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Serum Heat Shock Protein 90 Alpha: A New Marker of Hypertension-Induced Endothelial Injury?*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of article

Abstract

Background. Hypertension-induced endothelial dysfunction is associated with an impaired bioavailability of nitric oxide regulated through interactions between nitric oxide synthase and heat shock protein-90 (Hsp-90). The role of Hsp-90 in the development of arterial hypertension remains unclear.

Objectives. The objective of the study was to evaluate serum concentrations of Hsp-90 α in patients with arterial hypertension in comparison to their normotensive counterparts.

Material and Methods. The study was performed on 49 adults (mean age 55.6 years) with an elevated waist circumference. The individuals presented no subjective feeling of any disease, admitted no drug treatment for any condition, and had not previously been diagnosed with the metabolic syndrome. Patients were screened for arterial hypertension and other component disorders of the metabolic syndrome. Hsp-90 α concentrations were evaluated by enzyme-linked immunosorbent assay (ELISA).

Results. Twenty-eight subjects were diagnosed with arterial hypertension, while 21 individuals had normal blood pressure. Twenty-five patients satisfied the metabolic syndrome diagnostic criteria. Hsp-90 α concentrations were significantly higher ($p = 0.002$) in the individuals with arterial hypertension than in their normotensive counterparts (median \pm interquartile range): 19.42 ng/mL \pm 5.17 vs. 16.86 ng/mL \pm 3.18. The concentrations of Hsp-90 α correlated positively with systolic blood pressure ($R = 0.39$; $p = 0.005$) and diastolic blood pressure ($R = 0.29$; $p = 0.046$).

Conclusions. An increase in Hsp-90 α concentrations in patients with arterial hypertension may be a compensatory mechanism for the impaired bioavailability of nitric oxide. The role of Hsp-90 α as an early marker of hypertension-associated endothelial injury should be confirmed in further studies on a larger group of patients (*Adv Clin Exp Med* 2015, 25, 2, 255–261).

Key words: arterial hypertension, metabolic syndrome, endothelial dysfunction, nitric oxide, heat shock protein.

Arterial hypertension, as one of the most prevalent causes of cardiovascular events, is a widespread public health problem. The disorder results from an endothelial dysfunction associated with an impaired bioavailability of nitric oxide (NO), which is known to be one of the crucial factors for maintenance of normal blood pressure. Essential hypertension is linked to reduced endothelium-dependent vessel relaxation [1, 2]. Normal vasorelaxation is mediated by a number of endothelial

substances, including NO, prostaglandins (PGI₂ and PGE₂), and a family of endothelial-derived hyperpolarizing factors (EDHF), such as hydrogen sulfide, hydrogen peroxide, potassium ions, carbon monoxide, epoxyeicosatrienoic acids, and C-natriuretic peptide, which activate potassium channels of the vascular smooth muscle cells [2].

Arterial hypertension is associated with an impaired function of nitric oxide [1, 2]. This gaseous lipophilic free radical is generated by three isoforms

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of nitric oxide synthases (NOS): type 1 or neuronal (nNOS), type 2 or inducible (iNOS) and type 3 or endothelial NOS (eNOS). The release of nitric oxide by the endothelial layer can be up-regulated (by estrogens, exercise, and positive dietary factors) or down-regulated (by oxidative stress, smoking, and oxidized low-density lipoproteins) [3].

The expression of eNOS is altered in many cardiovascular diseases, including hypertension, atherosclerosis, and diabetes mellitus [4]. The enzyme's activity is regulated through its interactions with such protective proteins as the 90 kDa heat shock protein (Hsp-90) and caveolin-1 [1].

The chaperone Hsp-90 is one of the most abundant proteins in eukaryotic cells. Under non-stress conditions it constitutes about 1–2% of all cellular proteins. Hsp-90 is essential for the maturation and activity of proteins involved in signal transduction, cell-cycle regulation, protein folding and degradation, and morphological evolution [5, 6]. Hsp-90 can be detected in the cytosol in two isoforms – Hsp-90 α and β [7]. Hsp-90 α is a highly inducible protein, while Hsp-90 β seems to be a constitutive isoform [7]. High expression of Hsp-90 α is associated with enhanced regulation of the cell cycle [8], tumor progression [9], and signaling of tyrosine kinases mediated by growth factors [10]. Hsp-90 β is involved in processes of long-term cellular adaptation, including the development of drug resistance [11]. In 2002, an isoform called Hsp90N, associated with cellular transformation, was added to the Hsp90 family [12].

The functional activity of Hsp-90 requires a complex interaction with other co-chaperones playing a crucial role in the folding of newly formed proteins and the stabilization of denatured proteins following cellular stress. Apart from co-chaperones, Hsp-90 also binds to hundreds of client proteins, predominantly involved in signal transduction [6].

One of the key client proteins of Hsp-90 is endothelial NOS. Hsp-90 associates with eNOS, thereby determining its proper folding and function [5]. Hsp-90 has also been implicated in the balance between the production of nitric oxide and superoxide anion by eNOS [13]. Impaired interaction between Hsp-90 and eNOS may result in an altered expression of eNOS, which is observed in numerous cardiovascular diseases, including hypertension. This suggests that Hsp-90 may be involved in the pathogenesis of hypertension.

Arterial hypertension is one of the crucial components of the metabolic syndrome, a complex pathology with rising prevalence worldwide [14]. Currently, the metabolic syndrome is diagnosed according to the Joint Interim Statement issued by the International Diabetes Federation

in 2009 [14]. It has been reported that the metabolic syndrome (diagnosed according to the former NCEP-ATP III criteria updated in 2005) can be observed in 23% and 20% of Polish adult males and females, respectively [15].

Material and Methods

A total of 49 non-smoking individuals with an elevated waist circumference (equal to or greater than 80 cm in females and 94 cm in males) were enrolled in the study (Table 1).

None of the individuals presented any subjective feeling of any disease (particularly any cardiovascular disorder), nor had they been previously diagnosed with any component disease of the metabolic syndrome. Furthermore, none admitted to drug treatment for any acute or chronic condition.

All of the patients were screened for arterial hypertension according to the following criteria: 1) systolic blood pressure equal to or higher than 140 mm Hg; and/or 2) diastolic blood pressure equal to or higher than 90 mm Hg. Ambulatory blood pressure measurements were repeated at least twice on different days. The final diagnosis of arterial hypertension was confirmed by the results of blood pressure self-monitoring at home. The patients were also screened for other component diseases of the metabolic syndrome on the basis of an assessment of triglycerides, HDL-cholesterol and fasting glucose serum levels.

Hsp-90 α concentrations were evaluated by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's procedure (Stressgen, Hsp-90 α ELISA Kit). All samples were analyzed in duplicate.

All of the patients understood the general nature of the study and gave their written informed consent for participation in the project. The research was conducted according to the guidelines set forth in the Declaration of Helsinki. All procedures involving patients were approved by a local Research Ethics Committee (KE-0254/110/2007).

Statistical Analysis

The values for the analyzed parameters were presented as arithmetic mean, standard deviation, minimal and maximal values, lower and upper quartiles, and median. The Shapiro-Wilk test was used to assess the distribution conformity of the parameters with a normal distribution, the Mann-Whitney *U*-test to compare the type of distribution and variance homogeneity between two groups, and Spearman's rank correlation coefficient test to assess whether two parameters were correlated.

A p value of less than 0.05 was considered statistically significant. The data was analyzed using STATISTICA 8.0 software (StatSoft, the USA).

Results

Arterial Hypertension and Metabolic Syndrome

Among all the patients enrolled in the study, 28 individuals (57.1% of the total 49; 23 females and 5 males) were diagnosed with arterial hypertension, while 21 (42.9% of 49; 17 females and 4 males) had normal blood pressure. A detailed characterization of the two groups is presented in Table 2.

In the group of patients with arterial hypertension (n = 28), 19 (67.9%) were diagnosed with the metabolic syndrome. In the group of subjects with normal blood pressure (n = 21), the metabolic syndrome was present in 6 patients (28.6%). In total, 25 individuals (51%) among all those examined met the metabolic syndrome diagnostic criteria. The distribution of other components

of the metabolic syndrome (triglycerides, HDL-cholesterol or fasting glucose serum levels) in the groups of patients with arterial hypertension or normal blood pressure is presented in Table 3.

Hsp-90α Concentration

The concentration of Hsp-90α was significantly higher ($Z = 3.02$, $p = 0.002$) in the group of patients with arterial hypertension than in the group of individuals with normal blood pressure (control): $19.42 \text{ ng/mL} \pm 5.17$ vs. $16.86 \text{ ng/mL} \pm 3.18$ (Fig. 1).

The concentration of Hsp-90α was significantly higher ($Z = -2.47$, $p = 0.01$) in the group of patients with the metabolic syndrome than in the group of individuals not diagnosed with this pathology ($18.98 \text{ ng/mL} \pm 5.6$ vs. $17.26 \text{ ng/mL} \pm 3.43$) (Fig. 2).

The concentrations of Hsp-90α correlated positively with systolic blood pressure ($R = 0.39$; $p = 0.005$), diastolic blood pressure ($R = 0.29$; $p = 0.046$) and mean arterial pressure ($R = 0.34$; $p = 0.017$), and negatively with HDL-cholesterol serum levels ($R = -0.31$; $p = 0.029$).

Table 1. Basic characteristics of patients (n = 49, 40 females and 9 males)

Parameter	Mean	SD	Median	Lower quartile	Upper quartile	Min-Max
Age (years)	55.6	6.8	54	52	59	41–72
Waist circumference (cm)	106.1	14.1	105	95	117	80–130
Body weight (kg)	91.2	15.2	86	79	105	62–130
BMI (kg/m ²)	33.5	4.3	32.6	30.6	36.7	26.7–43.9

BMI – body mass index; SD – standard deviation; min-max – minimal-maximal value.

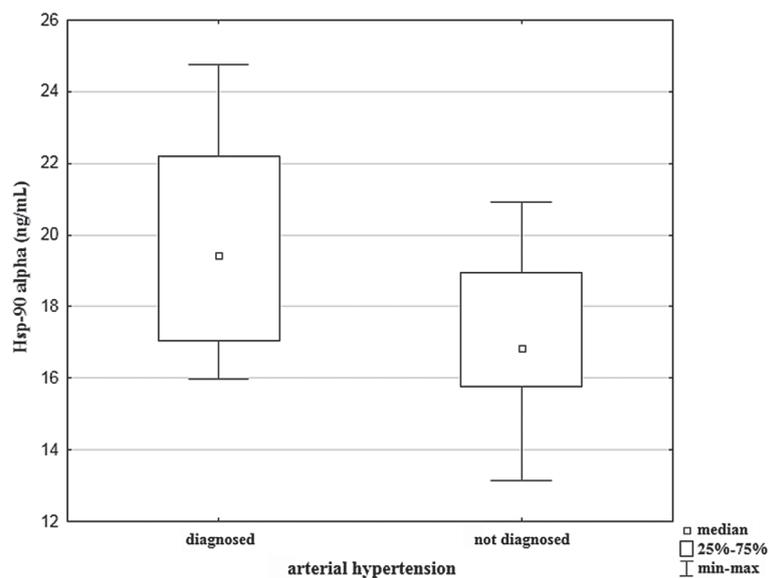
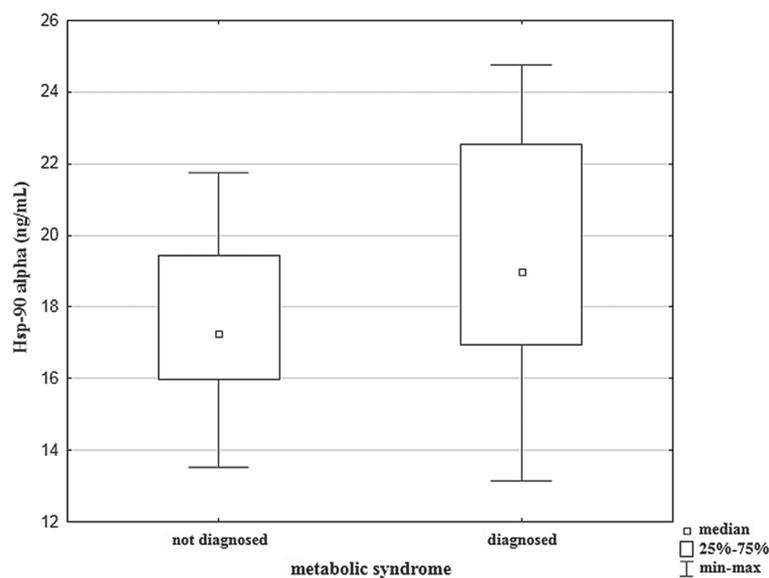
Table 2. Characteristics of subjects with arterial hypertension or normal blood pressure (control)

Parameter	Arterial hypertension (n = 28)	Control group (n = 21)	Mann-Whitney U-test
Age (years)	53.5 ± 7.5	57 ± 8	p = 0.37
Body weight (kg)	86.5 ± 27.5	86 ± 23	p = 0.82
BMI (kg/m ²)	32.6 ± 7.1	32.6 ± 5.3	p = 0.82
Waist circumference (cm)	103.5 ± 24	105 ± 22	p = 0.88
Systolic pressure (mm Hg)*	145 ± 10	125 ± 15	p < 0.001
Diastolic pressure (mm Hg)*	90 ± 5	75 ± 5	p < 0.001
Mean arterial pressure (mm Hg)*	108.3 ± 5	91.7 ± 8.3	p < 0.001
HDL-cholesterol (mg/dL)*	48 ± 16	69 ± 25	p = 0.003
Triglycerides (mg/dL)*	154 ± 35	117 ± 33	p = 0.01
Fasting glucose (mg/dL)	96 ± 25.5	90 ± 10	p = 0.12

* Statistically significant difference ($p < 0.05$); data is presented as median ± interquartile range.

Table 3. Distribution of metabolic syndrome components in relation to arterial hypertension diagnosis

Parameter		Arterial hypertension diagnosed	Arterial hypertension not diagnosed
		no. of patients	
HDL-cholesterol (mg/dL)	< 50 mg/dL (females) < 40 mg/dL (males)	14	6
	≥ 50 mg/dL (females) ≥ 40 mg/dL (males)	14	15
		total 28	total 21
Triglycerides (mg/dL)	≥ 150 mg/dL	16	5
	< 150 mg/dL	12	16
		total 28	total 21
Fasting glucose (mg/dL)	≥ 100 mg/dL	12	4
	< 100 mg/dL	16	17
		total 28	total 21

**Fig. 1.** Concentration of Hsp-90α in the groups of patients with arterial hypertension or normal blood pressure**Fig. 2.** Hsp-90α concentrations in relation to the presence of the metabolic syndrome

There was no significant correlation between the concentration of Hsp-90 α and age, waist circumference, body weight, BMI, triglycerides, or fasting glucose levels. No significant difference was noted in the concentration of Hsp-90 α between premenopausal ($n = 19$) and postmenopausal ($n = 21$) females.

Discussion

Our study indicates a positive correlation between serum Hsp-90 α concentration and arterial blood pressure, which may implicate Hsp-90 α in the pathogenesis of arterial hypertension. The potential role of Hsp-90 α in the development of arterial hypertension remains unclear, although several studies have been undertaken in an attempt to elucidate this relationship [16–22].

In an animal model of spontaneously hypertensive rats, Hsp-90 expression was higher in the left ventricles in comparison to control animals with normal blood pressure. Interestingly, all the isoforms of NOS were initially down-regulated, but expression was ultimately substantially increased in the left ventricles of spontaneously hypertensive rats. The authors concluded that changes in the expression of NOS isoforms and Hsp-90 may constitute a compensatory mechanism maintaining the formation of bioactive nitric oxide. It has also been suggested that variations in the expression of NOS isoforms are linked to the duration of hypertension [16].

In 2012, Oyama et al. reported that repetitive hyperthermia attenuates progression of cardiac hypertrophy and delays the transition from hypertensive cardiomyopathy to heart failure in hypertensive rats. This phenomenon resulted from both induction of heat shock proteins (Hsp-90, Hsp-60 and Hsp-70) and activation of eNOS [17].

Overexpression of Hsp-90 has also been described in cerebral microvessels in an animal model of rats with spontaneous hypertension [18].

Data regarding enhanced Hsp-90 expression in the mesenteric nerves of portal hypertensive rats have been presented as well [19]. Hsp-90 has been shown to mediate the increased nitrergic vasorelaxation observed in portal hypertension – a pathology resulting from decreased splanchnic vascular resistance, an initiator of the development of hyperdynamic circulatory syndrome.

In 2013, Hassanpour et al. reported that Hsp-90 and Hsp-60 gene expression is up-regulated in the hearts of chickens that develop pulmonary hypertension syndrome. It has been suggested that this phenomenon may be an early compensatory mechanism for a pathological process. Surprisingly,

hearts of chickens with fully developed pulmonary hypertension syndrome presented considerable reductions in Hsp-90, Hsp-60, and Hsp-70, which has provided evidence for a loss of compensatory responsiveness in the dilated heart. Variations in Hsp-90 expression during the course of pulmonary hypertension confirm the chaperone's significant role in the pathogenesis of the disease [20].

In our study, increased Hsp-90 α serum concentration is correlated with an increase in the magnitude of arterial blood pressure. Moreover, Hsp-90 α concentrations are higher in overweight or obese patients with newly diagnosed arterial hypertension than in overweight or obese patients with normal blood pressure. These observations are concordant with previous findings of increased Hsp-90 expression in animal models of hypertension [16–20]. It should be emphasized that there is as yet no data on Hsp-90 α expression in human subjects with spontaneous hypertension. The only available human study reported that Hsp-90 overexpression can be observed in the renal tubular cells of patients with hypertension-induced renal failure [21]. However, in the cited research, the elevation of Hsp-90 expression might have resulted from a number of other disturbances associated with chronic renal failure, and therefore the conclusion that increased Hsp-90 expression results from arterial hypertension seems premature and not fully convincing.

Another noteworthy study, conducted by Tiss et al., demonstrated that obesity triggers a change in the heat shock response pattern of expression at protein and mRNA levels [22]. Obese adult non-diabetic normotensive human subjects displayed significantly increased expression of Hsp-60, Hsp-72 and Hsp-90 in comparison to their lean counterparts. The heat shock response expression pattern was concomitant with enhanced inflammatory response in the adipose tissue. Three-month moderate physical exercise reduced the heat shock protein expression in the obese individuals to the normal levels observed in lean subjects, with a parallel reduction in the inflammatory response [22].

The significant increase in Hsp-90 α concentration observed in our study in the group of overweight or obese patients with newly diagnosed arterial hypertension is probably an early compensatory mechanism for the disease process. The findings suggest a protective role of Hsp-90 α in early endothelial dysfunction leading to the development of arterial hypertension. Increased expression of Hsp-90 α compensates for the impaired function of endothelial NOS (eNOS) associated with the reduced bioavailability of nitric oxide. Therefore, a potential role of Hsp-90 α as an early marker of endothelial injury associated with hypertension should be considered and assessed in

further studies conducted on a larger group of patients, focusing on Hsp-90 α expression at protein and mRNA levels.

Finally, it should also be mentioned that an additional goal of our study was to screen for arterial hypertension and other component diseases of the metabolic syndrome in overweight or obese patients. It must be emphasized that none of the subjects enrolled in the study presented any subjective feeling of any disease (particularly any cardiovascular disorder). Therefore, diagnosis of the metabolic syndrome in more than half of the 49 enrolled individuals indicates an urgent social need for broad and active screening for all of the component diseases of this dangerous and insidious pathology.

The authors concluded that a significant increase in Hsp-90 α concentration in the group of overweight or obese patients with newly diagnosed arterial hypertension may be an early compensatory mechanism for the disease. The findings also suggest a protective role of Hsp-90 α in early endothelial dysfunction leading to the development of arterial hypertension. Therefore, a potential role of Hsp-90 α as an early marker of endothelial injury associated with hypertension should be considered. The metabolic disturbances diagnosed in most of the patients examined, who had presented no subjective feeling of any disease prior to the study, indicate an urgent social need for wide and active screening for the metabolic syndrome.

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