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# Effect of GPX1 Gene Polymorphism Distribution on the Indices of Cellular Adhesion and Functional Condition of the Endothelium in Patients with Chronic Diffuse Liver Diseases

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**The aim.** To study peculiarities of the indices of the cellular adhesion and endothelial functional state in patients with CDLD (chronic diffuse liver diseases**)** depending on Pro197Leu polymorphism GPX1 gene.

**Methods.** 28 patients with CDLD aged were examined. We have determined Pro197Leu polymorphism GPX1 gene, the level of soluble intercellular adhesion molecule – 1 type (ICAM-1), desquamated endothelial cells and the content of nitrogen monoxide (NO) metabolites in the blood serum.

**Results.** 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 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.

**Conclusions.** It was establish dependence of level cellular adhesion and endothelial function in patients with chronic diffuse liver disease from gene polymorphism distribution Pro197Leu GPX1.

**Key words:** chronic diffuse liver disease, polymorphism, intercellular adhesion molecule, endothelium

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## БАУЫРДЫҢ СОЗЫЛМАЛЫ ДИФФУЗДЫ АУРУЫ БАР НАУҚАСТАРДА GPX1 ГЕНІ ПОЛИМОРФИЗМІ ДИСТРИБУЦИЯСЫНЫҢ ЭНДОТЕЛИЙДІҢ ҚЫЗМЕТ ЖАҒДАЙЫ МЕН ЖАСУШАЛЫҚ АДГЕЗИЯ КӨРСЕТКІШТЕРІНЕ ӘСЕРІ

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**Зерттеудің мақсаты.** Бауырдың созылмалы диффузды ауруы бар науқастарда GPX1 гені полиморфизмі дистрибуциясы кезінде эндотелийдің қызмет жағдайы мен жасушалық адгезия көрсеткіштерін зерттеу.

**Әдістері.** Бауырдың созылмалы диффузды ауруы бар 28 науқас тексерілді**.** GPX1 генінің полиморфизмінің Pro197Leu, десквамацияланған эндотелиоциттердің бірінші типті жасушааралық адгезияның молекулалық деңгейі және көктамыр қан сарысуындағы нитроген монооксидінің метаболиттері құрамы зерттелді.

**Нәтижесі.** Бауырдың созылмалы диффузды ауруы бар науқастарда GPX1 генінің полиморфизмінің Pro197Leu экспрессиясы мен жасуша адгезиясының көрсеткіштері арасындағы байланыс анықталды. Ол Leu-аллеляның гомозиготты тасымалдаушыларының қан сарысуында 1-ші типті жасушааралық адгезиясының жоғары деңгейімен көрініс тапты. Сонымен қатар, бауырдың созылмалы диффузды ауруы бар науқастарда эндотелийдің қызмет жағдайының бұзылуы GPX1 генінің Pro197Leu полиморфизмімен байланысы байқалды. Бұл LeuLeu-генотипті тасымалдаушыларда азот оксидінің метаболиттерінің төмендігі мен десквамацияланған эндотелиоциттердің санының жоғарылауымен көрініс тапты.

**Қорытынды.** Бауырдың созылмалы диффузды ауруы бар науқастарда GPX1 генінің полиморфизмінің Pro197Leu экспрессиясы мен жасуша адгезиясының көрсеткіштері арасындағы байланыс анықталды.

**Маңызды сөздер:** Бауырдың созылмалы диффузды аурулары, полиморфизм, жасушааралық адгезия молекуласы, эндотелий

**ВЛИЯНИЕ ДИСТРИБУЦИИ ПОЛИМОРФИЗМА ГЕНА GPX1 НА ПОКАЗАТЕЛИ КЛЕТОЧНОЙ АДГЕЗИИ И ФУНКЦИОНАЛЬНОГО СОСТОЯНИЯ ЭНДОТЕЛИЯ У БОЛЬНЫХ ХРОНИЧЕСКИМИ ДИФФУЗНЫМИ ЗАБОЛЕВАНИЯМИ ПЕЧЕНИ**

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**Цель исследования.** Изучить особенности клеточной адгезии и функционального состояния эндотелия у больных хроническими диффузными заболеваниями печени в зависимости от Pro197Leu полиморфизма гена GPX1.

**Методы.** Обследовано 28 больных хроническими диффузными заболеваниями печени. Определен Pro197Leu полиморфизма гена GPX1, уровень молекулы межклеточной адгезии 1-го типа, десквамированных эндотелиоцитов и содержание метаболитов монооксида нитрогена в сыворотке венозной крови.

**Результаты.** У больных хроническими диффузными заболеваниями печени установлена связь между экспрессией Pro197Leu полиморфизма гена GPX1 и показателями клеточной адгезии, которая проявляется достоверно высоким содержанием молекулы межклеточной адгезии 1-го типа в сыворотке крови у гомозиготных носителей Leu-аллеля. Изменения функционального состояния эндотелия у больных хроническими диффузными заболеваниями печени ассоциируются с Pro197Leu полиморфизмом гена GPX1, о чем свидетельствует достоверно выше показатель количества десквамированных эндотелиоцитов и низкий уровень метаболитов оксида азота у носителей LeuLeu-генотипа.

**Выводы.** Установлено зависимость показателей клеточной адгезии и функционального состояния эндотелия у больных хроническими диффузные заболевания печени от дистрибуции Pro197Leu полиморфизма гена GPX1.

**Ключевые слова:** хронические диффузные заболевания печени, полиморфизм, молекула межклеточной адгезии, эндотелий.

## INTRODUCTION

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 [2,12,15].

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 [12,16]. 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.

Due to recent scientific research both of Ukrainian and foreign scientists the concept of relations between indices of cytokine regulation, endothelium functional state and expression of various genes is beyond any doubt [15]. Although dependence of the above indices upon GPX1 Pro-197Leu gene polymorphism in patients with CDLD remains above the attention of researchers.

**The aim**: Peculiarities of the indices of the cellular adhesion and endothelial functional state in patients with CDLD depending on Pro197Leu polymorphism in GPX1 gene.

## MATERIALS AND METHODS

28 patients with CDLD aged from 25 to 74 (an average age – 52,3±6,09) were included into the study. There were 19 men (67,9%) and 9 women (32,1%), an average duration of the disease was 5,9±1,30 years. The control group included 20 practically healthy individuals (an average age - 52,2±12,15), 13 men (65,0%) and 7 women (35%) among them.

The diagnosis of chronic hepatitis (CH) was made in 13 individuals (46,4%) with an average age of 49,6±8,59. There were 7 men (53,8%) and 6 women (46,2%) among them, an average duration of the disease was 6,0±2,10 years. A mild form of CH was found in 8 patients (28,6%) and moderate form - in 5 patients (17,8%).

Liver cirrhosis (LC) was diagnosed in 15 patients (53,6%) with an average age of 55,0±7,43. Men constituted 11 patients (73,3%), women – 4 (26,7%), an average duration of the disease was 5,7±1,80 years. A mild form of LC was found in 9 patients (32,2%) and moderate form - in 6 (21,4%).

The study was conducted on the basis of the Department of Gastroenterology, Chernivtsi Regional Clinical Hospital.

The diagnoses of CH and LC were made according to the Classification of the World Congress of Gastroenterologists (Los Angeles, 1994, with additions of V. Desmet et al, 1995) and specifications of the International Classification of Diseases (ICD) of the 10$^{th}$ revision [4].

CH and LC were verified on the basis of complaints, anamnesis, objective status, common laboratory methods 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), aspartateaminotranspherase (AsAT), gammaglutamyltranspherase (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 CH and LC was found on the basis of clinical manifestations and biochemical signs – AlAT, AcAT activity, thymol test, bilirubin level in the blood [5].

The degree of LC compensation was estimated by the criteria of C.G. Child and J.G. Turcotte (1964) in the modification of K.N.H. Pugh (1973). The levels of bilirubin, albumins, prothrombin were detected in the blood serum, the presence of ascites and encephalopathy was found [13].

The degree of portal hypertension was determined on the basis of varix dilatation of the lower esophageal portion, subcutaneous veins of the anterior abdominal wall, umbilical veins, splenomegaly, ascites and hepatic encephalopathy [7].

Inclusion criteria were: the age from 25 to 76, diagnosed CH and LC (of a mild and moderate activity) verified by means of clinical, laboratory and instrumental examinations, informed written concern of the patient to participate in the study.

Patients with decompensated LC (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 etiology, Wilson's disease, congenital $\alpha_1$-antitripsin insufficiency ($\alpha_1$-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.

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 Pro197Leu regions 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 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' |

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$^0$C. Samples for PCR were prepared by means of "AmplySense – 200 – 1" set (Russian).

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 – $NO_2$ and the content of total final metabolites NO (nitrates+nitrites). The method to detect $NO_2$ content in the venous blood plasma is based on the photocolorimetric detection of optic density of $NO_2$ stained complex by Griess test [11]. The amount of desquamated endothelial cells (EC) in the blood was estimated by J.Hladovec method in N.Petrishchev et al. modification [6].

The protocol of examination of the patients was approved during the proceedings on Biomedical Ethics at Bukovinian State Medical University. The document is compiled according to the requirements stipulated by the 6$^{th}$ chapter of CH GCP (1996). While compiling the protocol, the main principles of the Helsinki Declaration on Biomedical Research (1974) adapted during the 41$^{st}$ International Assembly in Gong Kong (September, 1989) were followed. The protocol corresponds to the basic principles of proper medical practical work such as respect of a personality, awareness of the patient, estimation of the risk of harm and benefit [17,18,19].

Primary findings of patients' examinations were included into the data base in the system of MicrosoftExcel. Formalization, standardizing of the results and statistical analysis were included into the further processing of our findings.

Before checking statistical hypotheses the analysis of regular distribution of the values in randomized surveys by means of detection of asymmetry and excess coefficients with the help of Khan-Shapiro-Wilcky criterion was conducted.

Probability of the difference of an average arithmetic and its errors between the groups of the study was calculated by means of double odd Student t-criterion.

For the data corresponding to normal distribution with equality of general dispersions of sampling checked by means of Fisher-criterion a probable difference was with $p<0,05$ [8].

Mathematical calculation of the results obtained was conducted on IBM PC Pentium III by means of computer program Primer of Biostatistics, Version 4.03 (S.Glantz, USA) and the standard package of statistical programs of Microsoft Office Excel 2007 [1].

## RESULTS

The indices of cellular adhesion and functional endothelial state in patients with CDLD did experience reliable changes depending on polymorphism of GPX1 gene and were statistically different from the group of practically healthy individuals (table 2).

**Table 2. 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<br>$P_1$<0,01 | 351,38±18,274<br>$P_1$<0,001<br>$P_2$>0,05 | 387,41±20,108<br>$P_1$<0,001<br>$P_2$<0,01<br>$P_3$>0,05 |
| Stable NO metabolites ($NO_2$, $NO_3$, mcmol/L) | 18,14±0,684 | 13,45±1,002<br>$P_1$<0,01 | 11,45±1,139<br>$P_1$<0,001<br>$P_2$>0,05 | 10,24±1,012<br>$P_1$<0,001<br>$P_2$<0,05<br>$P_3$>0,05 |
| Endothelial cells, x $10^4$/L | 3,04±0,204 | 4,63±0,320<br>$P_1$<0,001 | 5,83±0,549<br>$P_1$<0,001<br>$P_2$>0,05 | 6,27±0,625<br>$P_1$<0,001<br>$P_2$<0,05<br>$P_3$>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

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% ($P_1$<0,001), ProLeu-genotype – on 25,4% ($P_1$<0,001) and 49,2% ($P_1$<0,001) for the patients with LeuLeu-genotype. LeuLeu-genotype carriers presented the value of this index on 25,3% higher ($P_1$<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 ($P_1$<0,01) in comparison with the control value, Leu-allele ones – in 1,8 times correspondingly ($P_1$<0,001), ProLeu-heterozygotes – in 1,6 times ($P_1$<0,001). Reliably lower level of NO metabolites (on 23,9%, $P_1$<0,001) was found in the blood of LeuLeu-genotype carriers as compared with the patients of ProPro-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).

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.

## DISCUSSION

Obtained data is consistent with the results of the last year's research and shows that genetic variation in genes that codes enzymes of the glutathione family affect susceptibility to the occurrence of CDLD, one of which is the pathogenesis of endothelial dysfunction [12,20]. Most researchers have found that certain allelic variants of glutathione peroxides gene may increase the likelihood of oxidative stress.

It is well-known that oxidative stress that accompanies the intensification of peroxidation processes and antioxidant imbalance background of activation is the leading mechanism of liver disease [2,10].

Certain researchers [9,15] indicate that in patients with CDLD homozygotic carrying of Leu-allele gene GPX1 is connected with reliably higher level of general bilirubin and its indirect fraction, as well as bigger activity of aminotranspherases.

These results can be explained by increasing free radical oxidation processes in Pro197Leu polymorphism of GPX1 gene carriers [14]. Free radicals are able to directly destroy NO [3]. Increased lipid peroxidation of membranes is the result of damaging the structure of the endothelium and its violation NO-producing ability. Developing an absolute or relative deficiency of NO, is required for normal regulation of vascular tone. The weakening of the NO-dependent vasodilatatory reactions, in its turn, leads to increased vascular tone, increased blood clots, and as a result, tissue hypoxia. In addition, the reduced inhibitory effect of NO on platelet aggregation, leukocyte adhesion to the endothelium and smooth muscle cell proliferation of the vascular wall, creates prerequisites for vascular disorders. Important role in the pathogenesis of endothelial dysfunction plays adhesion cell growth. Of particular importance in cell migration is given intercellular adhesion molecule type 1 - ICAM-1 [12,14]. It is known that cell adhesion is a violation of not only the development but also to the further progression of endothelial damage [16]. Progression endothelial dysfunction, in turn, leads to tissue hypoxia, disruption of metabolism and infiltration of macrophages subendothelial space, induction of endothelial apoptosis [3,15], which is consistent with our results with respect to the growing number desquamated (circulating) endothelial cells in peripheral blood of patients studied.

## CONCLUSIONS

1. 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.

2. 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.

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