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The relationship of allelic polymorphism of cytokine genes, parameters of the immune and cytokine profile in patients with chronic hepatitis B

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Abstract. ***The aim*** is to conduct a comprehensive assessment of immunogenetic and biochemical parameters in patients with chronic viral hepatitis B (CVHB) living in the Odesa region. ***Materials and methods.*** A total of 82 patients with CVHB were examined. The traditional biochemical and immunological parameters, the content of cytokines IL-4, IL-10, TNF-α and the gene polymorphism of cytokines interleukin (IL)-4, IL-10, tumor necrosis factor (TNF)-α were determined. The patients were divided into groups in accordance with the identified polymorphisms of cytokine genes for assess the relationship of non-parametric and parametric indicators (changes in the immunological status, biochemical parameters and cytokine profile) ***Results.*** With the use of the Spearman's rank correlation coefficient, certain relationships have been established. In patients with homozygous *CC IL-4* polymorphism, more pronounced changes in the cytokine profile, immunological status and a higher level of alanine aminotransferase and aspartate aminotransferase activity were noted. ***Conclusion.*** The revealed relationship between the alanine aminotransferase and aspartate aminotransferase levels, the immunological status and cytokine profile indicators with certain TNF-α and IL-4 genotypes suggests a genetic predisposition of certain patients with CVHB to a more severe course. The information obtained can be applied as one of the additional criteria for the activity of inflammatory changes in the liver tissue.

Key words: chronic hepatitis B, gene polymorphism, liver fibrosis, cellular immunity.

Introduction

Viral hepatites is one of the biggest threats public health, since, despite the significant spread, most people do not know that they are infected, there is no access to treatment sufficient, and the complications caused by them lead to cirrhosis and hepatocellular carcinoma. According to the evaluations data, in the world 256 million are infected with viral hepatitis B (VHB). People infected by VHB develop cirrhosis of the liver in a 5-year cumulative incidence is estimated at 8–20%; by in the presence of liver cirrhosis, the annual risk of liver decompensation and the incidence of hepatocellular carcinoma varies from <1% to 5%; only 15–40% of patients with decompensated liver cirrhosis have a chance to survive within 5 years [1, 2].

Current research shows that malignancy (hepatocarcinoma formation) is possible at the stage of the chronic inflammatory process of the liver of viral etiology before the onset of cirrhotic changes [3, 4].

Numerous research works show that the outcomes of the viral chronic inflammatory process in the liver are determined by the genetic features of the virus, as well as the human body. The search for genetic markers that will make it possible to accurately predict the rate of formation of fibrotic changes in the liver tissue, assess the possibility of transformation of fibrosis into cirrhosis, and also predict the likelihood of a stable biochemical and virological response to antiviral treatment is ongoing.

Cytokines influence the development of the immune response by transmitting signals between different cells. Of certain importance is the polymorphism of the genes of cytokines and their receptors.

The works of a number of authors show that polymorphism of a number of human genes determines changes in blood biochemical parameters and the amount of cytokines [5, 6].

However, the study of allelic polymorphism of cytokine genes, the relationship with clinical signs and laboratory parameters was carried out in patients with chronic hepatitis of various etiologies, and not only in patients with chronic VHB.

It seems appropriate to evaluate the complex of allelic polymorphism of several cytokine genes, clinical and immunological changes in patients infected with hepatitis B virus alone.

The aim of the work: to conduct a comprehensive assessment of immunogenetic and biochemical parameters in patients with chronic VHB living in the Odesa region.

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 Clinical Infectious Hospital. The patients are residents of the Odesa region, the study group was dominated by men (68%). The duration of the disease was no more than 10 years. Patients infected with the HIV virus, other hepatotropic viruses, and drug abusers were not included in the study. 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 study participants signed a written voluntary informed consent for the study. The methodology for conducting this clinical observation complied with the requirements of the Bioethics Committee of Odesa National Medical University (protocol No. 179 dated November 19, 2010).

In all patients, the dynamics of the general clinical analysis of blood and urine, the concentration of total bilirubin in the blood serum and its fractions, and the activity of alanine aminotransferase (ALT) were studied.

In order to confirm the final diagnosis and determine the stage of the disease, routine biochemical tests were used

(an increase in the activity of ALT and aspartate aminotransferase (AST), the concentration of total bilirubin and the predominance of its direct fraction, the concentration of total protein and its fractions, the level of the prothrombin index).

Traditional serological markers (HBeAg and HBsAg antigens, aHBe antibodies) were detected by ELISA, and the quantitative content of HBV DNA was determined using polymerase chain reaction.

The methodology for molecular genetic tests of the pilot project and immunological studies is described in our previous works [7].

The determination of cytokines in blood serum was carried out by enzyme-linked immunosorbent assay using reagent kits for the quantitative determination of the concentration of IL-4, IL-10, TNF-α in human biological fluids in accordance with the instructions. The evaluation of the results was carried out by photometric method (microplate enzyme immunoassay analyzer «Stat Fax-2100», USA).

The obtained parametric and non-parametric indicators were processed by statistical methods in MS Excel.

Research results and discussion

The clinical course of chronic VHB in the studied patients was characterized by next changes in the general condition: asthenovegetative syndrome was observed in all patients (100%), dyspeptic — in 68 patients (83%), arthralgic — in 34 (41%). Jaundice was registered in 21% of patients, it was weak and short-lived. Most patients had hepatomegaly (93%) and splenomegaly (47%).

The clinic of the disease in the group of patients with chronic VHB was not characterized by a cyclical process. All patients noted various manifestations of asthenovegetative syndrome (100%), a significant part had various dyspeptic symptoms (83%), arthralgic — in 41 (41%). Jaundice was rarely recorded (11%), was short-lived and mild. Only 39% of patients had pain in the joints. The majority of patients with chronic VHB were found to have enlarged liver (93%) and spleen (47%).

Analysis of changes in biochemical indicators in patients with chronic VHB showed definite changes: compared to healthy individuals, the level of total bilirubin was 1.9 times higher (19.9±1.23 μmol/l), the content of total protein was 1.2 times lower (64.09±0.68 g/l), albumin — 1.3 times (35.45±0.55 g/l) and γ-globulins — 1.7 times (7.27±0.17 g/l). The activity of ALT and AST was also increased — 5.8 times (3.29±0.91 mmol/l) and 6.8 times (2.27±0.74 mmol/l), respectively — compared to healthy subjects.

Comparative pilot studies of allelic polymorphism of *IL-10 (rs1800896), IL-4 (rs2243250)* and *TNF-α (rs1800620)* genes in patients with chronic VHB and healthy people were published in our previous work [7].

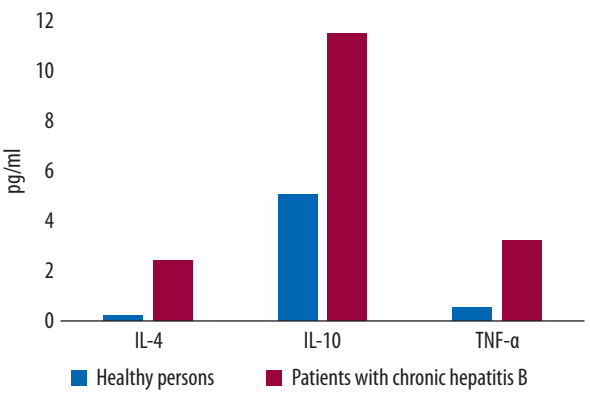
The relationship between the degree of liver fibrosis and certain genotypes of *IL-4 (rs2243250)* and *TNF-α (rs1800620)* was revealed, which may allow using this information as one of the criteria for the rate of progression of liver fibrosis. The severity of changes in the parameters of the immune response is an additional criterion for the degree of morphological disorders in the liver tissue. It has been suggested that it is expedient to further study the polymorphism of genes of other cytokines and their quantitative content in the blood serum of patients in order to create a personalized approach to the treatment and prediction of outcomes of viral hepatitis.

The results of the study of the immunological status of patients with chronic VHB in comparison with healthy individuals are presented in our previous work also [8].

The study of the subpopulation composition of peripheral blood lymphocytes in patients with chronic VHB revealed a significantly low expression of CD3+, CD4+, CD16+ and an increase in the number of cells expressing CD8+ and CD19+ antigens compared with healthy individuals ($p<0.05$).

A comparative analysis of the cytokine profile in healthy individuals and patients with chronic VHB revealed significant differences in the content of IL-4, IL-10 and TNF-α, as well as individual fluctuations. The results are shown in Figure.

Figure Level of cytokines IL-4, IL-10 and TNF-α in patients with chronic hepatitis and healthy people



Patients were divided into groups in accordance with the identified polymorphisms of cytokine genes to assess the relationship between nonparametric and parametric parameters (changes in immunological status, biochemical parameters and cytokine profile). There are not patients with mutant homozygotes of AA *IL-10 (rs1800896)* and AA *TNF-α (rs1800620)* were identified in the project, only 7 groups of patients were identified.

In patients with homozygous CC *IL-4 (rs2243250)* IL-4 polymorphism, more pronounced changes in the cytokine profile, immunological status and a higher level of ALT and AST activity were noted. Using the Spearman rank correlation coefficient, the following relationships were established:

- moderate negative relationship between the allelic variant of IL-4 and the relative content of CD3+ (carriers of the *CC IL-4 (rs2243250)* have a smaller amount of IL-4, carriers of the genotype *CT IL-4 (rs2243250)* have a greater amount), $p\leq0.05$;
- moderate negative relationship between the allelic variant of *IL-4 (rs2243250)* and the relative content of CD4+ (carriers of the *CC IL-4 (rs2243250)* have a smaller amount of IL-4, carriers of the genotype *CT IL-4 (rs2243250)* have a larger amount), $p\leq0.05$;
- moderate negative relationship between the allelic variant of *IL-4 (rs2243250)* and the relative content of CD16+ (carriers of the *CC* genotype have a smaller amount of IL-4, carriers of the genotype *CT IL-4 (rs2243250)* have a larger amount), $p\leq0.05$;
- moderate positive relationship between the allelic variant of *IL-4 (rs2243250)* and the activity of AST and ALT (carriers of the *CC IL-4 (rs2243250)* genotype have a higher activity, carriers of the *CT IL-4 (rs2243250)* genotype have a lower activity), $p\leq0.05$;
- moderate positive relationship between the allelic variant of *IL-4 (rs2243250)* and the content of IL-4 and TNF-α cytokines (carriers of the *CC IL-4 (rs2243250)* have a greater amount of IL-4, carriers of the *CT IL-4 (rs2243250)* have a smaller amount), $p\leq0.05$;
- moderate negative relationship between the allelic variant of *IL-4 (rs2243250)* and the content of IL-10 (carriers of the

CC IL-4 (rs2243250) have a smaller amount of IL-4, carriers of the genotype *CT IL-4 (rs2243250)* have a larger amount), p≤0.05.

In carriers of various *IL-10 (rs1800896)* genotypes, such patterns were not found.

In patients with homozygous *GG TNF-α (rs1800620)* polymorphism, there were more pronounced changes in the cytokine profile, immunological status, and a higher level of ALT and AST activity. Using the Spearman rank correlation coefficient, the following relationships were established:

- a strong positive relationship between the allelic variant of TNF-α and the relative content of CD3+ (carriers of the *GG TNF-α (rs1800620)* have more TNF-α, carriers of the *GA TNF-α (rs1800620)* have less TNF-α), p≤0.05;
- moderate positive relationship between the TNFα allelic variant and the relative content of CD4+ (carriers of the *GG TNF-α (rs1800620)* have more TNF-α, carriers of the *GA TNF-α (rs1800620)* have less TNF-α), p≤0.05;
- moderate positive relationship between the TNF-α allelic variant and the relative content of CD16+ (carriers of the *GG TNF-α (rs1800620)* have more TNF-α, carriers of the *GA TNF-α (rs1800620)* have less TNF-α), p≤0.05;
- moderate negative relationship between the TNFα allelic variant and ALT and AST activity (carriers of the GG *TNF-α (rs1800620)* have lower activity, carriers of the GA *TNF-α (rs1800620)* have higher activity), p≤0.05;
- moderate negative relationship between the TNF-α allelic variant and the content of IL-4 and TNF-α cytokines (carriers of the *GG TNF-α (rs1800620)* have less TNF-α, carriers of the *GA TNF-α (rs1800620)* have more TNF-α), p≤0.05;
- weak positive relationship between the allelic variant of TNF-α and the content of IL-10 (carriers of the *GG TNF-α (rs1800620)* have a larger amount of TNF-α, carriers of the *GA TNF-α (rs1800620)* have a smaller amount), p≤0.05.

Conclusions

The revealed relationship between ALT and Asat activity, immunological status and cytokine profile parameters with TNF-α and IL-4 genotypes suggests a genetic predisposition of patients with chronic VHB (carriers of certain alleles) to a more severe course of the disease.

This information can be used as one of the additional criteria for the activity of inflammatory changes in the liver tissue and possible a possible criterion for the rapid progression of fibrotic changes in the liver tissue.

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Взаємозв'язок алельного поліморфізму генів цитокінів, показників імунного та цитокінового профілю у хворих на хронічний вірусний гепатит В

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Анотація. Мета: провести комплексну оцінку імуногенетичних та біохімічних показників у хворих на хронічний вірусний гепатит В (ХВГВ), які проживають в Одеському регіоні. Об'єкт і методи дослідження. Обстежено 82 хворих на ХВГВ. Визначено традиційні біохімічні та імунологічні показники, вміст інтерлейкіну (ІЛ)-4, ІЛ-10, фактора некрозу пухлини (ФНП)-α та поліморфізм генів цитокінів ІЛ-4, ІЛ-10, ФНП-α. Результати. Для оцінки взаємозв'язку непараметричних та параметричних показників (змін імунологічного статусу, біохімічних показників та цитокінового профілю) хворі розподілені на групи відповідно до виявлених поліморфізмів генів цитокінів. Із застосуванням коефіцієнта рангової кореляції Спірмена встановлено певні зв'язки. У пацієнтів з гомозиготним поліморфізмом *CC IL-4* відзначали більш виражені зміни цитокінового профілю, імунологічного статусу і більш високий рівень активності аланінамінотрансферази і аспартатамінотрансферази. Висновок. Виявлений взаємозв'язок рівня аланінамінотрансферази і аспартатамінотрансферази, показників імунологічного статусу та цитокінового профілю з певними генотипами ІЛ-4, ІЛ-10, ФНП-α дозволяє припустити генетичну схильність певних хворих ХВГВ до більш тяжкого перебігу. Отримана інформація може бути застосована як один із додаткових критеріїв активності запальних змін печінкової тканини.

Ключові слова: хронічний вірусний гепатит В, поліморфізм генів, фіброз печінки, клітинний імунітет.

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