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COMPARATIVE CHARACTERISTICS OF ALLELIC POLYMORPHISM OF CYTOKINE GENES IN PATIENTS WITH CHRONIC HEPATITIS C AND CHRONIC HEPATITIS B

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Hepatitis B virus and hepatitis C virus are common causes of chronic hepatitis; from 5 to 10% of cases of HBV infection with or without co-infection with the hepatitis D virus, and in about 75% of cases of HCV infection turn into a chronic form.

The aim of the study is a comparative analysis of the frequency of detection of certain alleles of *IL-4(rs2243250), TNFα (rs1800620)* and *IL-10(rs1800896)* in patients with chronic hepatitis B and chronic hepatitis C.

The most pronounced differences in the studied groups were found in allelic polymorphism of *TNFα (rs1800620)*. In patients with chronic hepatitis B the homozygous variant *GG TNFα (rs1800620)* prevailed – 85,37 %, and in patients with chronic hepatitis C this genotype was found only in 19,0 % of patients. Heterozygous variant *GA TNFα (rs1800620)* prevailed in patients with chronic hepatitis C (77,0 %), in patients with chronic hepatitis B it was observed only in 14,67 % of patients. A small number of patients with chronic hepatitis C (4,0%) had a homozygous variant of *AA TNFα (rs1800620)* (mutation), which was not found in patients with chronic hepatitis B. The difference between these indicators is statistically significant.

Significant differences in gene polymorphism *TNFα (rs1800620)* in patients with chronic hepatitis C and B showen the possibility of individual the genetic profile in chronic hepatitis. The absence of a significant difference in the frequency of *IL-4 (rs2243250)* and *IL-10(rs1800896)* genotypes may be a confirmation of the important role of these cytokines in the immunological segment of the patients' genetic profile.

Key words: chronic hepatitis B, chronic hepatitis C, allelic polymorphism of cytokine genes.

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ПОРІВНЯЛЬНА ХАРАКТЕРИСТИКА АЛЬНОГО ПОЛІМОРФІЗМУ ГЕНІВ ЦИТОКІНІВ У ХВОРИХ НА ХРОНІЧНИЙ ГЕПАТИТ С ТА ХРОНІЧНИЙ ГЕПАТИТ В

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Вірус гепатиту В та вірус гепатиту С є частими причинами хронічного гепатиту; від 5 до 10% випадків HBV-інфекції з або без коінфекції з вірусом гепатиту D, і в близько 75% випадків HCV-інфекції переходять в хронічну форму.

Метою дослідження є порівняльний аналіз частоти виявлення алелів *IL-4(rs2243250)*, *TNFα (rs1800620)* та *IL-10(rs1800896)* у пацієнтів з ХГВ та ХГС.

Суттєві відмінності в поліморфізм генів *TNFα (rs1800620)* у хворих на хронічні гепатити С та В вказують на можливість індивідуалізації генетичного профілю при різних гепатитах. Відсутність суттєвої різниці частоти генотипів *IL-4(rs2243250)* та *IL-10(rs1800896)* може бути підтвердженням важливої ролі цих цитокінів в імунологічному сегменті генетичного профілю пацієнтів.

Ключові слова: хронічний гепатит В, хронічний гепатит С, алельний поліморфізм генів цитокінів.

Introduction

Hepatitis B virus and hepatitis C virus are common causes of chronic hepatitis; from 5 to 10% of cases of HBV infection with or without co-infection with the hepatitis D virus, and in about 75% of cases of HCV infection turn into a chronic form.

In 2010, WHO recognized viral hepatitis B and C as one of the leading health problems in the world. In 2014, the WHO Assembly established a global strategy for combating viral hepatitis ("Global Health Sector Strategy on Viral Hepatitis"), including the mortality rate from them among 10 key indicators for assessing the state of the health care system and setting the goal of reducing mortality associated with with infections caused by hepatitis B and C viruses by 65% by 2030.

According to the WHO, in 1990-2015, approximately 1.3-1.5 million people died annually in the world from infection associated with hepatitis B and C viruses, and 96% of them from chronic viral hepatitis, including liver cirrhosis and hepatocellular cancer. However, the same report emphasized that all countries have problems with statistical accounting of mortality from these diseases [1, 2, 3].

The current treatment strategy is based on genotype and virological response during treatment. However, the possibility of prescribing antiviral drugs is limited by various contraindications, significant side effects (anemia, thrombocytopenia, depression), as well as high cost. The heterogeneity of the response to antiviral therapy in chronic HCV infection patients necessitates the search for prognostic factors of response to treatment [4, 5].

In recent years, a number of studies have been carried out to study the genetic factors that determine the nature of the interaction of the pathogen and the macroorganism, affect the processes of chronicity of HCV infection and the rate of fibrogenesis. Given that cytokines are the most important participants in the immunopathogenesis of viral hepatitis, the study of the functional significance of polymorphism in the promoter region is an urgent issue. The study of immunogenetic factors that determine the susceptibility to chronicity of the process in viral hepatitis B is of particular importance, since it is the mechanisms of the immunological response that determine the course and outcome of the disease.

A number of groups of human genes have been identified, for which the relationship between their polymorphism and chronic HCV infection course variants has been shown. The dependence of chronic HCV infection progression on allelic variants of the cytokine genes IL-1β, IL-10, IL-6, TNFα, TNFβ has been established [6].

It is likely that it is the combination of several immunity defects due to the presence of polymorphism in several genes simultaneously that affects the course of a chronic viral infection.

Studies of the frequencies of genotypes and alleles of cytokine genes in patients with HBV are few. In addition, it has not been established whether polymorphic variants of some cytokine genes are common for chronic HCV infection and chronic HCB infection also [7].

The aim of the study– to conduct a comparative analysis of the frequency of occurrence of polymorphisms of the *IL-4(rs2243250)*, *TNFα (rs1800620)* and *IL-10(rs1800896)*, genes in patients with chronic hepatitis B and chronic hepatitis C living in the Odesa region, to improve the quality of diagnosis based on the obtained genetic criterias.

Materials and methods

82 patients with chronic hepatitis B and 100 patients with chronic hepatitis C aged 18 to 62 years were examined. All examined patients were under monitoring in the hepatological center of the Odesa Municipal Infectious Hospital.

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 C and chronic hepatitis B based on the routine biochemical parameters (activity of AST and ALT, level of bilirubin and the predominance of its direct fraction), qualitative and quantitative determination of HCV RNA and HBV DNA by PCR.

Molecular genetic studies included the determination of polymorphic variants of the *IL-4(rs2243250), TNFα (rs1800620), IL-10(rs1800896)* 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 are described in the GenBank genomic database. The studies were carried out on the basis of the German Diagnostic Center. St. Paul (Odesa).

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

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 in an ethnically homogeneous group of the Odessa region. Various mechanisms of action of hepatitis C and B viruses, as well as the features of the antiviral response of the human body, suggest different variants of certain polymorphisms.

The frequency of variants of allelic polymorphisms of the cytokine genes *IL-4(rs2243250), TNFα (rs1800620), IL-10(rs1800896)* in patients with different hepatites presented in Table 1.

When studying the occurrence of allelic polymorphisms of *IL-10(rs1800896)*, *IL-4(rs2243250)* and *TNFα (rs1800620)*, certain differences were revealed in the studied groups of patients with CHC and CHB.

The study of the polymorphic region of *IL-4 (rs2243250)* revealed the predominance of the homozygous variant *CC IL-4 (rs2243250)* both in the group of patients with hepatitis B and in the group of patients with hepatitis C, which amounted to 60,89% and 66,0%, respectively. The heterozygous variant *CT IL-4(rs2243250)* genotype was almost equally identified: 39,02% in patient with chronic hepatitis B patients and in 30,0% patients with chronic hepatitis C. However, in the study group of patients with chronic hepatitis B, the homozygous variant *TT IL-4(rs2243250)* (mutation) was not detected, and the frequency of its detection in the group of patients with chronic hepatitis C was not high – 4,0%. No statistically significant difference was found.

When studying the polymorphic region of *IL-10(rs1800896)*, a certain predominance of the homozygous variant *GG IL-10 (rs1800896)*) in the group of patients with hepatitis B (31,7%) was revealed in comparison with the group of patients with hepatitis C (19,0%). The heterozygous variant of the *GA IL-10(rs1800896)* genotype was almost equally identified: 60,0% in patients with chronic hepatitis B and 53,0% in patients with chronic hepatitis C. In the study group of patients with chronic hepatitis C, the homozygous variant of *AA IL-10(rs1800896)* (mutation) was detected much more often (28,0%) than in the group of patients with hepatitis B (7,3%). Statistically significant difference wasn't found.

The most pronounced differences in the studied groups were found in the study of *TNFα (rs1800620)* allelic

Table 1

Comparative frequency occurrence of allele polymorphisms in patients with chronic hepatitis C and patients with chronic hepatitis B

genotype/ allele	patients with hepatitis C (n=100)	frequency (%)	patients with hepatitis B (n=82)	frequency (%)	χ2	significance level
1	2	3	4	5	6	7
TNFα (rs1800620)						
AA	4,0	4,0	-	-		
GA	77,0	77,0	12	14,63	46,71	p < 0,001
GG	19,0	19,0	70	85,37	54,20	p < 0,001
IL-10(rs1800896)						
AA	28	28,0	6	7,3	7,25	p < 0,01
GA	53	53,0	50	60,0	0,75	p > 0,1
GG	19	19,0	26	31,7	2,68	p > 0,1
1	2	3	4	5	6	7
IL-4(rs2243250)						
TT	4	4,0	-	-		
CT	30	30,0	32	39,02	1,08	p > 0,1
CC	66	66,0	50	60,98	0,32	p > 0,001

polymorphism. In patients with chronic hepatitis B, the homozygous variant *GG TNFα (rs1800620)* prevailed – 85,37%, and in patients with chronic hepatitis C this genotype was found only in 19,0% of patients. Heterozygous *GA TNFα (rs1800620)* variant prevailed in patients with CHC (77%), in patients with CHB it was observed only in 14,63 % of patients. A small number of patients with chronic hepatitis C (4,0%) had a homozygous variant of *AA TNFα (rs1800620)* (mutation), which was not found in patients with chronic hepatitis B. The difference between these indicators is statistically significant.

Conclusions

1. In ethnically homogeneous groups of residents of the Odesa region between patients with chronic hepatitis B and chronic hepatitis C, differences were found in the frequencies of alleles of the *TNFα (rs1800620)* ($p<0.05$).

2. Some differences were found in the studied groups of patients when studying the allelic polymorphism of *IL-10 (rs1800896)*, however, the difference is not statistically significant.

3. Comparative analysis of *IL-4 (rs2243250)* allelic polymorphism in patients with chronic hepatitis C and chronic hepatitis B did not reveal a statistically significant difference.

Thus, the absence of a significant difference in the frequency of *IL-4(rs2243250)* and *IL-10(rs1800896)* genotypes may confirm the important role of these cytokines in the immunological segment of the genetic profile of patients. Significant differences in the polymorphism of the *TNFα (rs1800620)* genes in patients with chronic hepatitis C and chronic hepatitis B indicate the possibility of individualization of the genetic profile in various hepatitis. It can be assumed that the genetic characteristics of the patient can be the basis for predicting the rate of development of fibrotic changes in the liver, as well as a criterion for the timely administration of antifibrotic therapy.

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