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EFFECT OF DISTRIBUTION DIO1 AND GPX1 GENE POLYMORPHISM ON THE FUNCTIONAL CONDITION OF THE ENDOTHELIUM IN PATIENTS WITH CHRONIC DIFFUSE LIVER DISEASES

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Abstract. In patients with chronic diffuse liver diseases there is a relation between the expression of Pro197Leu polymorphism of GPX1 gene and indices of cellular adhesion, which is revealed by a reliably higher content of soluble intercellular adhesion molecule – 1 type in the blood serum in homozygotic carriers of Leu-allele. Changes of the endothelial functional state in patients with chronic diffuse liver diseases are associated with Pro197Leu polymorphism of GPX1 gene, which is proved by a reliably higher index of desquamated endotheliocytes amount and lower level of NO metabolites in the carriers of LeuLeu-genotype.

Examination of the indices of cellular adhesion and functional endothelial state in patients with chronic diffuse liver diseases did not reveal their dependence on the distribution of A/C polymorphism of DIO1 gene.

Key words: chronic diffuse liver disease, gene polymorphism, endothelium.

Genetic polymorphism is the basis of a phenotype difference of peculiarities, and it can stipulate congenital susceptibility to various diseases. The study of this question draws much attention to the gene coding factors involved in the development of variable pathology [5, 7].

The analysis of genetic associations plays an important role in the examination

of the role of genetic factors involved in the development of polymorphic diseases and chronic diffuse liver disease (CDLD) in particular. The difference of marker allele frequency in patients with certain pathology and healthy individuals gives the evidence to draw a conclusion about the link between a particular allele and corresponding pathology [1,7; 3,15]. The information available concerning the links of CDLD pathogenesis allows detecting the range of genes-candidates which potential relation with this pathology needs further investigation [6,29; 8,797].

Due to recent scientific research both of Ukrainian and foreign scientists the concept of relations between indices of cytokine regulation, endothelium functional state, fibrinolysis and expression of various genes is beyond any doubt [7, 875;9, 321]. Although dependence of the above indices upon DIO1 and GPX1 Pro197Leu gene A/C polymorphism in patients with CDLD remains above the attention of researchers.

Objectives: to study peculiarities of the indices of the cellular adhesion, endothelial functional state and fibrinolysis in patients with CDLD and disorders depending on A/C polymorphism in DIO1 and GPX1 Pro197Leu gene.

Materials and methods. 28 patients with CDLD aged from 34 to 72 were examined. Depending on the distribution of DIO1 gene A/C polymorphism the patients were divided into three groups: AA-genotype carriers – 9 patients, AC-genotype – 11, CC-genotype – 8. Depending on GPX1 gene Pro197Leu polymorphism there were 12 homozygotes by Pro-allele, 8 – by Leu-allele and 8 ProLeu-heterozygotes.

The diagnosis of CDLD was made on the basis of anamnesis, generally accepted complex of clinical-laboratory and instrumental investigation methods, USD of the abdominal organs. Patients with chronic hepatitis and cirrhosis of a viral etiology, Wilson-Konovalov disease, congenital insufficiency of α-antitripsin (α-inhibitor of proteinase), idiopathic (genetic) hemochromatosis, autoimmune hepatitis were excluded from the study.

Alleles of A/C regions in DIO1 gene and Pro197Leu in GPX1 gene were studied by means of excretion of genome DNA from leukocytes of the peripheral blood with further amplification of a polymorphic region by means of polymerase chain reaction (PCR) on the programmed amplificatory "Amply-4L" ("Biocom", Moscow) with individual temperature program for the parameters of every gene. Table 1 presents succession of oligonucleotides in primers and their calculation positions on chromosomes.

Table 1

Succession of oligonucleotides in primers used for polymerase chain reaction (PCR) to identify A/C polymorphism of DIO1 gene and Pro197Leu of GPX1 gene

Gene name	*Gene localization on chromosome*	*Primer*	*Succession of oligonucleotides in primers*
DIO1	1 p33-p32	Direct	5'-GAACTTGATGTGAAGGCTGGA-3'
		Reverse	5'-TAACCTCAGCTGGGAGTTGTTT-3'
GPX1	3p21	Direct	5'-TCGAAGCCCTGCTGTCTCA-3'
		Reverse	5'-CGAGACAGCAGCACTGCAA-3'

DNA extraction was conducted by means of "DNA-sorb-B" reagents, variant 100 (Russian) according to the instruction. Purified DNA was kept under the temperature of 20±20C. Samples for PCR were prepared by means of "AmplySense – 200 – 1" set (Russian). Bcl I restriction endonuclease produced by "SibEnzyme" firm (Russian) was used to discriminate DIO1 gene alleles.

The content of soluble intercellular adhesion molecule – 1 type (ICAM-1) in the blood serum was detected by immunoenzymatic method with the use of commercial test system "BenderMedSystems" (Austria).

Functional endothelial state was estimated by the content of NO metabolites and the amount of desquamated endothelial cells in the blood. NO content in the blood serum was estimated by the concentration of its final stable metabolite – NO2 and the content of total final metabolites NO (nitrates+nitrites). The method to detect NO2 content in the venous blood plasma is based on the photocolorimetric detection of optic density of NO2 stained complex by Griess test [2, 4]. The amount of desquamated endothelial cells (EC) in the blood was estimated by J.Hladovec method in N.Petrishchev et al. modification [4, 51].

Total non-enzymatic and enzymatic fibrinolysis of citrated blood plasma was estimated by asofibrinolysis (Simko Ltd., Ukraine).

The results obtained are calculated by means of Biostat program with the use of Student t-criterion.

Results and Discussion.

Table 2

Indices of cellular adhesion, functional endothelial state and fibrinolysis in patients with chronic diffuse liver disease depending on A/C polymorphism of DIO1 gene (M±m)

Index	*Control group n=20*	*Genotypes of DIO1 gene, n=28*		
		AA n=9	*AC n=11*	*CC n=8*
ICAM-1, ng/mL	259,60+10,324	346,17±18,532 P1<0,001	325,82±21,491 P1<0,01 P2>0,05	361,06±24,796 P1<0,001 P2<0,01 P3>0,05
Stable NO metabolites (NO2, NO3), mcmol/L	18,14±0,684	12,23±1,713 P1<0,01	11,12±0,438 P1<0,0001 P2>0,05	12,43±1,785 P1<0,01 P2>0,05 P3>0,05
Endothelial cells, x 104/L	3,04±0,204	5,58±0,753 P1<0,01	5,35±0,254 P1<0,001 P2>0,05	5,75±0,649 P1<0,001 P2>0,05 P3>0,05

Total fibrinolyitc activity, mcmol azofibrin/1mL per hour	1,63±0,041	1,33±0,072 P1<0,001	1,27±0,047 P1<0,001 P2>0,05	1,32±0,050 P1<0,001 P2>0,05 P3>0,05
Non-enzymatic fibrinolytic activity, mcmol azofibrin/1mL per hour	0,51±0,019	0,67±0,038 P1<0,001	0,68±0,029 P1<0,001 P2>0,05	0,70±0,020 P1<0,001 P2>0,05 P3>0,05
Enzymatic fibrinolytic activity, mcmol azofibrin/1mL per hour	1,12±0,051	0,66±0,084 P1<0,001	0,59±0,072 P1<0,001 P2>0,05	0,62±0,065 P1<0,001 P2>0,05 P3>0,05

Notes: n – numbers of obseravtions;

P1 – probability of changes concerning the control

P2 – probability of changes concerning the group of patients with AA-genotype

P3 – probability of changes concerning the group of patients with AC-genotype

The content of ICAM-1 in the serum of homozygotic carriers of A-allele increases in 33,3% (P1< 0,001) concerning the value in the control group against in 25,5% (P1<0,01) and 39,1% (P1<0,001) respectively in the groups of AC- and CC-genotype carriers.

With AA-genotype a reliable 1,5 decrease (P1<0,01) of NO stable metabolites content and 1,8 increase (P1<0,01) of desquamated endotheliocytes were observed in the blood of patients in comparison with the indices of the control group. For AC-heterozygotes it was in 1,6 and 1,8 times correspondingly (P1<0,001), and for CC-homozygotes – in 1,5 and 1,9 times (P1<0,001).

Examination of fibrinolytic blood activity showed that total fibrinolytic activity (TFA) of the blood plasma in all the group of patients was reliably lower than that of the control indices: CC-genotype – on 19% (P1<0,001), AC-genotype and AA-genotype – on 22,1% and 18,4% correspondingly (P1<0,001) without reliable intergroup difference. At the same time non-enzymatic fibrinolytic activity (NEFA) in all groups of patients increases in comparison with the control on 37,3% (P1<0,001), 33,3% and 31,4% correspondingly (P1<0,001). TFA index in patients with AA-genotype was reliably lower than that of the control in 1,7 times (P1<0,001), CC-genotype – in 1,8 times (P1<0,001), while for the patients with AC-genotype a maximal inhibition of TFA was registered – in 1,9 times (P1<0,001).

Table 3 presents the results of examination of cellular adhesion, functional endothelial state and fibrinolysis in patients with CDLD depending on the distribution of Pro197Leu polymorphism of GPX1 gene.

Reliable increase of ICAM-1 content in the blood serum of all the groups concerning the control values was found: for the carriers of ProPro-genotype – on 19,1% (P1<0,001),

ProLeu-genotype – on 25,4% (P1<0,001) and 49,2% (P1<0,001) for the patients with LeuLeu-genotype. LeuLeu-genotype carriers presented the value of this index on 29,9% higher (P1<0,001) than that of the patients with ProPro-genotype.

Pro-allele homozygotes revealed reliable decrease of stable NO metabolites in the blood in 1,3 (P1<0,01) in comparison with the control value, Leu-allele ones – in 1,8 times correspondingly (P1<0,001), ProLeu-heterozygotes – in 1,6 times (P1<0,001). Reliably lower level of NO metabolites (on 23,9%, P1<0,001) was found in the blood of LeuLeu-genotype carriers as compared with the patients of ProPro-genotype.

Table 3

Indices of the endothelial function and fibrinolysis in patients with CDLD depending on Pro197Leu polymorphism of GPX1 gene (M±m)

Index	*Control group n=20*	*Genotypes of GPX1 gene, n=28*		
		ProPro, n=12	ProLeu, n=8	LeuLeu, n=8
ICAM-1, ng/mL	259,60+10,324	309,23±12,463 P1<0,01	351,38±18,274 P1<0,001 P2>0,05	387,41±20,108 P1<0,001 P2<0,01 P3>0,05
Stable NO metabolites (NO2, NO3, mcmol/L)	18,14±0,684	13,45±1,002 P1<0,01	11,45±1,139 P1<0,001 P2>0,05	10,24±1,012 P1<0,001 P2<0,05 P3>0,05
Endothelial cells, x 104/L	3,04±0,204	4,63±0,320 P1<0,001	5,83±0,549 P1<0,001 P2>0,05	6,27±0,625 P1<0,001 P2<0,05 P3>0,05
Total fibrinolytic activity, mcmol azofibrin/1mL per hour	1,63± 0,041	1,27±0,049 P1<0,001	1,31±0,062 P1<0,001 P2>0,05	1,33±0,055 P1<0,01 P2>0,05 P3>0,05
Non-enzymatic fibrinolytic activity, mcmol azofibrin/1mL per hour	0,51±0,019	0,64±0,034 P1<0,001	0,69±0,022 P1<0,001 P2>0,05	0,72±0,024 P1<0,001 P2>0,05 P3>0,05
Enzymatic fibrinolytic activity, mcmol azofibrin/1mL per hour	1,12±0,051	0,61±0,083 P1<0,001	0,68±0,077 P1<0,001 P2>0,05	0,56±0,074 P1<0,001 P2>0,05 P3>0,05

Notes: n – numbers of obseravtions;

P1 – probability of changes concerning the control

P2 – probability of changes concerning the group of patients with ProPro-genotype

P3 – probability of changes concerning the group of patients with ProLeu-genotype

While comparing the index of desquamated endotheliocytes amount in the blood of patients with CDLD depending on Pro197Leu of GPX1 gene with the control value its index has been found to increase in 1,5 times (P<0,001) in the group with ProPro-genotype, in 1,9 times (P<0,001) in the group of patients with ProLeu-genotype, and in 2,1 times (P<0,001) in the group with LeuLeu-genotype. The value of the given index in the blood of LeuLeu-genotype carriers was found to be reliably higher than that in patients with ProPro-genotype on 35,4% (P<0,05).

Examination of fibrinolytic blood activity showed that TFA of the blood plasma in patients of all the groups was reliably lower than that of the control values: in patients with ProPro-genotype – on 22,1% (P<0,001), with ProLeu-genotype and LeuLeu-genotype – on 19,6% (P<0,001) and 18,4% (P<0,01) respectively without reliable difference between the groups.

NEFA in patients of all the groups elevated, and increasing of this index in comparison with the control group was indicative of it: on 25,5% (P<0,001), 35,3% (P<0,001), and 41,2% (P<0,001) in the carriers of ProPro-, ProLeu- and LeuLeu-genotype respectively

Thus, homozygotic carrier of Leu-allele in patients with CDLD is associated with a reliable higher level of ICAM-1 in the blood serum, index of endotheliocytemia and lower level of NO stable metabolites.

Perspectives of further study. The results obtained are indicative of the necessity to create a differentiated approach concerning the diagnostics and correction of the disorders of cellular adhesion and functional endothelial state in case of chronic diffuse liver diseases depending on the expression of A/C polymorphism of DIO1 gene and Pro197Leu of GPX1 gene.

CONCLUSIONS

Examination of the indices of cellular adhesion, functional endothelial state and fibrinolysis in patients with CDLD did not reveal their dependence on the distribution of A/C polymorphism of DIO1 gene.

In patients with CDLD there is a relation between the expression of Pro197Leu polymorphism of GPX1 gene and indices of cellular adhesion, which is revealed by a reliably higher content of intercellular adhesion molecules of the 1 type (ICAM-1) in the blood serum in homozygotic carriers of Leu-allele.

Changes of the endothelial functional state in patients with chronic diffuse liver diseases are associated with Pro197Leu polymorphism of GPX1 gene, which is proved by a reliably higher index of desquamated endotheliocytes amount and lower level of NO metabolites in the carriers of LeuLeu-genotype.

Pro197Leu polymorphism of GPX1 gene does not influence upon the indices of the system of fibrinolysis in patients with chronic diffuse liver diseases.

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