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CLINICAL STUDY

HSP60, oxidative stress parameters and cardiometabolic risk markers in hypertensive and normotensive Slovak females

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Abstract: *Background:* The aim of our study was to analyse the relationships between hypertension, HSP60, oxidative stress, lipid profile and cardiometabolic risk in 126 females with arterial hypertension (AH^+) and 39 normotensive females (AH^-).
Results: Females with AH^+ were significantly older and more frequently suffered from ischemic heart disease, angina pectoris, prior MI, abdominal obesity, obesity, metabolic syndrome and diabetes mellitus. On the other hand, normotensive females smoked significantly more often.
Plasma levels of HSP60 were similar in both AH^+ and AH^- groups. However, hypertensive females exhibited almost two times lower values of oxidative glutation and lower levels of carbonyl protein, but significantly higher levels of homocysteine. In normotensive females, the total glutathione was the only parameter predicting females with the plasma level of HSP60 = 60 ng/ml. The independent predictors in hypertensive females were angina pectoris, triglycerides and the mean arterial pressure (MAP). MAP had also a borderline significance in normotensive females suggesting an association between HSP60 and blood pressure. MAP formed a J shaped curve with HSP60.
Conclusion: Results suggest the association of blood pressure and heart shock protein 60 Kda in form of the J curve *(Tab. 11, Fig. 3, Ref. 29).* Full Text in free PDF *www.bmj.sk.*
Key words: atherosclerosis, HSP60, hypertension, mean arterial pressure, metabolic syndrome, oxidative stress.

Heat shock proteins (HSPs) belong to endogenous danger signals released by stressed cells called alarmins and function in cell protection mainly as molecular chaperones. They allow cells to adapt to gradual changes in their environment and help them to limit the damage caused by stress. Thus they facilitate cellular recovery and help to survive in otherwise lethal conditions (1, 2).

The role of heat shock proteins (HSPs) in the etiopathogenesis of cardiovascular diseases is unclear despite the two decades of an intensive research. Individual HSPs subtypes differ by various effects and several clinical studies reported contrast results (3, 4). Human HSP60 has been studied in detail in patients with atherosclerosis and related clinical complications. HSP60 significantly supports the inflammatory process of atherosclerosis (5), associates with markers of inflammation as well as of oxidative stress (6, 7), predicts not only the early symptoms of coronary heart diseases (8) and heart failure, but also the late serious clinical events such as myocardial infarction (1) or chronic heart failure (9). Equally important is the association between the increased plasma levels of HSP60 and the markers of an increased psycho-emotional load (10).

The relation of HSP and arterial hypertension is more complex and involve atherosclerotic process as the mechanism participating in progression of arterial hypertension (10) or HSP could influence the blood pressure due to its upregulation of RT1 gene expression, which contribute to the development of hypertension (11). Zhang et al (12) recently described a strong synergic effect between the concentration of HSP60 and hypertension and the risk of development of ischemic heart disease and even emphasise their combination as a detrimental risk of IHD. On the other hand, Jastrzebski (13) did not find a relation between the end-organ damage and plasma levels of HSP60 in patients with arterial hypertension. Intersexual differences in HSPs were not studied and the most cited studies recruited significantly less females, who are still incorrectly underestimated for the risk of atherosclerotic process and its clinical complications.

The main goal of our study was to analyse the relationship between the blood pressure, or arterial hypertension, HSP60, oxidative stress, lipid profile and the cardiometabolic risk in female population.

Materials and methods

We randomly selected both male and female Caucasian patients aged between 35–75 years with known IHD from mixed

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Tab. 1. Recruitment process in individual districts.

Nové Zámky, Bratislava		Veľký Lom	
	No. subjects		No. subjects
Registry	6228	Inhabitants	325
Age 35-75 yrs	3627	Age 35-75 yrs	223
Selected	700		
Randomised	350		
Examined	247	Examined	186
Females	99	Females	66
Hypertensive	85	Hypertensive	41
Normotensive	14	Nornotensive	25

urban/rural district of Nové Zámky, city of Bratislava and invited adult residents from the village of Velký Lom and surrounding area (district Velký Krtíš) aged between 35–75 years. The age range was chosen to examine patients in the age of expected high risk of cardiovascular events. Patients suffering from megaloblastic anemia, malabsorption or inflammatory bowel disease, serum positive arthritis, drug addiction, toxicomania and alcoholism (Jelinek IV), chronic renal insufficiency stage III and IV according to Sarré, extensive trauma less than 3 months before the study, maniodepressive psychosis, gastric surgery, immobility, acute MI or coronary intervention less than 3 month before study, and on cancer chemotherapy or radiotherapy less than 3 months before study, vegan diet, on polyvitamine substitution, were excluded from the study.

IHD patients were selected from the registry of two district cardiologists in Nové Zámky and one in Bratislava. Arterial hypertension was defined as blood pressure = 140/90 mmHg or lower blood pressure on antihypertensive therapy. IHD was defined as MI requiring hospitalization and/or typical angina pectoris (AP) or proven and treated silent IHD documented by medical report and ECG signs of ischemia. Metabolic syndrome was defined according to the ATP III criteria (14). Height, weight, waist and hip circumferences were measured according to standard protocols. The recruitment process according to the inclusion and exclusion criteria in individual districts is shown in Table 1.

Finally we studied 126 females with arterial hypertension and 39 normotensive controls. The selected patients and controls had a brief medical examination and were interviewed by a physician using a standardized questionnaire. The questionnaire recorded personal and family history, nutritional habits assessing the basic pattern of meat, vegetable, fruit and salt intake, smoking status (current, former smoker, never smoked), physical activity, and the evaluation of risk factors. The IHD part of the questionnaire was based on the Rose´s Protocol for Angina Pectoris (15)**.** Blood pressure was measured in a separate quiet room after a 5 minute rest in sitting position three times on the right arm using a standard mercury sphygmomanometer. Only the last two measurements were recorded for the study.

Venous blood samples were collected in EDTA-Potassium tubes to obtain plasma and in standard tubes to obtain serum after an overnight fasting without cubital compression. Blood for plasma was immediately centrifuged in a cooled centrifuge (4 °C) for 30 minutes; blood for serum was allowed to clot for 15 minutes at room temperature at 22–25 °C and underwent the same centrifugation procedure. EDTA vacutainers for homocysteine measurement were kept on ice and centrifuged within 15 minutes after the drawing of blood in a cooled centrifuge (3000 rpm/30 min/4 °C). Plasma levels of total cholesterol (TC) and triglycerides (TG) were measured enzymatically, ApoB and Apo-AI levels were measured by an immunoturbidimetric method, HDL cholesterol (HDL-C) was determined directly by commercial kit (Genzyme) on the autoanalyser (Hitachi 911, Roche, Switzerland). LDL-C was evaluated by a direct method for LDL-C measurement (Randox, UK) and calculated using the Friedewald formula only if the triglyceride concentration was below 4.5 mmol/l. Basic laboratory screening was performed using standard methods. Homocysteine analysis was performed by high-performance liquid chromatography (HPLC) using a standard kit (CHROMSYSTEMS Instruments & Chemicals GmbH, Munich). Vitamin B_6 levels in serum were measured using the HPLC method (CHROMSYSTEMS Instruments & Chemicals GmbH, Munich, Germany), serum vitamin B12 and folic acid levels by ELISA (Roche Diagnostics Corp., USA). The blood cell count and urine analysis was determined by standard methods.

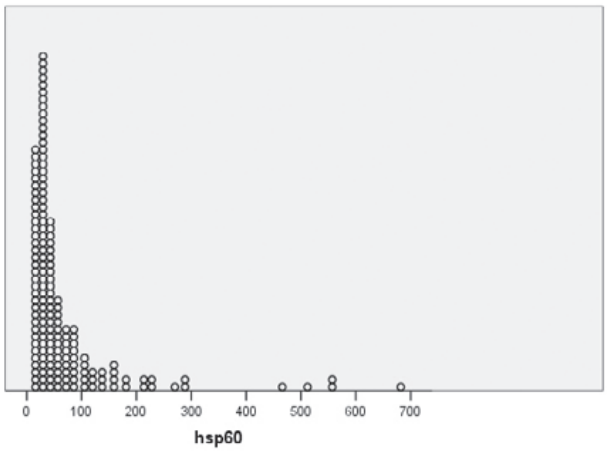
The concentration of uric acid was measured spectrophotometrically at 456 nm (Hitachi 911, Roche, Switzerland), plasmatic total antioxidant status (TAS) was stated colorimetrically using the original kit for TAS (Randox, Cat no NX 2332, Randox Laboratories, Crumlich, UK). Blood for total glutathione (tGSH) and oxidized glutathione (GSSG) measurement was deproteinized by 10 % sulfosalicylic acid (SSA) (400 µl 10 % SSA, 750 µl blood), centrifuged at 4 °C at 10 000 rpm for 12 minutes and the supernatant was stored in cryovials at -70 °C until spectrophotometrical analysis at 410 nm using microplate reader (TECAN Spectra Fluor, Austria) in kinetic-type reaction (16). The Schiff base substance was measured fluorometrically. All measurements done for this study were performed in certified laboratories.

The study was approved by the Ethics Committee of the Faculty of Medicine Comenius University in Bratislava and all subjects signed an informed consent.

Statistical analysis

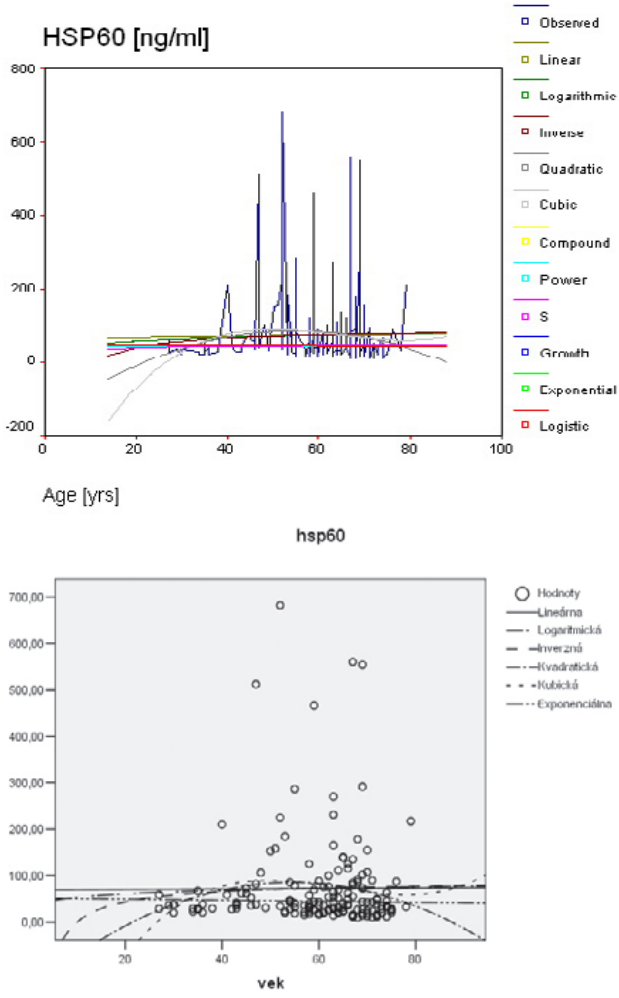
All data from questionnaires and the results of the laboratory tests were input into databases and underwent a three step control.

Data were analyzed using the SPSS 10.0 for Windows. The independent samples t-tests and chi-square analyses were used to compare participants recruited from the three sites on demographic variables. No significant differences were revealed between the participants recruited from the three sites, therefore these participants were used to form one group of patients with arterial hypertension for the purposes of all analyses. Normal distributions of parametric variables were examined by using the



Abbreviations: HSP60 – the level of heat shock protein given in ng/ml

Fig. 1. Histogram of distribution of the HSP60 plasma level in whole group.



Abbreviations: HSP60 – the level of heat shock protein given in ng/ml

Fig. 2. Displaing curve estimation of various mathematical models between age as independ variable and HSP60 as dependent variable.

one-sample Kolmogorov-Smirnova test. Homogenously distributed data were compared using the independent 2-samples Student t-test. Heterogeneously distributed data were either analysed after a log-transformation or they were compared using the non-parametrical Mann-Whitney U test for the two independent groups using the Willcox or McNemar test for two dependent groups. A post hoc analysis by Dunnet or Duncan test was performed, when applicable.

Binomial data were analyzed using chi-square tests to compare differences between AH^+ and AH^- control groups. Odds ratio was calculated to estimate the risk of various clinical conditions for the presence of an increased plasma level of HSP60. A correlation between the plasma level of HSP60 and individual clinical or laboratory parameters was tested using the two-tailed bivariate correlation according to the Pearson or Spearman, considering the type of analysed data – categoric, interval or binomial variables. The relationships between the plasma level of HSP60 and other clinical or laboratory parameters were tested by a multivariable linear correlation analysis for HSP60 as categorical variable or by a hierarchic binomial regression for HSP60 given as binomial parameter.

The Cox´s analysis and the Kaplan-Meier curves for the proportion of subject in the risk for an increased HSP60 were used to estimate the effect of age on plasma concentration of HSP60.

The statistical analysis and presentation was performed according to the standard recommendation (17–21). Statistical significance was considered at the level of $p < 0.05$ with power = 0.80.

Results

Statistical characteristics

Possible sources of biases

There is a significant difference in the number of participants in the group of AH^+ females and normotensive controls (126 vs 39 subjects). An asymmetric proportion of groups caused an absence of statistical significance in several analyses, in which a clear between-group difference (even about 50 % of mean or median values) was observed.

To demonstrate a trend of statistical analysis we reported an actual P value (or other corresponding estimates) and not only the statement "statistically significant" $p < 0.01$ or $p < 0.001$). Another important source of bias is the non-gaussian distribution of HSP60 values with significant skewness to the right (Fig. 2). The standard deviation exceeded 150 % of the mean value. An

Tab. 2. Critical cut off points for HSP60 quartiles.

Quartile	Range [ng/ml]
I	0 – 25.85
II	25.86 – 37.10
III	37.11 – 74.20
IV	74.21 – maximal

Abbreviations: HSP60 – the level of heat shock protein given in ng/ml

Tab. 3. Estimation curve of various mathematical models between the age as independent variable and HSP60 as dependent variable.

Equation	Model summary					Parameter estimates			
	R Square	F	df1	df2	Sign.	Constant	b1	b2	b3
Linear	0.000	0.007	1	163	0.93	69.05	0.06		
Logarithmic	0.001	0.097	1	163	0.76	28.53	10.80		
Inverse	0.002	0.293	1	163	0.59	88.20	-892.77		
Quadratic	0.016	1.284	2	162	0.28	-148.43	8.58	-0.08	
Cubic	0.019	1.019	3	161	0.39	-498.81	30.16	-0.50	0.003
Exponential	0.001	0.170	1	163	0.68	51.81	-0.002		

Legend: R squared – correlation between parameters; F – value of F– score; df1 and df2 – degree of freedom; Sign. – statistical significance

Tab. 4. Basic clinical and risk characteristics.

Number	126	39	
Age [years]	62.8 ± 8.8	48.8 ± 13.6	0.001
BMI	31.1 ± 5.4	25.9 ± 5.1	0.001
IHD	110 (87.3%)	16 (41.0%)	0.001
AP	68 (50.0%)	11 (28.2%)	0.018
MI	24 (19.0%)	2 (5.1%)	0.043
MS	75 (59.5%)	4 (10.3%)	0.001
DM	31 (24.6%)	4 (10.3%)	0.071
Smokers	10 (7.9%)	11 (28.2%)	0.002
Abdominal obesity	117 (92.9%)	21 (53.8%)	0.001
BMI = 30	69 (54.8%)	7 (17.9%)	0.001
Menopausa	17 (43.6%)	21 (53.8%)	0.001

Abbreviations: AH^+ – hypertensive females; AH^- – normotensive females; BMI – body mass index; IHD – ischemic heart disease; AP – angina pectoris; MI – myocardial infarction; AH – arterial hypertension; MS – metabolic syndrom; DM – diabetes mellitus

Tab. 5. Heamatological parameters in hypertensive and normotensive females.

Number	AH^+ 126	AH^- 39	Sign.
WBC [$*10^9$/l]	6.49 ± 1.84	7.11 ± 1.70	0.06
RBC [$*10^{12}$/l]	4.68 ± 0.36	4.62 ± 0.37	0.43
HGB [g/dl]	138.6 ± 11.1	139.3 ± 10.9	0.74
HCT [%]	0.42 ± 0.04	0.42 ± 0.03	0.88
MCV [fl]	88.7 ± 4.27	89.9 ± 3.14	0.10
PLT [$*10^9$/l]	250.2 ± 63.8	272.0 ± 64.3	0.064

Abbreviations: AH^+ – hypertensive females; AH^- – normotensive females; WBC – leucocyte count; RBC – erythrocyte count; HGB – hemoglobin concentration; HCT – hematocrit; MCV – mean corpuscular volume of erythrocyte; PLT – platelet count

Tab. 6. Biochemical parameters in hypertensive and normotensive females.

Number	*AH*$^+$ 126	AH^- 39	Sign.
Bi [mmol[l]	13.1 ± 4.17	12.3 ± 4.96	0.32
Glycaemia [mmol/l]	6.63 (3.84–17.63)	5.83 (4.08–13.87)	0.009
GMT [µmo/l]	0.44 (0.11–10.3)	0.31 (0.11–1.9)	0.072
ALP [µmo/l]	3.36 ± 1.00	2.73 ± 0.84	0.001
AST [µkat/l]	0.38 ± 0.17	0.34 (0.17–0.82)	0.006
ALT [µkat/l]	0.28 ± 0.18	0.20 (0.05–0.56)	0.16
Creatinine [µmo/l]	86.5 ± 12.8	81.6 ± 11.2	0.035
UA [µmo/l]	305.0 ± 82.5	251.5 ± 70.4	0.001

Abbreviations: AH^+ – hypertensive females; AH^- – normotensive females; Bi – total bilirubine; GMT – gamagluthamyltranspherase; ALP – alcalic phosphatase; AST – aspartateaminotranspherase; ALT – alaninaminotranspherase; UA – uric acid

Tab. 7. Lipid spectrum in hypertensive and normotensive females.

No	AH^+ 126	AH^- 39	Sign.
TCH [mmol/l]	6.17 ± 1.15	5.86 ± 1.12	0.15
TG [mmol/l]	1.62 ± 0.83	1.26 (0.53–3.17)	0.004
HDL-C [mmol/l]	1.37 ± 0.32	1.42 ± 0.24	0.33
LDL-C [mmol/l]	4.09 ± 1.01	3.88 (2.30–6.70)	0.18
Apo-A1 [g/l]	1.17 ± 0.16	1.17 ± 0.12	0.99
Apo-B [g/l]	1.05 ± 0.20	0.97 ± 0.21	0.025

Abbreviations: AH^+ – hypertensive females; AH^- – normotensive females; TCH – total cholesterol; TG – triglycerides; HDL-C – HDL cholesterol; LDL-C – LDL cholesterol; Apo-A1 – apolipoproteine A1; Apo-B – apolipoproteine B

adjustment of the non-gaussian distribution by a log-transformation improved the skewness in all groups; however, a slight skewness persisted in the subgroup analysis.

The non-gaussian distribution of HSP60 values determined a non-parametric method in most of the analyses, or it determined an adjustment of data using the quartiles of HSP60. The critical cut--off points for individual HSP60 quartiles are presented in Table 2.

An equally important source of statistical bias is the fact that increased skewness decrease the robustness of linear correlation, which is the base for the correlation methods. Table 3 and Figure 1 present an estimation curve for different mathematical models between the age and the plasma level of HSP60. Although the age could be a significant source of bias, the Table 3 documents a lack of correlation between HSP60 and age. A linear regression did not correlate at all, and the quadratic and cubic models, which have shown the highest correlation coefficient, did not reach a statistical significance. A logarithmic model was also non-significant, which may explain a decreased effectiveness of log-transformation of HSP60 values (Fig. 1).

Tab. 8. Oxidative stress parameters and cardiometabolic markers in hypertensive and normotensive females.

No	AH+ 126	AH– 39	Sign.
HSP60 [ng/ml]	73.9 (9.3–682.0)	67.4 (15.9–512.0)	0.54
TAS [umol/l]	1.33 ± 0.19	1.30 (0.91–1.69)	0.52
uCRP [mg/dl]	2.63 (0.00–9.8)	1.87 (0.00–10.9)	0.124
oLDL [mmol/l]	3.60 ± 1.19	3.86 (1.18–6.35)	0.23
CARBO [pg/ml]	63.8 ± 27.4	68.6 (34.0–107.2)	0.014
SBS [ěmol/l]	23.8 ± 5.81	23.0 ± 6.33	0.46
TGSH [umol/l]	1005.9 ± 503.7	1113.9 ± 464.2	0.27
GSSG[umol/l]	19.7 ± 16.8	32.2 (3.62–140.4)	
0.0001			
GSHRATIO	0.02 (0.001–0.172)	0.03 (0.007–0.23)	0.29
Hcy [µmo/l]	12.0 ± 5.52	9.25 (4.47–24.8)	0.001

Abbreviations: AH+ – hypertensive females; AH– – normotensive females; HSP60 – heat shock protein 60 kDa; TAS – total antioxidative status; uCRP – ultrasensitive C-reactive protein; oLDL – oxidised LDL cholesterol; CARBO – carbonyl protein; SBS – Shiff base substance; TGSH – total glutathion; GSSG – oxidised glutathion

Tab. 9. Risk ratio of individual clinical cardiometabolic markers for increased HSP60 ? 80 ng/ml.

No	AH+ 126		39	AH–
IHD	1.48 [0.39–5.58]	0.56	0.55 [0.08–3.06]	0.68
AP	1.84 [0.80–4.20]	0.15	1.02 [0.17–6.26]	0.99
MI	2.76 [1.07–7.07]	0.038	5.17 [0.28–94.5]	0.33
MS	1.59 [0.68–3.75]	0.30	0.80 [0.68–0.94]	0.05
DM	1.68 [0.68–4.10]	0.34	0.80 [0.68–0.94]	0.05
Smokers	0.74 [0.15–3.69]	0.71	2.52 [0.41–12.28]	0.38
Abdominal obesity	0.89 [0.36–2.18]	0.79	1.92 [0.36–10.32]	0.65
BMI = 30	1.42 [0.62–3.26]	0.40	0.72 [0.07–7.17]	0.78
Menopausa	1.39 [1.21–1.50]	0.05	2.50 [0.42–14.82]	0.42

Abbreviations: AH+ – hypertensive females; AH– – normotensive females; IHD – ischemic heart disease; AP – angina pectoris; MI – myocardial infarction; MS – metabolic syndrome; DM – diabetes mellitus; BMI – body mass index

Tab. 10. Independent predictors of increased HSP60 > 60 ng/ml in hypertensive and normotensive females.

	HSP60 > 60 ng/ml AH–		AH+	
Variable	OR [95%CI]	Sign.	OR [95%CI]	Sign.
tGSH	1.002 (1.0–1.003)	0.047		
AP			3.44 (1.15–10.38)	0.028
MAP			1.04 (1.002–1.09)	0.039
TG			3.56 (1.07–11.85)	0.038
UA			0.986 (0.977–0.995)	0.03

Abbreviations: AH+ – hypertensive females; AH– – normotensive females; tGSH – total glutathione; AP – angina pectoris; MAP – mean arterial pressure; TG - triglycerides> 1.70; UA – uric acid

Tab. 11. Variables tested in logistic regression analysis with HSP60 > 60 ng/ml as dependent variable, which did not reach statistical significance.

AH-	Score	Sign.	AH+	Score	Sign.
MI	0.105	0.746		0.530	0.467
IHD	2.692	0.101		0.006	0.938
DM	2.254	0.133		1.121	0.290
MetSY	**2.909**	**0.088**		2.810	**0.094**
BMI	2.208	0.137		0.688	0.407
AP	0.426	0.514		2.508	0.113
MAP	**3.341**	**0.068**		3.172	0.075
TG	0.003	0.955		3.564	0.038
UA	0.328	0.567		5.407	0.020
SBS	0.285	0.593		1.432	0.231
GSSG	2.553	0.110		0.577	0.448
Carbo	2.014	0.156		0.205	0.651
TAS	1.317	0.251		0.000	0.999
Waist88	0.447	0.504		0.138	0.710
ApoA1	0.442	0.506		0.235	0.628
ApoB	0.064	0.800		1.983	0.159
uCRP	0.992	0.319		1.949	0.163
Platelets	0.802	0.371		0.001	0.969
Smoking	**3.405**	**0.065**		1.395	0.238
	22.50				

Abbreviations: AH+ – hypertensive females; AH– – normotensive females; MI – myocardial infarction; IHD – ischemic heart disease; DM – diabetes mellitus; MetSy – metabolic syndrome; BMI – body mass index; AP – angina pectoris; MAP – mean arterial pressure; TG – triglycerides > 1.7 mmol/l; UA – uric acid; SBS – Shiff base substance; GSSG – oxidised glutathione; CARBO – carbonyl protein; TAS – total antioxidative status; Waist88 – waist circumference > 88 cm; ApoA1 – apolipoprotein A1; ApoB – apolipoprotein B; uCRP – ultrasensitive CRP

Basic clinical and risk characteristics

Study group consisted of 126 females with arterial hypertension (AH^+) and 39 normotensive females (AH^-). Females with AH^+ were significantly older and more frequently suffered from IHD, AP, prior MI, abdominal obesity, obesity (BMI = 30), metabolic syndrome and diabetes mellitus. Hypertensive females were more often postmenopausal. On the other hand, normotensive females smoked significantly more often (Tab. 4).

Safety parameters comprised blood count and basic biochemical screening. Slight differences in blood cell counts were statistically non-significant, but normotensive females had a bordeline higher level of leucocytes and thrombocytes (Tab. 5).

A comparison of basic biochemical characteristics showed several significant differences between the groups, which at least partially reflected a higher age in hypertensive females and more clinical pathologies. Higher glyceamia reflected a higher number of diabetic females and higher creatinine reflected mild impairment of glomerular filtration rate in hypertensive females. AH^+ women had also a significantly higher level of ALP. The level of ALT was also significantly higher; however, the mean value did no exceed the reference range (Tab. 6).

An estimation of lipid spectrum revealed a higher plasma triglycerides and apolipoprotein B in AH^+ females (Tab. 7).

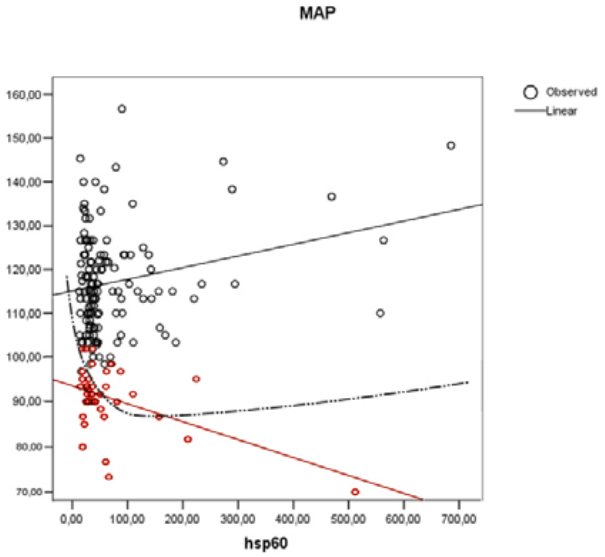


Fig. 3. The correlation between HSP60 and blood pressure in AH^+ and AH^-.

HSP60 and markers of oxidative stress

Plasma levels of HSP60 were similar in both AH^+ and AH^- groups: 73.9 vs 67.4 ng/ml; (p = 0.54). However, hypertensive females exhibited almost two times lower values of oxidative glutation (p = 0.001) and slightly lower levels of carbonyl protein (p = 0.014). Surprisingly, the level of ultrasensitive CRP (uCRP), oxidative LDL cholesterol and Shiff base substance were similar in both groups as well as another marker of oxidative stress – the total antioxidative status.

AH^+ group of patients exhibited significantly higher levels of homocysteine, however, the levels of total glutation, another marker of cardiometabolic risk, did not reach a statistically significant difference (Tab. 8).

Analysing the individual clinical cardiometabolic markers for risk of increased HSP60 = 80 ng/ml (Tab. 9), the only significant parameters were prior MI (OR = 2.76 [CI 95% 1.07–7.07] and menopausa (OR = 1.35 [CI 95% 1.21–1.50]).

Using a binary logistic regression we analysed HSP60 with cut off points = 60 ng/ml as dependent variables, on the association of cardiometabolic risk and oxidative stress markers as independent variables in the group of hypertensive and normotensive females. In normotensive females, dichotomised according to the level of HSP60 = 60 ng/ml, the only statistically significant parameter was total glutathione, however, with a low predictive value. Strong independent predictors in hypertensive females were angina pectoris and the level of triglycerides.

An increased mean arterial pressure (MAP) was weak but significant predictor of an increased HSP60 in hypertensive females (Tab. 9) and had a borderline significance (p=0.068) in normotensive females (Tabs 10–11), suggesting an association between HSP60 and blood pressure.

However, the risk ratio below value 1 suggests an association of increased HSP60 with a decreased mean arterial pressure in AH^-. Performing a linear correlation between the blood pressure and HSP60 in both groups and superimposed both relationships, the final graph formed a J-shaped curve between the mean arterial pressure and the level of HSP60 with an indirect correlation in normotensive females and a direct correlation in hypertensive females (Fig. 3).

On the other hand, an increased plasma level of uric acid predicted lower levels of HSP60. (Tab. 10). There is a trend for the association of HSP60 > 60 ng/ml with metabolic syndrome (p=0.088) as well as smoking (p=0.065) as the major risk factors (Tab. 11).

Discussion

Endothelial cells in the vessel wall can be damaged not only by classic risk factors, but also by (auto)immune reactions to antigens present in the cell surface, e.g heat shock protein 60 (22). HSP60 normally located in mitochondria can be translocated into the cell membrane in response to stress stimuli. HSPs are released by stressed or injured cells but also by activated monocytes and macrophages (23). HSPs in host can also be derived by microorganisma during infection (24). Some persistent infections may cause immune responses that promote atherogenesis. Cellular immunity (Th1) against Helicobacter pylori derived HSP60 cross reacts with endogeneous HSP60 to cause cardiovascular disease likely by molecular mimicry (24).

Atherosclerosis and its complications represent the leading cause of mortality and morbidity and the role of HSPs in atherosclerosis is controversial. HSP60 probably acts as autoantigen and may trigger both the cell and antibody mediated immune response (25). High levels of circulating HSP60 and antibody to human HSP60 have been associated with a higher risk of coronary heart disease and acute myocardial infarction induces HSP60 release (26). Elevated levels of HSP60 were also found in plasma of patients with heart failure, disease accompanied by chronic low grade inflammation and tissue injury. Increased levels of Hsp60 were probably driven by NFkappaB activation (27).

Experimental studies in previous decades have also proved a direct participation of abnormal production of HSP in the etiopathogenesis of hypertension. Hamet et al (11) demonstrated a higher transcription rate of HSP70 gene in spontaneously hypertensive rats detecting increased accumulation of messenger RNA for HSP70. HSP70 gene is located in the area of histocompability complex RT1 gene, which may participate alone or in cooperation with other genes on expression of hypertension. The whole mechanism is regulated by enviromental factors, what may explain individual propagation of hypertension in the presence of given gene(s).

Ellins et al (28) found an independent association between the high plasma levels of HSP60 and increased carotid stiffness. They also found that HSP60 is a potent activator of vascular endothelial and smooth muscle cells and they concluded that long-term stimulation of these cells by HSP60 might drive blood vessel to changes resulting in decreased arterial elasticity. Pockley

and all (29) followed the dynamics of atherosclerosis defined as changes in the maximum intima-media thickness (maxIMT) of common carotide artery in arterial hypertension patients recruited into ELSA study (European Lacidipine Study on Atherosclerosis) for four years. They found a decreased progression of atherosclerosis in patients belonging to the highest quartile of HSP70. A protective effect of HSP70 reached 0R=0.42, and similar non-significant trend was observed for HSP60.

In the robust HOPE study, anti- HSP60 and HSP65 antibodies were analysed in high cardiovascular risk patients with arterial hypertension. HSP60 failed to identify patients suffered by MI, stroke or death due to cardiovascular events during 5 year follow-up. On the other side, HSP65 alone or in association with increased fibrinogene level or increased cytomegalovirus antibodies represented a significantly increased risk for the cardiovascular events. Despite of clear correlation with clinical events, neither the HSP65 levels nor the less predictive HSP60 levels correlated with inflammatory or infectious markers (4).

Our data showed a weak but significant correlation of HSP60 plasma levels with blood pressure. The increased mean arterial pressure mildly predicted risk for elevated HSP60. However, angina pectoris, a clinical parameter reflecting coronary atherosclerosis, had a substantially higher predictive value for elevated HSP60 in comparison to the mean arterial pressure (OR 3.54 vs 1.04) in hypertensive females.

The analysis of the relationship between HSP60 and inflammatory parameters (number of leucocytes, thrombocytes, and level of uCRP) did not find any correlation in accordance with other studies (13). Jastrzebski et al (13) found increased inflammatory markers in hypertensives with a target organ damage, but not in uncomplicated hypertension. Similarly to our finding, there were no differences between groups.

In the testing of oxidative stress markers only two of variables – uric acid and Shiff base substance exhibited a significant correlation with HSP60 (rho = -0.21; p = 0.020, resp. rho = –0.21; p = 0.016). Other markers, oxidative LDL cholesterol, carbonyl protein, oxidised glutathione, total antioxidative status was not associated with HSP60.

Conclusion

Significant differences were found in oxidative glutathione and carbonyl protein as markers of oxidative stress between hypertensive and normotensive females, but not in the heat shock protein 60 kDa. In normotensive females, the total glutatione was the only significant parameter predicting females with the plasma level HSP60 = 60 ng/ml. Significant independent predictors in hypertensive females were angina pectoris, level of triglycerides and the mean arterial pressure (MAP). The MAP formed a J shaped curve with HSP60. This is the first report suggesting an association between the blood pressure and heart shock protein 60 Kda.

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