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EFFECT OF DISTRIBUTION PRO197LEU POLYMORPHISM OF GLUATHIONE PEROXIDASE-1 GENE ON THE INDICES OF THE SYSTEM OF BLOOD PLASMA FIBRINOLYSIS IN PATIENTS WITH CIRRHOSIS

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This research aimed at studying peculiarities of the indices of fibrinolysis system in patients with liver cirrhosis depending on Pro197Leu glutathione peroxidase-1 gene polymorphism. Methods. 28 patients with liver cirrhosis were involved into the study. We assessed Pro197Leu polymorphism gluathione peroxidase-1 gene, total, non-enzymatic, and enzymatic fibrinolysis of blood plasma. Results. Estimation of the indices of fibrinolytic blood activity in patients with liver cirrhosis showed that total fibrinolytic activity and enzymatic fibrinolytic activity were reliably lower than that of the control indices at the same time non-enzymatic fibrinolytic activity increased and did not reveal their dependence on the distribution of Pro197Leu polymorphism of glutathione peroxidase-1 gene. Conclusions. Pro197Leu polymorphism of glutathione peroxidase-1 gene does not influence upon the indices of the system of fibrinolysis in patients with liver cirrhosis.

Key words: liver cirrhosis, polymorphism, glutathione peroxidase-1 gene, fibrinolysis.

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Introduction

The data available concerning the components of chronic diffuse liver disease pathogenesis allow us to detect the range of genes-candidates, whose potential relations with this pathology need further investigation [6, 11]. The difference of marker allele frequency in patients with certain pathology and healthy individuals gives the evidence to draw a conclusion about the correlation between a particular allele and the pathology studied [2, 4, 6, 11]. The analysis of genetic associations plays an important role in the evaluating genetic factors involved in the development of polymorphic diseases, and liver cirrhosis in particular [6, 10, 11].

Due to recent scientific research both of Ukrainian and international scientists the concept of relations between indices of fibrinolysis and expression of various genes is beyond any doubt [1, 3, 5, 9]. Nevertheless, the dependence of the above indices upon Pro197Leu glutathione peroxidase-1 (GPX1) gene in patients with liver cirrhosis deserves more detailed study.

This research aims at studying peculiarities of the indices of the fibrinolysis in patients with liver cirrhosis depending on Pro197Leu polymorphism in GPX1 gene.

Materials and methods

30 patients with liver cirrhosis aged from 39 to 68 were examined. Depending on the distribution of GPX1 gene Pro197Leu polymorphism the patients were divided into three groups: ProPro -genotype carriers – 12 patients, ProLeu -genotype – 9, LeuLeu -genotype – 9.

The diagnoses of liver cirrhosis were verified on the basis of complaints, anamnesis, objective status, and standard laboratory techniques of examination (general clinical blood and urine analyses, biochemical blood test – general bilirubin and its fractions, sublimate and thymol tests, ionogram, proteinogram, coagulogram). The activity of the following blood enzymes was examined: alaninaminotranspherase (AlAT), aspartate amino transpherase (AsAT), gamma glutamyl transpherase (GGT), alkali phosphatase (AP). The levels of urea, creatinine were detected in the blood as well as serum markers of hepatitis B and C viruses. Instrumental examinations were conducted (USD of the abdominal organs, esophagogastroduodenofibroscopy (EGDFS)).

The degree of activity of liver cirrhosis was assessed by the clinical manifestations and biochemical signs as AlAT, AcAT activity, thymol test, blood bilirubin level [8]. The degree of liver cirrhosis compensation was estimated by the criteria of C.G. Child and J.G. Turcotte (1964) modified by K.N.H. Pugh (1973). The levels of bilirubin, albumins, prothrombin were evaluated in the blood serum, the presence of ascites and encephalopathy was found [7].

The patients with decompensated liver cirrhosis (III degree of hepatic-cellular failure, hypoalbuminemia less than 30%, III-IV degree of hepatic encephalopathy, resistant ascites, systemic hypotension), chronic hepatitis of a viral aetiology, Wilson's disease, congenital α_1-antitripsin insufficiency (α_1-inhibitor of proteinases), idiopathic (genetic) hemochromatosis, autoimmune hepatitis, diabetes mellitus, III-IV degree of chronic heart failure with ejection fraction of the left ventricle less than 45%, acute disorders of the cerebral circulation and acute coronary syndrome, psychic disorders, residents of the III-IV zones of radiation contamination, individuals during pregnancy or lactation period or those receiving oral contraceptives, with any acute inflammatory processes, other concomitant decompensated diseases or acute conditions able to affect the results of the study, were excluded from the investigation.

Alleles of Pro197Leu regions in GPX1 gene

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

DNA extraction was performed by "DNA-sorb-B" reagents, variant 100 (Russia) according to the instruction. Purified DNA was kept under the temperature of 20±2^{0}C. Samples for PCR were prepared by using "AmplySense – 200 – 1" (Russia).

Table 1

Succession of oligonucleotides in primers used for polymerase chain reaction (PCR) to identify Pro197Leu polymorphism of GPX1 gene

Gene name	Gene localization on chromosome	Primer	Succession of oligonucleotides in primers
GPX1	3p21	Direct	5'-TCGAAGCCCTGCTGTCTCA-3'
		Reverse	5'-CGAGACAGCAGCACTGCAA-3'

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

The results obtained are calculated by applying Biostat program with ranking by Student t-criterion.

Results and Discussion

Examination of blood fibrinolytic activity demonstrated a reliable decrease in total fibrinolytic activity index by 16,8% (P<0,001) due to reduced enzymatic portion of fibrinolysis (on 42,1%, P<0,001). The index of non-productive non-enzymatic, being by 43,1% higher (P<0,001) than that of the control, increased against this ground.

Thus, the patients with liver cirrhosis demonstrate inhibition of fibrinolytic blood plasma activity occurring due to the inhibition of enzymatic fibrinolysis as well as compensatory increase in non-enzymatic fibrinolytic activity.

Thus, endothelial dysfunction caused by pathological mechanisms such as oxidant stress and increased cellular adhesion is likely to inhibit fibrinolytic blood activity in the examined patients.

Table 2 presents the results of examination of fibrinolysis in the patients with liver cirrhosis 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 the patients of all the groups was reliably lower than that of the control values: in the patients with Pro-Pro-genotype by 19,0% (P<0,001), with ProLeu-genotype and LeuLeu-genotype by 16,6% (P<0,001) and 14,7% (P<0,01) respectively without reliable difference between the groups.

Table 2

Indices of the fibrinolysis in patients with live

r cirrhosis 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=9	LeuLeu, n=9
Total fibrinolyitc activity, mcmol azofibrin/1mL per hour	1,63± 0,041	1,32±0,049 P_1<0,001	1,36±0,058 P_1<0,001 P_2>0,05	1,39±0,051 P_1<0,01 P_2>0,05 P_3>0,05
Non-enzymatic fibrinolytic activity, mcmol azofibrin/1mL per hour	0,51±0,019	0,69±0,032 P_1<0,001	0,74±0,018 P_1<0,001 P_2>0,05	0,76±0,021 P_1<0,001 P_2>0,05 P_3>0,05
Enzymatic fibrinolytic activity, mcmol azofibrin/1mL per hour	1,12±0,051	0,65±0,081 P_1<0,001	0,71±0,070 P_1<0,001 P_2>0,05	0,59±0,072 P_1<0,001 P_2>0,05 P_3>0,05

Notes: *n- numbers of observations; P_1 – probability of changes relating the control; P_2 – probability of changes relating the group of the patients with ProPro-genotype; P_3 – probability of changes relating the group of the patients with ProLeu-genotype.*

Non-enzymatic fibrinolytic activity in the patients of all the groups elevated, and increasing of this index in comparison with the control group was indicative of the following: by 35,3% (P<0,001), 45,1% (P<0,001), and 49,0% (P<0,001) in the carriers of ProPro-, ProLeu- and LeuLeu-genotype respectively.

Reliable decrease of enzymatic fibrinolytic activity of all the groups concerning the control values was found: for the carriers of ProPro-genotype – on 42,0% (P_1<0,001), ProLeu-genotype – on 41,3% (P_1<0,001) and 51,2% (P_1<0,001) for the patients with LeuLeu-genotype.

Thus, Pro197Leu polymorphism of GPX1 gene does not influence upon the indices of the system of fibrinolysis in patients with liver cirrhosis.

Conclusions

Examination of the indices of fibrinolytic blood activity in patients with liver cirrhosis 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.

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

The prospects of further investigation require in-depth studies of the pathogenetic peculiarities of liver cirrhosis in order to find out the mechanisms of

their occurrence and progression and substantia-substantiation of the improved methods to correct and prevent the disease.

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Реферат

ВПЛИВ ДИСТРИБУЦІЇ PRO197LEU ПОЛІМОРФІЗМУ ГЕНА ГЛУТАТІОНПЕРОКСИДАЗИ 1-ГО ТИПУ НА МАРКЕРИ СИСТЕМИ ФІБРИНОЛІЗУ ПЛАЗМИ КРОВІ У ХВОРИХ НА ЦИРОЗ ПЕЧІНКИ

Чимпой К.А.

Ключові слова: цироз печінки, поліморфізм, глутатіонпероксидаза 1-го типу, ген, фібриноліз.

Мета дослідження. Вивчити особливості показників системи фібринолізу у хворих на цироз печінки залежно від Pro197Leu поліморфізму гена GPX1. Матеріали та методи. Обстежено 30 хворих на цироз печінки. Визначено Pro197Leu поліморфізм гена GPX1, загальну фібринолітичну, ферментативну та неферментативну фібринолітичну активність плазми крові. Результати. У хворих цирозом печінки встановлено порушення фібринолізу зі зниженням сумарної і ферментативної фібринолітичної активності плазми крові на тлі зростання непродуктивної неферментативної фібринолітичної активності, а також відсутність залежність показників системи фібринолізу від дистрибуції Pro197Leu поліморфізму гена GPX1. Висновки. Дистрибуція Pro197Leu поліморфізму гена GPX1 не впливає на показники системи фібринолізу у хворих на цироз печінки.

Реферат

ВЛИЯНИЕ ДИСТРИБУЦИИ PRO197LEU ПОЛИМОРФИЗМА ГЕНА ГЛУТАТИОНПЕРОКСИДАЗЫ 1-ГО ТИПА НА ПОКАЗАТЕЛИ СИСТЕМЫ ФИБРИНОЛИЗА ПЛАЗМЫ КРОВИ У БОЛЬНЫХ ЦИРРОЗОМ ПЕЧЕНИ

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Ключевые слова: цирроз печени, полиморфизм, глутатионпероксидаза 1-го типа, ген, фибринолиз.

Цель исследования. Изучить особенности показателей системы фибринолиза у больных циррозом печени в зависимости от Pro197Leu полиморфизма гена глутатионпероксидазы 1-го типа. Материалы и методы. Обследовано 30 больных с циррозом печени. Определено Pro197Leu полиморфизм гена GPX1, общая фибринолитическая, ферментативная и неферментативная фибринолитическая активность плазмы крови. Результаты. У больных циррозом печени установлено нарушение фибринолиза со снижением суммарной и ферментативной фибринолитической активности плазмы крови на фоне роста непродуктивной неферментативной фибринолитической активности, а также отсутствие зависимости показателей системы фибринолиза от дистрибуции Pro197Leu полиморфизма гена глутатионпероксидазы 1-го типа. Выводы. Дистрибуция Pro197Leu полиморфизма гена глутатионпероксидазы 1-го типа не влияет на показатели системы фибринолиза у больных циррозом печени.