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THE ROLE OF INTERLEUKIN-17 GENE POLYMORPHISM (RS612242 C11139G) IN THE FORMATION OF THE CRYOGLOBULINEMIC SYNDROME IN PATIENTS WITH CHRONIC HEPATITIS C

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Abstract

Background. Chronic hepatitis C (CHC) is characterized by the development of extrahepatic symptom manifestations, which leads to an increased risk of mortality in these patients. Today, the factors contributing to the development of extrahepatic manifestations of CHC are being studied. Particular attention is paid to determining the clinical significance of cytokine gene polymorphism. Therefore, the **aim of our work** was study the role of interleukin-17 gene polymorphism (rs612242 C11139G) in the mixed cryoglobulinemia (MC) formation and manifestation of cryoglobulinemic syndrome in patients with chronic hepatitis C.

Materials and methods. The study included 149 patients with CHC and 45 healthy people. Determination of single-nucleotide IL-17 gene polymorphism (rs 612242 C11139G) was performed by real-time polymerase chain reaction. Statistical processing was performed in program "STATISTICA for Windows 13" (StatSoft Inc., No. JPZ804I382130ARCN10-J).

Results. We found that most often in patients with CHC (140 - 94.0%) and in healthy people (39 - 86.7%) was detected CC-genotype. It was found that the GG genotype was found to be significantly more likely in healthy subjects (χ^2=20.5, p<0.0001). It was determined that patients with MC were significantly more likely to have a CC-genotype compared with healthy people (χ^2=5.08, p<0.05), but the frequency of detection of this genotype was not statistically different compared with patients without MC (p>0.05). In patients with CHC carriers of the CC genotype of the IL-17 gene polymorphism significantly more likely (p<0.05), clinical signs associated with MC, namely weakness (65.6% vs. 40.0%), arthralgia (58.9% vs. 30.0%), vasculitis (14.4% vs. 2.0%) with the formation of the Meltzer's triad (14.4% vs. no formation). It was showed that type 2 diabetes was significantly more likely to be detected in patients without MC (χ^2=5.52, p<0.05).

Conclusion. Allele C of the IL-17 gene polymorphism has an effect on the chronicity of hepatitis C according to a multiplicative model of inheritance. The CC-genotype plays a role in the formation of HCV-associated MC. Weakness, arthralgia and vasculitis with the formation of the Meltzer's triad are the most common manifestations of cryoglobulinemic syndrome.

Key words: **chronic hepatitis C; HCV; Genetic Polymorphism; interleukin-17; cryoglobulinemia.**

Introduction

In the course of chronic hepatitis C (CHC), not only the hepatic manifestations of the disease but also the extrahepatic lesions of the organism are of great importance [1]. This is primarily due to the proven influence of hepatitis C virus on the activation of autoimmune processes and the start of the production of mixed cryoglobulins with the formation of cryoglobulinemic syndrome [2]. Laboratory serum mixed cryoglobulins are found in 40-70% of patients with CHC, but clinically cryoglobulinemic syndrome is manifested only in 10-15% of cases [3]. Even when treating CHC patients with specific antiviral therapy, cryoglobulinemic vasculitis, as one of the three manifestations of cryoglobulinemic syndrome (weakness, arthralgia, vasculitis and Meltzer's triad formation), increases the odds of a 25% chance of death [4, 5].

The immunopathogenesis of HCV-associated cryoglobulinemic syndrome is explained by polyclonal stimulation of B-cells by virus antigens and a decrease in their activation threshold, leading to the uncontrolled production of autoantibodies and mixed cryoglobulins (MC) [10]. One of the factors regulating the activity of B-lymphocytes is interleukin-17 (IL-

17), which is able to stimulate epithelial, endothelial and fibroblastic cells to secrete inflammatory regulators (IL-1, IL-8, tumor necrosis factor) [6]. The role of IL-17 in the induction of proinflammatory cytokines and antimicrobial peptides in infectious and autoimmune diseases has only been studied [7].

Cytokine production and the course of infectious diseases are affected by the genes that encode them. For example, genotype TT conditioned reactivation Varicella Zoster virus in patients with herpes zoster and clinical manifestation of the disease [22]. There is evidence of the effect of IL-17 gene polymorphism (rs2275913 G152A) on the development of bronchial asthma in children, namely the presence of the A allele and AA homozygotes [8]. Another study found an association of IL-17 gene polymorphism (rs81933036), namely its TT genotype, with a greater likelihood of developing an intestinal Behcet disease [9]. According to the results of a meta-analysis [11], a strong relationship between the probability of development and progression of ulcerative colitis with the IL-17 polymorphism was established. Similar data were obtained in another meta-analysis [12] regarding the effect on carcinogenesis in general. Articles that included studies of IL-17A polymorphism (rs2275913 G197A) and IL-17F (rs763780 T7488C) were analyzed, and it was determined that both polymorphisms have a strong influence on the development of tumors of different localization. The GG genotype of the IL-17A polymorphism (rs2275913) was more commonly observed in patients with collateral cancer, and the genotype CC of IL-17F (rs763780) was observed in patients with gastric cancer [12].

In our opinion, the study [13], in which the authors found an association of IL-17 polymorphism (rs2275913 G197A) with the development of hepatocellular carcinoma in patients with CHC, deserves special attention. According to them, the GG and GA + GG genotypes increased the risk of this complication, while the AA genotype reduced it. However, in the literature available to us, we have not found any work on the effect of IL-17 gene polymorphism on the course of CHC and the formation of HCV-associated mixed cryoglobulinemia.

Aim of the work - to study the role of interleukin-17 gene polymorphism (rs612242 C11139G) in the mixed cryoglobulinemia formation and manifestation of cryoglobulinemic syndrome in patients with chronic hepatitis C.

Materials and methods. The study involved 149 patients who were examined at the hepatology center of the municipal institution "Zaporizhzhya Regional Clinical Infectious Hospital" of the Zaporizhzhya Regional Council. The volume of examinations was performed according to the protocol of the Ministry of Health of Ukraine №729 of 18.07.2016. The age

of patients ranged from 24 to 73 years, the median was 42.0 [37; 51] year. There were 98 males and 51 females. Most patients had infection with 1 genotype (101 - 67.8%) and 3 genotype (45 - 30.2%) of the virus. The degree of liver fibrosis was determined using non-invasive methods (Fibrotest or Elastometry), the results of which revealed F0 in 11 (7.4%), F1 - in 23 (15.4%), F2 - in 53 (35.5%), F3 - in 50 (33.6%), F4 - in 12 (8.1%) patients.

Genotyping of IL-17 gene polymorphism (rs 612242 C11139G) was performed on the basis of the Department of Molecular Genetic Studies of the Training and Laboratory Center of the ZSMU (headed by Prof. Kamyshny O. M.) with the help of a polymerase chain reaction detection system CFX-96 Touch (BIO-RAD, USA) using NP-524-100 (RF) kits. Spectrophotometric method determined the content of mixed cryoglobulins in serum. The control group consisted of 45 healthy people.

Statistical processing of data was performed in STATISTICA for Windows 13 (StatSoft Inc., No. JPZ804I382130ARCN10-J). To assess the significance of differences between qualitative traits, the χ^2 method is used. To analyze the distribution of genotypes between patients and the healthy, we used the odds ratio (OR) method in the Case-Control online calculator (http://gen-exp.ru/calculator_or.php). The OR level was considered greater than one to be significant for influencing the trait. Impact was also considered highly probable with a confidence interval (95% CI) greater than 1 when the unit did not enter the specified interval.

Results. According to the results of frequency analysis of different genotypes registration of IL-17 gene polymorphism in patients with CHC and healthy people, we found that most often in patients with CHC (140 - 94.0%) and in healthy people (39 - 86.7 %) was detected CC-genotype. The CG genotype was detected only in patients with CHC (9 - 6.0%), and the GG genotype, on the contrary, was detected only in healthy people and was not detected in patients with CHC. To assess the impact of each allele (C and G) on the likelihood of chronic hepatitis C, a multiplicative model of inheritance was created. According to this model, the allele C (χ^2=14.36, p<0.005, OR=4.94, 95% Cl=2.01-12.15) has the greatest influence on the formation of hepatitis C chronic cases (Table 1).

Table 1. Multiplicative model of alleles inheritance of the IL-17 gene polymorphism

Alleles	Patients with CHC (n=149)	Healthy people (n=45)	χ^2	p	OR (odd ratio)	
					Value	95% Cl
Allele C	0,970	0,867	14,36	0,0002	4,94	2,01-12,15
Allele G	0,030	0,133			0,20	0,08-0,50

Accordingly, healthy individuals are more characterized by the presence of the G-allele of the IL-17 gene polymorphism, which is confirmed by our observations, namely the presence of the GG genotype exclusively in the control group. In the following study, we paid attention to carriers of the CC-genotype, so for further calculations, two groups of patients were formed: carriers of the CC-genotype and carriers of the CG/GG-genotype. It was found that the GG genotype was found to be significantly more likely in healthy subjects, whereas this genotype was not observed at all in patients (χ^2=20.5, p<0.0001). This may indicate a favorable effect of the genotype to counteract the chronicity of hepatitis C (Table 2).

To determine the role of IL-17 gene polymorphism (rs612242 C11139G) in the formation of HCV-associated MC, we compared the frequency of detection of this extrahepatic onset in patients with CHC depending on the genotype of the IL-17 gene. The incidence of mixed cryoglobulins in the serum of patients with CHC was 62.4% (93 out of 149). It should be noted that carriers of the CC genotype were 90 patients (96.8%). It was determined that patients with MC were significantly more likely to have a CC-genotype compared with healthy people (χ^2=5.08, p<0.05), but the frequency of detection of this genotype was not statistically different compared with patients without MC (p>0.05). The above indicates a certain effect of the IL-17 gene polymorphism (rs 612242 C11139G) on the formation of MC in patients with CHC, namely the CC genotype (Table 2).

Table 2. Detection frequency of different IL-17 gene polymorphism genotypes (rs 612242 C11139G) in patients with CHC depending on the presence of MC

	Healthy people (n=45)	Patients with CHC (n=149)	Patients with CHC (n=149)	
			with MC (n=93)	without MC (n=56)
Genotype CC	39 (86,7%)	140 (94%)	90 (96,8%) *****	50 (89,3%)
Genotype CG	0 (0%)	9 (6%)	3 (3,2%)	6 (10,7%) *
Genotype GG	6 (13,3%)	0 (0%) *	0 (0%) *	0 (0%) *

Note: * - significant difference compared to healthy people (p<0.05).

The onset of clinical manifestations of cryoglobulinemic syndrome did not occur in all patients with CHC. Weakness was most commonly reported among CG/GG genotype carriers (66.7%, 6 of 9) and CC genotype carriers (56.4%, 79 of 140). The second most frequent manifestation was arthralgia, which was detected in 48.6% (68 of 140) patients with the CC genotype and in 33.3% (3 of 9) patients with CG/GG genotypes. We reported vasculitis and Meltzer's triad formation only in patients with CC genotype in 10% (14 of 140) and 9.3% (13 of 140) cases, respectively. Among the carriers of CG/GG genotypes, these manifestations of cryoglobulinemic syndrome have not been identified at all.

For a deeper study of the effect of the CC-genotype of the IL-17 gene polymorphism on the manifestation of cryoglobulinemic syndrome, we examined the frequency of clinical manifestations associated with the manifestation of cryoglobulinemic syndrome in carriers of CC-genotype in the presence of mixed cryoglobulins. We found that weakness was observed in 65.6% (59 of 90), which was significantly more frequent than carriers of the same genotype, but without MC - in 40% (20 of 50) (χ^2=8.54, p<0.005). Arthralgia were also more commonly observed in patients with MC: 58.9% (53 of 90) versus 30% (15 of 50) (χ^2=10.74, p<0.005). Vasculitis was reported in patients with MC in 14.4% (13 of 90) and without MC in 2% (1 of 50), but this difference was statistically significant (χ^2=5.53, p<0.05). The formation of the Meltzer's triad was determined only in patients with MC among carriers of the CC genotype - in 14.4% (13 of 90) patients (χ^2=7.96, p<0.005) (Fig. 1).

Analysis of the frequency of other extrahepatic manifestations of CHC in patients with CC-genotype, depending on the presence of MC, showed that type 2 diabetes was significantly more likely to be detected in patients without MC (χ^2=5.52, p<0.05). It should be noted the presence of thyropathies, which had a subclinical course and was confirmed only by changes in the content of thyroid hormones in the serum, both in patients with CHC with MC and without this extrahepatic manifestation, but no statistically significant difference was found (p>0,05) (Fig. 1).

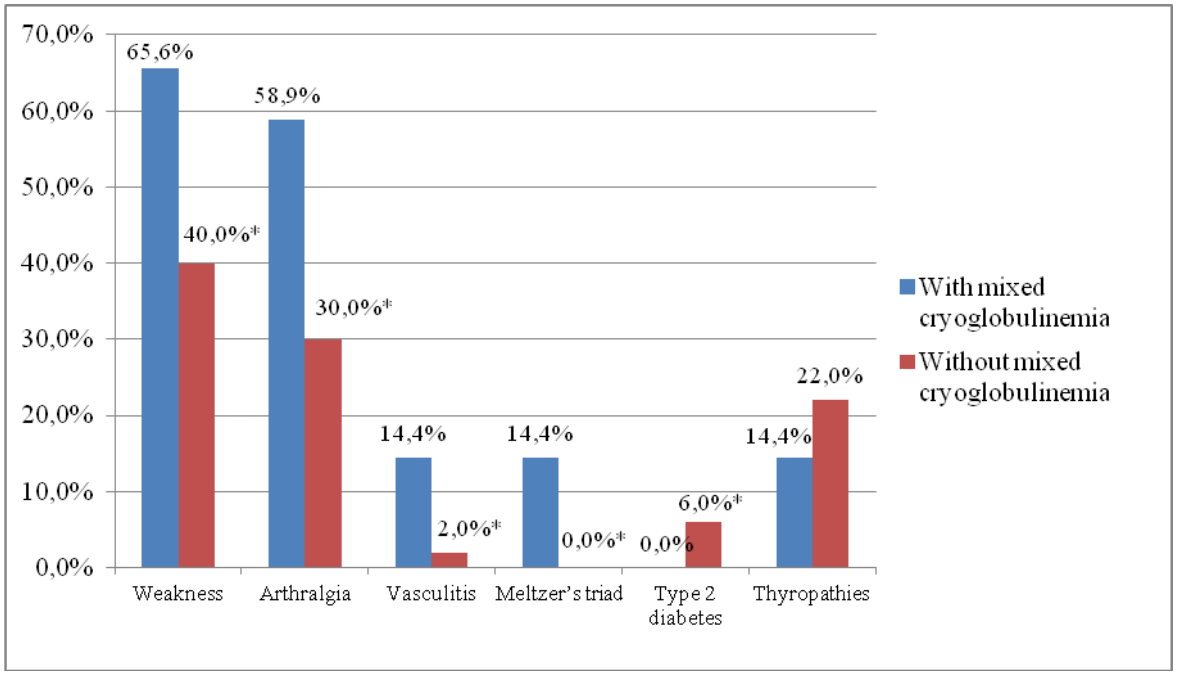

Fig. 1. Frequency of clinical manifestations detection of cryoglobulinemic syndrome and other extrahepatic signs of CHC in carriers of CC genotype depending on the presence of MC

Note: * - significant difference compared to CHC patients with MC ($p<0.05$).

Discussion

As a result of the study, we found a certain effect of the IL-17 gene polymorphism on the process of chronic hepatitis C virus. We found that carriers of the C-allele of the IL-17 gene polymorphism have a significantly higher probability of HCV chronicity compared to G-allele carriers ($\chi^2=14.36$, $p<0.005$, OR=4.94, 95% Cl=2.01-12.15). In the study of the frequency of detection of genotypes of the IL-17 gene polymorphism, we found a higher frequency of GG-genotype among healthy people, compared with patients with CHC ($\chi^2=20.5$, $p<0.0001$). In the present study [14], no significant difference was found in the incidence of IL-17 gene polymorphism in healthy and sick people, but an equally high incidence of CC genotype was determined in both groups. In the available sources, we were unable to find data regarding the effect of each allele on the chronicity of hepatitis C.

In the analysis of the relationship between IL-17 gene polymorphism and MC formation, we found that in patients with cryoglobulinemia, the CC genotype was significantly more likely to be compared with healthy humans ($\chi^2=5.08$, $p<0.05$). At the same time, we found no statistical difference between patients with and without MC. Therefore, it can be argued that the CC genotype can contribute to the formation of MC in patients with

CHC. We have already published work on the polymorphism of the IL-6 gene, where we found that the carrier of the G-allele (namely CG/GG genotypes) had a significant difference (χ^2=8.94, p=0.003) for the formation of MC [15]. Other researchers have also studied this problem, but they have drawn attention to the human leukocyte antigen (HLA) complex, resulting in data on the effect of HLA-B51 and HLA-B35 on autoimmune disorders in CHC patients [16]. In this study [17], it was found that the HLA-B8-DR3 haplotype was associated with a higher frequency of detection of mixed cryoglobulins in the blood of patients with CHC. We found individual studies related to the study of the relationship between polymorphism of interleukin genes and the development of MC.

We examined the frequency of cryoglobulinemic syndrome manifestation and found that significantly more frequent clinical manifestations were observed in carriers of the CC genotype with MC, namely weakness (65.6%), arthralgia (58.9%), vasculitis (58.9%)) and the Meltzer's triad formed (14.4%). In world practice, these manifestations of cryoglobulinemic syndrome are very different among researchers: from 38% to 98% [18, 19, 20, 21]. But all studies indicate that weakness, arthralgia and vasculitis with the formation of the Meltzer's triad are the most common manifestations of cryoglobulinemic syndrome.

Conclusion

1. Allele C of the IL-17 gene polymorphism (rs 612242 C11139G) has an effect on the chronicity of hepatitis C according to a multiplicative model of inheritance (χ^2=14.36, p<0.005, OR=4.94, 95% Cl=2.01-12.15).

2. The CC-genotype of the IL-17 gene polymorphism (rs 612242 C11139G), which is more frequently detected in these patients compared to healthy people (χ^2=5.08, p<0.05) plays a role in the formation of HCV-associated MC.

3. In patients with CHC carriers of the CC genotype of the IL-17 gene polymorphism (rs 612242 C11139G) significantly more likely (p<0.05), clinical signs associated with MC, namely weakness (65.6% vs. 40.0%), arthralgia (58.9% vs. 30.0%), vasculitis (14.4% vs. 2.0%) with the formation of the Meltzer's triad (14.4% vs. no formation).

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