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

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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.

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-
The study of the polymorphic region of the IL-4(rs2243250), IL-10(rs1800896), TNFα (rs1800620) genes in 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 an ethnically homogeneous group of patients with chronic hepatitis B living in the Odessa region.

Materials and methods

We examined 82 patients with chronic hepatitis B aged 18 to 54 years. All examined patients were under dispensary observation in the hepatological center of the Odessa 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 Odessa 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 (Odessa).

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 χ2 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 Odessa 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>polymorphism</th>
<th>primer name</th>
<th>nucleotide sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>C589T</td>
<td>rs2243250</td>
<td>5’-TAAACTTGGAGAACATGCTT-3’</td>
</tr>
<tr>
<td>IL-10</td>
<td>G1082A</td>
<td>rs1800896</td>
<td>5’-CTTATCCTACTTCCTC-3’</td>
</tr>
<tr>
<td>TNFα</td>
<td>G308A</td>
<td>rs1800620</td>
<td>5’-AGGCAAAGGTGTTGAGGCC-3’</td>
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</tbody>
</table>
Polymorphism frequency of IL-4 (rs2243250) in the control and study groups

<table>
<thead>
<tr>
<th>genotype / allele</th>
<th>control group (n=30) frequency (%)</th>
<th>patients with hepatitis B (n=82) frequency (%)</th>
<th>χ²</th>
<th>significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>26</td>
<td>50</td>
<td>60,98</td>
<td>5,65</td>
</tr>
<tr>
<td>CT</td>
<td>2</td>
<td>32</td>
<td>39,02</td>
<td>9,59</td>
</tr>
<tr>
<td>TT</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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</table>

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

<table>
<thead>
<tr>
<th>genotype / allele</th>
<th>control group (n=30) frequency (%)</th>
<th>patients with hepatitis B (n=82) frequency (%)</th>
<th>χ²</th>
<th>significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>14</td>
<td>26</td>
<td>31,7</td>
<td>1,645</td>
</tr>
<tr>
<td>GA</td>
<td>8</td>
<td>50</td>
<td>60,0</td>
<td>8,197</td>
</tr>
<tr>
<td>AA</td>
<td>8</td>
<td>6</td>
<td>7,3</td>
<td>4,954</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>genotype / allele</th>
<th>control group (n=30) frequency (%)</th>
<th>patients with hepatitis B (n=82) frequency (%)</th>
<th>χ²</th>
<th>significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>27</td>
<td>70</td>
<td>85,37</td>
<td>0,336</td>
</tr>
<tr>
<td>GA</td>
<td>2</td>
<td>12</td>
<td>14,63</td>
<td>1,1</td>
</tr>
<tr>
<td>AA</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 2**

**Table 3**

**Table 4**

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) was 3,3% 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 criteria and the speed of development of the chronic inflammatory process in the liver tissue will be the subject of further research.

REFERENCES

Надійшла до редакції 03.12.2022 р.
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