had exclusion criteria such as poorly controlled diabetes, severe hypertension, overt signs/symptoms of CVD or established CVD, endocrine abnormalities, pregnancy and who refused to participate at any point were withdrawn from the study. None of the subjects had a previous history of myocardial infarction or angina pectoris. The subjects were aged between 18 and 55 years with BMI between 25 and 45 kg/m^2. Each patient gave informed written consent to participate in the study, which was approved by the Mashhad University of Medical Science Ethics Committee.

Anthropometric measurements and blood sampling

For all patients height and body weight were measured with the subjects dressed in light clothing after an overnight fasting. The body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Hip circumference was measured at the levels of the major trochanters through the pubic symphysis, and waist circumference was measured mid-way between the lateral lower rib margin and the iliac crest with the scale to the nearest ±0.1 cm. Blood pressure was measured twice while the patients were seated and rested for 15 min, using a standard mercury sphygmomanometer calibrated by the Iranian Institute of Standards and Industrial Research. The interval between each blood pressure measurement was at least 30 min, and the average of the two measurements was taken as the blood pressure. Blood samples were taken from each patient for analysis after a 12 hour fasting. Hemolyzed samples were excluded from analysis. After separation, aliquots of serum were frozen at −80 °C until analysis.

Routine biochemical analysis

Full fasted lipid profile comprising total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) was determined for each subject. Serum lipid and fasting blood glucose (FBS) concentrations were measured enzymatically using commercial kits.

Serum anti-HSP-27 antibody titers measurements

Serum Hsp27 antibody titers were measured using an in-house ELISA assay, as we have previously described [4]. Briefly, Serum Hsp27 antibody titers were measured using an in-house ELISA assay. Microtiter plates (Nunc Maxisorp, 3Nunc) were coated with 100 ng per well recombinant human Hsp27 dissolved in 50 μL carbonate buffer pH 9.6 incubated for 18 hours at 4 °C under humidified conditions. The wells were washed three times in wash buffer (PBS 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 added to the each well in duplicate and incubated for 30 min at room temperature. After washing (four times in wash buffer and two times in PBS), 100 μL peroxidase conjugated-goat anti-human IgG (Sigma-Aldrich, 4Inc., USA) 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 wash buffer and two times in PBS), 100 μL of TMB substrate (200 μl of 6 mg/mL TMB in DMSO was added to 10 mL of 50 mM acetate buffer, pH 4.5, containing 6 μL H_2O_2) was added per well and plate incubated for 15 min in the dark at room temperature. The reaction was terminated by adding 50 μL 2 M HCl per well. Optical density at 450 nm was measured using a Labsystems iEMS Reader MF Microtiter plate reader with a reference wavelength of 620 or 570 nm. The within-assay and between-assay precision values were 3.5% and 5.2% respectively. After correction for the non-specific 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

XLSTAT software, 2011 edition, was used to perform the statistical analysis. Data were checked for normality using Shapiro–Wilk test. Values were expressed as mean ± SD or, in the case of non-normally distributed data, as median and inter-quartile range. The proportion of females and presence of CVD risk factors were compared by using chi-square test. In the univariate study, data that were normally distributed were analyzed using one-way analysis of variance (ANOVA). Data found to be non-normally distributed were analyzed using the non-parametric Kruskal–Wallis test (for 3 groups). For multiple comparisons of parameters Bonferroni corrections were made. Stepwise multiple regression analysis was used to investigate the relationship between the anti-Hsp-titers and individual factors including age, total-cholesterol, triglyceride, HDL-C, LDL-C, BMI, systolic and diastolic blood pressure, fasting blood glucose, and waist and hip-circumferences. A two-sided *P*-value of <0.05 was considered statistically significant.

Results

Demographic data

Among 250 subjects who were involved in the study; 50 subjects were of normal-weight (24 females), 100 subjects were over-weighted (63 females) and finally 100 subjects were entered as the obese group (51 females). Normal weight, overweight and obese subjects did not differ with respect to age, gender, smoking status, and presence of other CVD risk factors such as diabetes and hyperlipidemia ($P>0.05$), except hypertension. As would be expected obese subjects had significantly higher levels of waist and hip-circumferences compared with overweight and normal-weight subjects ($P<0.05$). The same results were observed when normal weight and overweight subjects were compared ($P<0.05$). Moreover, triglyceride, HDL-C and LDL-C were significantly different between control subjects and overweight and obese subjects. The demographic data of 3 groups have been summarized in Table 1. Plasma antibody titers to Hsp-27 did not differ significantly between male and female subjects ($P>0.05$). Nor did titers differ significantly between subjects who were smokers and those who were non-smokers ($P>0.05$).

Plasma levels of anti-Hsp27 antibody levels

The analysis showed that overall obese subjects had higher levels of anti-Hsp27 antibody levels [0.34 (0.20–0.39) absorbency unit] when compared with the 2 other groups ($P<0.05$). Moreover, there was a significant difference between antibody levels to anti-Hsp27 in overweight subjects [0.29 (0.15–0.34) absorbency unit] when compared with control group [0.18 (0.10–0.23) absorbency unit] ($P<0.05$) (Fig. 1).

Multiple linear regressions

Little of the variation in the antibody titers to Hsp-27 could be explained by the best-fitting models derived from stepwise multiple

Table 1
Comparison of clinical and biochemical characteristics of subjects.

Groups	Control	Overweight	Obese
N	50	100	100
Female (%)	24 (48)	63 (63)	51 (51)
Smoker (%)	8 (16)	23 (23)	20 (20)
Diabetics (%)	0 (0)	3 (3)	5 (5)
Hyperlipidemic (%)	16 (32)	28 (28)	29 (29)
Hypertensive (%)	12 (24%)	20 (20)	32 (32)[a]
Age (year)	54.20 ± 10.98	51.29 ± 10.38	52.31 ± 10.06
BMI (kg/m^2)	22.57 ± 1.86	27.77 ± 1.82[b]	33.63 ± 3.34[a,b]
WC (cm)	86.57 ± 8.51	95.88 ± 8.37[b]	107.40 ± 13.53[a,b]
HC (cm)	95.82 ± 6.12	102.71 ± 8.45[b]	114.51 ± 8.91[a,b]
WC/HC ratio	0.90 ± 0.08	0.93 ± 0.07	0.94 ± 0.12
FBS (mg/dL)	89.20 ± 26.08	89.10 ± 14.55	88.65 ± 23.78
Total cholesterol (mg/dL)	201.09 ± 45.74	179.80 ± 38.62	180.94 ± 43.30
Triglycerides (mg/dL)	114.51 ± 54.28	145.12 ± 88.29[b]	129.79 ± 53.25
HDL-C (mg/dL)	46.50 ± 8.35	41.50 ± 8.32[b]	42.54 ± 8.16[b]
LDL-C (mg/dL)	128.68 ± 41.88	107.94 ± 31.24[b]	106.71 ± 32.28[b]
DBP (mm Hg)	76.78 ± 11.05	78.15 ± 10.39	79.26 ± 11.01
SBP (mm Hg)	120.82 ± 21.25	116.49 ± 19.79	118.91 ± 16.12

BMI, body mass index; WC, waist circumferences; HC, hip circumferences; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol, DBP; diastolic blood pressure, SBP; systolic blood pressure. Values are expressed as mean ± SD, or median and interquartile range. Chi-square, one-way analysis of variance (ANOVA), and Kruskal–Wallis tests were used to compare qualitative and quantitative (normal and non-normal) variables, respectively.
[a] Comparison with overweight group. $P<0.05$.
[b] Comparison with control group. $P<0.05$.

linear regressions: in this model the variation in anti-Hsp27 antibody levels was explained by age ($\beta=-0.311$), BMI ($\beta=0.316$), systolic blood pressure ($\beta=-0.184$) and LDL-C ($\beta=-0.278$); other variables did not have any significant association with anti-Hsp27 antibody. We found the following equation after linear regression analysis:

Total anti-Hsp27-antibody titers $= -0.311^{*}(\text{age}) - 0.184^{*}(\text{systolic blood pressure}) + 0.316^{*}(\text{BMI}) - 0.278^{*}(\text{LDL-C})$

Discussion

In the present study, we found that obesity, a risk factor for CVD, was associated with the levels of anti-Hsp27 antibody titers. Consistent with our hypothesis, the obese subjects had the highest levels of anti-Hsp27 antibody titers and after those, overweight subjects had the higher levels compared with normal subjects. The antibody levels were correlated with age, BMI, LDL-C and systolic blood pressure.

Among different subjects, we [5] have previously found that anti-Hsp-60, 65, and 70 were associated with obesity and obese subjects had significant higher levels of anti-Hsp antibodies when compared with overweight and normal weight subjects; however there was no significant difference between the latter 2 groups.

In this study, anti-Hsp-60 antibody levels were found to be more strongly positively associated with the indices of obesity ($P<0.001$) when compared with anti-Hsp-65 and -70. While most of the previous studies have focused on Hsp-65 and -70, there have been recent interest and investigations of the possible role of the smaller HSPs, such as Hsp-27 in atherogenesis [1]. However, to our knowledge the exact role of anti-Hsp-antibodies has not been well defined, nor has there been a systematic study of the different subtypes of antibody. Several studies have reported that patient with higher levels of CVD risk factors have higher levels of anti-Hsp-antibodies as we have reviewed previously, and have concluded that these anti-Hsps may have a pathologic role in atherosclerosis, while it may also be possible that these Hsps have a protective role in the process of atherosclerosis.

In addition, in dyslipidemic patients higher levels of several anti-Hsp antibodies have been found [6]. Anti-Hsp-27 antibody levels have been associated with cardio/cerebrovascular events and it has been suggested that high levels of anti-Hsp-27 antibody levels could be the potential risk factor for occurrence of vascular events [4,7]. Interestingly, it has been reported that weight loss is able to reduce the levels of anti-Hsp27 antibody levels in children [8] and adults [unpublished data]. In another study, it was found that IgG anti-Hsp-27 concentrations were strongly associated with age, gender, and hypertension and weakly with diabetes in patients with acute coronary syndrome; however, other cardiovascular risk factors were not associated with anti-Hsp-27 IgG antibody concentrations [9]. Furthermore, it has also been reported that anti-Hsp-27 antibody titers were inversely related to age but unrelated to several other established cardiovascular risk factors [10]. We were unable to demonstrate an association between anti-Hsp27 antibody levels and several other coronary risk factors in an Iranian cohort [4].

In conclusion, the high antibody titers to Hsp-27 in obese subjects without overt signs/symptoms of CVD or established CVD may be related to a heightened state of immunoactivation associated with obesity. It could be hypothesized that raised anti-Hsp antibody titers develop relatively early in the atherogenic process; however a prospective study would be required to confirm this. It may be interesting to investigate whether control of CVD risk factors such as obesity, diabetes and hypertension is able to reduce anti-Hsp antibody levels.

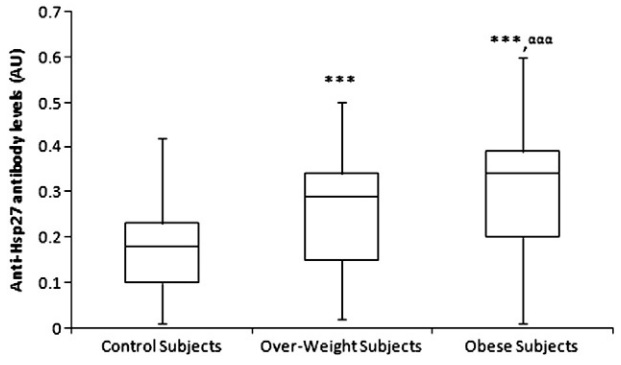

Fig. 1. Levels of anti-Hsp27 antibody among different groups [median (inter-quartile range)]. AU, *** and $^{\alpha\alpha\alpha}$ mean absorbency units, significant difference with control subjects ($P<0.05$) and significant difference with overweight subjects ($P<0.05$). Normal weight, overweight and obesity were defined as BMI<25, 25≤BMI<30, and a BMI of ≥30 respectively.

Conflict of interest

The authors declare no conflict of interest.

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