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Evaluating The Association Between Serum Hsp27 Antibody and Hypertension in Patients without Underlying Cardiovascular Disease

Mahdieh Khazaee[1] , Mahdi Taheri Bonakdar [2], Mahmoud Ebrahimi [3], Mohammad Reza Aarpazhooh[3], Hamideh Ghazizadeh [2,3,4] , Mohsen Mouhebati[5] , Maryam Shahi [2], Fatemeh Haji shamsaei [6], Zahra Alizadeh hassani [6], Faezeh Ghasemi [7], Maryam Tayefi [8] , Shima Tavallaie [9], Amirhossein Sahebkar [10,11], Gordon A . Ferns [12], Seyed Mohammad Reza Parizadeh [2], Majid Ghayour-Mobarhan * [2,4]

[1] Immunology Research Center, BuAli Research Institute, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[2] Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
[3] Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran.
[4] International UNESCO Center for Health-Related Basic Sciences and Human Nutrition, Department of Nutrition, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[5] Cardiovascular Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[6] Department of Biology, Faculty of Sciences, Mashhad Branch, Islamic Azad University, Mashhad, Iran.
[7] Department of Medical Biotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[8] Norwegian Center for E-Health Research, University Hospital of North Norway, Tromsø, Norway.
[9] Department of Biochemistery and Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[10] Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran.
[11] Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
[12] Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex BN1 9PH, UK.

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ABSTRACT

Introduction: An association between heat shock protein 27 (Hsp27) antigen with cardiovascular risk factors has been shown previously. Furthermore, higher levels of serum anti-HSP27 antibodies are also related to higher cardiovascular morbidity and mortality. In the current study, we looked at the relationship between serum Hsp27 antibodies and hypertension, as an important cardiovascular risk factor, in individuals without evidence of cardiovascular disease (CVD) .

Methods: A sub-population of hypertensive patients (HTN+) without underlying CVD were recruited from the Mashhad stroke and atherosclerosis heart disease (MASHAD) study to assess the association between serum Hsp27 antibodies and hypertension; independent of other cardiovascular risk factors. A total of 1599 people were studied of whom 288 individuals had hypertension and 1311 were used as controls (HTN-) .

Results: Mean serum Hsp27 antibody titers were 0.20 (0.27) OD in the whole population sample and was not significantly different in the normotensive (HTN-) compared to HTN+ individuals with different degrees of hypertension .

Conclusion: There were no significant associations between serum anti-Hsp27 concentrations and either the presence or severity of hypertension. Future studies are warranted to explore the association of anti-Hsp27 antibody and antigen levels and other cardiovascular risk factors.

► *Khazaee, M., Taheri Bonakdar, M., Ebrahimi, M., Aarpazhooh, M.R., Ghazizadeh, H., Mouhebati, M., Shahi, M., Haji shamsaei, F., Alizadeh hassani, Z., Ghasemi,F., Tayefi , M., Tavallaie, S., Sahebkar, A., Ferns, G.A., Parizadeh, A.M.R., Ghayour-Mobarhan, M. Evaluating The Association Between Serum Hsp27 Antibody and Hypertension in Patients without Underlying Cardiovascular Disease. J Cardiothorac Med. 2023; 11(1): 1109-1116. Doi: 10.22038/jctm.2023.70690.1411*

*Corresponding Author: Majid Ghayour-Mobarhan MD, PhD, Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. 99199-91766, Mashhad, Iran; Tel:+985138002288, Fax: +985138002287; Email: ghayourm@mums.ac.ir.

Introduction

Heat shock proteins (HSPs) are a family of molecular chaperones that are typically intracellular proteins, but can be released following exposure to a variety of stressful stimuli including: free radicals, heat, toxins, ischemia, oxidants and cytokine stimulation. HSPs protect cells from damage and allow denatured proteins to adopt their natural structure (1). Hsp27 is a member of the small HSP family with a molecular weight of around 27 kDa, however, it can oligomerize to form large 800 kDa aggregates in the cytosol (2). Hsp27 is available at high levels in numerous adult tissues including the lung, heart, and uterus (3). In addition to its role as a chaperone, Hsp27 is involved in several other important cellular processes including cell migration (4, 5), apoptosis (6-8), endothelial barrier function (9-12), oxidative stress protection, and inflammation (13-16).

Recently, it has been reported that serum concentrations of HSPs are correlated with the development of atherosclerosis, and that HSPs have a pivotal role in maintaining vascular function (17). Martin-Ventura et al. showed that Hsp27 is reduced in atherosclerotic plaques (18). Wick and colleagues confirmed Hsp27 downregulation in atherosclerotic plaques, and furthermore, they have suggested that normal adjacent tissue expressed higher levels of Hsp27 (19). We have previously hypothesized that reduced HSP27 expression favors smooth muscle growth and plaque formation (20). Park et al. showed that Hsp27 could also restrict the atherosclerotic inflammatory response by inhibiting NF-κB activation (21). It has been shown that high serum anti-HSP27 titers are associated with increased cardiovascular morbidity and mortality (22). Several studies have shown that some CVD risk factors are directly associated with higher anti-HSP titers (23). Shams et al. suggested that antibodies against Hsp27 are significantly associated with hypertension, and age, but not with other CVD risk factors in a UK population sample (24). We have evaluated the association between Hsp27 antibodies and hypertension as a major risk factor for atherosclerosis in an Iranian population sample. Previous studies have assessed the relationship between Hsp27 and atherosclerosis and even if they concluded a relationship between Hsp27 and hypertension it was not their primary purpose and none of them were designed to assess the relationship between Hsp27 antibodies and hypertension specifically. Moreover, populations recruited into earlier studies comprised patients with CVD with or without hypertension. In the present study, we used a sample population from the hypertensive patients (HTN+) without underlying CVD to evaluate the relationship between serum antibodies to Hsp27 and hypertension independent of cardiovascular disease.

Materials and Methods

Sample population

In this nested case-control study, we compared serum anti-Hsp27 antibodies in untreated hypertensive patients (n=288) with healthy volunteers (HTN-) (n=1311).

Samples were selected from the study population of MASHAD study (25). The levels of serum anti-Hsp27 antibody were measured on the blood sample which were already collected in the MASHAD study at baseline in 2007. The subjects with hypertension were divided into two subgroups: 223 individuals with stage I hypertension and 65 patients with stage II hypertension without any underlying CVD and were not treated for their hypertension at the time of sampling. The control group were age- and sex-matched non-hypertensives that comprising 580 subjects with normal blood pressure and 731 persons in the pre-hypertension stage without any underlying CVD. Also combined dyslipidemia was identified based on the NCEP ATPIII criteria (26).

Inclusion criteria for the recruited subjects were:

1-Ages of 35 to 65

2-Those without history of CVD

Furthermore, the inclusion criteria for the healthy control subjects were:

1-Not being in any stages of hypertension according to JNC8 guidelines (۴)

2-Not taking any medication

Exclusion criteria for all participants were:

1-Patients on treatment for hypertension.

2-Patients positive for transmittable viral infections including HIV, HBV, HCV, and HTLV

3-Patients with CVD or a positive family history of CVD

4-A history of cancer, or collagen vascular illnesses

5-Treatment with immunosuppressive medication

6-Pregnancy

Laboratory testing

At the beginning of the "MASHAD Study" in 2007, 5 ml blood sample was taken from all participants after 12 hours fasting. Also, at that time, blood pressure was checked in "MASHAD Study" three times by a specialist nurse and the mean value was recorded. Anthropometric parameters were assessed at baseline. Laboratory tests were undertaken on both patient and control group on serum samples from "MASHAD Study" using autoanalyzer device and kits from Pars Azmoon, and consisted of HDL-cholesterol, LDL-cholesterol, fasting blood sugar, triglycerides, and total cholesterol (27).

Blood pressure measurements

Blood pressure was measured twice for each participant by a standard mercury manometer and in the sitting position, regarding the standard protocol released by American Heart Association (28). The participants were classified as hypertensive if the systolic and/or diastolic blood pressure values were ≥140 and ≥90 mmHg, respectively. Pre-HTN was defined as an SBP 120 to <140 mm Hg and/or DBP 80 to <90 mm Hg. Stage 1 of HTN was defined as SBP was 140 to159 mmHg and/or DBP was 90 to 99 mmHg. Stage 2 of HTN was classified as the participants had SBP≥160 mmHg or DBP≥100 mmHg (29).

Anti-Hsp27 antibody measurement

Serum Hsp27 antibody titers (Stressgen, Canada) were measured using an enzyme-linked immune sorbent assay (ELISA), as described previously (30). A 50-ng recombinant human Hsp27 was dissolved in 50 µL carbonate buffer (pH = 9.6) and was added to each well of the 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 bindings, 2% goat serum in PBS were added to each well and after incubation time (30 minutes at 37 ° C and 30 minutes at 25 ° C) they were washed three times with PBS. After 30 min incubation with serum (diluted 1:50) in room temperature, wells were washed three times. Then, wells 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 dark room. After adding 50 µL 3M HCl to each well, optical density at 450 nm was read using a Lab systems iEMS Reader MF microtiter plate reader. Optical density ratio was compared to a reference wavelength of 620 or 570 nm and results were given in optical density units.

Statistical Analysis

All data were analyzed using SPSS version 20. Normality of distribution of all quantitative variables were examined by Kolmogorov-Smirnov test. Anti-Hsp27, triglyceride and Hs-CRP variables had abnormal distribution and analyzed by Kruskal wallis H test.

Pearson and Spearman's tests were used to show a significant difference between normal and abnormal variables, respectively. Independent T-test was used to compare a normal variable between patients and control group. P values <0.05 were considered significant.

Results

A total of 1599 people were studied, of whom 37.7% were male. The demographic features of the study groups are shown in Table 1. Participants were categorized into two groups and four subgroups, based on their blood pressure. The hypertensive group, which consisted of 288 patients, had a significantly higher waist and hip circumference, weight, body mass index, waist to height, and waist to hip ratio in comparison with the non-hypertensive group. The healthy control group (1311 individuals) had significantly lower fasting

blood glucose, total cholesterol, triglyceride, and Hs-CRP.

There was not significant difference in dyslipidemia between hypertensive and non-hypertensive groups and their subgroups (Table 1).

Subjects were further categorized into four groups based on their blood pressure according to JNC8 guidelines (31). Mean serum anti-Hsp27 was around 0.2 OD in our sample population and was not significantly different between normotensive subjects and hypertensive group and their subgroups (Table 1). Moreover, no significant difference was seen in serum anti-Hsp27 levels between subjects with isolated diastolic or systolic hypertension compared with the non-hypertensive group in the population (Figure 1). Furthermore, there was no significant difference in serum anti-Hsp27 levels among subgroups at different stages of hypertension (Figure 2).

Table 1. Comparison of the baseline characteristics between hypertensive (HTN+) and non-hypertensive (HTN-) group.

		HTN-		HTN+	
Different stages of hypertention		**Normotension**	**Pre HTN**	**Stage 1 HTN**	**Stage 2 HTN**
Frequency	Male	190 (32.8%)	302 (41.3%)	87 (34.8%)	24 (36.9%)*
	Female	390 (67.2%)	429 (58.7%)	163 (65.2%)	41 (63.1%)*
	Total	580	731	223	65
Age (year)	Male	44.80±7.18	47.86±7.72	48.77±8.19	50.95±9.65**
	Female	45.69±7.76	48.78±7.29	50.53±6.26	53.05±5.47**
Waist circumference (cm)		93.62±11.95	97.79±12.17[a]	97.79±12.17[a]	100.07±12.52[a]
Weight		69.83±12.72	74.63±13.06[a]	76.17±13.75[a]	75.35±16.44[a]
Height (m)		159.59±8.54	160.79±9.33	159.69±9.30	160.49±8.42
Hip circumference (cm)		104.12±10.04	105.75±10.37[a]	107.51±10.53[a]	105.34±9.94
BMI (kg/m2)		26.56±6.78	27.54±7.56	29.17±6.39[ab]	26.50±9.03
Waist/Weight		1.36±0.17	1.33±0.18	1.33±0.18	1.37±0.21
Waist/Height		0.58±0.08	0.61±0.08[a]	0.62±0.08[ab]	0.62±0.07[a]
Waist/Hip		0.89±0.07	0.92±0.10[a]	0.93±0.08[a]	0.95±0.07[a]
SBP (mmHg)		105.92±8.50	123.94±10.24[a]	138.99±14.31[ab]	162.26±22.74[abc]
DBP (mmHg)		69.04±6.75	80.70±4.41[a]	93.06±5.10[ab]	104.19±10.54[abc]
FBG (mg/dl)		93.33±45.17	95.08±35.17	101.55±40.43	101.34±39.24
LDL-C (mg/dl)		113.29±34.95	115.35±37.52	119.67±37.77	122.57±39.89
HDL-C (mg/dl)		44.58±11.55	44.70±11.66	45.09±10.3	44.65±9.54
Cholesterol (mg/dl)		189.96±41.23	196.16±40.42	202.16±39.84[a]	212.18±47.00[ab]
Triglyceride (mg/dl)		112(86)	126(87)	135(85)	158(106)**
Hs-CRP (m/dl)		1.78(2.69)	2.02(3.34)	2.37(3.72)	2.43(4.60)**
Anti-Hsp27 (OD)		0.21(0.26)	0.21(0.28)	0.19(0.24)	0.22(0.33)
Diabetes %		8.3	9.6	14.4	9.2
Smoking %		22.3	19.1	17.2	16.9
Dyslipidemia %		84.2	84.6	87.6	89.2

Abbreviations: HTN: Hypertension**; FBG:** Fasting Blood Glucose**; SBP:** Systolic Blood Pressure**; DBP:** Diastolic Blood Pressure**; BMI:** Body Mass Index**; Hs-CRP:** High-Sensitivity C-reactive Protein**; Anti-Hsp27:** Anti Heat Shock Protein 27**; OD:** Optical Density**.**

Letter "a" show a significant association between normotension group and other groups (including: Pre HTN, Stage 1 HTN and Stage 2 HTN groups).

Letter "b" show a significant association between Pre HTN group and Stage 1 HTN and Stage 2 HTN groups.

Letter "c" show a significant association between Stage 1 HTN and Stage 2 HTN groups.

* and ** show $p<0.05$ and $p<0.01$, respectively.

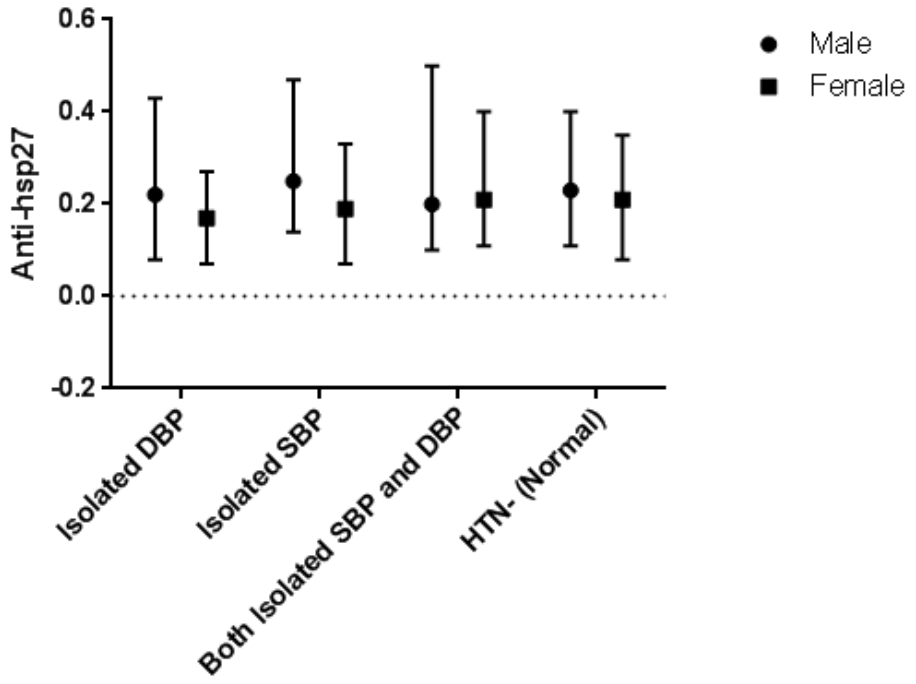

Figure 1. Comparison of serum anti-Hsp27 antibody titers in patients with Isolated Systolic Blood Pressure (SBP): (SBP≥140 & DBP<90), Isolated Diastolic Blood Pressure (DBP): (SBP<140 & DBP≥90), Both Isolated SBP and DBP: (SBP≥140 & DBP≥90), Hypertension (HTN)-: (SBP<140 & DBP<90).

Discussion

The relationship between hypertension and cardiovascular disease is persistent and independent of other risk factors; myocardial infarction, heart failure, cerebrovascular accidents and kidney diseases are more prevalent in hypertensive patients than the normal population (32). It is estimated that 8% of CVD burden and mortality is due to hypertension. Furthermore, the global prevalence of hypertension is increasing especially in developing countries.

Our results showed that mean serum anti-Hsp27 concentrations were not significantly different between untreated hypertensive patients and non-hypertensive subjects. No significant difference was observed in anti-Hsp27 levels between 4 subgroups after further categorizing hypertension group (HTN+) into 2 stages (Stage 1 HTN and stage 2 HTN) and healthy subjects (HTN-) to normotension and pre HTN groups (Figure 2). These findings suggest that the increase in serum anti-Hsp27 levels in patients with CVD is independent of baseline blood pressure.

Anthropometric factors including, weight, waist circumference, BMI, waist to height ratio, waist to hip ratio, FBG, cholesterol, triglyceride, and Hs-CRP were significantly different between HTN+ and HTN- groups. And these results are consistent with previous studies (33,34).

Many studies have suggested that patients with cardiovascular disease (CVD) have higher serum levels of anti-Hsp27 antibodies (24, 25, 35). Also, CVD is strongly related to increased blood pressure (36, 37). Keeping these two findings in mind, using a relatively large sample size we found that, unlike some of the previous studies, increase in serum levels of anti-Hsp27 antibodies in CVD patients is not associated with increased blood pressure in these patients.

Many studies have sought to assess the relationship between HSPs and atherosclerosis pathogenesis and CVD risk factors, however, only a handful of them have concentrated exclusively on hypertension. Hsp70 and Hsp65 are the most extensively studied HSPs in cardiovascular diseases. Kunes et al. demonstrated increased induction of Hsp70 in lymphocytes of

hypertensive subjects (38). Pockley et al. reported that anti-Hsp70 and anti-Hsp65 antibody levels were elevated in hypertensive patients, independent of age, smoking habits and blood lipids, whereas Hsp70 and Hsp60 and anti-Hsp60 antibody levels were similar in hypertensive and non-hypertensive groups (39).

Molvarec et al. indicated that Hsp70 levels in serum are elevated in transient hypertension of pregnancy, further clarifying the role of HSPs in hypertension (40). It is suggested that HSPs are elevated in acute hypertension, though they are not significantly different in the patients with established hypertension and non-hypertensive patients (17,39).

The relationship between anti-Hsp27 antibodies and hypertension is still controversial. Shams et al. study on 60 patients with CVD reported that anti-Hsp27 antibodies were higher in patients with acute chest pain and this increase in antibody levels was associated with blood pressure and age (24).

The differences in results may be due to the sample size of some of these previous studies, the presence of other co-morbidities, such as CVD, or the effects of treatment. Our study was on hypertensive patients without any other underlying CVD, or a family history of hypertension, moreover, the recruited subjects were not previously aware of their high blood pressure therefore they were not under any anti-hypertensive treatment. We studied the independent role of hypertension on the circulating concentrations of anti-Hsp27 antibodies without the potential confounding effects of drugs and underlying diseases.

Conclusion

The findings of the present study do not suggest any association between serum concentrations of anti-Hsp27 and hypertension. It seems likely that the association of anti-Hsp27 and CVD is independent of the presence of hypertension.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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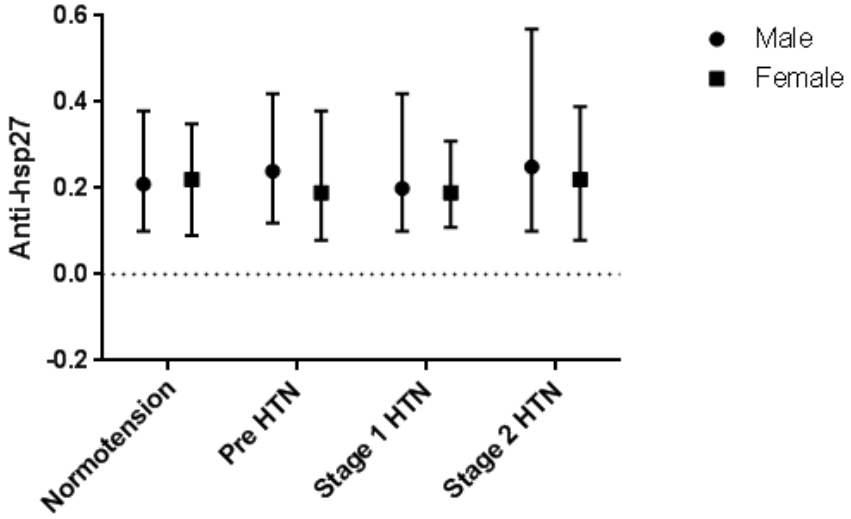

Figure 2. Comparison of serum anti-Hsp27 antibody titers between patients in different stages of hypertention (HTN).

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