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Association of Serum Anti-Heat Shock Protein 27 Antibody with Obesity Risk among Healthy Individuals in MASHAD Study

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ABSTRACT

Introduction: The association between the serum antibody titer of several heat shock proteins (HSPs) and cardiovascular disease (CVD) risk factors so far has been the subject of several previous studies .

Aim: To evaluate the association of adiposity indices and HSP-27 antibody titers in healthy individuals.

Materials and Methods: Overall 4823 individuals were recruited from Mashhad Stroke and Heart Atherosclerotic Disorders Study (MASHAD study), that included 1496 individuals with normal-weight (body mass index (BMI) <25 kg/m2), 1975 individuals with over-weight (BMI 25-30 kg/m2), and 1352 individuals with obesity (BMI≥ 30 kg/m2).

Results: The serum anti-HSP-27 antibody titers were not significantly different among obese individuals [0.21 (0.11 – 0.34) absorbency unit (AU)], over-weight individuals [0.19 (0.10 – 0.33) AU] and normal-weight individuals [0.19 (0.10 – 0.33) AU].

Conclusions: we have found no significant relationship among anti-HSP-27 antibody concentration and degrees of adiposity among Iranian adults.

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Introduction

Heat shock proteins (HSPS) are molecule chaperones that protect the cell against various forms of cellular stress including free radicals, high temperatures, cytokine-stimulating infections, and various cellular functions. HSPS play a role in many pathogenesis conditions, particularly atherosclerosis (1). It is found that HSP-27 expression, small heat shock proteins with 27 kDa molecular weight, is increased by cardiomyocytes in response to ischemia (2). HSP-27 has also been shown to protect the cell from inflammation and atherogenesis via binding to scavenger receptor-A (3). Reaction of the immune system against HSP-27 antigen leads to the construction of anti-HSP-27 antibodies. The role of pathogenesis in several disorders has been proposed for anti-HSP-27 antibodies (4).

Recent studies conducted over the past two decades have shown the association among HSP-27 and cardiovascular disease (CVD) (5). Positive associations of serum anti-HSP-27 antibody concentration and CVD have been indicated in recent clinical studies (3, 5, 6). Obesity is associated with CVD events, possibly due to inflammation induced by adipose tissue (7). Therefore, the objective of our investigation was to demonstrate the association between anti-HSP-27 antibody titers and adiposity indices as CVD risk factors.

Methods and Material

Study Samples

Overall, 4823 individuals including 1971 male and 2852 female, 35-64 years, were chosen from the Mashhad Stroke and Heart Atherosclerotic Disorders Study (MASHAD study) (8). The participants with CVD or any signs/symptoms of CVD, poorly controlled diabetes mellitus, severe hypertension, infectious disease, endocrine impairments, and pregnant or breastfeeding women were excluded. This study was confirmed by Mashhad University of Medical Sciences Ethics Committee and informed consent form was obtained from all subjects participated in our project.

Anthropometric Assessments

Anthropometric indices for all participants were measured according to standard procedure which explained, previously (8). Body mass index (BMI) was measured by division of weight (kg) into height square meters (m2). Therefore, individuals were classified to three groups: normal-weight cases (BMI < 25 kg/m2), over-weight cases (25 ≤ BMI < 30 kg/m2) and obese subjects (BMI ≥ 30 kg/m2). We also excluded the over-weight and obese individuals who took any medication or had weight control in the last three months before participating in the study.

Biochemical Analysis

After 12-14 hours fasting, blood samples were taken from all individuals. The serum levels of fasting blood glucose (FBG) and lipid profile including, triglycerides (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) (8) and also high sensitive C-reactive protein (hs-CRP) was measured through using Pars Azmoon kits and an Alycon auto analyzer (ABBOTT, Chicago, IL, USA). Blood pressure was evaluated twice with 30-minute interval for all participants in sitting and resting mode by a standard sphygmomanometer and the average reported as the final blood pressure.

Serum anti-HSP-27 antibody titer assay

Serum anti-HSP-27 antibody levels was evaluated by in-house enzyme linked immunesorbent assay (ELISA) (4), explained previously in detail (3).

Statistical Analysis

SPSS version 18 was used for statistical analysis. Data were checked for normal distribution. The results are showed as mean ± SD and inter-quartile range for normal and non-normal distributed data, respectively. To compare baseline and clinical individual characteristics, t-test and Mann-Whitney test were used for parametric and non-parametric variables, respectively. 3 groups comparison of Normally distributed variables and Non-parametric variables were analyzed by one-way ANOVA and Kruskal–Wallis test respectively. Chi-squared test was used to compare categorical variables. Bonferroni corrections were performed for multiple comparisons of parameters.

Analysis of multiple regression was used to explore the association between the anti-HSP-27 antibody concentrations and personal factors including age, TC, TG, LDL-C, HDL-C, FBG, height, weight, waist and hip circumferences, BMI, systolic and diastolic blood pressure (SBP and DBP). P-value of < 0.05 was statistically considered significant.

Results

In this study among 4823 participants, 1496 participants were normal-weight (females=670), 1975 participants were over-weight (females=1123) and 1352 participants were obese (females=1059). The prevalence of obesity and over-weight were increased in women compared to men in the current study (P<0.05).

The three categories were different in terms of all variables except for age and diabetes mellitus prevalence (P>0.05). Normal-weight individuals were more likely to be a current smoker or ex-smoker (P<0.001). As would be expected, increased anthropometric measurements including weight, BMI, waist and hip-circumferences and decreased height were observed in the obese participants compared to the normal-weight subjects (P<0.001). Similar results were obtained when over-weight and normal-weight individuals were compared (P<0.001). In addition, serum FBG, TG, TC, HDL-C, LDL-C, hs-CRP, SBP, DBP and prevalence of hypertension and hyperlipidemia were significantly different in three studied groups (P<0.001).

Table 1 presents the demographic data of the three groups. Male and female individuals didn't show significant different between serum anti-HSP-27 antibody levels (P>0.05) (Figure 1). However, serum anti-HSP-27 antibody titers were different in smokers compared to non-smokers (P<0.05).

Serum anti-HSP-27 antibody concentration

The findings revealed that serum titers of anti-HSP-27 antibody was not different between obese individuals [0.21 (0.11 – 0.34) AU] and normal-weight individuals [0.19 (0.10 – 0.33) AU]. Serum titers of anti-HSP-27 antibody was not also different in individuals who were over-weight [0.19 (0.10 – 0.33) AU] and those who were normal-weight (P>0.05), (Table 2).

Multiple linear regressions

This model explained the variation of anti-HSP-27 antibody titers only by weight (β = 0.048) and smoking habit (β = – 0.031) while the other variables have no significant association with anti-HSP-27 antibody titers. The following equation has been found after linear regression analysis;

Total anti-HSP-27 antibody level = 0.191 ± 0.048*(weight) – 0.032*(smoking [yes (present or past habit) = 1/no = 0)]

Y = 0.191+0.048 (weight)– 0.032 (smoking)

Discussion

Despite the positive association among antibody titers to HSP-27 and atherosclerosis proposed in previous studies (3, 6, 9), this relationship is inconsistent. In the current project we intended to evaluate the association of anti-HSP-27 antibody concentration with adiposity among Iranian adults. No significant relationship was found among anti-HSP-27 antibody titers and degrees of adiposity. There wasn't any significant difference between three BMI groups and levels of antibody. The antibody levels were correlated with weight and smoking habits.

Although the majority of previous studies have examined the role of HSP-65 and HSP-70, recent investigations considered the role of small HSPs like HSP-27 in atherogenesis (5). Xu et al., demonstrated increased concentration of anti-HSP-65 antibodies after several years in subjects with progressive atherosclerosis in a follow up study (10). Anti HSP-70 antibodies were associated with less calcification of blood vessels (11). A prospective case-control study among women hasn't found any significant association between serum HSP-27 concentration of baseline and CVD incidence in 5.9 years of follow-up (12). Ghayour-Mobarhan et al., showed that serum anti-HSP-27 titers increased in individuals with acute coronary syndrome (ACS) in the first 12h, and decreased to approximately normal concentration in the second 12h (6).

Shams et al. reported that higher anti-HSP-27 antibody titers in participants with chest pain compared to control group. Individuals with myocardial infarction (troponin I positive) and those with troponin I negative did not show significance differences in anti-HSP-27 antibody titers (13). This anti-HSP-27 antibody have concluded to be protective against cardiovascular disease and also a potential biomarker (14). The other study showed that the level of anti-HSP-27 concentration was decresed in individuals with moderate and high physical activity levels (PAL) (15). In a study conducted by Tavana et al., in 2020, on 6447 subjects, serum antibody titers to HSP27 were not significantly different between four studied groups consist of nondiabetic, diabetic, undiagnosed diabetes and impaired fasting glucose subjects (16). Seham et al. assessed association of serum anti-HSP-27 antibody concentration in volunteer' β-thalassemia with the clinical and biochemical parameters in a case control study. There is no significant relationship between anti-HSP-27 antibody titers with height and weight in control groups that is opposed with our finding (17). Sadabadi et al. revealed that serum anti-HSP27 antibody were not statistically different between individuals with and without metabolic syndrom (18) .

Table 1. Comparison of clinical and biochemical characteristics between subgroups of BMI.

	Normal-weight	Over-weight	Obese
N	1496	1975	1352
Female (%)	44.8 (670)	56.9 (1123) [b]	78.3 (1059) [a,b]
Age (year)	46.93 ± 8.35	47.01 ± 7.96	47.55 ± 7.61
Smoker (%)	36.7 (549)	28.9 (499) [b]	27.7 (374) [a,b]
Height (cm)	163. 23 ± 0.09	161.06 ± 0.09 [b]	157.34 ± 0.08 [a,b]
Weight (kg)	59.76 ± 8.45	71.34 ± 8.23 [b]	82.63 ± 11.02 [a,b]
BMI Kg/m2	22.38 ± 2.06	27.43 ± 1.38 [b]	33.31 ± 3.01 [a,b]
WC (cm)	84.80 ± 8.80	94.31 ± 8.33 [b]	105.06 ± 10.34 [a,b]
HC (cm)	95.50 ± 5.68	102.64 ± 5.73 [b]	112.87 ± 8.06 [a,b]
WHR	0.89 ± 0.08	0.92 ± 0.08 [b]	0.93 ± 0.08 [a,b]
FBG (mg/dl)	83.66 ± 27.66	86.35 ± 26.42 [b]	88.47 ± 26.13 [b]
Serum LDL-C (mg/dl)	113.17 ± 33.23	115.79 ± 33.13	118.04 ± 34.90 [b]
Serum HDL-C (mg/dl)	44.42 ± 10.17	42.31 ± 9.85 [b]	42.77 ± 9.36 [b]
Serum TG (mg/dl)	93 (69 – 133)	121 (85.75 – 171) [b]	130 (96 – 179) [a,b]
Total cholesterol (mg/dl)	182.71 ± 36.89	190.06 ± 36.04 [b]	194.66 ± 37.90 [a,b]
Serum hs-CRP (mg/dl)	1.22 (0.75 – 1.96)	1.51 (0.92 – 2.86) [b]	2.36 (1.30 – 5) [a,b]
SBP (mm Hg)	115(104.33–123.33)	120 (110 – 129.33) [b]	120.67(110.67–133.33) [a,b]
DBP (mm Hg)	76.67 (70 – 80)	80 (70 – 83.33) b	80 (74.58 – 86.75) [a,b]
Diabetes (%)	3.3 (49)	3.8 (75)	4.4 (60)
Hypertension (%)	4.2 (63)	7.9 (156) [b]	11.6 (157) [a,b]
Hyperlipidemia (%)	5.1 (76)	12.2 (241) [b]	15.4 (208) [a,b]

Abbreviations: BMI:body mass index; **WC**: waist circumference, **HC**: hip circumference, **WHR:** waist-hip ratio, **LDL_C:** low-density lipoprotein cholesterol, **HDL-C:** high-density lipoprotein cholesterol; **Hs-CRP:** high-sensitivity C-reactive protein, **SBP:** systolic blood pressure, **DBP:** diastolic blood pressure.

Values are expressed as mean ± SD or median and interquartile range. Categorical data were compared by Chi-square tests. Between-group comparisons were assessed by Kruskal–Wallis for non-normal distribution data (serum triglycerides, hs-CRP, SBP and DBP) and by one-way ANOVA for normally distributed data.

a Comparison with over-weight group. P<0.05.

b Comparison with control group. P<0.05.

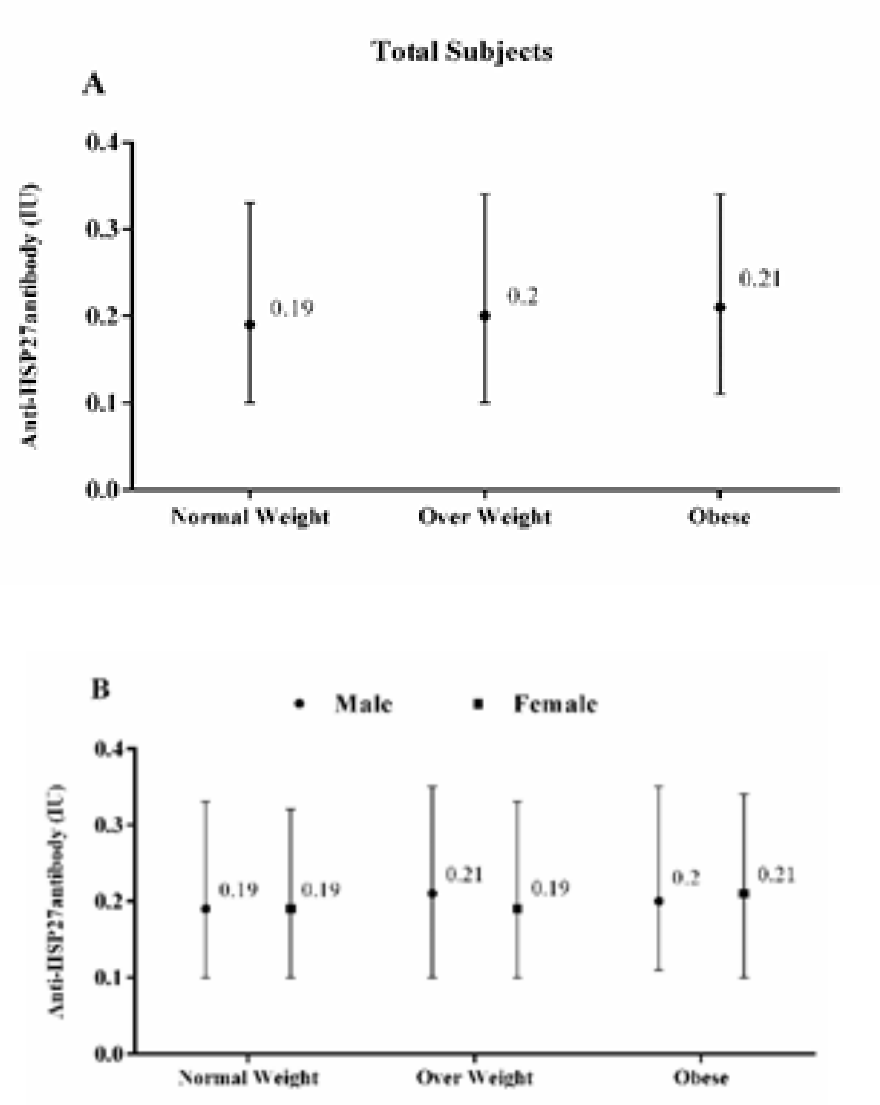

Figure 1. Levels of anti-HSP-27 antibody; A: among total individuals, B: according to Male and Female. Data presented as median (inter-quartile range). Normal-weight, over-weight and obesity were defined as BMI<25, 25≤BMI <30 and BMI ≥30 respectively.

Burut et al., measured the HSP-27 antigen and anti-HSP-27 antibody in various groups of diabetic individuals with and without CVD event. No association founded among BMI and HSP-27 antigen and anti-HSP-27 antibody in these groups which is in agreement with current findings (4). Pourghadamyari et al., demonstrated that serum anti-HSP-27 antibody concentration were related with CAD severity. As well as they found significant correlations between serum anti-HSP-27 level with weight (p = 0.005), and BMI (p = 0.014) (3).

Table 2. Association between subgroups of BMI and serum anti-HSP-27 antibody titers in MASHAD study participants.

		Normal-weight	Over-weight	Obese	P-value
Anti-HSP-27 antibody	**Males**	0.19 (0.10 – 0.33)	0.21 (0.10 – 0.35)	0.20 (0.11 – 0.35)	0.371
	Females	0.19 (0.10 – 0.32)	0.19 (0.10 – 0.33)	0.21 (0.10 – 0.34)	0.118
	Total	0.19 (0.10 – 0.33)	0.20 (0.10 – 0.34)	0.21 (0.11 – 0.34)	0.203

Values are expressed as median and inter-quartile range. Between-group comparisons were assessed by Kruskal–Wallis as they are non-normal distribution data.

We have previously reported that anti-HSP-60, HSP-65 and HSP-70 antibody titers were significantly increased among obese individuals compared with normal and over-weight subjects; while there wasn't any significant difference between normal-weight and over-weight participant (19). Tavallaie et al., reported highest levels of anti-HSP-27 antibody among obese individuals, moreover, those over-weight individuals had elevated titers compared to the normal-weight participants. The exclusion criteria in Tavallaie's study is similar with our project but they recruited only 250 individuals [including obese subject (n=100), over-weight (n=100) and normal-weight (n=50)] aged 18 to 55 years (20). Kargari et al., demonstrated a positive association between anti-HSP-27 antibody titers and obesity. Overall, 283 obese, 393 over-weight and 256 normal-weight individuals were enrolled and their results opposed with ours (21). It seems that different results of two recent studies with current study could be because of the different sample size and age of subjects.

Conclusion

Serum anti-HSP-27 antibody titer were not correlated with adiposity in healthy individuals from the MASHAD study population and it could be better to assess of HSP27 antigen for all participants.

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