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The Association Study of Antioxidant Status and Antioxidant Genes Polymorphisms in Patients with Ischemic Heart Disease in the Republic of Tatarstan

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Abstract: Ischemic heart disease (IHD) is a multifactorial disease caused by the interplay of environmental risk factors with multiple predisposing genes. The present study was undertaken to evaluate the role of genetic polymorphism, oxidative stress and antioxidant status in IHD patients. IHD patients are characterized by a change of the blood serum's antioxidant status, which is expressed in significant increases/decreases of the total antioxidant activity, lipid hydroperoxides and ceruloplasmin indicators in comparison with normal values and also in significant increases of the malondialdehyde for the patients with acute myocardial infarction. This study also showed that the level of some of the examined antioxidant system indicators depends on polymorphism of the genes, which encode the enzymatic antioxidant system pool (genes of catalase, extracellular and mitochondrial superoxide dismutase and glutathione peroxidase).

Key words: Acute myocardial infarction · Exertional angina · Antioxidative defense · Antioxidant status · Gene polymorphism · Genetic predisposition

INTRODUCTION

Recently, genetic variation of antioxidant system enzymes has become one of the most studied subjects regarding the aetiopathogenesis of cardiovascular diseases (CVD) [1]. It is known that active oxygen species are the cause of ischemic heart disease (IHD), which is one of the most widespread cardiovascular system (CVS) diseases. Under the influence of active oxygen species, peroxidation begins, resulting in the accumulation of low-density lipoproteins (LDL) in the blood. In turn, these low-density lipoproteins involve macrophages, which take and store cholesterin, ultimately leading to the formation of atherosclerosis plaques on blood vessel walls and the development of thrombotic occlusion [2].

The complex mechanism that forms the disease's clinical phenotype is caused by a great number of genes involved in the pathogenesis of IHD. However, the role of the genes, which encode antioxidant system enzymes, in developing the clinical forms of IHD and their relation with antioxidant protection indicators, are studied insufficiently. Additionally, the results of the research are frequently contradictory [3].

The purpose of this study is to characterize the antioxidant status of blood serum and to assess the influence of the genetic polymorphism of some of the antioxidant system genes on changes to the antioxidant status of people with ischemic heart disease. The specific changes observed in this study are acute myocardial infarction (AMI) and exertional angina (EA), with the subject pool being individuals within the population of the Republic of Tatarstan.

MATERIALS AND METHODS

Assessment of antioxidant status in blood serum during IHD (total antioxidant activity (AOA), lipid hydroperoxides (LHP), malondialdehyde (MDA) and ceruloplasmin (CP)) has been carried out for 128 patients by the Central Research Laboratory of the Kazan State Medical University (Kazan), utilizing standard methods [4-7].

The examined patients have undergone genetic typing, using polymorphous loci of catalase CAT (rs1001179) genes, glutathione peroxidase-1 GPx-1 (rs1050450) genes, mitochondrial and extracellular

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Table 1: Average values of the antioxidant system indicators in the IHD group

Antioxidant system indiAator	Me ± SE*	Norm**
AOA, %	49.20 ± 2.08	41.5 ÷ 44.5
AP, mg%	62.75 ± 0.81	25 ÷ 48
LHP, relative units	9.33 ± 0.32	2 ÷ 2.3
MDA, µmol/L	3.09 ± 0.22	2.2 ÷ 4.8

* Average value ± standard error.

** Normal antioxidant system indicators are specified according to A.A. Kishkun [13].

Table 2: Antioxidant system indicators for the patients with various clinical IHD forms

		AMI (n=110)		EA (n=18)	
Antioxidant system indicator		% of patients	Me ± SE	% of patients	Me ± SE
AOA, %	< N*	31.8	21.53±2.18	55.6	20.88±2.56
	N	0	-	0	-
	> N	68.2	61.56±1.08	44.4	63.35±3.01
CP, mg%	< N	1.8	21.09±2.15	0	-
	N	7.3	43.36±1.66	5.6	47.16
	> N	90.9	63.21±0.82	94.4	61.47±1.35
LHP, relative units	< N	0.9	1.72	0	-
	N	0.9	2.1	0	-
	> N	98.2	9.04±0.37	100	9.05±0.84
MDA, µmol/L	< N	47.3	1.38±0.31	50	1.23±0.12
	N	33.6	3.15±1.13	38.9	2.39±1.1
	> N	19.1	7.8±1.56	11.1	4.41±0.72

* < N, N, > N: separate antioxidant system indicators below normal, normal and above normal, respectively.

superoxide dismutase SOD2 and SOD3 genes (rs4880 and rs699473, respectively) and NADPH oxidase genes (rs699473). The products of these genes are involved in the metabolism of prooxidants and antioxidants.

The group of examined patients was developed through the Cardiologic Department #1 of the Municipal Emergency Hospital #1 (Kazan). The selection was carried out by sex, age, nationality and hereditary IHD load. All of the patients consented to participate in this research.

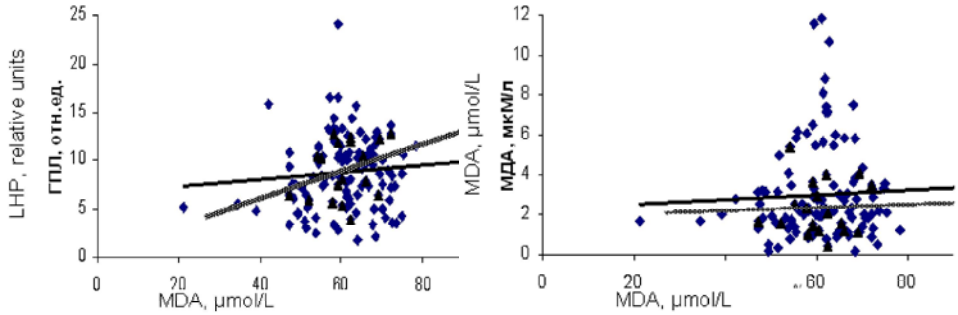
Extraction of the genomic DNA was carried out by the standard two-stage phenolic-chloroform extraction method [8]. Genetic typing of DNA polymorphisms was carried out by the PCR method, with subsequent restriction analysis according to the reports described in the literature [9, 10, 11], using the thermocycler "MyCycler" (Bio-Rad Laboratories, U.S.A.). The restriction products were fractionated in polyacrylamide gel, colored with ethidium bromide and visualized using the gel documentation system ChemiDoc^{Ti} XRS (Bio-Rad Laboratories, U.S.A.).

Differences between the groups deemed significant were assessed by the chi-square test at significance level p=0.05. For objective assessment, the average value and standard error (SE) [12] were calculated based on the obtained data regarding biochemical indicators in both the patient groups and the control group. Interrelation between the indicators was estimated using the Spearman correlation coefficient. Statistical analysis was carried out with the help of the software package "Excel Office 2000."

Research Results: During the assessment of the antioxidant status of IHD patients' blood serum, a sharp increase in average ceruloplasmin (CP) and lipid hydroperoxides (LHP) values and also a slight increase in the total antioxidant activity (AOA) value, were observed; at the same time, malondialdehyde (MDA) content was within normal values (Table 1).

In the AMI and EA patient groups, a sharp increase in CP and LHP content was observed in more than 90% of cases; however, average values of these indicators had no significant differences in the examined groups ($p > 0.05$) (Table 2).

During the comparative analysis of the average AOA values, an increase in the percentage of patients with a statistically significa group, the percentage of these patients was less than half (44.4%). That being said, the average AOA values did not differ between the examined groups ($p = 0.0912$). nt increase of this indicator in the AMI group (68.2%) was observed. At the same time, in the EA



Picture 1: Correlation analysis between LPO and ceruleoplasmin indicators in the AMI and EA groups.

A significant decrease in the MDA level was observed in 50% of the IHD cases. Other patients were noted to have significant increases in the AOA level in comparison with normal values. In the AMI group, in comparison with the EA group, a significant increase of this indicator (more than 1.7 times ($p < 0.05$)) was observed.

Correlation analysis between the antioxidant status indicators showed no dependence between the AOA, CP indicators and the LHP, MDA lipid peroxidation products and lipid peroxidation (LPO) products in the AMI and EA groups. In all examined groups, a positive correlation between the LPO and CP products (Fig. 1) was observed.

During this study, there was an average degree of positive correlation between LHP and CP content, which is more pronounced for the EA patients (R_S=0.41, p=0.0006). A low degree of correlation between the MDA and CP indicators (R_S=0.22, (p=0.034) and R_S= 0.39 (p=0.003) for the AMI and EA groups, respectively) was observed for both groups.

Thus, IHD patients are characterized by a change of the blood serum's antioxidant status, which is expressed in significant increases/decreases of the AOA, LHP and CP indicators in comparison with normal values and also in significant increases of the MDA content for the AMI patients.

Since antioxidant system functioning depends directly on the expression of the genes which encode production of certain products, we have carried out an assessment of the influence of polymorphous variants of some antioxidant genes (CAT, GPx-1, SOD2, SOD3) and the NADPH oxidase gene, whose product is the main basic source of free radical particles, on the level of the antioxidant status indicators.

We have carried out a comparative analysis regarding the influence of the genotypes on the antioxidant status indicators only for the AMI group due to the fact that the EA group was small. During the analysis, we showed that carriers of AT and TT genotypes of polymorphous variants of the CAT and SOD3 genes are characterized by a reduced AOA value that is still near a normal value, as well as by relatively reduced LHP indicators compared with carriers of other genotypes of these markers (Table 3). There was also a decrease in the average CP level values for carriers of the TT polymorphism genotype of the GPx-1 gene. For polymorphisms of the SOD2 and NADPH genes, no influence on the antioxidant status indicators for the AMI patients was found. There were no significant differences between the average MDA values for carriers of the various genotypes for all polymorphous loci.

Discussion of the results. According to the peroxy concept of atherosclerosis development, when there is an influence of certain risk factors, active oxygen formation increases. This formation initiates free radical oxidation mechanisms that lead to the deformation of the membrane lipoprotein complex, membrane permeability changes and damage to the enzymes that participate in ion transport and oxidative phosphorylation. To regulate these processes, the body has an antioxidant system, which can be of two types: specific, which is aimed at the destruction of formed active oxygen species (this type includes enzymatic pool (catalase, superoxide dismutases, glutathione peroxidase and some others) and nonenzymatic pool (ceruloplasmin, transferring)). It is nonspecific, which prevents conditions and possibilities for electron leakage that lead to the generation of active oxygen species during oxidation-reductions [14].

IHD is characterized by increased content of lipid peroxidation (LPO) products (such as LHP (primary LPO product) and MDA (one of the end LPO products)) in blood serum and by decreased activity of antioxidant agents (e.g., the total AOA indicator and the CP content in blood serum) [15]. The AMI patients had the highest degree of activity of the LPO indicators and the patients with large-focal infarction had more pronounced changes than the patients with small-focal infarction [16].

Table 3: Comparative characteristics of the average antioxidant system indicators, depending on the genotype of polymorphism of the antioxidant system genes

Genotype	AOA, %	CP, mg%	LHP, relative units	MDA, µmol/L
Polymorphism -262A/T of the CAT gene				
AA	54.02±4.81	59.93±2.95	8.74±0.63	3.09±0.62
AT	45.48±3.31*	60.84±1.55	7.71±0.65	2.91±0.34
TT	51.88±2.97	62.56±1.24	9.71±0.53	3.14±0.34
Polymorphism +593C/T of the GPx-1 gene				
CC	48.92±4.27	61.3±2	9.49±0.57	3.24±0.51
CT	49.40±3.14	62.65±1.47	8.84±0.52	2.96±0.31
TT	49.44±3.49	59.07±1.63	8.77±0.92	3.01±0.39
Polymorphism +9T/C of the SOD2 gene				
TT	51.43±3.70	61.37±1.63	8.92±0.61	3.05±0.5
TC	48.66±4.46	60.43±1.9	9.49±0.58	3.12±0.42
CC	48.82±2.93	61.85±1.49	8.73±0.6	2.98±0.31
Polymorphism +186C/T of the SOD3 gene				
CC	52.39±3.92	60.43±2.1	9.7±0.64	3.14±0.36
CT	53.41±2.58	61.73±1.08	9.25±0.57	3.2±0.39
TT	40.60±4.61	62.42±2.45	7.67±0.67	2.36±0.32
Polymorphism +242C/T of the NADPH gene				
CC	55.63±3.48	61.89±1.5	9.49±0.86	3.12±0.55
CT	55.55±2.55	60.74±1.28	8.97±0.61	2.97±0.38
TT	49.81±3.92	61.10±2.33	7.88±0.65	3.23±0.42

* boldface text indicates significant differences in the average values ($p < 0.05$).

In the case of IHD, we have noted a negative correlation between LPO level and activity of the antioxidant system for the IHD patients. This correlation, when presented in healthy people, has an inverse character. A number of research studies confirm a statistically valid interrelation between high LHP and MDA levels and low CP level and IHD development risk [15, 17].

The results of our research contradict the data for main antioxidant system indicators for IHD patients from prior studies. In the patient groups examined by us, an increase in the percentage of patients with increased total antioxidant system activity and ceruleoplasmin content was observed and can be connected to the intensification of free radical processes in the body and the appearance of tissue necrosis foci during IHD and, especially, AMI.

Most of the examined group (more than 80%) is characterized by decreased and normal MDA content in blood serum and considerably increased LHP content (more than 90%). This can be explained by the LPO process abnormality. As a result, the oxidation of macromolecules does not reach end products, (one of which is MDA) and the accumulated primary product (LHP) exceeds its indicator's normal value by several times.

In both the AMI and EA groups, almost 100% increased the CP content. The CP content performs a number of important biological functions (e.g., increasing cell membrane stability, participation in immune responses, ion exchange, LPO inhibition and hemopoiesis stimulation) in the body and was observed. The CP can act as a prooxidant or as an antioxidant, depending on the presence of other factors. In the presence of superoxide (e.g., in an inflamed vascular endothelium), it can destroy superoxide radicals, thereby preventing LPO activation. A direct correlation between the content of the LPO and CP products was observed and could indicate antioxidant protection "overload."

Currently, there are a fair number of studies that assess the influence of the antioxidant system genes' polymorphism on activity of the products encoded by them and various antioxidant system indicators during many multifactor diseases [18, 19]. For example, it is established that the replacement of C by T in the -9 position of the SOD2 gene (GCT/GTT) changes the structural organization of the enzyme considerably [20].

In this work, it is shown that the level of some of the examined antioxidant system indicators depends on polymorphism of the genes, which encode the enzymatic antioxidant system pool (genes of catalase, extracellular and mitochondrial superoxide dismutase and glutathione peroxidase). Certain genotypes of the polymorphism -262 A/T of the CAT gene (localized in the promotor area) and the polymorphism +186C/T of the SOD3 gene (consisting of the replacement of arginine by glycine in 213th position of the polypeptide chain) are characterized, in comparison with other genotypes, by somewhat low AOA and LHP indicators. The TT genotype of the polymorphism

+593C/T of the GPx-1 gene (replacement of proline by leucine in 197th position of the amino acid sequence) is characterized by a decrease in the CP indicators in the AMI group.

Therefore, carriers of these genotypes can have rather low antioxidant system indicators against the background of pronounced changes in antioxidant system work. It is necessary to take this into account when interpreting biochemical analysis results.

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