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Predictors of Coronary Atherosclerosis: HSP70, Markers of Oxidative Stress and Endothelial Dysfunction

Julia A. Kotova, PhD*; Anna A. Zuikova, PhD, ScD; Aleksander N Pashkov, PhD, ScD; Natalia V. Strahova, PhD; Olga N. Krasnorutskaya, PhD

Voronezh State Medical University named after N.N. Burdenko
Voronezh, Russian Federation

Abstract

The aim of this study was to evaluate the role of HSP70, and markers of oxidative stress and endothelial dysfunction, as determinants of the severity of coronary atherosclerosis. The study revealed significant differences between patient groups with and without coronary atherosclerosis in terms of HSP70, superoxide dismutase, total homocysteine (tHcy) and markers of oxidative modification of proteins. Significant correlations between Gensini score, lipid profile parameters and studied markers were determined. The results of multiple linear regression analysis allow us to consider the levels of HSP70, tHcy, LDL-C and ketone derivative of 2.4-dinitrophenylhydrazine as factors associated with the risk of coronary atherosclerosis.(**International Journal of Biomedicine. 2019;9(2):97-101.**)

Key Words: coronary atherosclerosis • heat shock protein 70 • superoxide dismutase • homocysteine

Abbreviations

ADPH, aldehyde derivative of DNPH; **CHD**, coronary heart disease; **CAG**, coronary angiography; **CA**, coronary atherosclerosis; **DNPH**, 2.4-dinitrophenylhydrazine; **ED**, endothelial dysfunction; **GS**, Gensini score; **HSP**s, Heat shock proteins; **HSP70**, heat shock protein 70; **HDL-C**, high-density lipoprotein cholesterol; **KDPH**, ketone derivative of DNPH; **LDL-C**, low-density lipoprotein cholesterol; **MLRA**, multiple linear regression analysis; **OS**, oxidative stress; **OMP**, oxidative modification of proteins; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase; **TC**, total cholesterol; **TG**, triglycerides; **tHcy**, total homocysteine.

Introduction

Coronary artery disease (CAD) is the leading cause of death and disability worldwide.[1,2] Atherosclerosis, which is the primary pathophysiologic mechanism for the development of plaque leading to CAD, is a multifactorial and multifaceted process. In this process, fundamental roles for inflammation and OS have been established.[3-6] In CHD, a decrease in intracellular protection against reactive oxygen species, primarily due to a decrease in the level of SOD—the key enzyme of the antioxidant system—has been demonstrated by a number of researchers.[7] The imbalance between pro-oxidants and antioxidants leads to oxidative damage of proteins—an early indicator of the cell damage,[8,9] including the endothelium.In contrast to their inherent harms, ROS also function as signaling molecules, inducing stress tolerance mechanisms. OS can be responsible for the increased expression of HSPs. HSPs have been reported to work together with the antioxidant system to inhibit or neutralize the cellular effects of ROS.[10-12] OS is one of the most important factors that produce ED. ED contributes to atherogenesis at every phase of atherosclerosis.[13] In addition, Hcy is an established biomarker for ED and vascular disease, and is linked to increased OS.[14] Elevated Hcy promotes atherosclerosis through increased OS, impaired endothelial function, and induction of thrombosis.[15]

In this regard, the aim of this study was to evaluate the role of HSP70, and markers of OS and ED, as determinants of the severity of CA.

***Corresponding author:** Julia A. Kotova, PhD. Voronezh State Medical University n. a. N.N. Burdenko. Voronezh, Russia. E-mail: kotova_u@inbox.ru*

Materials and Methods

We examined 354 CHD patients (175 women and 179 men aged between 47 and 75 years, mean age of 61.8±8.1 years) who had CA of varying degrees, according to CAG.

All patients underwent the following examinations: assessment of traditional risk factors, physical examination, clinical and biochemical laboratory methods, 12-lead ECG, echocardiography, Holter ECG monitoring, treadmill test, and coronary angiography. Blood samples were obtained in the morning after a 12 h overnight fast. The levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) were determined in the blood plasma using "Daytona» analyzer (RANDOX, Ireland).

CAG was performed by the Judkins technique using General Electric Innova 3100 (GE Healthcare, USA).

The severity of CAD was evaluated by the Gensini score (GS).[16] The severity of the disease is expressed as the sum of the scores for individual lesions and the functional importance index of the area of each lesion in the coronary tree. According to the calculated GS, patients were divided into two groups: GS0 – 152 patients with normal coronary artery and GS>1 – 202 patients with mild to severe coronary stenosis. In Group GS0, 53(35%) patients received statins for more than 6 months before the study, 99(65%) patients did not receive statins for 6 months before the study. In Group GS>1, 79(39%) patients received statins for more than 6 months before the study, 123(61%) patients did not receive statins for 6 months before the study.

The determination of OMP in the blood serum was carried out using the methods by Dubinina et al.[17] The assay is based on the spectrophotometric detection of the reaction between 2.4-dinitrophenylhydrazine (DNPH) with protein carbonyl to form protein hydrazone. The optical density of 2,4-dinitrophenylhydrazones derivates was recorded on an SF-36 spectrophotometer. The optical density of aldehyde- and ketone derivatives of a neutral character was recorded at 356 nm and 370 nm, respectively (ADPHn and KDPHn). The optical density of aldehyde- and ketone derivatives of a basic character was recorded at 430 nm and 530 nm, respectively (ADPHb and KDPHb).

The SOD activity was determined by the spectrophotometric method. The serum level of tHcy was determined by EIA using «Axis-Shield» test kit. Extracellular Hsp70 was measured by ELISA (Elisa Kit for Hsp70, Cloud-Clone Corp.) in blood samples.

Statistical analysis was performed using statistical software package SPSS version 20.0 (SPSS Inc, Chicago, IL). Median values are presented with interquartile (IQ) ranges (IQR; 25th to 75th percentiles). The Mann-Whitney test was used to compare median values. The frequencies of categorical variables were compared using Pearson $\chi 2$. The Spearman correlation coefficient (r_s) was used to assess the relationship between variables. Stepwise multiple linear regression analysis (MLRA) was done to determine the variables with independent significant association with the severity of coronary atherosclerosis, and included all variables (biochemical markers and lipid metabolism indicators) with significant relationship with coronary atherosclerosis in univariate analyses ($P<0.05$ after correction for multiple comparisons). Probability values less than 0.05 were considered statistically significant. A probability value of $P<0.05$ was considered statistically significant.

The study was approved by the Voronezh State Medical University Ethics Committee. Written informed consent was obtained from all patients.

Results

The main characteristics of the patients are presented in Table 1. The patients of the two groups were comparable in age. In Group GS>1, with a predominance of men, an average statistical power was found between the presence of CA, determined by the GS index, and the gender of patients ($\chi^2=14.174$, $P=0.0001$; $\varphi=0.459$, $P=0.0001$). In addition, the statistical relationship between gender and the number of affected vessels was determined: An insignificant lesion was more common in women (83%), two-vessel lesions were common in men (58.7%) and women (41.3%), and the three-vessel or multivessel lesions were predominant in men (61.6%) ($\chi^2=8.116$, $P=0.017$). The blood levels of TC, LDL-C and HDL-C also differed significantly between the two groups. GS>1 patients had higher levels of tHcy and OMP and lower levels of HSP70 and SOD activity (Table 2).

Table 1.

The main characteristics of the patients (Me, IQR [P_{25}; P_{75}])

Variable	GS0	GS1	*P*-value
Men	51	128	
Women	101	74	
Age, years	59.1 [51;67.5]	60.59 [57;65]	0.742
TC, mmol/l	4.54 [4.2;3.8]	5.82 [5.15;6.8]	0.001
TG, mmol/l	1.2 [1.1;1.3]	1.43 [1.1;1.57]	0.259
LDL-C, mmol/l	2.44 [2.05; 2.76]	2.94 [2.37; 2.94]	0.05
HDL-C, mmol/l	1.19 [1.1;1.3]	1.01 [0.9;1.1]	0.003

Table 2.

The blood levels of studied markers (Me, IQR [P_{25}; P_{75}])

Variable	GS0	GS1	*P*-value
HSP70, ng/ml	4.47 [4.12;4.98]	3.11 [2.63;3.71]	0.000
SOD, %	39.10 [39.10;43.89]	34.99 [32.26;36.14]	0.000
tHcy, μmol/l	8.47 [8.47;9.97]	11.46 [10.36;12.0]	0.000
ADPHn, IU/mg	21.88 [21.54;22.29]	25.9 [24.30;27.31]	0.000
KDPHn, IU/mg	20.32 [19.88;20.32]	21.43 [20.51;22.74]	0.003
ADPHb, IU/mg	10.73 [10.54; 10.88]	11.07 [10.67; 11.71]	0.075
KDPHb, IU/mg	2.54 [2.34;8.72]	6.87 [6.34;8.50]	0.006

A correlation analysis revealed direct correlations between GS and tHcy, indicators of OMP, as well as inverse correlations between GS and SOD activity. In addition, inverse

correlations were found between HSP70 and TC and LDL-C; SOD activity and TC; ADPHn, KDPHn and HDL-C. Direct correlations were found between HSP70 and SOD activity and HDL-C; ADPHn, KDPHn, ADPHb, KDPHb and TC; ADPHn and TG, LDL-C. We also found significant correlations between the GS and lipid profile parameters (TC [r_s=0.61, *P*=0.000], TG [r_s=0.27, *P*=0.04], LDL-C [r_s=0.45, *P*=0.001], and HDL-C [r_s=-0.46, *P*=0.000]).

On the first step of MLRA, the inclusion of HSP70 into the regression model (negative regression coefficient) explained 67% of the variance of the dependent variable (Table 3). On the second step, with the tHcy inclusion into the model, the relationship between HSP70 level and the presence of coronary atherosclerosis was maintained, and this model explained 73.1% of the variance of the dependent variable. The third predictor in the model was LDL-C. With the continued contribution of HSP70 and tHcy to the development of coronary atherosclerosis, this model explained 75.3% of the variance of the dependent variable. On the next step, the input variable was found to be KDPHb, and the model explained 76.7% of the variance of the dependent variable. At this step, the regression analysis was completed, which confirms the greatest importance of 4 markers in predicting coronary atherosclerosis: HSP70, tHcy, LDL-C, and KDPHb. The forced inclusion of other biochemical markers did not improve the model: The regression coefficients for them, as independent predictors, were insignificant, the standardized regression coefficients were low, the incremental R^2 was insignificant, and the partial F-test did not reveal significant differences among the models.

Table 3.

Results of the multiple linear regression analysis

Variable	B±Std. Error	β	*P*	adR²	F	*P*
Model 1				0.670	106.632	0.000
HSP70	-0.851 ±0.082	-0.822	0.000			
Model 2				0.731	71.785	0.000
HSP70	-0.524 ±0.118	-0.506	0.000			
tHcy	3.290 ±0.926	0.406	0.001			
Model 3				0.753	53.702	0.000
HSP70	-0.537 ± 0.114	-0.519	0.000			
tHcy	2.660 ±0.930	0.328	0.006			
LDL-C	2.210 ±0.962	0.173	0.026			
Model 4				0.767	39.025	0.000
HSP70	-0.511 ±0.019	-0.490	0.000			
tHcy	2.576 ±0.956	0.312	0.032			
LDL-C	1.982 ±0.908	0.150	0.047			
KDPHb	0.903 ±0.254	0.164	0.049			

Analyzing the ratios of standardized regression coefficients included in the regression analysis, it can be noted that the relative unique prognostic importance of HSP70, as an independent predictor of coronary atherosclerosis, is about -0.5. That is, when other independent predictors remain unchanged, and the HSP70 level increases by 1 standard deviation, then the severity of coronary atherosclerosis decreases by 0.5 standard deviation. This once again confirms the high importance of HSP70 as a protective factor for coronary atherosclerosis within these regression models.

Discussion

Identifying patients at early stages of coronary atherosclerosis is still a major problem. In the past two decades, numerous studies have demonstrated the importance of oxidative stress in the development of atherosclerosis. Elevated concentrations of a variety of oxidative stress markers were linked to a more frequent occurrence of cardiac events.[18] The study performed by Y Huo et al.[19] revealed metabolic disturbances in the model of long-term hyperhomocysteinemia together with vascular remodeling. Authors suggested that OS, ED, and decreased PPARγ expression in the vessel wall could be underlying mechanisms. Activation of ROS transduce matrix metalloproteinase, renders eNOS ineffective and promotes endothelial-smooth muscle disconnection/uncoupling by antagonizing PPARγ.[20] Elevated levels of plasma Hcy cause endothelial dysfunction and vascular diseases. [21,22] The importance of homocysteine in vascular function and arteriosclerosis was discovered by demonstration of arteriosclerotic plaques in children with homocystinuria caused by inherited enzymatic deficiencies of cystathionine synthase, methionine synthase, or methylene-tetrahydrofolate reductase.[23,24] Nonfasting plasma tHcy levels were independently associated with increased rates of all-cause and CVD mortality in the elderly Framingham men and women.[25] The elevated levels of homocysteine and indicators of oxidative stress were also found in our study. At the same time, a significant difference between GS0 and GS>1 precisely in ketone derivatives indicates the duration of oxidative stress and the degree of destruction of the protein molecule.

The activation of HSP70 may play a role in protecting the cells against oxidative stress and inflammatory damage.[26] When assessing the level of HSP70, we found a decrease in its level in the presence of coronary atherosclerosis, as well as a high association with GS. The first evidence that high levels of human HSP70 are associated with low CAD risk, probably through its multiple protective effects on a cell's response to stress, was provided by Zhu et al.[27] However, there are conflicting reports that preclude assigning HSP70 a definite role in atherosclerosis at present. Plasma levels of HSP70 have been found to have an inverse [27,28] as well as a direct association [29,30] with the severity of atherosclerosis. HSP70 is presently a matter of debate.[31,32] High levels of circulating HSP70 (HSPA1A) are associated with low risk of CAD;[27] they appear in hypertensive subjects with a lesser intima media, thickening after 4 years of follow-up.[33] In the study by E. Dulin, extracellular HSP70 and anti-HSP70 antibody concentrations have been proposed as biomarkers for the progression of atherosclerotic disease.[34] At least 4 studies have demonstrated that the transgenic overexpression of HSP70 in the heart of mice significantly protects against ischemia/reperfusion injury.[35-38]

Thus, our findings emphasize the significance of the studied markers in the pathogenesis of coronary atherosclerosis and make it possible to use the indicators of HSP70, tHcy, LDL-C and KDPHb in screening the risk for the development of coronary atherosclerosis.

Conclusion

The study revealed significant differences between groups with and without coronary atherosclerosis in terms of HSP70, superoxide dismutase, tHcy and markers of oxidative modification of proteins (except ADPHb). Significant correlations between Gensini score, lipid profile parameters and studied markers were determined. The results of multiple linear regression analysis allow us to consider the levels of HSP70, total homocysteine, low-density lipoprotein cholesterol and KDPHb as factors associated with the risk of coronary atherosclerosis.

Competing Interests

The authors declare that they have no competing interests.

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