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THE SIGNIFICANCE OF GENE'S POLYMORPHISM *TNF α* (*rs1800620*) IN THE DEVELOPMENT OF FIBROSIS IN PATIENTS WITH VIRAL HEPATITIS OF VARIOUS ETIOLOGY

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Abstract

Recently it has been established that cytokines are involved in the regulation of the body's defense reactions in response to a pathogen and form an independent system for regulating homeostasis. **The aim of this work** is to study the relationship of the polymorphic marker *TNF α* (*rs1800620*) with its quantitative content and the degree of liver fibrosis in patients with various chronic hepatitis (VHC, VHB, VHB+C). The 82 patients with chronic hepatitis B, 62 patients with hepatitis B+C, and 100 patients with chronic hepatitis C were examined. *GGTNF α* (*rs1800620*) genotype is protective for patients with various viral hepatitis, and the *GATNF α* (*rs1800620*) and *AATNF α* (*rs1800620*) genotypes are profibrogenic for patients with chronic hepatitis B, chronic hepatitis B+C and chronic hepatitis C, respectively, since a high degree of fibrosis than in carriers of the homozygous *GGTNF α* (*rs1800620*) genotype. It is possible that the rate of fibrosis progression is influenced not only

by the qualitative content of TNF α , but also by a certain genotype.

Key words: chronic hepatitis; gene's polymorphism; fibrosis progression.

Introduction

In recent years, it has been established that cytokines are a group of polypeptide mediators of intercellular interaction that are involved in the regulation of the body's defense reactions in response to a pathogen, and also communicate between the immune, endocrine, hematopoietic and other systems. In this regard, cytokines are isolated into an independent system for regulating homeostasis [1, 2].

The study of immunoregulatory cytokines in chronic hepatitis B is of particular importance, since the mechanisms of the immunological response influence the course and outcome of the disease, and developing metabolic processes are the main factors that regulate the immune response to the antigen [3, 4]. The severity of chronic viral hepatitis is determined by the severity of fibrosis and the development of liver cirrhosis. However, there is still no clear understanding of the reasons that lead to different rates of formation of liver fibrosis in patients with chronic viral hepatitis.

There is information in the literature that polymorphism of a number of cytokine genes affects the processes of fibrogenesis and the progression of chronic viral hepatitis. Studies by a number of authors have revealed an association of polymorphic variants of *TNF α (G308A)* with the stage of liver fibrosis, as well as a connection of the TNF α gene with the level of TNF α in the blood serum [5].

However, the results of studying the association of genetic markers in patients with chronic viral hepatitis are often contradictory. In addition, studies of TNF α polymorphism have been largely carried out in patients with chronic hepatitis C.

Thus, it seems relevant to further study the polymorphism of cytokine genes, in particular TNF α , to clarify their influence on the course and outcome of chronic viral hepatitis.

The aim of this work is to study the relationship of the polymorphic marker *TNF α (rs1800620)* with its quantitative content and the degree of liver fibrosis in patients with various chronic hepatitis (VHC, VHB, VHB+C).

Materials and research methods

The 82 patients with chronic hepatitis B, 62 patients with hepatitis B+C, and 100 patients with chronic hepatitis C were examined. All of them were residents of Odessa region. The number of women included in the study was 93 (38.1%), men - 151 (61.9%). 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 confirmation of the diagnosis based on ELISA method identified traditional serological markers (HBeAg and HBsAg antigens, aHBe antibodies; aHCV total and IgM). Using polymerase chain reaction, the quantitative content of HBV DNA and HCV RNA was determined.

Determination of the concentration of the cytokine TNF α in blood serum was carried out by solid-phase ELISA analysis using reagent kits in human biological fluids in accordance with the attached instructions. The results were assessed photometrically (enzyme-linked immunosorbent microplate analyzer "Stat Fax-2100", USA).

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.

Polymorphism *TNF α (rs1800620)* 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 results obtained were subjected to statistical processing in MicroSoft Excel and Statistica programs.

Results and discussions

At the moment of admission to the hospital, asthenovegetative syndrome was observed in all patients with chronic hepatitis C (100%), dyspeptic syndrome in 86 patients (86%), and arthralgic syndrome in 41 patients (41%). Jaundice was registered rarely (11%) and was weak and undisturbed. The majority of patients had hepatomegaly (92%) and splenomegaly (45%).

The clinical progression of chronic hepatitis B in the following patients was also characterized by similar changes in the background: asthenovegetative syndrome in all patients (100%), dyspeptic syndrome in 68 patients (83%), arthralgic – in 34 (41%). Jaundice was registered in 21% of patients, it was mild and non-trivial. The majority of patients had hepatomegaly (93%) and splenomegaly (47%).

Asthenovegetative syndrome was also associated with chronic hepatitis B+C in all

patients (100%), dyspeptic syndrome in 60 patients (98%), and arthralgic syndrome in 25 patients (40%). Jaundice was registered more often, less in patients with other hepatitis (31%). The majority of patients had hepatomegaly (96%) and splenomegaly (68%).

The clinical progression of chronic hepatitis of viral etiology is characterized by periods of intensification and subsidence of painful signs, however, all patients before the start of antiviral treatment reinforced the meaning of the change in the cold state, as and reduced the bitterness of life. The frequency of occurrence of various *TNFα (rs1800620)* in patients with chronic hepatitis and healthy individuals differs significantly. The results are presented in Figure 1.

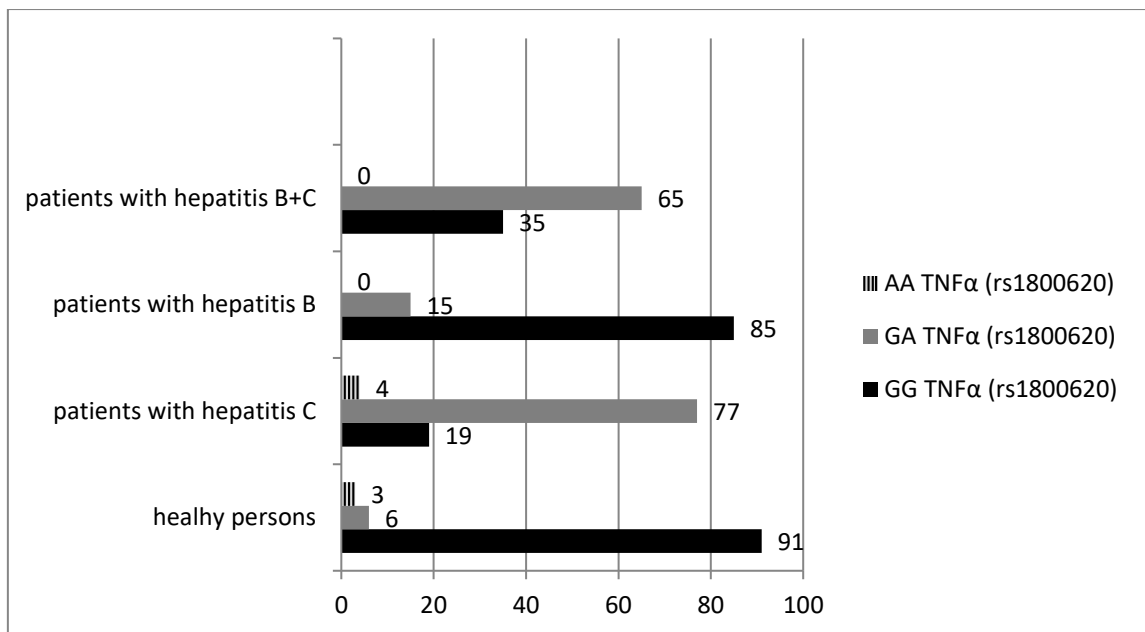


Fig. 1. Distribution of frequencies of *TNFα (rs1800620)* genotypes in patients with hepatitis and healthy individuals.

As can be seen from the presented figure, in healthy individuals the homozygous genotype *GGTNFα (rs1800620)* predominates (90,0%), the frequency of occurrence of other genotypes *GATNFα (rs1800620)* and *AATNFα (rs1800620)* is insignificant (6,7% and 3,3%, respectively).

In patients with chronic hepatitis C, the heterozygous genotype *GATNFα (rs1800620)* predominates (77,0%). The frequency of occurrence of homozygous genotypes *AATNFα (rs1800620)* and *GGTNFα (rs1800620)* was 19,0% and 4,0%, respectively.

The frequency of occurrence of the homozygous *GGTNFα (rs1800620)* genotype in patients with chronic hepatitis B is higher in comparison with other allelic variants. The normal homozygous *GGTNFα (rs1800620)* genotype occurs in 85.37% of patients, the

heterozygous *GATNFα (rs1800620)* genotype in 14.63%. The mutant homozygous variant *AATNFα (rs1800620)* was not found in patients with chronic hepatitis B.

In patients with chronic hepatitis B+C, the frequency distribution of TNFα genotypes was similar to the group of patients with chronic hepatitis C. The homozygous *GGTNFα (rs1800620)* genotype was detected in 35.5% of patients. The heterozygous variant *GATNFα (rs1800620)* was dominant and was found in 64.5% of patients with chronic hepatitis B+C. The mutant homozygous variant *AATNFα (rs1800620)* was not identified in this group of patients.

When studying the cytokine profile in healthy individuals and patients with chronic hepatitis, significant fluctuations in the concentration of TNFα were revealed. In healthy people, the concentration of TNFα varied from 0 to 6 pg/ml, the average value was 0.5 ± 0.05 pg/ml.

To assess the cytokine profile and evaluate its relationship with TNFα genotypes, patients were divided into 3 groups in accordance with a specific variant of the studied polymorphic marker G308A of the TNFα gene (Table 1).

Table 1. Concentration of TNFα in patients with different hepatitis depending on the allelic polymorphism of the *G308A* gene

Genotype	Chronic hepatitis C n=100	Chronic hepatitis B n=82	Chronic hepatitis B+C n=62
<i>GGTNFα (rs1800620)</i>	11.30±0.07 pg/ml	2.57±0.11 pg/ml	1.52±0.03 pg/ml
<i>GA TNFα (rs1800620)</i>	16.64±0.05 pg/ml	6.02±0.08 pg/ml	4.20±0.19 pg/ml
<i>AA TNFα (rs1800620)</i>	19.68±0.08 pg/ml	-	-

To assess the relationship between the identified parameters of the immunogenetic profile and the severity of morphological changes, all patients underwent FibroScan.

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.

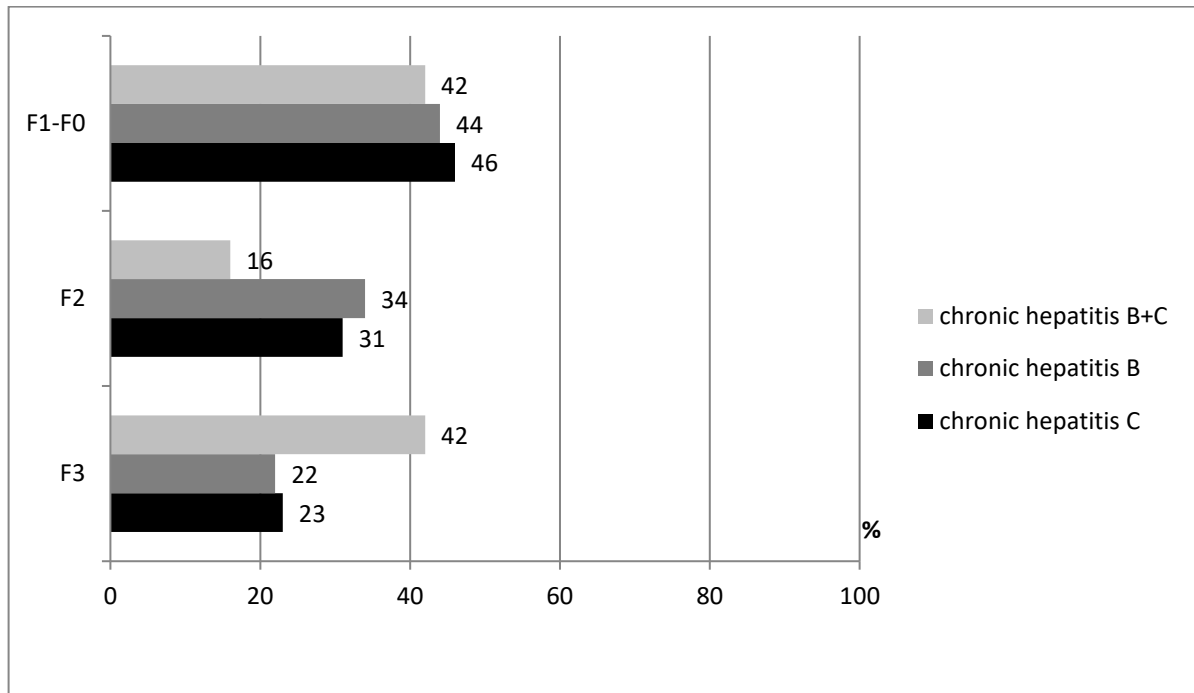


Fig. 2. Distribution of patients with chronic viral hepatitis according to the degree of liver fibrosis

As follows from Figure 2, the number of patients with a minimal degree of liver fibrosis was almost the same in the groups of patients with viral hepatitis. The number of patients with a degree of fibrosis F2 in the group of patients with chronic hepatitis B+C was smaller (16%) than in patients with chronic hepatitis C and chronic hepatitis B (31% and 34%, respectively). The number of patients with a degree of fibrosis F3 in the group of patients with chronic hepatitis B+C (42%) was almost 2 times higher than the number of such patients with chronic hepatitis B (22%) and chronic hepatitis C (23%).

To assess the relationship between the fibrotic process in the liver and TNF α genotypes, patients were divided into 3 groups in accordance with a specific variant of the studied polymorphic marker *TNF α (rs1800620)*(table 2).

To assess the relationship between nonparametric (polymorphic marker G308A of the TNF α gene, stage of liver fibrosis) and parametric (concentration of the TNF α cytokine in the patient's blood) data, the Spearman rank correlation coefficient was used.

The presence of the following patterns has been established:

Table 2. Relationship between liver fibrosis and TNF α genotypes

Polymorphism	F0-F1	F2	F3
<i>chronic hepatitis C</i>			
<i>GGTNFα (rs1800620)</i>	15	1	2
<i>GA TNFα (rs1800620)</i>	30	29	19
<i>AA TNFα (rs1800620)</i>	-	1	3
<i>chronic hepatitis B</i>			
<i>GGTNFα (rs1800620)</i>	34	22	8
<i>GA TNFα (rs1800620)</i>	-	6	12
<i>AA TNFα (rs1800620)</i>	-	-	-
<i>chronic hepatitis B+C</i>			
<i>GGTNFα (rs1800620)</i>	18	2	2
<i>GA TNFα (rs1800620)</i>	8	8	24
<i>AA TNFα (rs1800620)</i>	-	-	-

- moderate negative (inverse) relationship between the degree of fibrosis and *TNF α (rs1800620)* genotypes in patients with chronic hepatitis C, $p < 0.01$ (a lower degree of fibrosis is observed in carriers of the *GGTNF α (rs1800620)*, a higher degree of fibrosis is observed in carriers of the *AATNF α (rs1800620)*);

- moderate negative (inverse) relationship between the degree of fibrosis and *TNF α (rs1800620)* in patients with chronic hepatitis B, $p < 0.01$ (a lower degree of fibrosis is observed in carriers of the *GGTNF α (rs1800620)*, a higher degree of fibrosis is observed in carriers of the *GATNF α (rs1800620)*);

- moderate negative (inverse) relationship between the degree of fibrosis and *TNF α (rs1800620)* genotypes in patients with chronic hepatitis B+C, $p < 0.01$ (a lower degree of fibrosis is observed in carriers of the *GGTNF α (rs1800620)* genotype, a higher degree of fibrosis is observed in carriers of the *GATNF α (rs1800620)* genotype).

Conclusions

As a result of the pilot studies, it can be assumed that the *GGTNF α (rs1800620)* genotype is protective for patients with various viral hepatitis, and the *GATNF α (rs1800620)* and *AATNF α (rs1800620)* genotypes are profibrogenic for patients with chronic hepatitis B, chronic hepatitis B+C and chronic hepatitis C, respectively, since a high degree of fibrosis than in carriers of the homozygous *GGTNF α (rs1800620)* genotype. It is possible that

the rate of fibrosis progression is influenced not only by the qualitative content of TNF α , but also by a certain genotype.

References

1. Мороз Л. В., Бондарук І. Ю. Діагностична роль неінвазивних маркерів фіброзу печінки у хворих на хронічний вірусний гепатит С. Гепатологія. 2019. № 2. Вип. 44. С. 28-34 [Moroz L. V., Bondaruk I. Yu. Diagnostic role of non-invasive markers fibrotic liver in patients with chronic viral hepatitis C. *Hepatology*. 2019. No. 2. Issue 44. P. 28-34 J.]
2. Федорченко С.В. Коинфекція HCV/HBV: монографія. Київ: ВСИ «Медицина»; 2018. – 120 с. [Fedorchenko S.V. *HCV/HBV co-infection: monograph*. Kyiv: VSY "Medicine"; 2018. – 120 p.]
3. Monaco C., Nanchahal J., Taylor P., Feldmann M. Anti-TNF therapy: past, present and future. *Int Immunol*. 2015. №27(1). P. 55-62. doi: 10.1093/intimm/dxu102.
4. Karsdal M.A., Daniels S.J., Holm N. S. [et al]. Collagen biology and non-invasive biomarkers of liver fibrosis. *Liver Int*. 2020; 40: 736– 750. <https://doi.org/10.1111/liv.14390>.
5. Mourtzikou A., Alepakia M., Stamoulic M. [et al]. Evaluation of serum levels of IL-6, TNF- α , IL-10, IL-2 and IL-4 in patients with chronic hepatitis. *Immunology*. 2014. Vol. 33. Issue 2. P. 41-50. DOI: [10.1016/j.inmuno.2014.01.001](https://doi.org/10.1016/j.inmuno.2014.01.001)