was evaluated in terms of changes in the level of the main stable metabolites of nitric oxide (NO2 and NO3), in the membranes of red blood cells in the blood plasma and in exhaled breath condensate (EBC). All the patiens underwent spirometry as well. The evaluation of the drug effectiveness was carried out before and after the treatment.

Results. It has been established that during the period of asthma exacerbation, high levels of nitric oxide in the blood, red blood cells and EAC can be found, which is the sign of endothelial dysfunction. Interdependency between the indicators of respiratory function (degree of obstruction) and the content of nitric oxide in the SSC has been found: the more severe the obstruction, the higher the rates of nitrogen oxide in the SSC. The results show the positive effect of L-arginine on endothelial system performance and spirometry along with a basic therapy.

Conclusions. The use of L-arginine has a positive effect on the endothelial system and spirometry in patients with asthma against pathogenic therapy.

Key words: Asthma, L-arginine, SSC, endothelial dysfunction.

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PECULIARITIES OF CARBOHYDRATE METABOLISM OF PATIENTS WITH ARTERIAL HYPERTENSION AGAINST THE BACKGROUND OF ABDOMINAL OBESITY DEPENDING ON PRO197LEU POLYMORPHISM OF THE GPX1 GENE

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Abstract. Pro197Leu polymorphism of the gene GPX1 in 102 patients with arterial hypertension and concomitant abdominal obesity and 97 healthy individuals have been studied. Disorders of distribution of genotype frequencies comparing with the control group at the expense of the reduction of Pro/Pro genotype frequency have been found in the main group. Analyzing the data, the increasing risk of disorders 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. Analyzing the changes of carbohydrate metabolism depending on the Pro197Leu polymorphism of the gene GPX1, it was established that in patients with Leu/Leu genotype the production of immunoreactive insulin, leptin, C-peptide, HOMA-IR increased significantly. Thus, Pro-allele possesses protective properties as to the reduction in the activity of glutathione peroxidase. Insulin and leptin resistance develop in the carriers of Leu-allele, wich causes disturbances in carbohydrate metabolism.

Key words: Pro197Leu polymorphism of the gene GPX1, carbohydrate metabolism, insulin resistance, abdominal obesity, metabolic syndrome.

Introduction. 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 it catalyzes the interaction of reduced glutathione with these substances [3, 8, 5, 10]. Numerous pathologic processes in the organism are known to develop as a result of disorders in the mechanisms of antioxidant protection. In particular, the patients with insulin resistance, accompanied by hyperglycemia and increased production of cytokines, aquired oxidant stress. The accumulation of free radicals activates factors of transcription such as NFkB, which initiate the process of proinflammatory cytokines release[6]. The accumulation of free radicals results in lipid peroxidation of cellular membranes, causes atherosclerosis and endothelial dysfunction [11]. 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 have been known, but the Pro197Leu polymorphism has been under our study, at which in the position 593 the amino acid cysteine (C) is replaced with thymine (T) (C593T), resulting in substitution of the amino acid proline for leucine in the 197 codon. This mutation refers to missens - functional polymorphisms [1]. 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].

Thus, Bastaki et al. discovered that GPX 1 activity 6 times slows down in homozygous patients for the Leu-allele[3]. Zelkova T.V. et al. found out that the homozygous for mutant allele more often

suffered from coronary artery disease and myocardial infarction at the age before 50 [12].

The aim of the study. To investigate the dependence of carbohydrate metabolism in patients with arterial hypertension and concomitant abdominal obesity depending on Pro197Leu polymorphism of the GPX1gene.

Material and methods. Pro197Leu polymorphism of the gene GPX1 have been studied in 102 patients and 97 healthy individuals by isolating genomic DNA from peripheral blood leukocytes, after that amplification of the polymorphic area in the state of polymerase chain reaction (PCR) was performed on the programmed PCR thermal cyclers «Amply-4L» («Biocom», Moscow) at individual temperature response. Reagents "DNA-sorb-B" option 100 were used for DNA isolation from lymphocytes according to instructions. PCR samples were prepared by means of the set «АмплиСенс-200-1» (Russia). 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 transiluminator in the presence of a marker of molecular mass 100-1000 bq (Fermentas^{R} », USA).

Pearson's X^2 criterion 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.

To evaluate the dependence of carbohydrate metabolism depending on Pro/Leu polymorphism of the gene GPX1 we divided the patients into groups in the following way: 18 patients with Pro/Pro, 59 with Pro/Leu and 25 with Leu/Leu genotypes, the control group consisted of 20 healthy individuals. Disorders of carbohydrate metabolism were diagnosed according to WHO criteria (1999). Fasting immunoreactive insulin (IRI), C-peptide were determined by immunoassay method, glucose content by glucose oxidase method, the content of glycated hemoglobin (HbA_{1C}) was studied by the method of microcolumn chromatography to evaluate the compensation of carbohydrate metabolism.

To assess the degree of insulin resistance there was used small model of homeostasis (Homeostasis model assessment – HOMA [Matthew DR, 1985].

Statistical analysis of the data was carried out using the Student's t-test and Pearson's rank correlation coefficient using 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 GPX1, it has been found that in the group of patients with abdominal obesity against the background of arterial hypertension there takes place a significant reduction of the frequency of Pro/Pro genotype as compared with the control group ($X^2=7,0$, $p<0,05$), while there hasn't been found out a reliable difference between the frequencies of Pro/ LeuandLeu/Leu genotypes in the main and control groups ($X^2=1,9$, $p>0,05$ and $X^2=2,6$, $p>0,05$).

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 metabolic syndrome compared with a group of healthy subjects (table 1). Thus, it has been found out that in patients with Pro/Leu polymorphism the risk of disturbance of GPX1 activity increases 5,2 times ($p<0,05$, OR=1,65, CI=0,95 % 0,94-2,90; table 1), 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; table 1).

So, the risk of reduction of GPX 1 activity 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 qualities concerning the development of redox system violation.

When studying the dependence of indices of carbohydrate metabolism onPro197Leu polymorphism of GPX1 gene, a significantly higher level of IRI in homozygousgroup for the mutant allele in relation to the heterozygous group for this allele and homozygous ones for wild allele has been received, 62,8 % and 37,8 % higher respectively ($p<0,05$) (table 2). A credible growth of IRI in patients with Pro/Pro, Pro/Leu and Leu/Leu genotypes in relation

Table 1

The distribution of genotype frequencies depending on GPX Pro197Leu polymorphism gene 1 in patients with hypertension and concomitant abdominal obesity and the control group

Genotypes	Cases	Controls	X^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

Note. X^2 - Pearson criterion, OR - odds ratio, CI - confidence interval

Table 2

Peculiarities of indicators of carbohydrate metabolism and anthropometric features in hypertensive patients with concomitant abdominal obesity according to Pro197Leu polymorphism of the gene GPX 1

Index	GenotypesGPX 1 , n=102			Control group, n=20
	Pro/ Pro	Pro/ Leu	Leu/ Leu	
Glucose, mmol/l	6,32±0,156*	7,49±0,112*	8,21±0,168*	4,73±0,174
Immuno-reactive insulin, IU/ml	15,79±2,438 */**	18,648±2,362 */***	25,69±2,108*	6,11±1,314
HOMA-IR	4,35±0,124*/***	4,187±0,183*/***	8,97±0,367*	0,97±0,035
C-peptide, ng/ml	3,98±0,183 */**/***	5,23±0,149*	5,72±0,218*	1,286±0,124
Leptin, ng / ml	16,22±4,106*/***	20,22±3,768*	30,28±4,357*	4,72±0,153
HbA_{1C}, %	6,55±0,326*	7,69±0,085*	8,23±0,962*	4,42±0,577

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

to the group of healthy individuals was found 2,6, 3,1 and 4,2 times higher. The content of leptin was significantly 1,9 times higher in the group with Leu/ Leu genotype compared with the group whith Pro/ Pro genotype and corresponding 3,4, 4,3 and 6,4 times higher in the groups with Pro/Pro, Pro/Leu and Leu/Leu genotypes in relation to the control group ($p<0,05$).

The level of C-peptide in the groups with Pro/ Leu and Leu/Leu genotypes was significantly 28,9 % and 43,8 % higher than the value of this indicator in the group with Pro/Pro genotype. The level of C-peptide in all groups of the main group, namely in Pro/Pro, Pro/Leu and Leu/Leu patients compared with the control group was 3,1, 3,9 and 4,5 times higher respectively.

A significant rise in glucose level in all patients of the main group compared with the control one, namely in the groups with Pro/Pro, Pro/Leu and Leu/ Leu genotypes was established to be 33,5 %, 58,4 % and 73,5 % higher correspondingly without credible intergroup differences ($p<0,05$).

A significantly higher value of HOMA-IR has been obtained in the group of patients homozygous for the mutant allele compared with groups with Pro/ Leu and Leu/Leu genotypes 2,1 and 2,2 times respectively. HOMA-IR value was credibly 4,5, 4,3 and 9,3 times higher in patients with Pro/Pro, Pro/ Leu and Leu/Leu genotypes compared with the control group respectively.

The level of HbA_{1c} was significantly 1,5, 1,8 and 1,9 times higher in patients with Pro/Pro, Pro/ Leu and Leu/Leu genotypes in the main group in relation to the control group respectively ($p<0,05$). There wasn't any reliable group difference depending on Pro/Leu polymorphism of GPX1.

These results coincide with the data by Hironori Kobayashi and co-authors, who revealed GPX1 in adipocytes and described the reduction in activity of this enzyme in hypertrophied adipocytes of patients with type 2 diabetes. The authors believe that against the background of the free radical processes activation in adipocytes in patients with diabetes mellitus type 2 and as a result of reduced GPX1 production, inhibition of phosphorylation of insulin receptors with subsequent development of insulin resistance develops [9].

Conclusions

1. In patients with arterial hypertension against the background of abdominal obesity the risk of reduction of glutathione peroxidase1 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 arterial hypertension against the background of abdominal obesity is connected with the disorder of carbohydrate metabolism as a result of insulin and leptin resistance development.

Prospects for further research. The survey results indicate the necessity of development of effective measures for carbohydrate metabolism correction in hypertensive patients against the background of abdominal obesity.

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ОСОБЕННОСТИ УГЛЕВОДНОГО ОБМЕНА У ПАЦИЕНТОВ С АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИЕЙ НА ФОНЕ АБДОМИНАЛЬНОГО ОЖИРЕНИЯ В ЗАВИСИМОСТИ ОТ PRO197LEU ПОЛИМОРФИЗМА ГЕНА GPX 1

Н.О. Абрамова, Н.В. Пашковская

Резюме. Нами исследовано Pro197Leu полиморфизм гена GPX1 у 102 больных с артериальной гипертензией и сопутствующим абдоминальным ожирением и 97 практически здоровых лиц. В основной группе выявлено нарушение распределения частот генотипов по сравнению с группой контроля за счет снижения частоты Pro/Pro генотипа. При анализе полученных данных мы обнаружили рост риска нарушения активности GPX1 у лиц с Pro/Leu и Leu/Leu вариантами полиморфизма по сравнению с гомозиготами по «дикому» аллелю в 4,7 и 6,9 раза соответственно. При анализе изменений углеводного обмена в зависимости от полиморфизма Pro197Leu гена GPX1, мы установили, что у лиц с Leu/Leu генотипом достоверно возрастала продукция имунореактиного инсулина, лептина, С-пептида и соответственно повышался HOMA - IR. Итак, Pro-аллель обладает протекторными свойствами по снижению активности глутатионпероксидазы. У носителей Leu-аллеля развивается инсулино- и лептинорезистентнисть, что приводит к возникновению нарушений углеводного обмена.

Ключевые слова: Pro197Leu полиморфизм гена GPX1, углеводный обмен, инсулинорезистентность, абдоминальное ожирение, метаболический синдром.

ОСОБЛИВОСТІ ВУГЛЕВОДНОГО ОБМІНУ У ПАЦІЄНТІВ ІЗ АРТЕРІАЛЬНОЮ ГІПЕРТЕНЗІЄЮ НА ТЛІ АБДОМІНАЛЬНОГО ОЖИРІННЯ ЗАЛЕЖНО ВІД PRO197LEU ПОЛІМОРФІЗМУ ГЕНА GPX 1

Н.О. Абрамова, Н.В. Пашковська

Резюме. Нами досліджено Pro197Leu поліморфізм гена GPX1 у 102 хворих на артеріальну гіпертензію із супутнім абдомінальним ожирінням та 97 практично здорових осіб. В основній групі виявлено порушення розподілу частот генотипів порівняно із групою контролю за рахунок зниження частоти Pro/Pro генотипу. При аналізі отриманих даних ми виявили зростання ризику порушення активності GPX 1 у осіб із Pro/Leu та Leu /Leu варіантами поліморфізму порівняно із гомозиготами за «диким» алелем у 4,7 та 6,9 раза. Під час аналізу змін вуглеводного обміну залежно від поліморфізму Pro197Leu гена GPX1, ми встановили, що в осіб із Leu /Leu генотипом вірогідно зростала продукція імунореактиного інсуліну, лептину, С-пептиду та відповідно підвищувався HOMA-IR. Отже, Pro-алель володіє протекторними властивостями щодо зниження активності глутатіонпероксидази. У носіїв Leu-аллеля розвивається інсуліно- та лептинорезистентність, що призводить до виникнення порушень вуглеводного обміну.

Ключові слова: Pro197Leu поліморфізм гена GPX1, вуглеводний обмін, інсулінорезистентність, абдомінальне ожиріння, метаболічний синдром.

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