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# FEATURES OF ALLELIC POLYMORPHISM OF CYTOKINE GENES IL-10, IL-4, TNFA IN PATIENTS WITH CHRONIC HEPATITIS B

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In the world approximately 250 million people are recognized as chronic carriers of HBV surface antigen (HBsAg) with a large regional variation in the level of endemicity of HBsAg positive patients from 2 to 8%. All patients with chronic HBV infection have an increased risk of liver cirrhosis and hepatocellular carcinoma, depending on the host's response to infection and viral factors. Studies of interrelationship between polymorphisms of cytokine genes and variants of chronic hepatitis B are not numerous, often, are estimated in one group with chronic hepatitis C.

The method of this study was the equalization of the frequency of polymorphisms of the genes *IL-10 (rs1800896), IL-4 (rs2243250)* and *TNF α (rs1800620)* in healthy individuals and patients with chronic hepatitis B, which are residents of the Odessa region.

Polymorphism of genes in cytokines investigated by PCR. The stage of liver fibrosis was determined by the results of the non-invasive FibroScan method. The frequencies of alleles and genotypes in the groups were compared according to Pearson's chi-squared test with Yates' correction for continuity with the number of freedom steps being 1.

There are different frequencies of the *IL-4(rs2243250*) and *IL-10* (*rs1800896*) gene alleles in groups of healthy persons and patients with chronic hepatitis B. According to the established stage of liver fibrosis, the presence of the CC *IL-4(rs2243250)* allele is more resistant to hepatitis B, lower heterozygous CT of the *IL-4(rs2243250)* gene ($p<0.05$). Also, heterozygous carriers of the GA *IL-10(rs1800896)* allele are more susceptible to chronic hepatitis B compared with the mutant AA *IL-10(rs1800896)* allele.

Identification of frequencies of gene`s polymorphism in groups of healthy persons and patients with chronic hepatitis B can serve as one of the genetic criteria for the progression of hepatitis B.

**Key words:** chronic hepatitis B, cytokine gene polymorphism, liver fibrosis.

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**ОСОБЛИВОСТІ АЛЕЛЬНОГО ПОЛІМОРФІЗМУ ГЕНІВ ЦИТОКІНІВ IL-10, IL-4, TNFα У ХВОРИХ НА ХРОНІЧНИЙ ГЕПАТИТ В**

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У світі приблизно 250 мільйонів осіб є хронічними носіями поверхневого антигену HBV (HBsAg) з великою регіональною варіацією мільйонів ендемічних рівнів HBsAg позитивних пацієнтів від 2 до 8%. Усі пацієнти з хронічною HBV-інфекцією мають підвищений ризик виникнення цирози печені та гепатоцелюлярної карциноми (ГЦК), залежно від відповіді організму господаря на інфекцію та вірусні фактори.

Метою даного дослідження було порівняння частоти поліморфізмів генів *IL-10 (rs1800896), IL-4 (rs2243250)* та *TNF α (rs1800620)* у здорових осіб і хворих на ХГВ. Поліморфізм генів цитокінів вивчався шляхом ПЛР. Ступінь фіброзу печінки встановлювався за результатами неінвазивного методу FibroScan. Було виявлено суттєві відмінності частот поліморфізму зазначених генів у групах здорових та хворих на ХГВ, що може надалі служити одним із генетичних критеріїв прогресування гепатиту В.

**Ключові слова:** хронічний гепатит В, поліморфізм генів цитокінів, фіброз печінки.

**Introduction**. Infection caused by the hepatitis B virus (HBV infection) is a global public problem with changing epidemiological data that occurs as a result of the influence of several factors, including measures that are carried out in connection with vaccinations, as well as population migration.

Approximately 250 million people are recognized as chronic carriers of HBV surface antigen (HBsAg) with a large regional variation in the level of endemicity of HBsAg positive patients from 2 to 8%. There is a tendency to decrease the level of HBV prevalence in several countries with high endemicity due to the improvement of socioeconomic status, vaccination programs and, possibly, effective antiviral treatment. Currently, in some European countries, the prevalence of HBV infection has increased due to population migration. Even with the help of universal vaccination programs, it is impossible to significantly prevent cases of acute HBV infection, especially in high-risk groups. All patients with chronic HBV infection have an increased risk of liver cirrhosis and hepatocellular carcinoma, depending on the host's response to infection and viral factors [1; 2].

Despite some progress in the study of the pathogenesis of chronic viral hepatitis, the causes of long-term persistence of viruses in the body have not been fully studied, and many details of the mechanism for the formation of chronic hepatitis and its further progression to cirrhosis are still not known. It is known that liver damage in CHB is immune-mediated, since the hepatitis B virus does not have a direct cytopathic effect. The persistence of HBV is associ-

ated with the inability of virus-specific cytotoxic lymphocytes to remove viruses from the body, which leads to a chronic necroinflammatory reaction in the liver, and later to the development of cirrhosis and hepatocarcinoma [3; 4].

In chronic hepatitis B, the mechanisms of the immunological response largely determine the course and outcome of the disease. Data have been obtained on the possible involvement of NK cells when activated by cytokines in the process of cell damage and exacerbation of chronic hepatitis B. It has now been proven that the development of hepatocarcinoma in CHB patients is possible even at the stage of chronic hepatitis, before the formation of liver cirrhosis [5; 6].

Recent studies have shown that an imbalance in the secretion of immunoregulatory cytokines and the regulation of cytokine-mediated cooperation, proliferation and differentiation of cells may be due to a complex of genes that determine the level of production of Th1 or Th2 type cytokines [7].

It has been established that the level of production of cytokines and their antagonists, the expression of receptors for various cytokines is determined by the set of allelic variants of cytokine genes inherited by the individual and their receptors.

However, studies on the relationship between polymorphisms of cytokine genes and the course of chronic hepatitis B are few and often evaluated in the same group with chronic hepatitis C [8].

In this regard, it seems relevant to evaluate the significance of the allelic polymorphism of the *IL-10 (rs1800896), IL-4 (rs2243250)* and *TNFα (rs1800620)* genes in an ethnically homogeneous group of patients with chronic hepatitis B living in the Odessa region.

**The aim of the study** – determination of the frequency of occurrence of polymorphisms of the *IL-4(rs2243250), IL-10(rs1800896), TNFα (rs1800620)* genes in patients with chronic hepatitis B compared with healthy individuals living in the Odessa region, to improve the quality of hepatitis B diagnosis according to the obtained genetic criteria.

**Materials and methods**

We examined 82 patients with chronic hepatites B aged 18 to 54 years. All examined patients were under dispensary observation in the hepatological center of the Odesa Municipal Infectious Hospital. The patients are residents of the Odessa region, the study group was dominated by men (70%). The duration of the disease was no more than 10 years.

The control group consisted of 30 practically healthy persons, the average age of which was 32±1.05 years. The number of women and men was the same (15 people each).

All patients included in the study were given free and informed consent. The methodology of this investigation is in accordance with the requirements of the Committee on Bioethics of the Odesa National Medical University (protocol 179 of 19.11.2010).

The confirmation of diagnosis of chronic hepatitis B based on the following biochemical parameters (increased AST and ALT activity, bilirubin concentration and the predominance of its direct fraction), serological markers (determination of HBsAg, HBeAg, aHBe, as well as HBV DNA) were studied.

Molecular genetic studies included the determination of polymorphic variants of the *IL-4(rs2243250), IL-10(rs1800896), TNFα (rs1800620)* genes. Polymorphism was studied by amplification of the corresponding regions of the genome by PCR. The structure of the primers used and the parameters of temperature cycles described in the literature and the genomic database. The studies were carried out on the basis of the German Diagnostic Center. St. Paul (Odesa).

The severity of the fibrotic process in the liver was determined by the METAVIR scale using the non-invasive FibroScan test. FibroScan is a non-invasive method for assessing the degree of liver fibrosis, which is implemented using a special apparatus. The FibroScan liver examination is based on the measurement of liver elasticity. The ultrasonic sensor of the instrument generates medium amplitude and low frequency oscillations. These vibrations pass through the skin, subcutaneous tissues and create in the liver.

The distribution of genotypes for the studied polymorphic loci was checked using Pearson's $\chi 2$ test. Allele and genotype frequencies in the groups were compared using Pearson's chi-squared test with Yates' correction for continuity with degrees of freedom equal to 1. Allele frequencies were calculated according to the Hardy-Weinberg law.

**Research results and discussion**

Considering that immune mechanisms play a significant role in the pathogenesis of chronic viral hepatitis, the regulation of which is determined by the balance of cytokines, it seems important to assess the frequency of occurrence of allelic variants of cytokine genes among healthy individuals and patients with chronic hepatitis B in an ethnically homogeneous group of the Odesa region.

Significant differences were found in the control and study groups of patients during studying the occurrence of allelic polymorphisms of *IL-10 (rs1800896), IL-4 (rs2243250) and TNF α (rs1800620)*. (Table 2, 3, 4).

The study of the polymorphic region of *IL-4 (rs2243250)* revealed the predominance of the homozygotes *CC IL-4 (rs2243250)* in the group both of patients with hepatitis B and in the control group, which amounted to 60,98% and 86,6%, respectively. In addition, in the group of patients with chronic hepatitis B, there was a significant predominance of the heterozy-

Table 1

**Characteristics of the studied polymorphisms**

| Gene | polymorphism | primer name | nucleotide sequence |
|---|---|---|---|
| IL-4 | C589T | rs2243250 | 5'-TAAACTTGGGAGAACATGGT-3' |
| IL-10 | G1082A | rs1800896 | 5'-CCT ATC CCT ACT TCC CCT-3' |
| TNFα | G308A | rs1800620 | 5′-AGG CAA TAG GTT TTG AGG GC-3′ |

Table 2

**Polymorphism frequency of IL-4 (rs2243250) in the control and study groups**

| genotype /allele | control group (n=30) | frequency (%) | patients with hepatitis B (n=82) | frequency (%) | $\chi^2$ | significance level |
|---|---|---|---|---|---|---|
| CC | 26 | 86,6 | 50 | 60,98 | 5,65 | p<0.05 |
| CT | 2 | 6,7 | 32 | 39,02 | 9,59 | p<0.01 |
| TT | 2 | 6,7 | - | - | | |

Table 3

**Polymorphism frequency of *IL-10 (rs1800896)* in the control and study groups**

| genotype/ allele | control group (n=30) | frequency (%) | patients with hepatitis B (n=82) | frequency (%) | $\chi^2$ | significance level |
|---|---|---|---|---|---|---|
| GG | 14 | 46,66 | 26 | 31,7 | 1,645 | p > 0,02 |
| GA | 8 | 26,67 | 50 | 60,0 | 8,197 | p < 0,005 |
| AA | 8 | 26,67 | 6 | 7,3 | 4,954 | p < 0,05 |

Table 4

**Polymorphism frequency of *TNF-α (rs1800620)* in the control and study group**

| genotype /allele | control group (n=30) | frequency (%) | patients with hepatitis B (n=82) | frequency (%) | $\chi^2$ | significance level |
|---|---|---|---|---|---|---|
| GG | 27 | 90,0 | 70 | 85,37 | 0,336 | p > 0,05 |
| GA | 2 | 6,7 | 12 | 14,63 | 1,1 | p > 0,05 |
| AA | 1 | 3,3 | - | - | | |

gous allele *CT IL-4 (rs2243250)* in comparison with the control group. In the study group of patients with chronic hepatitis B, homozygous *TT IL-4 (rs2243250)* variant (mutation) was not detected, while in the control group this genotype variant was found in 6% of people. The frequency allele C was 0.9 in the control group, and 0.8 in the group of patients with chronic hepatitis B. The frequency T allele was 0.1 and 0.2, respectively.

Thus, we can assume a probable relationship between the homozygous allele and a certain resistance to the hepatitis B virus, as well as the heterozygous variant *CT IL-4 (rs2243250)* and increased susceptibility to the hepatitis B virus (p<0,05).

In the study of *polymorphism IL-10 (rs1800896)* in patients with chronic hepatitis B, there was a significantly greater variability of the genotype than in people of the control group. In particular, the homozygous genotype *GG IL-10 (rs1800896)* in patients with chronic hepatitis B was much less common (31,7%) than in healthy individuals (46,66%). In the study group, the heterozygous variant *GA IL-10 (rs1800896)* prevailed (60%), while in the control group its occurrence was low (26,67%). The frequency of occurrence of the mutant variant of the genotype *AA IL-10 (rs1800896)* in the control and study groups had no significant difference (26,67% and 7,3% respectively). The difference in the frequency of occurrence of *GA IL-10 (rs1800896)* and *AA GG IL-10 (rs1800896)* genotypes in the study and control groups is significant (p>0.05). The frequency allele G was 0.53 in the control group, and 0.62 in the group of patients with chronic hepatitis B. The frequency allele A was 0.47 and 0.38, respectively.

The study of the polymorphic region *TNF-α (rs1800620)* revealed the predominance of the homozygous variant *GG TNF-α (rs1800620)* in the both group of patients with hepatitis B and in the control group, which amounted to 90% and 85,37%, respectively. However, in the group of patients with chronic hepatitis B, there was some predominance of the heterozygous allele *GA TNF-α (rs1800620)* in comparison with the control group. In the study group of patients with chronic hepatitis B homozygous variant *TT TNF-α (rs1800620)* (mutation) was not detected; in the control group, this variant of the genotype was found only in one person (3,3%). There was no statistically significant difference between the indicators.

The frequency allele G was 0.93 in the control group, and 0.89 in the group of patients with chronic hepatitis B. Allele frequency A was 0.07 and 0.11, respectively.

During the study, all patients were divided into 3 groups: absent or minimal fibrosis (F0-F1), moderate fibrosis (F2) and severe fibrosis of the liver (F3).

In the group of patients with chronic hepatitis B, the distribution of patients by the degree of fibrosis was as follows: the minimum degree of liver fibrosis (F0-F1) was detected in (41%) patients, moderate degree (F2) – in (34%) patients, distinct fibrosis (F3) – in (25%).

In patients with homozygous *CC IL-4(rs2243250)* genotype, there is a predominance of a minimal degree of fibrosis or its absence compared with patients who have a heterozygous *CT IL-4(rs2243250)* genotype.

In patients with homozygous variants of *GG IL-10 (rs1800896)* and *AA IL-10 (rs1800896)*, there is a predominance of the minimum degree of fibrosis in comparison with patients who have a heterozygous variant of *GA IL-10 (rs1800896)*.

**Conclusions.** In an ethnically homogeneous group of residents of the Odesa region, differences were found between patients with chronic hepatitis B and healthy

individuals in terms of the allele frequencies of the *IL-4(rs2243250)* and *IL-10 (rs1800896)* genes.

On the basis of the differences of the frequency of definite genotypes in patients with chronic hepatitis B and healthy persons ($p<0.05$), it is possible to assume a higher risk of developing the chronic form of hepatitis B in carriers of heterozygotes *CT IL-4 (rs2243250)* than in carriers of homozygotes *CC IL-4 (rs2243250)*.

On the basis of the differences of the frequency of definite genotypes in patients with chronic hepatitis B and healthy persons ($p<0.05$), it is possible to assume a higher risk of developing the chronic form of hepatitis B in carriers of heterozygotes *GA IL-10 (rs1800896)* than in carriers of homozygotes *AA IL-10 (rs1800896)*.

Thus, the obtained results indicate significant differences in *IL-4 (rs2243250)* and *IL-10 (rs1800896)* gene polymorphisms in patients with chronic hepatitis B and healthy individuals are marked. Determination of the genetic profile in such patients can be an important additional factor in the comprehensive assessment of the risk of developing chronic hepatitis B. The study of the relationship between genetic criterias and the speed of development of the chronic inflammatory process in the liver tissue will be the subject of further research.

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