Original Article

Serum anti-hsp27 antibodies concentration in diabetes mellitus; population-based case-control study

Mahshid Tavana[1], Naiemeh Varasteh[2], Mohadese Rohban[3], Hamideh Ghazizadeh[3], Mahsa Sadeghzade[3], Shima Tavalaie[3], Samaneh Abolbashari [4], Hossein Ramshini [1], Zahra Meshkat[5], Gordon A. Ferns[6], Majid Ghayour-Mobarhan[3,7,8*], Fatemeh Sadabadi [3*].

[1]Department of Biochemistry, School of Basic Science, Payame Noor University of Mashhad, Iran.
[2]School of Medicine, Islamic Azad University, Mashhad Branch, Iran.
[3]Metabolic Syndrome Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[4]Department of Medical Genetic, School of Medicine, Mashhad University of Medical Science, Mashhad, Iran.
[5]Department of Microbiology and Virology, School of Medicine, Mashhad University of Medical Science, Mashhad, Iran.
[6]Department of Medical Education, Brighton & Sussex Medical School, Brighton, BN1 9PH, UK.
[7]Department of Nutrition, School of Medicine, Mashhad University of Medical Science, Mashhad, Iran
[8]International UNESCO Center for Health-Related Basic Sciences and Human Nutrition, Department of Nutrition, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

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Abstract

Background: Diabetes mellitus is an important risk factor for cardiovascular disease. Different biomarkers have been investigated for the diagnosis of diabetes pathogenesis or its complications. There are also reports regarding an increased level of anti-HSP27 antibodies in atherogenesis. We aimed to evaluate serum anti-heat shock protein 27 antibodies level in subjects with diabetes mellitus and undiagnosed individuals. **Materials and Methods:** This cross-sectional study was conducted on 6447 MASHAD study subjects, including four groups with diabetes mellitus (n=610), undiagnosed diabetes (n=162), impaired fasting glucose (IFG) (n=619) and normal (n=5056) subjects. Demographic and anthropometric data were obtained from all participants. Fasting serum glucose (FSG) and other parameters were measured. In-house enzyme-linked immune sorbent assay method was used for measuring Anti-HSP27 antibodies levels. **Results:** There were significant differences in weight (p=0.034), body mass index, waist, and hip circumference, systolic and diastolic blood pressure, fasting serum glucose, lipid profile and high sensitive- C reactive protein (p<0.001) between four groups of diabetes mellitus, undiagnosed diabetes, impaired fasting glucose, and normal subjects. The serum anti-HSP27 antibody titer did not show a significant difference between studied groups. **Conclusion:** Serum antibody titers to HSP27 were not significantly different between four groups categorized based on their FSG levels in a large population.

Keywords: anti-HSP27 antibody, Diabetes mellitus, MASHAD study cohort.

***Corresponding Authors:** Majid Ghayour-Mobarhan. Tel: (+98) 51-18002475; Email: Ghayourm@mums.ac.ir; Fatemeh Sadabadi, Tel: (+98) 51-18002401; Email: Sadabadif2@mums.ac.ir

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Introduction

Diabetes mellitus (DM) is characterized by hyperglycemia due to impaired secretion of insulin or insulin action [1]. DM is associated with premature cardiovascular disease (CVD) and microvascular disease [2].

Some clinical and experimental documents

showed enhancement of oxidative stress (OS) resulting from reactive oxygen species (ROS) in type 2 diabetes mellitus. The ROS can trigger chain reactions leading to the development and progression of T2DM macro and microvascular complications[3].

Defective glucose homeostasis and insulin resistance in type 2 diabetes may be associated with an impairment in the expression and response of heat shock proteins [4].

Heat shock proteins (HSP) are families of proteins that have essential functions and are present in all organisms [5, 6]. HSPs are expressed by cells and respond to environmental stresses such as exposure to free radicals, high temperatures, infections, and toxins [7]. HSPs have been classified into several families based on their molecular weight: Hsp10, Hsp27, Hsp40, Hsp60, Hsp70, Hsp90, and Hsp110 [8]. Hsp27 can be detected in most cells especially in reacting to cellular stress status like oxidative stress. HSP27 can reduce the concentration of ROS[9]. The expression level is indistinguishable or relatively low in some cells, however, the expression of Hsp27 is abundant in some other cells [10]. Serum Hsp27 concentrations appear to be a biomarker for cardiac ischemia [11]. It has been shown that serum Hsp27 concentration was found to be correlated with type 1 diabetes complications [12], although anti-HSP27 antibody titers were unrelated to serum Hsp27 antigen concentrations in type 1 diabetic patients [13]. Anti-HSP27 antibody concentrations have been reported to be involved in the atherogenesis [14], and are correlated with CVD risk factors such as age and hypertension [15]. In our study, we aimed to evaluate serum anti-HSP27 antibody titers in the different groups based on glycemic status.

Methods

This cross-sectional study was conducted on subjects aged 35-64 years old who were categorized into four groups; diabetes mellitus (n=610), undiagnosed diabetes mellitus (n=162), impaired fasting glucose (IFG(n=619) and a normal fasting serum glucose (n=5056). Individuals were recruited as a part of the MASHAD Study cohort [16]. Exclusion criteria for all participants included pregnancy and breastfeeding women, subjects with cardiovascular disease, stroke, and systemic diseases. The research was approved by the Ethics Committee of Mashhad University of Medical Sciences. Individuals completed a written consent form for participation. Serum fasting glucose (FSG) was evaluated for the classification of subjects. Individuals with an FSG level above 126 (FSG≥126) were classified as having diabetes mellitus; those who had fasting blood glucose levels between 100–125 mg/dl were categorized in the IFG group [17]. Subjects whose diabetes have not been diagnosed previously, and were unaware of being diabetic, but whose FSG levels were ≥126 mg/dl in the screening tests termed undiagnosed diabetes [18].

Anthropometric and demographic data collection. For all individuals, anthropometric parameters including weight, height, waist circumference, and hip circumference were measured using a standard protocol. Body mass index (BMI) was calculated using the formula, weight divided to height squared (m2), for each person. Weight and height measurements were performed using standard scales, to an accuracy of 0.1 kg and 0.1 cm respectively. Systolic and diastolic blood pressures were performed twice without stress, and the mean of the two pressures was recorded as the final blood pressure.

Blood sampling and serum glucose level measurement. Blood samples were taken after fasting for at least 12 hours. Serum samples were obtained after centrifuging blood samples at 10,000 rpm for 15 min, and the obtained serum was stored at -80 ° C until analysis. For all subjects, FSG concentration was measured after 12 hours of fasting. Fasting serum glucose concentrations were evaluated by routine enzymatic kit by Alycon auto analyzer (ABBOTT, Chicago, IL, USA).

Serum anti-Hsp27 antibody concentration. Serum HSP27 antibody titers were measured using an enzyme-linked immune sorbent assay (ELISA). This method previously was described in detail [7].

Statistical analysis. All statistical analyses were performed using the SPSS version 16 software package. To evaluate the normality of data, we used the Kolmogorov-Smirnov test. Values were expressed as mean±SD or, in the case of non-normally distributed data, as median and inter-quartile range. To

illustrate the significant difference between two normal variables, independent t-test and between two non-normal quantitative variables from the Mann-Whitney test and to evaluate abnormal variables in more than two groups of Kruskal-Wallis test and for standard variables, ANOVA was used. The level of statistical significance was set to $p<0.05$.

Results

Baseline demographic, anthropometric, and biochemical characteristics between participants in four groups, categorized base on the FSG level, are shown in Table 1. As expected, there were significant differences in weight ($p=0.034$), BMI, waist and hip circumference, SBP, DBP, FBS, triglyceride, cholesterol, LDL-C, and Hs-CRP between studied groups ($p<0.001$). The results showed that there was no significant difference in height, HDL-C, and BUN ($p>0.05$) (Table1). Serum HSP27 antibody levels did not show a significant difference between four groups of the study including subjects with diabetes mellitus, undiagnosed diabetes, IFG, and normal ($p=0.513$) (Fig.1.A). Also, there is not any significant difference in anti-HSP27 antibody concentration between men and women (undiagnosed: $p=0.176$, IFG: $p=0.204$, Diabetes: $p=0.879$ Normal: $p=0.068$) (Fig.1.B).

Table1. Baseline clinical characteristic and Biochemical features of the study population

Characteristics	undiagnosed diabetes (162)	IFG (619)	Diabetes (610)	Normal (5056)	P-value
Weight (kg)	74.73±13.39	74.92±13.35	73.06±12.25[b]	71.12±12.81[abc]	<0.001
Height (cm)	1.60±0.09	1.59±0.08	1.59 ±0.08	1.6 ± 0.91[c]	<0.001
BMI (kg/m2)	29.15±5.36	29.31±4.83	28.76±4.48	27.58 ±4.69[abc]	<0.001
WC (cm)	99.21±12.53	98.86±11.96	98.90±11.07	94.22±11.95[abc]	<0.001
HC (cm)	105.82±10.28	105.76±9.98	104.18±9.20[b]	103.35±9.20[ab]	<0.001
SBP (mmHg)	126.61±18.90	128.42±20.76	128.81±19.77	119.97±17.62[abc]	<0.001
DBP (mmHg)	81.21±11.32	82.41±12.08	81.26±11.12	78.38±11.03[abc]	<0.001
FBS (mg/dl)	182.64±57.32	108.92±7.07[a]	164.68±74.55[ab]	79.15±9.76[abc]	<0.001
TC (mg/dl)	210.82±47.44	202.01±42.06[a]	199.86 ±45.03[a]	188.40±37.14[abc]	<0.001
TG (mg/dl)	154(98-224)	138(99-201)[a]	151(108-225)[b]	114(81-161)[abc]	<0.001
HDL-C (mg/dl)	42.35±10.32	43.44±10.88	42.06±9.78[b]	42.85±9.81	0.024
LDL-C (mg/dl)	126.52±41.91	122.84±37.11	120.26±37.95[a]	116.42±33.02[abc]	<0.001
hsCRP (mg/L)	2.59(1.41-6.16)	2.26(1.30-5.50)	2.33(1.30-4.74)	1.48(0.92-3.09)[abc]	<0.001
BUN (mg/dl)	12.37±4.38	13.05±4.23	13.15±4.31	12.99±4.20	0.224
Cr (mg/dl)	0.86 ±0.28	0.85 ±0.23	0.87 ±0.30	0.86 ±0.24	0.570

Data are shown as Mean ± SD for normal variables and interquartile range for non-normal variables. IFG impaired fasting glucose, BMI body mass index; WC waist circumferences; HC hip circumferences; SBP systolic blood pressure, DBP diastolic blood pressure, FSG fasting serum glucose, TC total cholesterol, TG triglycerides, HDL-C high-density lipoprotein, LDL-C low-density lipoprotein cholesterol, Hs-CRP high sensitivity C-reactive, BUN blood urea nitrogen, Cr creatinine.
The covariance analysis was used to examine the difference in the mean of data.
a: Differences between undiagnosed diabetes versus IFG, diabetes and normal groups
b: Difference between IFG versus diabetes and normal groups
c: Difference between diabetes and normal groups

Multivariate regression for anti-HSP27 and diabetes status was done in different models (Table 2).The effect of diabetes status on anti-HSP27 levels assessed in the model 1 (unadjusted), model 2 was adjusted for gender and age and model 3 was adjusted for gender, age, body mass index (BMI), smoking status and physical activity level (PAL) and has not been shown a significant association between anti-HSP27 level and diabetes in none of the models.

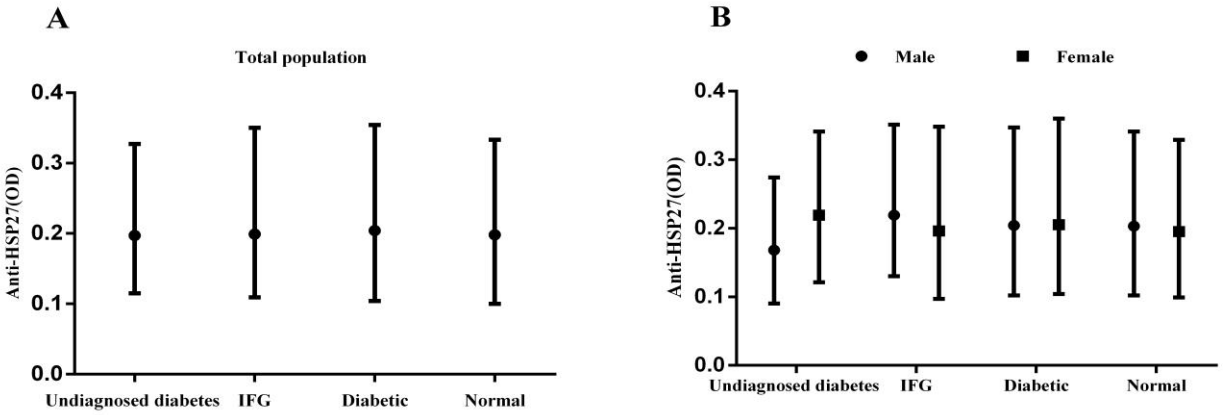

Fig. 1. The concentration of anti-HSP-27 antibody; A: among the total population, B: according to gender. Data presented as median (inter-quartile range).

Table2. Multivariate regression for anti-HSP27 and Diabetes status.

Variable	Model 1		Model 2		Model 3	
	Odds (CI95%)	P value	Odds (CI95%)	P value	Odds(CI95%)	p value
Undiagnosed diabetes	0.90(0.40-2.00)	0.806	0.89(0.40-1.98)	0.78	0.84(0.38-1.88)	0.68
Impair fasting glucose	1.31(0.88-1.97)	0.177	1.27(0.84-1.91)	0.25	1.15(0.76-1.73)	0.50
Diabetes	1.16(0.77-1.76)	0.459	1.15(0.76-1.75)	0.49	1.12(0.73-1.70)	0.59

Model 1: unadjusted; Model 2: adjusted gender and age; Model 3: adjusted for and gender, age, body mass index (BMI), smoking status, and Physical Activity Level (PAL).The normal group was considered as a reference for the analysis.

Discussion

The heat shock proteins are a family of proteins that have an antioxidant role and protect cells against various sources of stress. HSPs are primarily molecular chaperones that aid in protein folding, degrading of nonfunctional proteins, and preventing protein aggregation. A dual role is proposed for HSPs; an anti-inflammatory role inside and a pro-inflammatory role outside the cell [19].

A characteristic feature of diabetes mellitus is uncontrolled oxidative stress and production of ROS leading to the development of microvascular and macrovascular complications such as nephropathy, retinopathy, and neuropathy. Therefore, the antioxidant functions of HSPs can prevent these complications. However, it has been recognized that diabetes can impair HSPs expression and synthesis which contributes

to complications commonly observed in diabetics and this creates a vicious cycle [14].

Several studies have discussed whether HSP27 levels or Anti-HSP27 antibody titers could be served as a novel biomarker for diabetes complications [14].

A study by Habich et al. suggested that HSPs could be utilized in the development of novel therapeutic and diagnostic strategies for diabetes [20].

In this study, the serum anti-HSP27 antibody levels in patients with different stages of diabetes were analyzed using an enzyme-linked immune sorbent assay. The results indicated that no significant difference was found in anti-HPS27 levels between the four studied groups comprised of normal, diabetic, undiagnosed diabetes, and IFG patients.

Additionally, the findings of this study suggested that there was no significant difference in anti-HPS27 levels between females and males.

In a 2008 study conducted by Gruden et al. in Italy, the association of sHSP27 levels with diabetes complications was investigated by ELISA and they concluded that sHSP27 levels could be used as a novel marker for diabetic neuropathy [12].

Burut et al. evaluated the Hsp27 antigen and anti-HSP27 antibody (immunoglobulins M and G) levels of sera in individuals with various glycemic groups with or without cardiovascular events. They showed glucose intolerance patients with cardiovascular disease (CVD) had higher Hsp27 antigen titer compared glucose intolerance subject without CVD (p=0.03) and also normal glucose tolerance subjects with and without CVD (p=0.03 and p=0.02 respectively). There was a significant increased concentration of IgM anti-HSP27 antibody between glucose intolerance patients with CVD comparison to normal glucose tolerance subjects without CVD group (p=0.02). Immunoglobulins G titer against Hsp27 antigen were higher in glucose intolerance patients with CVD in the glucose intolerance subjects without (p=0.06). They concluded Hsp27 antigen and its antibody levels seem to be associated with CVD in glucose intolerance patients [14].

In a 2017 study which was performed on type 2 diabetes subjects without and with microvascular complications, Plasma Hsp27 levels were measured by ELISA and their results indicated that patients with diabetic neuropathy (DNe) had higher levels of Hsp27, thereby it could be served as a potential biomarker of DNe [2].

In a study conducted by Burt et al. in 2009, the association of HSP27 antibody levels with micro- and macrovascular complications of type 1 diabetes was investigated as part of the cross-sectional analysis of the EURODIAB Prospective Complications Study using an in-house enzyme-linked immunosorbent assay. Their results showed that Anti-HSP27 antibody levels could not be used as a marker of vascular complications in type 1 diabetes which supports the results of the present study [13].

A similar study by Kargari et al. was carried out on 933 subjects with metabolic syndrome (MetS) to investigate the anti-Hsp27 antibody in their serum samples. Their results are in agreement with the present study because no difference in serum anti-HSP27 concentrations was found between subjects with and without diabetes mellitus, metabolic syndrome, and gender [21].

In conclusion, our results revealed serum HSP27 antibody levels did not show a significant difference between 4 groups were categorized based on their fasting serum glucose levels. we believe Anti-HSP27 could not be an efficient method for the diagnosis of overt of latent diabetes.

Conclusion

The results of our investigation indicated that there was not a significant difference in serum anti-HSP27 antibody titers among nondiabetic, diabetic, undiagnosed diabetes, and impaired fasting glucose subjects.

Conflicts of Interest

The authors have no conflict of interest to disclose, Serum anti-HSP27 antibodies concentration in diabetes mellitus; population based case-control study.

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References

1. Association AD. Diagnosis and classification of diabetes mellitus. Diabetes care. 2014;37(Supplement 1):S81-S90.
2. Jakhotia S, Sivaprasad M, Shalini T, Reddy PY, Viswanath K, Jakhotia K, et al. Circulating levels of Hsp27 in microvascular complications of diabetes: Prospects as a biomarker of diabetic nephropathy. Journal of diabetes and its complications. 2018;32(2):221-5.
3. Wright Jr E, Scism-Bacon J, Glass L. Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. International journal of clinical practice. 2006;60(3):308-14.
4. Hooper PL, Hooper PL. Inflammation, heat shock proteins, and type 2 diabetes. Cell Stress and Chaperones. 2009;14(2):113-5.
5. Hightower LE. Heat shock, stress proteins, chaperones, and proteotoxicity. Cell. 1991;66(2):191-7.
6. Xu Q. Role of heat shock proteins in atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2002;22(10):1547-59.
7. Sahebkar A, Pourghadamyari H, Moohebati M, Parizadeh SMR, Falsoleiman H, Dehghani M, et al. 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. Clinical biochemistry. 2011;44(17-18):1390-5.
8. Rayner K, Chen Y-X, Siebert T, O'Brien ER. Heat shock protein 27: clue to understanding estrogen-mediated atheroprotection? Trends in cardiovascular medicine. 2010;20(2):53-7.
9. Vidyasagar A, Wilson NA, Djamali A. Heat shock protein 27 (HSP27): biomarker of disease and therapeutic target. Fibrogenesis & tissue repair. 2012;5(1):7.
10. Kostenko S, Moens U. Heat shock protein 27 phosphorylation: kinases, phosphatases, functions and pathology. Cellular and molecular life sciences. 2009;66(20):3289-307.
11. Park HK, Park E-C, Bae SW, Park MY, Kim SW, Yoo HS, et al. Expression of heat shock protein 27 in human atherosclerotic plaques and increased plasma level of heat shock protein 27 in patients with acute coronary syndrome. Circulation. 2006;114(9):886-93.
12. Gruden G, Bruno G, Chaturvedi N, Burt D, Schalkwijk C, Pinach S, et al. Serum heat shock protein 27 and diabetes complications in the EURODIAB prospective complications study: a novel circulating marker for diabetic neuropathy. Diabetes. 2008;57(7):1966-70.
13. Burt D, Bruno G, Chaturvedi N, Schalkwijk C, Stehouwer CD, Witte DR, et al. Anti–Heat Shock Protein 27 Antibody Levels and Diabetes Complications in the EURODIAB Study. Diabetes Care. 2009;32(7):1269-71.
14. Burut DFP, Borai A, Livingstone C, Ferns G. 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 and Chaperones. 2010;15(4):379-86.
15. Shams S, Shafi S, Bodman-Smith K, Williams P, Mehta S, Ferns GA. Anti-heat shock protein-27 (Hsp-27) antibody levels in patients with chest pain: association with established cardiovascular risk factors. Clinica Chimica Acta. 2008;395(1-2):42-6.
16. Ghayour-Mobarhan M, Moohebati M, Esmaily H, Ebrahimi M, Parizadeh SMR, Heidari-Bakavoli AR, et al. Mashhad stroke and heart atherosclerotic disorder (MASHAD) study: design, baseline characteristics and 10-year cardiovascular risk estimation. International journal of public health. 2015;60(5):561-72.
17. Bansal N. Prediabetes diagnosis and treatment: A review. World journal of diabetes. 2015;6(2):296.
18. Harris MI, Eastman RC. Early detection of undiagnosed diabetes mellitus: a US perspective. Diabetes/metabolism research and reviews. 2000;16(4):230-6.
19. Gomez-Pastor R, Burchfiel ET, Thiele DJ. Regulation of heat shock transcription factors and their roles in physiology and disease. Nature Reviews Molecular Cell Biology. 2018;19(1):4.
20. Habich C, Sell H. Heat shock proteins in obesity: links to cardiovascular disease. Hormone molecular biology and clinical investigation. 2015;21(2):117-24.
21. Kargari M, Parizadeh SMR, Karimian MS, Farahmand SK, Sahebkar A, Esmaeili H, et al. Serum anti-HSP27 antibody titers in patients with metabolic syndrome, with or without diabetes mellitus. Comparative Clinical Pathology. 2016;25(4):895-901.