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ASSOCIATION OF MARKERS OF THE SYSTEM OF FIBRINOLYSIS OF THE BLOOD PLASMA WITH POLYMORPHISM OF SELENOENZYMES GENES IN PATIENTS WITH CHRONIC DIFFUSE LIVER DISEASES

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Introduction. 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 in particular [2, 4, 5, 9]. 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 [3, 6]. The information available concerning the links of chronic diffuse liver disease pathogenesis allows detecting the range of genes-candidates which potential relation with this pathology needs further investigation [6, 8].

Due to recent scientific research both of Ukrainian and foreign scientists the concept of relations between indices of fibrinolysis and expression of various genes is beyond any doubt [1,7,8]. Although dependence of the above indices upon A/C polymorphism DIO1 and Pro197Leu GPX1gene in patients with chronic diffuse liver disease remains above the attention of researchers.

The aim of the study. To study peculiarities of the indices of the fibrinolysis in patients with chronic diffuse liver diseases depending on A/C polymorphism in DIO1 and GPX1 Pro197Leu gene.

Materials and methods. 28 patients with chronic diffuse liver disease 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 chronic diffuse liver disease 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 б-antitripsin (б-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.

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 ± 2^0C. Samples for PCR were prepared by means of "AmplySense – 200 – 1" set (Russian). BcI I restriction endonuclease produced by "SibEnzyme" firm (Russian) was used to discriminate DIO1 gene alleles.

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. The indices of fibrinolysis in patients with chronic diffuse liver disease did not experience reliable changes depending on polymorphism of DIO1 gene and were statistically different from the group of practically healthy individuals (table 2).

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'

Examination of fibrinolytic blood activity showed that total fibrinolytic activity 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 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). Total fibrinolytic activity 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 total fibrinolytic activity was registered – in 1,9 times (P1<0,001).

Table 3 presents the results of examination of fibrinolysis in patients with chronic diffuse liver disease depending on the distribution of Pro197Leu polymorphism of GPX1 gene.

Examination of fibrinolytic blood activity showed that total fibrinolytic activity 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.

Non-enzymatic fibrinolytic activity 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.

Table 2.

Indices of the 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
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;
P_1 – probability of changes concerning the control
P_2 – probability of changes concerning the group of patients with AA-genotype
P_3 – probability of changes concerning the group of patients with AC-genotype

Table 3.

Indices of the fibrinolysis in patients with chronic diffuse liver diseases 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
Total fibrinolyitc 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;
P_1 – probability of changes concerning the control
P_2 – probability of changes concerning the group of patients with ProPro-genotype
P_3 – probability of changes concerning the group of patients with ProLeu-genotype

Thus, A/C polymorphism of DIO1 gene and 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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CONCLUSIONS

1. Examination of the indices of fibrinolytic blood activity in patients with chronic diffuse liver disease showed that total fibrinolytic activity and enzymatic fibrinolytic activity was reliably lower than that of the control indices at the same time non-enzymatic fibrinolytic activity increases.

2. Pro197Leu polymorphism of GPX1 gene and A/C polymorphism of DIO1 gene does not influence upon the indices of the system of fibrinolysis in patients with chronic diffuse liver diseases.

The prospects of proceeding investigations will be further studies of pathogenetic peculiarities chronic diffuse liver diseases with the aim to find the mechanisms of their occurrence and progress and substantiation of the improved methods to correct and prevent the given pathology.

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АСОЦІАЦІЯ МАРКЕРІВ СИСТЕМИ ФІБРИНОЛІЗУ ПЛАЗМИ КРОВІ ЗІ ПОЛІМОРФІЗМОМ ГЕНІВ СЕЛЕНОЕНЗИМІВ У ХВОРИХ НА ХРОНІЧНІ ДИФУЗНІ ЗАХВОРЮВАННЯ ПЕЧІНКИ

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Резюме. Дистрибуція А/С поліморфізму гена DIO1 і Pro197Leu поліморфізму гена GPX1 не впливає на показники системи фібринолізу у хворих на хронічні дифузні захворювання печінки.

Ключові слова: хронічні дифузні захворювання печінки, поліморфізм генів, фібриноліз.

АССОЦИАЦИЯ МАРКЕРОВ СИСТЕМЫ ФИБРИНОЛИЗА ПЛАЗМЫ КРОВИ С ПОЛИМОРФИЗМОМ ГЕНОВ СЕЛЕНОЭНЗИМОВ У БОЛЬНЫХ ХРОНИЧЕСКИМИ ДИФФУЗНЫМИ ЗАБОЛЕВАНИЯМИ ПЕЧЕНИ

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Summary. Дистрибуция А/С полиморфизма гена DIO1 и Pro197Leu полиморфизма гена GPX1 не влияет на показатели системы фибринолиза у больных хроническими диффузные заболевания печени.

Ключевые слова: хронические диффузные заболевания печени, полиморфизм генов, фибринолиз.