Comp Clin Pathol (2016) 25:895–901
DOI 10.1007/s00580-016-2279-0

ORIGINAL ARTICLE

Serum anti-HSP27 antibody titers in patients with metabolic syndrome, with or without diabetes mellitus

Mehrdad Kargari[1] · Seyed Mohammad Reza Parizadeh[2] · Maryam Saberi Karimian[3] · Seyed Kazem Farahmand[4] · Amirhosein Sahebkar[5] · Habibollah Esmaeili[2,6] · Shima Tavallaee[2] · Mahmoud Ebrahimi[7] · Gordon A. Ferns[8] · Majid Ghayour-Mobarhan[2,7]

Received: 24 January 2016 / Accepted: 4 May 2016 / Published online: 16 May 2016

Abstract Metabolic syndrome (MetS) is characterized by clustering of clinical, physiological, biochemical, and metabolic factors that are associated with an increased risk of cardiovascular disease (CVD) and type 2 diabetes mellitus. Immune response to heat shock protein 27 (Hsp27) has been suggested to be implicated in atherogenesis. We aimed to investigate the association between serum anti-Hsp27 antibody concentrations and type 2 diabetes in patients with MetS. This was a cross-sectional observational study on groups of MetS and healthy subjects. The population sample was derived from MASHAD STUDY, a national cross-sectional study conducted by the Ministry of Health and Medical Education in 2004. Pregnant and breastfeeding women and patients who had cardiovascular disease, myocardial infarction, stroke, or systemic disease were excluded from the 9600 subjects of the MASHAD STUDY population. A total of 933 subjects including 477 women and 456 men were classified as having MetS, diabetes mellitus, or neither. Data including age, gender, and smoking habit collected using a questionnaire. MetS was diagnosed based on the International Diabetes Federation (IDF) definition. The serum anti-HSP27 antibody titers were measured by ELISA. There was no difference in serum anti-HSP27 concentrations between subjects with and without MetS, or diabetes mellitus, nor was there a significant difference in anti-HSP27 levels between men and women. There was no significant difference in anti-HSP27 antibody between diabetic MetS patients, normal population, non-diabetic MetS, and diabetic non-MetS patients (p value >0.05). Serum anti-Hsp27 antibody concentrations did not differ between individuals with or without MetS or diabetes mellitus.

Keywords Metabolic syndrome · Heat-shock proteins · Diabetes mellitus

Mehrdad Kargari and Seyed Mohammad Reza Parizadeh contributed equally to this work.

✉ Majid Ghayour-Mobarhan
ghayourm@mums.ac.ir

[1] Department of Biochemistry, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[2] Biochemistry and Nutrition Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad 99199-91766, Iran

[3] Student Research Committee, Department of Modern Sciences and Technologies, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[4] Department of Complementary and Chinese Medicine, School of Persian and Complementary Medicine, Mashhad university of Medical Sciences, Mashhad, Iran

[5] Biotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

[6] Department of Biostatistics, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran

[7] Cardiovascular Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[8] Brighton and Sussex Medical School, Division of Medical Education, Falmer, Brighton Sussex BN1 9PH, UK

Introduction

Metabolic syndrome (MetS) is characterized by clustering of clinical, physiological, biochemical, and metabolic factors that are associated with an increased risk of cardiovascular disease and type 2 diabetes mellitus. Its features include dyslipidemia, hypertension, glucose intolerance, and a proinflammatory and prothrombotic state (Kaur 2014). The prevalence of MetS varies from <10 % to as much as 84 % depending on the region, the population studied, and the definition of the syndrome used. Genetic background, diet, physical activity, smoking, family history of diabetes, and education can all affect the prevalence of the MetS (Cameron et al. 2004).

Insulin resistance is a consistent feature of metabolic syndrome and obesity and is increasing globally. Furthermore, metabolic syndrome and glucose intolerance are both important risk factors for cardiovascular disease (Burut et al. 2010). Type 2 diabetes may result in a vicious cycle in which a proinflammatory state is induced, resulting in further impairment in insulin responsiveness and loss of homeostatic signaling. This loss of homeostasis in insulin resistance and type 2 diabetes may be associated with impaired expression of heat-shock proteins (HSPs) by insulin-sensitive tissues and may lead to oxidative damage (Hooper and Hooper 2009). The family of heat shock proteins comprises a set of proteins that are expressed constitutively and have important functions in maintenance of intracellular integrity under normal as well as stressful conditions, and an insufficient HSP level might make someone less able to respond to cellular stress, potentially leading to severe cell damage (Young and Elliott 1989). The inflammatory state associated with obesity promotes insulin resistance, impairs insulin signaling which reduces the expression of HSPs, and makes tissues vulnerable to damage. The pancreatic beta-cell may be damaged and may lead to further losses in insulin signaling. Dieting, with weight loss, and exercise may lead to increased HSP expression, reduce inflammation, and improve insulin signaling (Hooper and Hooper 2009).

HSPs have been found in serum of CHD patients. They can form immune complexes with their corresponding antibodies which have a key role in the progression of atherosclerosis because of their proinflammatory properties. HSP27 has been reported in atherosclerotic plaque and plasma HSP27 antibodies have been reported at increased concentrations in patients with atherosclerosis (Riganò et al. 2007). Increased serum levels of HSP27 and anti-HSP27 have been reported in patients with unstable angina and myocardial infarction (Pauli et al. 1990). Previous studies have reported an increase of serum HSP27 or its corresponding antibodies with metabolic syndrome (Sahebkar et al. 2011).

In the current study, we have evaluated the serum levels of anti-HSP27 antibody in patients with metabolic syndrome with or without diabetes mellitus.

Methods

Subjects

This was a cross-sectional observational study on groups of metabolic syndrome and healthy subjects. This research was approved by the Mashhad University of Medical Sciences Ethics Committee. The population sample was derived from MASHAD STUDY, a national cross-sectional study conducted by the Ministry of Health and Medical Education in 2004 (Azimi-Nezhad et al. 2009). Pregnant and breastfeeding women and patients who had cardiovascular disease, myocardial infarction, stroke, or systemic disease were excluded from the 9600 subjects of the MASHAD STUDY population. The participants ($n=933$, aged 35–64 years old; 477 females, mean age 47.34 ± 7.98 years old and 456 males, mean age 47.37 ± 7.97 years old) had taken both oral and written informed consent. Data including age, gender, and smoking were collected using a questionnaire.

MetS was identified based on the International Diabetes Federation (IDF) definition, if three of the following five criteria were met: (1) abdominal obesity: waist circumference >94 cm in men and >80 cm in women; (2) hypertriglyceridemia ≥150 mg/dl or specific treatment; (3) low levels of HDL-C <40 mg/dl in men and <50 mg/dl in women or specific treatment; (4) high blood pressure (HBP) ≥130/85 mmHg or specific treatment; (5) high fasting glucose ≥100 mg/dl or treatment with antidiabetic drugs (Alberti et al. 2005). Diabetes mellitus was defined as fasting blood glucose ≥126 mg/dl and impaired fasting glucose defined as fasting blood glucose 100–125 mg/dl.

Subjects who had fasting blood glucose ≥126 mg/dl were classified as diabetic. Those who had fasting blood glucose of 100–125 mg/dl were classified as impaired fasting glucose (IFG) group and those with fasting blood glucose of less than 100 mg/dl were classified as normal group.

Patients who had heart, vascular, and brain atherosclerotic disease, systemic disease, pregnant women, and women who were breast feeding were excluded from the study.

Anthropometric and other measurements

Anthropometric parameters including weight, height, hip circumference, waist circumference, and BMI of participants were measured using a standard protocol. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2); and height and weight were measured with a standard scale to an accuracy of +0.1 cm and +0.1 kg, respectively. The systolic and diastolic blood pressure was measured three times with an interval of 30 min in participants and the average of the three measurements was taken as the blood pressure.

Blood sampling

After an overnight fast, blood samples were collected from each subject. These samples were then centrifuged at 10, 000*g* for 15 min at room temperature in order to separate serum. The obtained serum was stored at −80 °C.

Lipid profiles and blood glucose

For all participants, a fasted lipid profile included total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and fasting blood glucose concentrations were determined after 12 h fasting. Fasting blood glucose and serum lipid concentrations were measured enzymatically using commercial kits.

Anti-HSP27 antibody measurement

Serum HSP27 antibody titers (Stressgen, Canada) were measured using an enzyme-linked immune sorbent assay (ELISA), as described previously (Sahebkar et al. 2011). A 50-ng recombinant human HSP27 was dissolved in 50 μL carbonate buffer pH = 9.6 and was placed in per well plate (Nunc Maxisorp, Nunc) at 4 °C in a humidified chamber for overnight. The wells were washed three times in buffer phosphate saline (PBS), treated with 0.05 % Tween-20. To block non-specific binding, well plates were incubated in 2 % goat serum in PBS for 30 min in 37 °C and 30 min at room temperature. Wells were washed three times with PBS. After 30 min incubation in serum HSP27 (diluted1:50) in room temperature, wells were washed three times. Then, well plates were coated with 100 μL peroxide conjugated-goat anti-human IgG (Sigma-Aldrich, USA) for 30 min at room temperature. After being washed twice in PBS, each well was incubated at 100 μL of TMB substrate (100 μL of 6 mg/ml TMB in DMSO and 10 ml of 50 mM acetate buffer pH 4.5 containing 3 μL H2O2) for 15 min in a darkroom. After adding 50 μL of 3 M HCl for each well, optical density at 450 nm was read using a Lab systems iEMS Reader MF microtiter plate reader. The ratio of optical density was compared to a reference wavelength of 620 or 570 nm and results were given in optical density units.

Statistical analysis

All statistical analyses were performed with SPSS version 20 software package. Data were expressed as means ± SD (for normal distribution data) or median and interquartile range

Table 1 Comparison of the baseline characteristics between with or without MetS groups

	MetS+	MetS−	*p* value
n	333	600	–
Age (year)	49.41 ± 7.75	46.22 ± 7.87	0.001
Height (m)	1.61 ± 0.09	1.63 ± 0.09	0.001
Waist/height	63.87 ± 7.12	56.42 ± 7.94	0.001
Weight (kg)	78.74 ± 13.16	70.5 ± 12.19	0.001
Waist/weight	1.32 ± 0.17	1.32 ± 0.17	0.683
Fasting blood glucose (mg/dl)	102.52 ± 48.23	83.25 ± 22.67	0.001
BMI(kg/m^2)	30.34 ± 4.27	26.58 ± 4.47	0.001
Waist/hip	0.96 ± 0.11	0.9 ± 0.07	0.001
Waist circumference (cm)	102.56 ± 10.14	91.69 ± 11.58	0.001
Hip circumference (cm)	107.46 ± 10.03	101.64 ± 8.23	0.001
LDL-C (mg/dl)	120.06 ± 38.96	114 ± 35.22	0.008
HDL-C (mg/dl)	39.3 ± 7.96	44.16 ± 10.46	0.001
Triglyceride (mg/dl)	184.18	110.27	0.001
Hs-CRP (mg/dl)	5.36	4.5	0.001
Systolic blood pressure (mm Hg)	130.82 ± 18.1	116.19 ± 15.77	0.001
Diastolic blood pressure (mm Hg)	84.16 ± 10.7	76.3 ± 10.14	0.001
Diabetes %	16.27 % (54/332)	2.53 % (15/594)	0.001
Smoking %	23.12 % (77/333)	25.17 % (151/600)	0.486
Hypertension %	40 % (132/330)	12.03 % (71/590)	0.001
Hyperlipidemia %	49.55 % (165/333)	28.48 % (170/597)	0.001
Anti-Hsp27 (OD)	0.3 ± 0.27	0.31 ± 0.28	0.691

BMI body mass index, *LDL-C* low-density lipoprotein, *HDL-C* high-density lipoprotein cholesterol, *Hs-CRP* high sensitivity C-reactive protein

(in the case of triglycerides and Hs-CRP, for none normally distributed data). Group comparisons were performed using ANOVA, Kruskal-Wallis, and Bonferonni correction test. In order to assess the independent effects of age, sex, smoking, metabolic syndrome, and its components on measures of anti-HSP27 antibody, a multiple regression analyses was performed. The level of statistical significance was set to $p < 0.05$.

Table 2 Comparison of the baseline characteristics between diabetic and non-diabetic populations

	Diabetic	IFG	Normal	*p* value	Post hoc
n	69	82	775	–	–
Age (years)	50.04 ± 7.27	50.18 ± 8.69	46.82 ± 7.85	0.001	$P_1 = 0.969$
					$P_2 = 0.001$
					$P_3 = 0.001$
Height (m)	1.62 ± 0.09	1.61 ± 0.09	1.62 ± 0.09	0.496	–
Waist/height	63.08 ± 8.44	60.32 ± 8.87	58.58 ± 8.32	0.001	$P_1 = 0.094$
					$P_2 = 0.05$
					$P_3 = 0.001$
Weight (kg)	76.66 ± 12.93	75.24 ± 15.54	72.97 ± 12.88	0.063	–
Waist circumference/weight	1.35 ± 0.15	1.31 ± 0.18	1.32 ± 0.17	0.304	–
Fasting blood glucose (mg/dl)	187.68 ± 66.71	108.15 ± 6.5	79.58 ± 10.06	0.001	$P_1 = 0.001$
					$P_2 = 0.001$
					$P_3 = 0.001$
BMI(kg/m^2)	29.26 ± 4.96	28.96 ± 5.44	27.69 ± 4.65	0.015	$P_1 = 0.849$
					$P_2 = 0.026$
					$P_3 = 0.041$
Waist/hip circumference	0.97 ± 0.06	0.93 ± 0.09	0.92 ± 0.1	0.001	$P_1 = 0.011$
					$P_2 = 0.087$
					$P_3 = 0.001$
Waist circumference (cm)	101.9 ± 12	97.02 ± 13.64	94.84 ± 12	0.001	$P_1 = 0.038$
					$P_2 = 0.106$
					$P_3 = 0.001$
Hip circumference (cm)	105.64 ± 10.88	104.01 ± 10.1	103.51 ± 9.12	0.596	–
LDL-C (mg/dl)	118.17 ± 37.81	115.37 ± 33.45	116.2 ± 36.99	0.960	–
HDL-C (mg/dl)	40.71 ± 10.29	44.94 ± 12.29	42.32 ± 9.59	0.060	–
Triglyceride (mg/dl)	178.4952	143.8975	132.349	0.001	$P_1 = 0.03$
					$P_2 = 0.063$
					$P_3 = 0.001$
Hs-CRP (mg/dl)	7.45	7.14	4.07	0.001	$P_1 = 0.089$
					$P_2 = 0.002$
					$P_3 = 0.001$
Systolic blood pressure (mmHg)	128.2 ± 18.56	129.63 ± 19.19	119.92 ± 17.28	0.001	$P_1 = 0.923$
					$P_2 = 0.001$
					$P_3 = 0.001$
Diastolic blood pressure (mmHg)	80.85 ± 10.42	82.92 ± 11.88	78.56 ± 10.78	0.001	$P_1 = 0.591$
					$P_2 = 0.002$
					$P_3 = 0.023$
Smoking %	18.84 % (13/69)	23.17 % (19/82)	24.77 % (192/775)	0.531	–
Hypertension %	30.88 %	34.57 % (28/81)	19.9 % (152/764)	0.002	$P_1 = 0.633$
	(21/68)				$P_2 = 0.002$
					$P_3 = 0.032$
Hyperlipidemia %	43.48 % (30/69)	42.68 % (35/82)	34.58 % (268/775)	0.139	–
Anti-Hsp27 (OD)	0.27 ± 0.24	0.37 ± 0.31	0.30 ± 0.27	0.035	$P_1 = 0.048$
					$P_2 = 0.071$
					$P_3 = 0.890$

Values are expressed as mean ± SD. *P* value$_1$ is signed for significance of IFG and diabetes groups. *P* value$_2$ is signed for significance of normal and IFG groups. *P* value$_3$ is signed for significance of normal and DM groups

BMI body mass index, *LDL-C* low-density lipoprotein, *HDL-C* high-density lipoprotein cholesterol, *Hs-CRP* high sensitivity C-reactive protein

Table 3 Comparison between serum anti-Hsp27 antibody titers and different number of MetS components

	Number of metabolic syndrome components					
	0	1	2	3	4	5
Frequency	73	200	307	221	113	19
Anti-Hsp27 (OD)	0.26 ± 0.22	0.31 ± 0.31	0.33 ± 0.28	0.31 ± 0.27	0.29 ± 0.26	0.30 ± 0.28
p value	0.407	0.303	0.059	0.92	0.607	0.817

Comparison of anthropometric factors' correlation coefficient

We used spearman rank correlation coefficient if a variable is ordinal and the other is quantitative. We used polyserial correlation coefficient if a variable is nominal and the other is quantitative. We assess the correlation between smoking and diabetes by contingency coefficient.

Results

A total of 933 subjects including 477 women and 456 men with a previous history of either MetS ($n = 333$, 35.69 %) aged 49.41 ± 7.75 years or diabetes ($n = 69$, 7.45 %) aged 50.04 ± 7.27 years were recruited.

The baseline and biochemical characteristics

The baseline characteristics between with or without MetS groups is shown in Table 1. As reported in Table 2, there were significance differences in waist circumference, waist circumference/height, waist-to-hip circumference ratio, triglyceride, and Hs-CRP between normal and diabetes groups ($p = 0.001$, for all variables). There was a significant higher age, waist circumference/height ($p < 0.01$, for all), weight and BMI (p value < 0.05), waist/hip circumference, waist circumference, triglyceride, Hs-CRP, systolic and diastolic blood pressure ($p < 0.01$, for all variables), and hypertension ($p < 0.05$) in diabetes patients than in normal subjects. There was a significantly higher BMI ($p < 0.05$), Hs-CRP, systolic and diastolic blood pressure, hypertension ($p < 0.01$, for all variables), and anti-HSP27 antibody ($p < 0.05$), in individuals with IFG than in the normal group (Table 2). Fasting blood glucose had the most correlation ($r = 0.643$) and HDL-C had the least correlation ($r = -0.006$) with being diabetic status between all variables.

Table 4 Comparison between serum antiHsp27 antibody titers of men and women and also diabetic and non-diabetic subjects in with or without MetS population

Group		Frequency	AntiHsp27 antibody	*p* value
MetS+	Men	122	0.3 ± 0.23	0.666
	Women	211	0.3 ± 0.29	
MetS-	Men	334	0.31 ± 0.26	0.721
	Women	266	0.32 ± 0.3	
MetS+	DM+	54	0.27 ± 0.25	0.243
	DM−	278	0.31 ± 0.27	
MetS-	DM+	15	0.27 ± 0.19	0.930
	DM−	579	0.31 ± 0.28	

Anti-HSP27 antibody

There was no significant difference between anti-HSP27 antibody titers in with or without MetS groups (Table 1). We found that there were no significant differences between different number of MetS components and anti-HSP27 antibody titers (p value > 0.05) (Table 3).

The present results did not suggest any significant difference in serum anti-Hsp27 titers between diabetic and non-diabetic groups. On the other hand, it was significantly higher in those in the IFG group compared with the diabetes patients ($p < 0.05$) (Table 2).

Also, there was no significant difference between anti-HSP27 antibody of men and women and also diabetic and non-diabetic subjects with or without MetS ($p < 0.05$), (Table 4).

As shown in Table 5, there was no significant difference in anti-HSP27 antibody between the four groups of the study including diabetic MetS patients, normal population, non-diabetic MetS patients, and diabetic non-MetS patients ($p > 0.05$).

A multivariate analysis was performed for investigation of association of metabolic syndrome and its components with anti-Hsp27 antibody level by linear regression model. As shown in Table 6, waist circumference was significantly

Table 5 Comparison between serum anti-Hsp27 antibody between four groups of the study

Groups	Frequency	Anti-Hsp27 antibody (OD) ± SD
DM+MetS+	54	0.266 ± 0.253
DM−MetS−	579	0.311 ± 0.280
DM−MetS+	278	0.307 ± 0.271
DM+MetS−	15	0.270 ± 0.193

MetS metabolic syndrome, *DM* diabetes mellitus

Table 6 The multivariate analysis of association of metabolic syndrome and its components with anti-Hsp27 antibody by linear regression model

	Univariate			Multivariate[a]		
	CI for β		*p* value	CI for β		*p* value
MetS	−0.025	0.061	0.407	−0.077	0.025	0.323
Waist C (cm)	0.000	0.003	0.071	0.000	0.003	0.036
HDL-C (mg/dl)	−0.001	0.002	0.716	−0.002	0.002	0.948
TG (mg/dl)	0.000	0.000	0.236	0.000	0.000	0.330
FBG (mg/dl)	0.000	0.000	0.808	0.000	0.000	0.740
Hs-CRP (mg/dl)	0.000	0.002	0.490	−0.001	0.002	0.701
Systolic blood pressure (mmHg)	0.000	0.001	0.504	−0.001	0.002	0.505
Diastolic blood pressure (mmHg)	−0.001	0.002	0.719	−0.003	0.002	0.846

[a] In present of age, sex, smoking

MetS metabolic syndrome, *Waist C* waist circumference, *HDL-C* high-density lipoprotein cholesterol, *TG* triglyceride, *FBG* fasting blood glucose, *Hs-CRP* high sensitive C-reactive protein

related to anti-Hsp27 antibody level along with other metabolic syndrome components ($p < 0.05$).

Discussion

In the current study, we evaluated serum levels of anti-HSP27 in patients with metabolic syndrome with or without diabetes mellitus. We did not find any association between anti-HSP27 levels and either metabolic syndrome or diabetes. This result is in contrast to our previous study that indicated an elevation in anti-HSP27 titers in patients with documented coronary artery disease (CAD) and MetS compared to those with only CAD (Sahebkar et al. 2011). This inconsistency may be due to the difference in the nature of $MetS^-$ groups between the two studies. In this study, $MetS^-$ subjects did not have existing CAD while in our previous study, $MetS^-$ subjects were those who underwent angiography and, although CAD^-, might have had minimal CAD. It has been shown that serum HSP27 and anti-Hsp27 increase in unstable angina and myocardial infarction (Pauli et al. 1990). So, it seems that the $MetS^-$ group of the current study appears to be healthier than the $MetS^-$ group of our previous study.

The present results did not suggest any significant difference in serum anti-Hsp27 titers between diabetic and non-diabetic groups. It has been shown that heightened T-cell responses to Hsp60 in diabetic subjects may trigger an attack from the T cells of the immune system (Abulafia-Lapid et al. 1999). Moreover, diabetic patients have significantly higher anti-Hsp70 and anti-Hsp90 concentrations than non-diabetic individuals do (Sims et al. 2002). Previous in vitro studies have reported that a high ambient glucose level can lead to augmentation of intracellular reactive oxygen species (ROS) in endothelial cells (Giardino et al. 1996), and it can increase the expression of HSP27 in these cells (Dreher et al. 1995). Our results showed that serum anti-HSP27 titers were significantly higher in the IFG group compared with diabetes subjects. One explanation for our result may be the consumption of antihyperglycemic drugs (e.g., metformin) by diabetic patients. Metformin can suppress gluconeogenesis by inhibiting the glucose-6-phosphatase expression (Ota et al. 2009). It has been suggested that metformin can inhibit ROS production (Giardino et al. 1996), while IFG subjects are not receiving pharmacotherapy. Song et al. have shown that metformin upregulates SIRT3, a key regulator in cell metabolism. Augmented SIRT3 increases glucose uptake and decreases ROS production (Song et al. 2014).

Burut et al. (2010) did not find any significant difference with regard to Hsp27 IgG antibody level between the glucose intolerance CVD− and CVD+ groups and also among the normal glucose tolerance CVD and non-CVD subjects. However, they have shown that Hsp27 IgM antibody levels were significantly lower in normal glucose tolerance CVD patients compared with normal glucose tolerance non-CVD subjects. One explanation for this result may be that the HSP27 can be recognized by the immune system when released into the circulation. Therefore, IgM antibody levels decrease in patients with acute coronary syndromes as they react to HSP27. This result can be due to the immune complex formations that can facilitate the clearance of the released HSP27. Prolonged release of HSP27 can cause consumption of the IgM antibodies concentrated in the circulation (Burut et al. 2010). In the current study, we did not measure Hsp27 IgM antibody levels and did not find any significant difference in Hsp27 IgG antibody levels between the control and MetS or diabetes groups. A similar event may have happened to the IgG antibody.

There are certain limitations to our study. This is a cross-sectional study; hence, our ability is restricted to assess temporal relationships and identify causal biological mechanisms

underlying this association. Taking multiple medications by some patients limits our ability to detect independent effects of individual drugs on anti-HSP27 antibody concentration.

Conclusion

Our results showed that there was no difference in serum anti-HSP27 concentrations between subjects with and without MetS or diabetes mellitus. Nor was there a significant difference in serum anti-HSP27 levels between men and women.

Acknowledgments We thank all the patients and their family members who volunteered to participate in this study. Also, the authors would like to thank Mashhad University of Medical Sciences (MUMS) for financial support. The results presented in this work are part of Mr. Mehrdad Kargari's thesis in MUMS.

This work was supported by Mashhad University of Medical Science (MUMS), Iran.

Compliance with ethical standards

Funding This research is financially supported by the Mashhad University of Medical Sciences.

Ethical approval This research approved by the Mashhad University of Medical Sciences Ethics Committee.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

References

Abulafia-Lapid R, Elias D, Raz I, Keren-Zur Y, Atlan H, Cohen I (1999) T cell proliferative responses of type 1 diabetes patients and healthy individuals to human hsp60 and its peptides. J Autoimmun 12(2): 121–129

Alberti KGM, Zimmet P, Shaw J (2005) The metabolic syndrome—a new worldwide definition. Lancet 366(9491):1059–1062

Azimi-Nezhad M, Ghayour-Mobarhan M, Safarian M, Esmailee H, Parizadeh SMR, Rajabi-Moghadam M, Gholami A, Oladi MR, Ferns GA (2009) Anthropometric indices of obesity and the prediction of cardiovascular risk factors in an Iranian population. Sci World J 9:424–430

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 Chaperones 15(4):379–386. doi:10.1007/s12192-009-0152-7

Cameron AJ, Shaw JE, Zimmet PZ (2004) The metabolic syndrome: prevalence in worldwide populations. Endocrinol Metab Clin N Am 33(2):351–375

Dreher D, Vargas JR, Hochstrasser DF, Junod AF (1995) Effects of oxidative stress and Ca2+ agonists on molecular chaperones in human umbilical vein endothelial cells. Electrophoresis 16:1205–1214. doi:10.1002/elps.11501601201

Giardino I, Edelstein D, Brownlee M (1996) BCL-2 expression or antioxidants prevent hyperglycemia-induced formation of intracellular advanced glycation endproducts in bovine endothelial cells. J Clin Invest 97:1422–1428. doi:10.1172/JCI118563

Hooper PL, Hooper PL (2009) Inflammation, heat shock proteins, and type 2 diabetes. Cell Stress Chaperones 14(2):113–115

Kaur J (2014) A comprehensive review on metabolic syndrome. Cardiol Res Pract 2014:943162. doi:10.1155/2014/943162

Ota S, Horigome K, Ishii T, Nakai M, Hayashi K, Kawamura T, Kishino A, Taiji M, Kimura T (2009) Metformin suppresses glucose-6-phosphatase expression by a complex I inhibition and AMPK activation-independent mechanism. Biochem Biophys Res Commun 388(2):311–316

Pauli D, Tonka CH, Tissieres A, Arrigo AP (1990) Tissue-specific expression of the heat shock protein HSP27 during Drosophila melanogaster development. J Cell Biol 111:817–828

Riganò R, Profumo E, Buttari B, Tagliani A, Petrone L, D'Amati G, Ippoliti F, Capoano R, Fumagalli L, Salvati B, Businaro R (2007) Heat shock proteins and autoimmunity in patients with carotid atherosclerosis. Ann N Y Acad Sci 1107:1–10

Sahebkar A, Pourghadamyari H, Moohebati M, Parizadeh SM, Falsoleiman H, Dehghani M, Fazlinezhad A, Akhlaghi S, Tavallaie S, Paydar R, Ghayour-Mobarhan M, Ferns GA (2011) 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. Clin Biochem 44:1390–1395

Sims TJ, Lernmark A, Mancl LA, Schifferle RE, Page RC, Persson GR (2002) Serum IgG to heat shock proteins and Porphyromonas gingivalis antigens in diabetic patients with periodontitis. J Clin Periodontol 29(6):551–562

Song Y, Shi J, Wu Y, Han C, Zou J, Shi Y, Liu Z (2014) Metformin ameliorates insulin resistance in L6 rat skeletal muscle cells through upregulation of SIRT3. Chin Med J 127(8):1523–1529

Young RA, Elliott TJ (1989) Stress proteins, infection, and immune surveillance. Cell 59(1):5–8