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Endocrinology.

PECULIARITIES OF INDICES OF ENDOTHELIAL FUNCTION IN PATIENTS WITH METABOLIC SYNDROME DEPENDING ON PRO197LEU POLYMORPHISM OF THE GPX 1 GENE

Abramova N.O.
Candidate of medical sciences,
assistant of the clinical immunology, allergology and endocrinology department
State higher educational institution in Ukraine Higher state educational establishment of Ukraine «Bukovinian state medical university», Chernivtsi.

Pashkovska N.V.
Doctor of medical sciences, professor,
head of the department of clinical immunology, allergology and endocrinology
State higher educational institution in Ukraine Higher state educational establishment of Ukraine «Bukovinian state medical university», Chernivtsi.

Chympoy K.A.
Candidate of medical sciences, assistant of the internal medicine department
State higher educational institution in Ukraine Higher state educational establishment of Ukraine «Bukovinian state medical university», Chernivtsi.

Abstract. Pro197Leu polymorphism of the gene GPX1 have been studied in 102 patients with MS and 97 healthy individuals. The vascular endothelial growth factor (VEGF) and number of circulating desquamated endothelial cells were investigated in 102 patients, 20 healthy individuals made control group. Analyzing the data, the growth of risk of disorder in the GPX 1 activity in patients with Pro/Leu and Leu/Leu variants of polymorphism comparing with homozygotes for the «wild» allele at 4,7 and 6,9 times respectively had been revealed. Individuals with Leu/Leu genotype had significantly higher level of VEGF expression and endothelial desquamation intensity compared with the persons with Pro/Pro genotype. Consequently, in patients with with metabolic syndrome the risk of glutathione peroxidase 1 activity reduction is associated in a dose-dependent manner with the presence of «mutant» Leu-allele, while homozygous for the «wild» Pro-allele had a significantly lower risk of this disorder. The presence of Leu- allele in genotype of patients with metabolic syndrome is associated with functional state of the endothelium violation.

Introduction. Nowadays the way of people's life does not coincide with their physiological needs. The human body adapted to exhausting physical exertion, cold and hunger for thousands of years. The desire to achieve comfort was obviously the engine of progress. In industrialized countries people managed to create conditions that allow almost completely avoid physical activity. Thus, sedentary lifestyle, unbalanced diet against the background of genetic susceptibility caused rapid growth of overweight people amount [3, 9].

According to the data of the World Health Organization (WHO) the prevalence of overweight people at the age of 15 and over is recorded to be 2,3 billion and they predict growth of their amount by 700 million till 2015. About 30% (16,8% of women and 14,9% men) of the world population are overweight [2, 54].

Pathophysiological processes that accompany obesity are causal factors in the development of arterial hypertension, disorders of carbohydrate metabolism in form of diabetes mellitus type 2, dyslipidemia, which are components of metabolic syndrome (MS) [14, 79].

The prevalence of MS is about 25% of the world's population according to the criteria of IDF. It ranges from 10 to 84% depending on the region and the characteristics of the population being studied (sex, age, race, and ethnicity) [11, 2].

Cytoplasmic glutathione peroxidase *(GPX 1)* is one of the selenoenzymes important for the organism functioning, present in all tissues of the human body, which takes part in detoxication of hydrogen peroxide and products of lipid peroxidation, as catalyzes the interaction of redused glutathione with these substances [5, 826; 6, 2807; 10, 3021; 12, 1]. It is known that numerous pathologic processes in the organism develop in concequence of disorders in the mechanisms of antioxidant protection. Specially, in patients with insulin resistance accompanied by hyperglycemia and increased production of cytokines there arises oxidative stress. The accumulation of free radicals activates factors of transcription such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kb), which initiate the process of proinflammatory cytokines release [9, 1506]. The growth of free radicals results in lipid peroxidation of cellular membranes, causes atherosclerosis and endothelial dysfunction [13, 2].

We studied single nucleotide polymorphism of the gene *(GPX 1)* for going into the question of the dependence of these processes upon the disorders of redox homeostasis. The human gene *(GPX 1)* is localized in 3p21 chromosome and consists of two exons. Several single nucleotide polymorphism variants of this gene are known, but the *Pro-197Leu* polymorphism has been under our study, at which in the position 593 the amino acid cytosine (C) is replaced by thymine (T) (C593T), resulting in substitution of the amino acid proline for leucine in the 197 codon. This mutation refers to missense - functional polymorphisms. *Pro*-allele is «wild», while *Leu*- is a «mutant» allele. The presence of Leu-allele causes depression of *(GPX 1)* sensibility to stimulating factors [7, 17].

However, the dependence of functional state of endothelium parameters on *Pro-197Leu* polymorphism of the *(GPX 1)* gene against a background of MS remains unstudied.

The aim of the study. To investigate the dependence of functional state of endothelium parameters on *Pro197Leu* polymorphism of the *GPX 1* gene against a background of metabolic syndrome.

Material and methods. *Pro197Leu* polymorphism of the gene *GPX 1* have been studied in 102 patients with MS and 97 healthy individuals by isolation of genomic DNA from peripheral blood leukocytes, and then amplification of the polymorphic area in the state of polymerase chain reaction (PCR) was performed on the programmed PCR ther-

mal cyclers «Amply-4L» («Biocom», Росія) at individual temperature response. Reagents «ДНК-сорб-В» variant 100 (ФГУН ЦНИИЭ, Росія) were used for DNA isolation from lymphocytes according to instructions. PCR samples were prepared by means of the set «АмплиСенс-200-1» (ФГУН ЦНИИЭ, Росія). Products of PCR were separated using electrophoresis in 3% agarose gel in the presence of tetraborate buffer, concentrated with ethidium bromide. Fragments were visualized by transilyuminator in the presence of a marker of molecular mass 100-1000 bp («Fermentas®», USA).

Pearson's criterion (χ^2) was used to estimate the correspondence of the genotype frequencies under study to theoretically expected distribution at Hardy-Weinberg's equation. Odds ratio (OR) with determination of 95% confidence interval (CI) was calculated with the aim to establish the association of polymorphic variant of the gene with a pathological phenotype.

The diagnosis of MS was established according to criteria of the International Diabetes Federation (IDF) [3, 12].

Endothelial function indices were investigated in 102 patients, 20 healthy individuals made control group. The vascular endothelial growth factor (VEGF) level was established by immunoassay method using a set of firm «Vector-Best». The number of circulating desquamated endothelial cells was calculated by Hladovec J. method in Petrishchev N.N. et al. modification [4, 51].

Statistical analysis of the obtained data was carried out using the Student's t-test and Pearson's rank correlation coefficient by means of the software package Statistica 6.0 for Windows. The difference was considered reliable at $p < 0,05$.

Results and discussion. When assessing the distribution of genotype frequencies of the gene *GPX 1*, it has been found that in the group of patients with MS threre takes place a significant reduction of the frequency of *Pro/Pro* genotype as compared with the control group ($\chi^2 = 7,0$, $p<0,05$), while a reliable difference between the frequencies of *Pro/Leu* and *Leu/Leu* genotypes in the main and control groups ($\chi^2 = 1,9$, $p>0,05$ and $\chi^2 = 2,6$, $p>0,05$) has not been found out.

It has been revealed that *Pro/Leu* and *Leu/Leu* variants of polymorphism are associated with increased risk of violation of redox system in patients with MS as compared with a group of healthy subjects (table 1). Assessment of the relative risk was carried by odds ratio (OR) magnitude. The OR calculation showed that in patients with *Pro/Leu* polymorphism the risk of disturbance of *GPX 1* activity increases 5,2 times ($p<0,05$, OR = 1,65, CI = 0,95% 0,94 – 2,90), and in patients with *Leu/Leu* genotype the risk of such patology is 6,0 times higher than in persons with *Pro/Pro* genotype ($P<0,05$, OR = 1,92, CI 0,95% = 0,93 – 3,97).

So, the risk of *GPX 1* activity reduction in a dose-dependent way is associated with the presence of «mutant» *Leu*-allele, while homozygous for the «wild» *Pro*-allele had significantly lower risk of this disturbance development. *Pro*-allele has protective properties concerning the development of redox system violation.

Table 1

The distribution of genotype frequencies depending on *Pro197Leu* polymorphism of *GPX 1* gene in patients with metabolic syndrome and the control group

Genotypes	*Cases*	*Controls*	χ^2	p	OR	0,95% CI
	102	97				
Genotype frequency *Pro/Pro*	0,176	0,402	12,91	0,002	0,32	0,17-0,61
Genotype frequency *Pro/Leu*	0,578	0,454			1,65	0,94-2,90
Genotype frequency *Leu/Leu*	0,245	0,144			1,92	0,93-3,97

Notes: χ^2 – Pearson criterion, OR – odds ratio, CI – confidence interval.

When studying the dependence of functional state of the endothelium on *Pro-197Leu* polymorphism of *GPX 1* gene, a significantly higher level of VEGF in homozygous group for mutant *Leu*-allele and heterozygous group for this allele in comparison with homozygous ones for wild allele has been received, 45,3% and 62,8% higher respectively ($p<0,05$) (table 2). A credible growth of VEGF in patients with *Pro/Pro*, *Pro/Leu* and *Leu/Leu* genotypes in relation to the group of healthy individuals was found 1,9, 2,8 and 3,2 times higher ($p<0,05$).

Table 2

Peculitaries of functional state of endothelium indicators in patients with metabolic syndrome according to *Pro197Leu* polymorphism of the *GPX 1* gene

Index	Genotypes *GPX 1* , n = 102			Control group, n = 20
	Pro/Pro n = 18	*Pro/Leu* n = 59	*Leu/Leu* n = 25	
VEGF, pg / ml	146,3±16,83 */**/***	212,6±24,38 *	238,2±24,73 *	76,6±12,70
Endotheliocytes (104/l)	10,22±1,238 */***	13,8±2,214 *	18,25±2,964 *	2,99±0,423

Notes: 1. n – number of observations;
2. * – the probability of changes in relation to control;
3. ** – the probability of changes in relation to the group with *Pro/Leu* genotype;
4. *** – chance changes in relation to group with *Leu/Leu* genotype

VEGF is a cytokine, expression of which increases during hypoxia, hyperglycemia, it is activated in response to proinflammatory cytokines hyperproduction [8, 1].

In our investigation VEGF expression increases, probably due to transcription factors activation as a cause of free radicals accumulation against a background of reduced *GPX 1* activity. Free radicals start the process of proinflammatory cytokines release by activation of transcription factors such as NF-kb [9, 1509].

The number of circulating desquamated endothelial cells was established to be 7,9% higher in the group with *Leu/Leu* genotype as compared with the group whith *Pro/Pro* genotype ($p<0,05$) and respectively 3,4, 4,6 and 6,1 times higher in the groups with *Pro/Pro*, *Pro/Leu* and *Leu/Leu* genotypes in comparison with the control group ($p<0,05$). Small desquamation of endothelial cells also took place in the control group, which reflected the physiological process of intimal clearance from dead cells [1, 470].

The reason for the increased endothelium desquamation intensity is probably due to lipid peroxidation activation and increased cytokines expression, caused by accumulation of free radicals, which is more pronounced in homozygous for the «mutant» *Leu*-allele [5, 825; 7, 16; 9, 1512].

Thus, our data coincide with the results of other researchers. Bastaki M. et al. have discovered that *GPX 1* activity 6 times slows down in homozygous patients for the *Leu*-allele [5, 826]. Association of endothelial dysfunction with *Pro197Leu* polymorphism of *GPX 1* gene is reflected in the Zelkova T.V. et al. study. They found out that the homozygous for mutant *Leu*-allele more often suffered from coronary artery disease and myocardial infarction at the age of until 50 years old [15, 485].

Conclusions.

1. In patients with metabolic syndrome the risk of reduction of glutathione peroxidase 1 activity is associated in a dose-dependent manner with the presence of «mutant» *Leu*-allele, while homozygous for the «wild» *Pro*-allele had a significantly lower risk of this disorder.

2. The presence of *Leu*-allele in genotype of patients with metabolic syndrome is associated with functional state of the endothelium violation which is probably due to the decreased *GPX 1* activity.

Prospects for further research. The survey results indicate the necessity of effective measures for endothelial dysfunction correction development in patients with metabolic syndrome.

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