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Immunogenetic features in patients with chronic hepatitis C

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Abstract

The aim of this study was to investigate the gene IL-28B polymorphism and indices of the cellular immunity in patients with chronic hepatitis C (CHC) and healthy individuals belonging to ethnically homogeneous group of residents of Odessa region as well as to determine possible association of certain genotypes and the degree of liver fibrosis. Polymorphism was investigated using corresponding portions of the genome by PCR. The structure of the primers used and the parameters of temperature cycles are described in the literature and genomic database. Evaluation of morphological changes in the liver (the degree of activity and severity of fibrotic changes) was determined by the scale METAVIR using a non-invasive method Fibrotest. There was revealed a low inverse correlation between genotypes of IL-28B and the degree of liver fibrosis.

Keywords: chronic hepatitis C, allele gene polymorphism

Background

Chronic hepatitis C continues to be one of the most urgent medical problems. At present there are widely conducted researches to determine genetic determinants of development and progression of chronic hepatitis C as well as the response to drug therapy. Standard antiviral therapy with pegylated interferon in combination with ribavirin is most frequently used for the treatment of HCV patients. A sustained virologic response is observed in 40-50% of patients with genotype 1 HCV and in 70-80% with the genotypes 2 and 3 [1, 2, 3].

HCV genotype, baseline viral load and stage of liver fibrosis as well as gene IL-28B (rs12979860 and rs8099917) polymorphism [4, 5] are considered the most important predictors of sustained virologic response before treatment.

Single nucleotide polymorphisms of cytokine genes are one of the factors determining the individual features of the course and prognosis of HCV. However, results of the study of cytokine genes association are often contradictory, even in ethnically homogeneous group. Determination of polymorphism of IL-28B is carried out for two main loci: rs12979860 and rs8099917. CC genotypes for locus rs12979860 and rs8099917 locus for CT are associated with a greater likelihood of achieving sustained virologic response in patients with CHC in standard antiviral therapy. However, this regularity is typical mostly of patients with genotype 1 chronic hepatitis C [6, 7, 8].

It is proved that the effect of gene IL-28B polymorphism as an alternative response to antiviral therapy is estimated at the level of viral load. Thus, the patients with genotype 1 HCV and CC polymorphism rs12979860 allele were found to have a more pronounced and rapid reduction in viral load during the first 4 weeks of treatment. However, in patients with genotypes 2 and 3 HCV this regularity is not revealed [9, 10, 11, 12].

In the literature, there is evidence that the gene IL-28B polymorphism in the patients with genotype 1 HCV is associated with differential expression of intrahepatic genes stimulated by interferon. In addition, serum levels of IL-28A \ B were significantly higher in patients with a favorable allele of rs12979860 [13, 14, 15]. These data indicate the antiviral and immune-mediated effects of IL-28B, which is likely to depend on these polymorphisms [16].

A number of factors (age, gender, presence of other chronic diseases) can change the effect of the genotype of the patient on the efficacy of antiviral therapy, including peculiarities of the immune response of the organism to HCV [17, 18].

There was also shown a definite correlation of the virologic response to therapy of patients with chronic hepatitis C with pegylated interferon and ribavirin with the functional state of natural killer cells (EC) as well as with the level of specific T-cell immunity [19, 20, 21].

Therefore, it seems appropriate to examine the correlations between gene IL-28B polymorphism, indices of the immune status as well as with the degree of liver fibrosis in patients with CHC who live in Odessa region.

Purpose of the study - to determine the relationship between polymorphism of IL-28B gene (rs8099917) in the patients with chronic hepatitis C and a number of immunological indices depending on the degree of liver fibrosis.

Materials

There were examined 100 HCV patients aged 18 to 62, with a mean age of 42 ± 3.08. All patients were followed up in the Hepatologic Center of Odessa Municipal Clinical Infectious Hospital. The patients were residents of Odessa region, there were 44% of men and 56% of women in the study groups. The disease duration was not more than 10 years.

The control group for the study of immunological indices was made up of 30 healthy subjects with mean age 32 ± 1.05. The number of women and men was the same (15 persons).

All participants signed a written informed voluntary consent to be included in the study. The methodology of this clinical study was consistent with ONMedU Bioethics Committee requirements.

Verification of the diagnosis of HCV included: biochemical tests (increased activity of AST and ALT, bilirubin concentration and the dominance of its direct fraction) and serological markers (HCV-IgM, qualitative and quantitative determination of mRNA and the HCV PCR, virus genotyping).

Evaluation of morphological changes in the liver (the degree of activity and severity of fibrotic changes) was determined by the scale METAVIR using a non-invasive method Fibrotest. Assessment of the degree of fibrosis was determined by the level of such indices as alfa2macroglobulin, haptoglobin, apolipoprotein A1, general bilirubin, GGT, ALT and AST.

Molecular genetic study included determination of polymorphic variants of IL-28B gene (rs8099917). DNA extraction was performed using a set of "DNA-EXPRESS-blood" (SPC "Liteh", the Russian Federation). Polymorphism was investigated using amplification of the corresponding portions of the genome by PCR. The studies were conducted on the basis of the German Diagnostic Center after St. Paul (Odessa).

Determination of subpopulations of T and B lymphocytes (CD3 +, CD4 +, CD8 +, CD16 +, CD19 +) was carried out by the immunofluorescence method using monoclonal and polyclonal set of antibodies to determine differential human lymphocyte antigens by the immunofluorescence microscope "Eurostar".

In order to detect correlations between separate indices Spearman correlation coefficient was applied. The frequencies of alleles and genotypes in the groups were compared using Pearson's $\chi 2$ test with Yates correction for continuity in the number of degrees of freedom equal to 1.

Discussion

The distribution of allelic variants of the IL-28B (rs8099917) gene polymorphisms in the patients and healthy individuals is shown in Figure 1. To compare the frequency of occurrence of different genotypes in healthy individuals, the investigation of 226 persons living in the European region was found in the GenBank database.

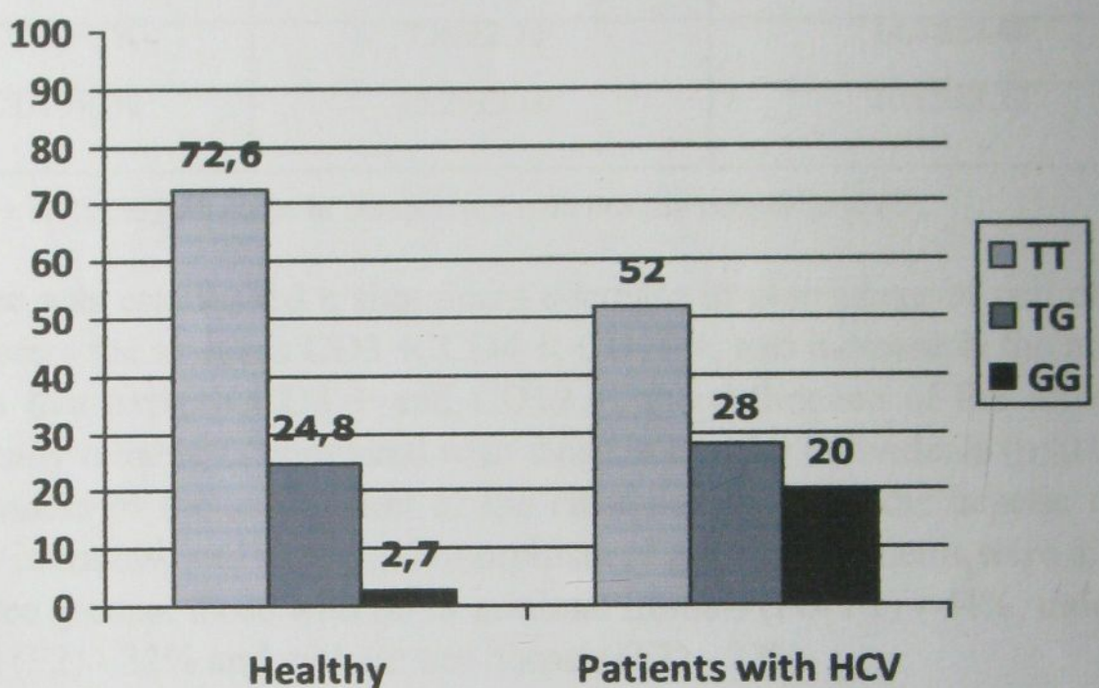

Fig.1 Occurrence frequency distribution of IL-28B genotypes in CHC patients and healthy individuals.

TT homozygous variant was found in 72.6% of individuals, heterozygous variant of TG - in 24.8%, and homozygous GG (mutation) - in 2.7% of patients.

When studying the allelic polymorphisms of IL-28B (rs8099917) in the group of HCV patients and in the group of healthy individuals there was revealed a predominance of homozygous genotype TT, 72.6% and 52%, respectively ($p < 0.001$, $\chi 2 = 13.12$), however, in the group of the patients this predominance was less significant. Almost an equal number of carriers had a heterozygous variant of TG: 28% in CHC patients, 24.8% in healthy individuals ($\chi 2 = 0.13$). There was noted the prevalence of heterozygous alleles of the GG (mutation) in the group of the patients with CHC - 20% compared with healthy individuals (2.7%) ($p < 0.05$, $\chi 2 = 28.4$). In healthy individuals the frequency of allele G was 0.85; A allele was 0.15. It was 0.66 and 0.44 respectively in the patients.

The findings of a number of indices of the immune status in the patients

with chronic hepatitis C and healthy individuals are presented in Table 2.

Table 2. Immunological indices in the patients with chronic hepatitis C and healthy individuals (M±m).

Subpopulations	Patients with HCV, n=100	Healthy individuals, n = 30
CD3+,%	32.29±4.66+	71.81±4.50
CD4+, %	27.97±4.49+	41.22±4.92
CD8+, %	24.07±4.28+	20.51±4.04
CD16+, %	7.89±2.70+	14.14±3.48
CD19+, %	15.29±3.60+	10.83±3.12

+ Reliable difference in comparison with healthy people ($p \leq 0.05$).

There was established a significant decrease in percentage of cell content that express the antigens CD3 +, CD4 +, CD16 +, and increase in the number of cells that express CD8 + and CD19 +, the difference of the indices is statistically reliable as compared with those in healthy individuals ($p \leq 0.05$).

To assess of the association of the changes degree of the hepatic tissue, cellular immunity and allelic polymorphism of genes, all patients were divided into three groups: those with no or minimal fibrosis (F0- F1) - 44%, moderate fibrosis (F2) - 32% and with severe fibrosis (F3) - 23%.

Analysis of the distribution of alleles of IL-28B (rs8099917) in the patients with chronic hepatitis C in accordance with the degree of fibrosis is shown in Figure 2.

While studying correlations of the fibrotic changes in the liver tissue, cellular immunity and allelic polymorphism of genotypes studied using Spearman's rank correlation coefficient there were established certain regularities.

Figure 2

	F	CD_{3+}	CD_{4+}	CD_{8+}	CD_{16+}	CD_{19+}
IL 28 GG, TG,TT	-0,402 **	0,795 **	0,771 **	-0,738 **	0,742 **	-0,767 **

** - Statistically significant association ($p < 0.01$)

There was revealed an inverse weak correlation ($r = - 0.402$) between the genotypes of IL-28B (rs8099917) and the degree of liver fibrosis. The carriers of genotype TT IL-28B (rs8099917) were revealed to have a lesser degree of liver fibrosis than the carriers of genotype GG IL-28B (rs8099917) ($p < 0.01$).

There was found a direct weak correlation between IL-28B genotype (rs8099917) and the number of certain cells. The carriers of the TT genotype IL-28B (rs8099917) were noted to have a higher content of CD3 +, CD4 +, CD16 + than the carriers of the GG genotype of IL-28B (rs8099917), Spearman rank correlation coefficient was 0.795, 0.771, 0.742 respectively ($p < 0.01$).

Furthermore, there was established an inverse correlation between the average number of CD8 + and CD19 +. The carriers of the TTIL-28B (rs8099917) genotype have smaller content of CD8 + ($r = - 0.738$) and CD19 + ($r = - 0.767$).

Conclusions

There was determined the presence of a weak inverse correlation between IL-28B genotype (rs8099917) and the degree of liver fibrosis; direct mean correlations between genotypes of IL-28B (rs8099917) and the content of CD3 +, CD4 +, CD16 +, and the average reverse correlation between genotypes of IL-28B (rs8099917) and the content of CD8 + and CD19 +. The established correlations described above allows to continue further search for more accurate genetic criteria of HCV progression. Changes of the immune status of the patients may serve as an additional criterion for predicting disease outcome.

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