International journal of endocrinology

Оригинальные исследования
/Original Researches/

УДК 616.33/.36:616.72-007.24]-085-092

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GENETIC DETERMINISM OF THE FUNCTIONAL CONDITION OF THE ENDOTHELIUM IN PATIENTS WITH CHRONIC DIFFUSE LIVER DISEASES AND DISORDERS OF THYROID HOMEOSTASIS

Summary. *Patients with chronic diffuse of liver disease have a violation of thyroid homeostasis in the form of reduction of free triiodothyronine in the blood serum, as well as thyroid stimulating hormone increasing content and of free thyroxin.*

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 type 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 nitric oxide metabolites in the carriers of LeuLeu-genotype.

Key words: *chronic diffuse liver disease, thyroid homeostasis, polymorphism, endothelium.*

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 [9, 11].

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, 3]. 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 [7, 10].

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 [11]. Although dependence of the above indices upon GPX1 Pro197Leu gene polymorphism in patients with CDLD remains above the attention of researchers.

The aim the study. 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 34 to 72 were examined. 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'

Table 2. Indices of the endothelial function 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	259.600 ± 10.324	309.230 ± 12.463 $p_1 < 0.01$	351.380 ± 18.274 $p_1 < 0.001$ $p_2 > 0.05$	387.410 ± 20.108 $p_1 < 0.001$ $p_2 < 0.01$ $p_3 > 0.05$
Stable NO metabolites (NO_2, NO_3, mcmol/L)	18.140 ± 0.684	13.450 ± 1.002 $p_1 < 0.01$	11.450 ± 1.139 $p_1 < 0.001$ $p_2 > 0.05$	10.240 ± 1.012 $p_1 < 0.001$ $p_2 < 0.05$ $p_3 > 0.05$
Endothelial cells, x 10^4/L	3.040 ± 0.204	4.630 ± 0.320 $p_1 < 0.001$	5.830 ± 0.549 $p_1 < 0.001$ $p_2 > 0.05$	6.270 ± 0.625 $p_1 < 0.001$ $p_2 < 0.05$ $p_3 > 0.05$

Notes: n — number of observations; probability of changes concerning: p_1 — the control; p_2 — the group of patients with ProPro-genotype; p_3 — the group of patients with ProLeu-genotype.

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 °C. Samples for PCR were prepared by means of AmplySense-200-1 set (Russian).

The content of soluble intercellular adhesion molecule type 1 (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 [2]. The amount of desquamated endothelial cells (EC) in the blood was estimated by J. Hladovec method in N. Petrishchev et al. modification [4].

Peculiarities of thyroid homeostasis were studied by the content of free thyroxin (fT_4), free triiodothyronine (fT_3) and thyroid stimulating hormone by means of immune-enzymatic method using the reagents ImmuneFa-TTH, IFA-SvT_3 and IFA-SvT_4-1 (JSC Immunotech) on the analyzer of immune-enzymatic reactions Uniplan calculating the coefficients fT_3/fT_4, fT_4/fT_3.

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

Results

Indicators studies of thyroid homeostasis at patients with CDLD established a probable reduction in fT_3 and increase of the concentration in fT_4 due to failure of peripheral monodeiodization against the increasing of thyroid stimulating pituitary function. As evidence of this assumption the probable reduction rate was observed in fT_3/fT_4 with a corresponding of indicator in fT_4/fT_3. However, in most of the examined cases the values of the studied parameters did not exceed the norm.

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).

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 % (p1 < 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.

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 [1, 10]. 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, 3, 5].

These results can be explained by increasing free radical oxidation processes in Pro197Leu polymorphism of GPX1 gene carriers [11]. Free radicals are able to directly destroy NO [6]. 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 [2]. It is known that cell adhesion is a violation of not only the development but also to the further progression of endothelial damage [6]. Progression endothelial dysfunction, in turn, leads to tissue hypoxia, disruption of metabolism and infiltration of macrophages subendothelial space, induction of endothelial apoptosis [5, 10], which is consistent with our results with respect to the growing number desquamated (circulating) endothelial cells in peripheral blood of patients studied.

Conclusions

1. Patients with chronic diffuse of liver disease have a violation of thyroid homeostasis in the form of reduction of blood serum of free triiodothyronine, as well as thyroid stimulating hormone increasing content and of free thyroxin.

2. 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 in the blood serum in homozygotic carriers of Leu-allele.

3. 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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Получено 17.01.14

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ГЕНЕТИЧЕСКАЯ ДЕТЕРМИНАЦИЯ ФУНКЦИОНАЛЬНОГО СОСТОЯНИЯ ЭНДОТЕЛИЯ У ПАЦИЕНТОВ С ХРОНИЧЕСКИМИ ДИФФУЗНЫМИ ЗАБОЛЕВАНИЯМИ ПЕЧЕНИ С НАРУШЕНИЕМ ТИРЕОИДНОГО ГОМЕОСТАЗА

Резюме. Установлено, что у пациентов с хроническими диффузными заболеваниями печени происходит нарушение тиреоидного гомеостаза в виде снижения содержания в сыворотке крови свободного трийодтиронина, повышения содержания тиреотропного гормона и свободного тироксина.

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

Ключевые слова: хронические диффузные заболевания печени, тиреоидный гомеостаз, полиморфизм, эндотелий.

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ГЕНЕТИЧНА ДЕТЕРМІНАЦІЯ ФУНКЦІОНАЛЬНОГО СТАНУ ЕНДОТЕЛІЮ В ПАЦІЄНТІВ ІЗ ХРОНІЧНИМИ ДИФУЗНИМИ ЗАХВОРЮВАННЯМИ ПЕЧІНКИ З ПОРУШЕННЯМ ТИРЕОЇДНОГО ГОМЕОСТАЗУ

Резюме. Установлено, що в пацієнтів із хронічними дифузними захворюваннями печінки відбувається порушення тиреоїдного гомеостазу у вигляді зниження вмісту в сироватці крові вільного трийодтироніну, підвищення вмісту тиреотропного гормону та вільного тироксину.

У пацієнтів із хронічними дифузними захворюваннями печінки встановлено зв'язок між експресією Pro197Leu поліморфізму гена GPX1 та показниками клітинної адгезії, що проявляється вірогідно вищим умістом молекули міжклітинної адгезії 1-го типу в сироватці крові в гомозиготних носіїв Leu-алелі. Зміни функціонального стану ендотелію в пацієнтів із хронічними дифузними захворюваннями печінки асоціюються з Pro197Leu поліморфізмом гена GPX1, про що свідчить вірогідно вищий показник кількості десквамованих ендотеліоцитів і нижчий рівень метаболітів оксиду азоту в носіїв LeuLeu-генотипу.

Ключові слова: хронічні дифузні захворювання печінки, тиреоїдний гомеостаз, поліморфізм, ендотелій.