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EFFECT OF PRO197LEU POLYMORPHISM OF THE GENE GPX1 ON CARBOHYDRATE METABOLISM AND ANTHROPOMETRIC INDICES OF PATIENTS WITH ARTERIAL HYPERTENSION AGAINST THE BACKGROUND OF ABDOMINAL OBESITY

Summary. *The aim of our study was to investigate the dependence of carbohydrate metabolism in patients with arterial hypertension and concomitant abdominal obesity depending on Pro197Leu polymorphism of the GPX1 gene. 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 on account of the reduction of Pro/Pro genotype frequency have been found in the main group. Analyzing the data, the growth of risk of disorder in the GPX1 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 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 increased significantly, HOMA-IR and BMI was significantly higher compared with the persons with Pro/Pro genotype. 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, which causes disturbances in carbohydrate metabolism.*

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

Introduction

Cytoplasmic glutathione peroxidase (GPX1) 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 reduced glutathione with these substances [4, 6, 8, 10]. It is known that numerous pathologic processes in the organism develop in consequence of disorders in the mechanisms of antioxidant protection. Specially, in patients with insulin resistance accompanied by hyperglycemia and increased production of cytokines, oxidative stress increases. The accumulation of free radicals activates factors of transcription such as NFkB, which initiate the process of proinflammatory cytokines release [7]. The growth of free radicals results in lipid peroxidation of cellular membranes, causes atherosclerosis and endothelial dysfunction [11]. We studied single nucleotide polymorphism of the gene GPX1 for going into the question of the dependence of these processes upon the disorders of redox homeostasis. The human gene GPX1 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 missense — functional polymorphisms [1]. Pro-allele is wild, while Leu- is a mutant allele. The presence of Leu-allele causes depression of GPX1 sensibility to stimulating factors [5].

Thus, Bastaki et al. discovered that GPX1 activity 6 times slows down in homozygous patients for the Leu-allele [4]. T.V. Zheykova et al. found out that the homozygous for mutant allele more often suffered from coronary artery disease and myocardial infarction at the age of up to until 50 years [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 GPX1 gene.

Material and Methods

Pro197Leu polymorphism of the gene GPX1 have been studied in 102 patients and 97 healthy individuals by isolation of 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 (Federal State Scientific Institution «Central Research Institute of Epidemiology» (FSSI CRIE), Russia) were used for DNA isolation from lymphocytes according to instructions. PCR samples were prepared by means of the set AmpliSens-200-1 (FSSI CRIE, 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 transilluminator in the presence of a marker of molecular mass 100–1000 Bq (Fermentas, USA).

Pearson's χ^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 of 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 (HbA1C) 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 D.R., 1985).

Anthropometric indices such as body mass index (BMI) were calculated according to Quetelet and waist to hip (W/H) ratio was measured [2].

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 ($\chi^2 = 7.0$, $p < 0.05$), while there hasn't been found out 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 $\chi^2 = 2.6$, $p > 0.05$).

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, 95% CI = 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, 95% CI = 0.93–3.97; Table 1).

So, the risk of reduction of GPX1 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 properties concerning the development of redox system violation.

When studying the dependence of carbohydrate metabolism on Pro197Leu polymorphism of GPX1 gene, a significantly higher level of IRI in homozygous group for mutant allele comparing with 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 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 with Pro/Pro genotype and, respectively 3.4; 4.3 and 6.4 times higher in the groups with Pro/Pro, Pro/Leu and Leu/Leu genotypes compared with 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, respectively 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 HbA1c 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

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

Genotypes frequency	Cases	Controls	χ^2	p	OR	95% CI
	102	97				
Pro/Pro	0.176	0.402	12.91	0.002	0.32	0.17–0.61
Pro/Leu	0.578	0.454			1.65	0.94–2.90
Leu/Leu	0.245	0.144			1.92	0.93–3.97

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 GPX1

Index	Genotypes GPX1, n = 102			Control group, n = 20
	Pro/Pro	Pro/Leu	Leu/Leu	
Glucose, mmol/l	6.320 ± 0.156*	7.490 ± 0.112*	8.210 ± 0.168*	4.730 ± 0.174
Immunoreactive insulin, IU/ml	15.790 ± 2.438*/***	18.648 ± 2.362*/***	25.690 ± 2.108*	6.110 ± 1.314
HOMA-IR	4.350 ± 0.124*/***	4.187 ± 0.183*/***	8.970 ± 0.367*	0.970 ± 0.035
C-peptide, ng/ml	3.980 ± 0.183*/**/***	5.230 ± 0.149*	5.720 ± 0.218*	1.286 ± 0.124
Leptin, ng/ml	16.220 ± 4.106*/***	20.220 ± 3.768*	30.280 ± 4.357*	4.720 ± 0.153
HbA1C, %	6.550 ± 0.326*	7.690 ± 0.085*	8.230 ± 0.962*	4.420 ± 0.577
BMI, kg/m^2	28.317 ± 4.140*/***	31.62 ± 4.68*	33.69 ± 4.75*	24.88 ± 2.98
Waist to hip ratio	0.937 ± 0.017*	1.050 ± 0.048*	1.159 ± 0.068*	0.680 ± 0.032

Notes: n — number of observations; * — the probability of changes in relation to control; ** — the probability of changes in relation to the group with Pro/Leu genotype; *** — chance changes in relation to group with Leu/Leu genotype.

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].

A credible 33.1% rise of BMI in patients with Leu/Leu genotype in relation to patients from the group with Pro/Pro genotype has been revealed by analyzing anthropometric indicators. At that, in all patients from the main group, namely with Pro/Leu and Leu/Leu genotypes, BMI value was credible 27.1 and 35.5 % higher compared with the group of healthy people, respectively. That corresponds with T.V. Zheykova results, she established a connection between Pro/Pro polymorphism and with significantly lower BMI [1]. BMI growth is probably connected with insulin resistance.

A reliable growth of W/H ratio in all patients of the main group, namely with Pro/Pro, Pro/Leu and Leu/Leu genotypes has been obtained 37.8; 54.3 and 70.5 % higher, respectively without any reliable intergroup difference.

Conclusions

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

3. Patients with arterial hypertension against the background of abdominal obesity, carriers of Leu-allele, are characterized by a higher body mass index and waist to hip ratio compared with homozygous for the Pro-allele.

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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ВЛИЯНИЕ PRO197LEU ПОЛИМОРФИЗМА ГЕНА GPX1 НА ПАРАМЕТРЫ УГЛЕВОДНОГО ОБМЕНА И АНТРОПОМЕТРИЧЕСКИЕ ОСОБЕННОСТИ У ПАЦИЕНТОВ С АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИЕЙ НА ФОНЕ АБДОМИНАЛЬНОГО ОЖИРЕНИЯ

Резюме. Целью нашего исследования было изучение зависимости показателей углеводного обмена у пациентов с артериальной гипертензией и сопутствующим абдоминальным ожирением от Pro197Leu полиморфизма гена GPX1. Полиморфизм Pro197Leu гена GPX1 изучен у 102 больных с артериальной гипертензией и сопутствующим абдоминальным ожирением и у 97 практически здоровых лиц. В основной группе выявлены нарушения распределения частоты 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 аллелей дикого типа в 4,7 и 6,9 раза. При анализе изменений углеводного обмена в зависимости от полиморфизма Pro197Leu гена GPX1 мы установили, что у лиц с Leu/Leu генотипом достоверно повышалась продукция иммунореактивного инсулина, лептина, С-белка, повышался и HOMA-IR, а также выявлены достоверно более высокие значения индекса массы тела по сравнению с лицами с Pro/Pro генотипом. Итак, Pro-аллель обладает протекторными свойствами в отношении снижения активности глутатионпероксидазы. У носителей Leu-аллели развивается инсулино- и лептинорезистентность, что приводит к возникновению нарушений углеводного обмена.

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

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ВПЛИВ PRO197LEU ПОЛІМОРФІЗМУ ГЕНА GPX1 НА ПОКАЗНИКИ ВУГЛЕВОДНОГО ОБМІНУ ТА АНТРОПОМЕТРИЧНІ ОСОБЛИВОСТІ ПАЦІЄНТІВ З АРТЕРІАЛЬНОЮ ГІПЕРТЕНЗІЄЮ НА ТЛІ АБДОМІНАЛЬНОГО ОЖИРІННЯ

Резюме. Метою нашого дослідження було вивчення залежності показників вуглеводного обміну в пацієнтів з артеріальною гіпертензією та супутнім абдомінальним ожирінням від Pro197Leu поліморфізму гена GPX1. 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 з групою контролю за рахунок зниження частоти Pro/Pro генотипу. При аналізі отриманих даних ми виявили зростання ризику порушення активності GPX1 в осіб із Pro/Leu та Leu/Leu варіантами поліморфізму порівняно із гомозиготами алелей дикого типу в 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 імунореактивного інсуліну, лептину, С-білка, підвищувався і HOMA-IR, а також виявлені вірогідно вищі значення індексу маси тіла порівняно з особами із Pro/Pro генотипом. Отже, Pro-алель має протекторні властивості щодо зниження активності глутатіонпероксидази. У носіїв Leu-алелі розвивається інсуліно- та лептинорезистентність, що призводить до виникнення порушень вуглеводного обміну.

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