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RELATIONSHIP OF CYTOKINE GENE POLYMORPHISM AND THE DEVELOPMENT OF LIVER FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS B&C

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Abstarct

One of the features of the spread of infectious diseases is an increase in the number of mixed infections caused by various types of microorganisms: bacteria, viruses, protozoa, fungi, mycoplasmas, chlamydia, etc. In mixed infections, the simultaneous presence of several pathogens leads to not only to the complex interaction of pathological processes, but also complicates the diagnosis and choice of treatment. Mixed hepatitis is characterized by high biochemical activity and histological changes, the presence of signs of portal hypertension. Infection with two viruses increases the risk of developing hepatocellular carcinoma. It should be noted that in patients with HBV infection, the development of hepatocellular carcinoma is possible at the stage of chronic hepatitis before the onset of liver cirrhosis. The **aim** of the study: to investigate a comprehensive assessment of the allelic polymorphism of the *IL-4 (rs2243250), IL-10 (rs1800896), TNFα (rs1800620), SMAD family member 7 (rs4939827)* and *eIF3h (rs16892766)* genes with the degree of fibrosis in patients with chronic hepatitis B&C. **Materials and methods.** In the course of the study, 62 patients

with chronic hepatitis B&C were examined before the start of treatment and after the end of the course of antiviral therapy. The degree of liver fibrosis was established by FibroScan. Molecular genetic studies included the determination of polymorphic variants of the *IL-4 (rs2243250), IL10 (rs1800896), TNFα (rs1800620), SMAD family member 7 (rs4939827), eIF3h (rs16892766)* genes. Polymorphism was studied by amplification of the corresponding regions of the genome by PCR. **Results and discussions.** Genotypes *CC IL-4 (rs2243250), GG IL-10 (rs1800896), GG TNFα (rs1800620), AA SMAD family member 7 (rs4939827), TT eIF3h (rs16892766)* have a protective effect on the course of chronic hepatitis B&C, as they are established in patients with chronic hepatitis B&C with a lower degree of fibrosis. Heterozygous *CT IL-4 (rs2243250), GA IL-10 (rs1800896), GA TNFα (rs1800620), CC SMAD family member 7 (rs4939827), CC eIF3h (rs16892766)* have a profibrotic effect on the course of chronic hepatitis B&C, therefore that they are established in patients with chronic hepatitis B&C with a greater degree of fibrosis.In patients with a low stage of fibrosis, the genotypes *CC IL-4 (rs2243250), GG IL-10 (rs1800896), GG TNFα (rs1800620), AA SMAD family member 7 (rs4939827), TT eIF3h (rs16892766)*, are most often determined. In patients with a degree of fibrosis, other genotypes were detected, namely *CT IL-4 (rs2243250), GA IL-10 (rs1800896), GA TNFα (rs1800620), CC SMAD family member 7 (rs4939827), CC eIF3h (rs16892766)*. **Conclusions.** The revealed differences in the frequency of occurrence of various gene polymorphisms in healthy subjects and patients with chronic hepatitis indicate a certain predisposition to the development of a chronic inflammatory process in the liver.

Key words: chronic hepatitis B&C; liver fibrosis; allelic polymorphism of cytokine genes *TNFα (rs1800620); IL-10 (rs1800896); IL-4 (rs2243250); SMAD family member 7 (rs4939827); eIF3h (rs16892766)*.

Relevance

Currently, one of the features of the spread of infectious diseases is an increase in the number of mixed infections caused by various types of microorganisms: bacteria, viruses, protozoa, fungi, mycoplasmas, chlamydia, etc. In mixed infections, the simultaneous presence of several pathogens leads to not only to the complex interaction of pathological processes, but also complicates the diagnosis and choice of treatment [1].

In the case of infection with different viruses, suppression of one virus or two viruses at the same time (interference phenomenon) is more often observed. However, there is contrary information that infection with several viruses is accompanied by their simultaneous reproduction with a cumulative effect and a progressive course of the pathological process in

the liver [2].

There are different points of view on the course of mixed hepatitis. Chronic mixed viral hepatitis is more often caused by a combination of HCV and HVB, or HDV+HBV. At the same time, the replication of more than one virus contributes to the progression of the chronic process with subsequent transformation into liver cirrhosis [3].

In case of chronic hepatitis B&C, two viral genomes are rarely detected at the same time. Mutual inhibition of two genomes is possible, which is later manifested by the dominance of one of the viruses. Simultaneous replication of several viruses contributes to the progression of chronic mixed hepatitis with further development of liver cirrhosis [4].

Mixed hepatitis is characterized by high biochemical activity and histological changes, the presence of signs of portal hypertension. Infection with two viruses increases the risk of developing hepatocellular carcinoma. It should be noted that in patients with HBV infection, the development of hepatocellular carcinoma is possible at the stage of chronic hepatitis before the onset of liver cirrhosis [5].

The existing developments have the disadvantage that they do not consider mixed infection (HBV+HCV) and the possibility of other combinations of cytokine genes than those given by the authors.

There is investigation in which genetic factors are studied that affect the interaction between the pathogen and the macroorganism, determine the rate of fibrogenesis and the consequences of viral hepatitis. Special attention is paid to the polymorphism of cytokine genes[6]. Our studies showed the functional significance of the polymorphism of the most important cytokines *IL-10 (rs1800896), IL-4 (rs2243250), TNFα (rs1800620)SMAD family member 7 (rs4939827)* and *eIF3h (rs16892766)* in the rate of progression of liver fibrosis in patients with chronic viral hepatitis (HBV, HCV).

The **aim** of the study: to investigate a comprehensive assessment of the allelic polymorphism of the *IL-4 (rs2243250), IL-10 (rs1800896), TNFα (rs1800620), SMAD family member 7 (rs4939827)* and *eIF3h (rs16892766)* genes with the degree of fibrosis in patients with chronic hepatitis B&C.

Materials and methods

In the course of the study, 62 patients with chronic hepatitis B&Cwere examined before the start of antiviral treatment and after the end of the course of therapy. All patients who participate in the study were registered at the Odessa Clinical Infectious Disease Hospital. There are 42 men and there only 20 women in group of studied patients. Patients diagnosed with HIV or other hepatotropic viruses were excluded from the study.

To compare sick and healthy people, a control group of 30 healthy middle-aged people was formed. The number of men and women was similar (15 people each).

The degree of liver fibrosis was established by FibroScan. FibroScan is a noninvasive 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.

Molecular genetic studies included the determination of polymorphic variants of the *IL-4 (rs2243250), IL10 (rs1800896), TNFα (rs1800620), SMAD family member 7 (rs4939827), eIF3h (rs16892766)*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) [7, 8].

Spearman's correlation coefficient was used to identify correlations between individual indicators.

Results and discussions

When comparing the frequency of allelic polymorphisms of the *IL-4 (rs2243250), IL10 (rs1800896), TNFα (rs1800620), SMAD family member 7 (rs4939827)* and *eIF3h (rs16892766)* cytokine genes in patients with chronic hepatites B&C and healthy persons, certain differences were revealed.

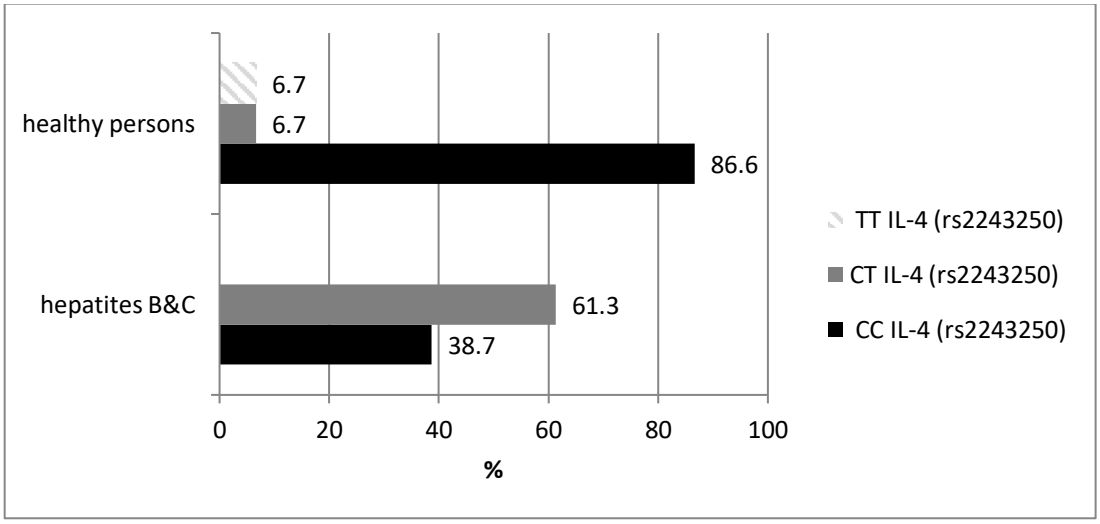

Fig. 1. Frequency distribution of *IL-4 (rs2243250)* in patients with viral hepatites B&C and healthy persons

In healthy individuals, the homozygous *CC IL-4 (rs2243250)* dominated – 88%. Only in patients with chronic mixed hepatitis B&C did the heterozygous *CT IL-4 (rs2243250)* predominate. A statistically significant difference in the prevalence of *CT* and *CC IL-4 (rs2243250)* in healthy persons and ill patients for chronic hepatitis B&C was revealed ($p < 0.001$).

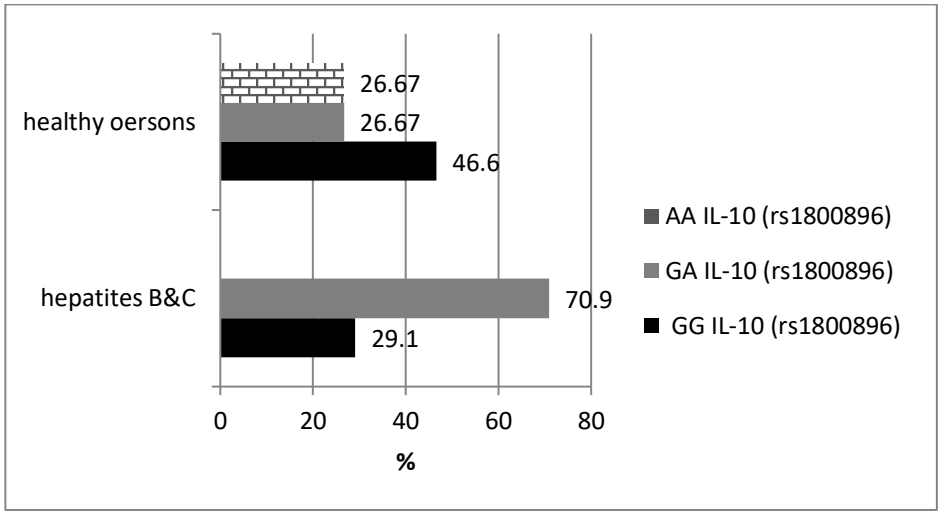

Fig.2. Frequency distribution of *IL-10 (rs1800896)* in patients with viral hepatites B&C and healthy persons

In the group of patients with hepatitis B&C, the heterozygous *GA IL-10 (rs1800896)* dominated in 70,9%, the homozygous *GG IL-10(rs1800896)* was found only in 28,19%, and the mutant homozygous genotype was not detected.

In healthy individuals, carriers of the homozygous *GG IL-10 (rs1800896)* prevailed, the number of carriers of the heterozygous *GA IL-10 (rs1800896)* and the mutant homozygous *AA IL-10 (rs1800896)* was the same – 26,67% each.

A statistically significant difference in prevalence of the *GA IL-10 - (rs1800896)* in healthy and ill patients for chronic hepatitis B&C was revealed ($p < 0.001$).

In patients with hepatitis B&C, the heterozygous *GA TNFα (rs1800620)* prevailed - 64.5%, patients with the homozygous *GG TNFα (rs1800620)* accounted for 35.5%, and patients with the mutant homozygous *AA TNFα (G308A)* were not identified.

In the group of healthy individuals, the homozygous *GG TNFα (rs1800620)* dominated – 80,0%, the number of patients with heterozygous *GA TNFα (rs1800620)* and mutant homozygous *AA TNFα (rs1800620) was* insignificant – 6.7% and 3.3%, respectively.

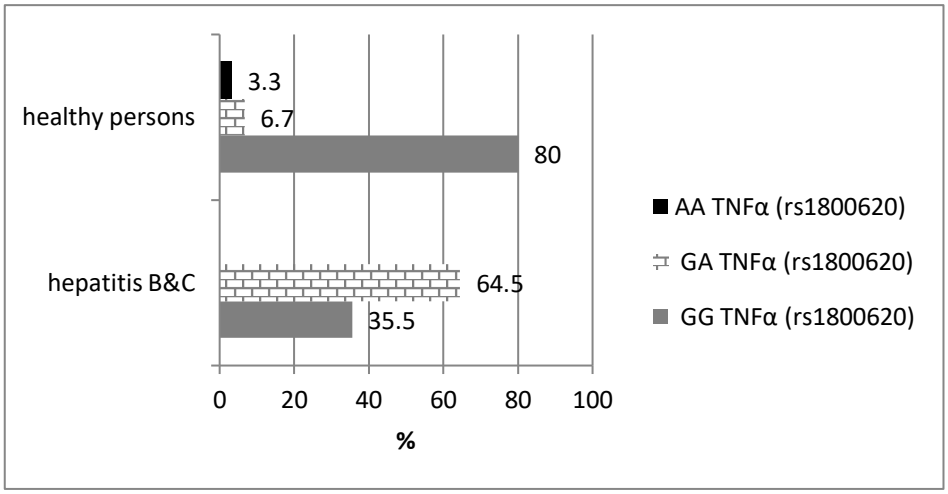

Fig. 3. Frequency distribution of *TNFα (rs1800620)* in patients with viral hepatitis B&C and healthy individuals

A statistically significant difference in the prevalence of the *GG TNFα genotype (rs1800620)* in healthy and ill patients for chronic hepatitis B&C was revealed ($p < 0.001$); as well as the difference in the incidence of heterozygous *GA TNFα (rs1800620)* in healthy and ill patients for hepatitis B&C ($p < 0.001$).

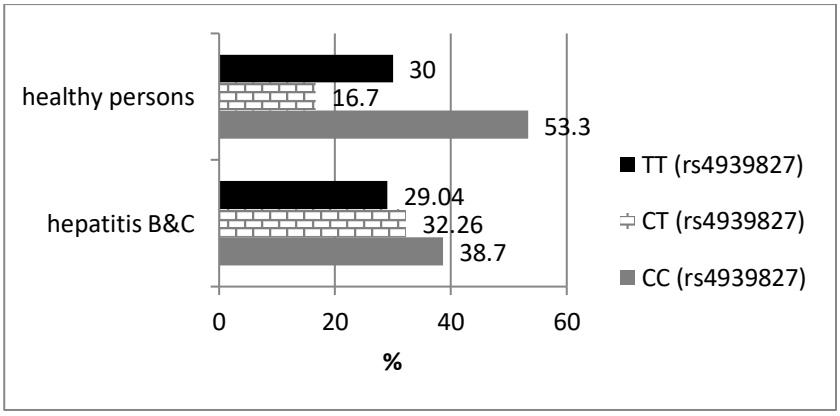

Fig. 4. Frequency distribution of *SMAD family member 7 (rs4939827)* in patients with viral hepatitis B&C and healthy individuals

In the group of healthy persons, the homozygous *CC SMAD family member 7 (rs4939827)* prevailed - it was determined in 53.3% individuals, the heterozygous *CT SMAD family member 7 (rs4939827)* and the mutant homozygous *TT SMAD family member 7 (rs4939827)* - in 16.7% and 30.0%, respectively.

No significant differences were found in the frequency of individual polymorphisms of

SMAD family member 7 (rs4939827) in the studied group of patients with hepatitis B&C. The homozygous *CC SMAD family member 7 (rs4939827)* was detected in 39% of patients, the *heterozygous CT of SMAD family member 7 (rs4939827)* – in 32.26% patients, and the mutant homozygous of *SMAD family member 7 (rs4939827)* – in 29,04% of patients. There was no statistically significant difference in the occurrence of genotypes in healthy and patients with hepatitis B&C.

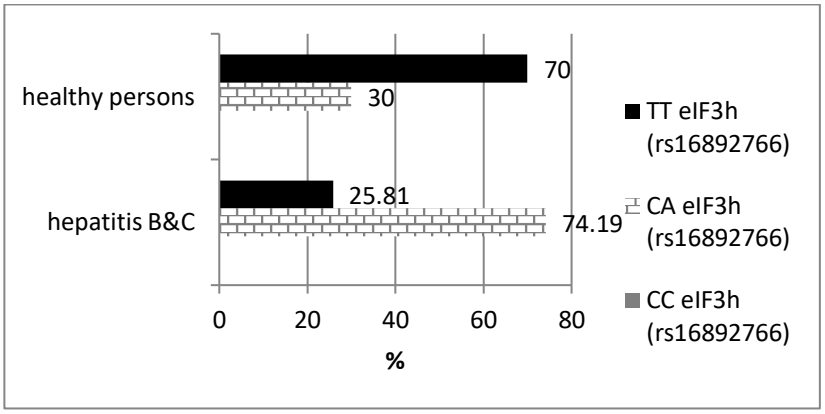

Fig. 5. Frequency distribution of *eIF3h (rs16892766)* in patients with viral hepatitis B&C and healthy individuals

In the control group, the homozygous mutant genotype *AA eIF3h (rs16892766)* prevailed - it was determined in 70% people, the heterozygous *CA eIF3h (rs16892766)* - only in 30% people. The homozygous *CC eIF3h (rs16892766)* was not detected in the control group.

In the studied group of patients with hepatitis B&C, the *CA eIF3h (rs16892766)* heterozygous prevailed, which was detected in 74.19% patients. Mutant homozygous *AA eIF3h (rs16892766)* was found only in 25.81%. The homozygous genotype of *CC eIF3h (rs16892766)* was not detected in the studied group. A statistically significant difference in the occurrence of *CA eIF3h (rs16892766)* genotype was revealed in healthy and patients with hepatitis B&C.

In the group of patients with hepatitis B&C, the distribution of patients according to the degree of fibrosis according to the results of FibroScan was as follows: the minimum degree of fibrosis (F0-F1) of the liver was detected in 40.3% (25 patients), moderate degree (F2) - in 17.8% (11 patients) and advanced fibrosis (F3) – in 41.9% (26 patients).

The relationship between the degree of liver fibrosis and allelic polymorphism of genes, as well as the relationship between individual cytokine genotypes, was assessed using Spearman's rank correlation coefficient (Table 1).

Table 1

Correlation between genetic parameters of patients with hepatitis B&C

	IL-4 (rs2243250)	*IL-10 (rs1800896)*	*TNFα (rs1800620)*	*SMAD familymember 7 (rs4939827)*	*eIF3h(rs16892766)*	Level of fibrosis, F
IL-4 (rs2243250)	1	-0,367*	-0,518**	-0,740*	0,375	0,910**
IL-10 (rs1800896)	-0,367*	1	0,120	0,595*	-0,244	-0,468**
TNFα (rs1800620)	-0,518**	0,120	1	0,425*	-0,219	-0,677**
SMAD familymember 7 (rs4939827)	-0,740*	0,595*	0,425*	1,000	-0,574*	-0,852*
eIF3h(rs16892766)	0,375	-0,244	-0,219	-0,574*	1,000	0,603*
Level of fibrosis, F	0,910**	-0,468**	-0,677**	-0,852*	0,603*	1

** - $p<0,01$; * - $p<0,05$

Thus, genotypes *CC IL-4 (rs2243250), GG IL-10 (rs1800896), GG TNFα (rs1800620), AA SMAD family member 7 (rs4939827), TT eIF3h (rs16892766)* have a protective effect on the course of chronic hepatitis B&C, as they are established in patients with chronic hepatitis B&C with a lower degree of fibrosis. Heterozygous *CT IL-4 (rs2243250), GA IL-10 (rs1800896), GA TNFα (rs1800620), CC SMAD family member 7 (rs4939827), CC eIF3h (rs16892766)* have a profibrotic effect on the course of chronic hepatitis B&C, therefore that they are established in patients with chronic hepatitis B&C with a greater degree of fibrosis.

In patients with a low stage of fibrosis, the genotypes *CC IL-4 (rs2243250), GG IL-10 (rs1800896), GG TNFα (rs1800620), AA SMAD family member 7 (rs4939827), TT eIF3h (rs16892766),* are most often determined. In patients with a degree of fibrosis, other genotypes were detected, namely *CT IL-4 (rs2243250), GA IL-10 (rs1800896), GA TNFα (rs1800620), CC SMAD family member 7 (rs4939827), CC eIF3h (rs16892766).*

According to the results of FibroScan, among patients with chronic hepatitis B&C with a low degree of fibrosis F0-F1, the protective genotype *CC IL-4 (rs2243250)* was detected in 92.31% patients with the specified degree of fibrosis, the protective genotype *GG TNFα (rs1800620)* - in 29.03% patients, protective genotype *GG IL-10 (rs1800896)* - in 22.58% patients. Among patients with a high degree of fibrosis F3, pro-fibrotic genotype *CT IL-4 (rs2243250)* was established in 41.94% patients with the specified degree of fibrosis,

genotype *GA TNFα (rs1800620)* - in 38.71%, genotype *GA IL-10 (rs1800896)* – in 41.94% patients (table 2).

Table 2

Distributionof genotypes *IL-4 (rs2243250), IL10 (rs1800896), TNFα (rs1800620), SMAD 7 (rs4939827), eIF3h (rs16892766)* in patients with varying degrees of fibrosis

	Patients with liver fibrosis F0-F1	Patients with liver fibrosis F2	Patients with liver fibrosis F3	Total
1	2	3	4	
CC IL-4 (rs2243250)	20	10	2	32
CT IL-4 (rs2243250)	2	14	16	22
TT IL-4 (rs2243250)	0	0	2	2
GG IL-10 (rs1800896)	2	10	2	14
GA IL-10 (rs1800896)	10	4	10	24
AA IL-10 (rs1800896)	8	10	4	22
GG TNFα (rs1800620)	6	0	4	10
GA TNFα (rs1800620)	16	22	6	44
AA TNFα (rs1800620)	0	2	6	8
CC SMAD familymember 7 (rs4939827)	16	0	0	16
CT SMAD familymember 7 (rs4939827)	6	18	16	40
TT SMAD familymember 7 (rs4939827)	0	6	0	6
CC eIF3h(rs16892766)	0	0	0	0
CA eIF3h(rs16892766)	4	4	4	12
AA eIF3h(rs16892766)	18	20	12	50

Thus, the proposed useful model due to the comprehensive assessment of allelic polymorphism of genes and the degree of liver fibrosis allows to assess the individual risk of progression of the disease, predict the course of the disease in the early stages and plan the tactics of patient management; the method is easy to use, can be automated and does not require large financial costs, allows you to create a personalized approach to the treatment of the patient.

Conclusions:

1. The revealed differences in the frequency of occurrence of various gene polymorphisms in healthy subjects and patients with chronic hepatitis indicate a certain predisposition to the development of a chronic inflammatory process in the liver.

2. The created prognostic scale makes it possible to make an individual treatment plan of the patient, in particular, andtiming of antifibrotic treatmentinitiation.

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