LABORATORY-BIOCHEMICAL FEATURES OF THE COURSE OF CHRONIC HEPATITIS C WITH POLYMORPHISM rs11536889 + 3725G/C OF TLR-4 GENE

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Abstract

Chronic HCV infection remains as a global health problem due to its wide prevalence, latent course without clinical manifestations, development of liver fibrosis with the eventual formation of cirrhosis and liver cancer, which largely leads to poor prognosis and short survival of patients. Therefore, it is necessary to study in more detail the risk factors for adverse events, as their modification which may improve the prognosis and clinical consequences for patients with CHC. The article considers the changes of the main biochemical markers of liver damage and their dependence with the rs11536889 + 3725G/C polymorphism of the TLR-4 gene.

Key words: HCV; chronic hepatitis C; cytolysis; cholestasis; hepatocellular insufficiency; TLR-4

BACKGROUND:

According to WHO data, was estimated that there are more than 170 million people in all over the world which were infected by hepatitis C virus. Every year this number increases by 2-3 million, despite the availability of effective drugs for managment. As a rule, the main clinical form of HCV infection is chronic hepatitis C (CHC), which develops in 60-80% of all

cases of infection. For a long time, most patients do not have any symptoms of the disease, which leads to late detection of the disease at the end stage of irreversible liver damage (cirrhosis, primary cancer) [1, 3, 4]. Liver cirrhosis, which usually develops in 15-30% of patients within 20 years, is a determining factor of mortality from CHC. According to the clinical course, cirrhosis can be compensated with minor liver dysfunction, or sub- and decompensated, which is usually an important precursor to the development of hepatocellular carcinoma, which develops in 2-7% of patients. Therefore, the main task in choosing of appropriate management strategy of patients with CHC is to assess the degree of necro-inflammatory changes and the stage of liver fibrosis [4, 6, 7, 8, 11].

The progression of CHC and the development of liver fibrosis are due to the diversity of the morphological response of the liver to damage (steatosis, pigment deposits, thrombosis, apoptosis, necrosis, adaptation, proliferation) of hepatocytes. After infection, the host's response for damaging is initiated by monocytoid and plasmocytoid dendritic cells, and in this case the big role belongs to signals, which are entering to these cells through Toll-like receptors (TLR). Stimulation of Toll-like receptors (TLRs) induces the production of interferon-type-1 (INF). In turn, HCV blocks the interferon response, disrupts the mechanisms of destruction of infected hepatocytes and inhibits antigen-presenting cells. HCV is able to come into direct contact with stellate liver cells, stimulating their proliferation and production of proinflammatory cytokines. Prolonged stimulation of stellate cells leads to the implementation of the phase of their fixation in collagen-producing myofibroblasts [3, 5, 7].

The standart for the diagnosis of fibrosis and cirrhosis is a puncture liver biopsy, which allows to establish the degree of inflammatory activity and the severity of liver fibrosis. But today the possibilities of non-invasive assessment and monitoring of liver fibrosis are widely studied. Many studies have been conducted on the diagnostic significance of serum markers of fibrosis, which allow to assess not only the stage of CHC, but also the activity of fibrogenesis in the liver. Various indices based on the ratio of a number of clinical and biochemical parameters are proposed: FibroTest and FibroMether, APRI, FIB-4 and a number of others. The method of liver elastography is also used [2, 8, 9, 10, 11].

Chronic HCV infection remains as a global health problem due to its wide prevalence, latent course without clinical manifestations, development of liver fibrosis with the eventual formation of cirrhosis and liver cancer, which largely leads to poor prognosis and short survival of patients. Therefore, accurate assessment of the severity of liver fibrosis and the degree of inflammation in chronic liver disease is crucial for predicting of the course of the disease [1, 4, 9, 11].

AIM:

To evaluate the effect of allelic polymorphism of +3725 G/C of TLR-4 gene and the activity of the liver inflammatory process in patients with CHC.

MATERIALS AND METHODS:

We examined 131 patients with CHC (diagnosis confirmed by qualitative and quantitative determination of HCV-RNA, HCV genotyping) aged 26 to 72 years (mean age $43,8 \pm 8,4$), which formed the main group. All patients were on outpatient follow-up and inpatient treatment at the Rivne Regional Hepatological and Diagnostic Center. All surveyed were residents of the Polissya region of Ukraine.

Changes in the main biochemical markers of liver damage were determined. Assessment of transaminase activity (alanine aminotransferase (ALT), aspartate aminotransferase (AST)) was performed by unified Reitman-Frenkel method and lactate dehydrogenase (LDH) by the Sevel-Tovarek method. To determine the total level of bilirubin, we used the unified method of Endrasic-Grof-Cleghorn, Ɣ-glutamyltransferase (GGT) - the rate of release of 4-nitroaniline from Ɣ-glutamylnitroanilide, and alkaline phosphatase (ALP) by the rate of releasing of p-nitrophenole, using calorimetric method. The content of total protein (microbiuret method), albumin level (unified method with bromocresol green), cholesterol level by unified Ilk method which were also determined. All mentioned laboratory examinations were performed by the laboratory of Rivne Central City Hospital.

To analyze the mononucleotide substitution of rs11536889 + 3725G/C of TLR-4 gene was used the technique of allele-specific PCR with two pairs of confronting primers (Hishida et. Al., 2009). The polymerase chain reaction was performed automatically on an iCycler thermal cycler manufactured by BIO-RAD (USA). PCR products were fractionated in 1,5–2% agarose gel and stained with 1% ethidium bromide solution. The presence of products was visualized using a UV transilluminator. The study was conducted by the Laboratory of Human Genomics at the Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine.

All obtained data were summarized by Microsoft Excel 2018 spreadsheets and analyzed using "Statistica 10" software. Statistical probability, which were analyzed according to Student's t-test, was regarded as valid at an error of value at $p < 0,05$.

RESULTS:

In order to determine the severity of CHC, the main indicators of liver damage syndromes, such as cytolytic, cholestatic, hepatocellular insufficiency, were evaluated.

According to our data, a significant increasing of serum ALT with the appearance of the C allele in the TLR-4 genotype was detected. Thus, the level of ALT in carriers of the GC genotype was 1,79 times higher than in monosynotic carriers of the G allele (genotype GG), and in monosynotic carriers of the C allele (genotype CC) 2,57 times higher. The same pattern was observed for AST. The level of this enzyme in the serum of carriers of genotypes GC and CC was 1,73 times and 2,26 times higher, respectively, than in carriers of genotype GG. There was no significant difference in the levels of ALT and AST between carriers of genotypes GC and CC (Table 1). Serum LDH level was also significantly higher in carrier of allele C of TLR-4 gene (p <0,05).

Table 1

Indicators of cytolytic syndrome in patients with CHC depending on the TLR4 genotype

Indicators	genotype GG	genotype GC	genotype CC	$p_1/p_2/p_3$
ALT (Un/l)	56,11±3,36	116,72±9,31	144,56±22,01	$p_1<0,001$ $p_2 = 0,3461$ $p_3<0,001$
AST (Un/l)	44,39±2,49	77,09±7,01	100,46±11,79	$p_1<0,001$ $p_2 = 0,2891$ $p_3<0,001$
LDH (Un/l)	218,23±1,12	258,73±1,08	289,66±9,08	$p_1<0,001$ $p_2<0,01$ $p_3<0,001$

Note: p_1 - the difference between GG and GC genotypes;
p_2 - the difference between GC and CC genotypes;
p_3 - the difference between GG and CC genotypes.

There is a significant difference between the number of patients with elevated levels of liver function tests of cholestasis syndrome depending on the presence of the C allele in the TLR-4 genotype. The level of total bilirubin in carriers of GC and CC genotypes was 1,55 and 1,68 times higher than in individuals with GG genotype. Similarly, the level of ALP was increased, which at the appearance of the C allele in heterozygous carriers was 1,67 times higher than in monozygotic carriers of the G allele. A significant difference between these indicators between the groups of carriers of genotypes GC and CC was not found. The level

of GGT in monozygotic allele carriers was determined to be 1,42 times higher than in heterozygous carriers and 2.7 times higher than in monozygotic carriers of the G allele (Table 2).

Indicators of cholestatic syndrome in patients with CHC depending on the TLR4 genotype

Indicators	genotype GG	genotype GC	genotype CC	$p_1/p_2/p_3$
Total bilirubin (μmol/l)	14,03±0,4	21,77±1,23	23,56±3,36	$p_1<0,001$ $p_2=0,6493$ $p_3<0,01$
GGT (Un/l)	57,56±3,67	109,38±12,4	155,53±18,38	$p_1<0,001$ $p_2<0,05$ $p_3<0,001$
ALP (Un/l)	88,18±3,03	147,33±10,43	146,86±19,88	$p_1<0,001$ $p_2=0,9885$ $p_3<0,01$

Note: p_1 - the difference between GG and GC genotypes;
p_2 - the difference between GC and CC genotypes;
p_3 - the difference between GG and CC genotypes.

Significant differences in hepatic insufficiency syndrome were observed between carriers of genotypes GG and GC, GG and CC. The presence of the C allele reduced the levels of total protein and albumin in carriers of the CC and GC genotypes by 1,1 and 1,05 times, respectively, compared with carriers of the GG genotype. Cholesterol level in individuals with the GC genotype were determined 1,23 times higher than in persons with the GG genotype (Table 3).

Evaluation of the direction, strength and reliability of correlations between CC/GC genotypes of the TLR-4 gene and indicators of liver function in patients with CHC was performed by in one-way analysis, using Spearman rank correlation method with defining correlation coefficient, reliably showed that the presence of the C allele in the genetic component of gene polymorphism, which coding TLR-4, revealed a direct correlation of moderate strength with the level of ALT (r=0,52; p<0,05) and the level of total bilirubin (r=0,56; p<0,05), a direct correlation of moderate strength between levels of AST (r=0,44; p<0,05), GGT (r=0,48; p<0,05) and cholesterol (r=0,44; p<0,05), inverse correlation of moderate strength with the level of total protein (r =-0,34; p<0,05) and the level of albumin (r =-0,51; p<0,05).

Table 3

Indicators of hepatocellular insufficiency syndrome in patients with CHC depending on the TLR4 genotype

Indicators	genotype GG	genotype GC	genotype CC	$p_1/p_2/p_3$
Total protein (g/l)	73,24±0.52	69,36±0,64	66,9±2,14	$p_1<0,001$ $p_2 = 0,2414$ $p_3<0,01$
Albumin (g/l)	45,06±0,4	40,06±0,68	37,3±1,03	$p_1<0,001$ $p_2=0,2035$ $p_3<0,001$
Cholesterol (μmol/l)	4,49±0,07	5,52±0,19	5,47±0,07	$p_1<0,001$ $p_2=0,9334$ $p_3<0,001$

Note: p_1 - the difference between GG and GC genotypes;
p_2 - the difference between GC and CC genotypes;
p_3 - the difference between GG and CC genotypes.

However, using a multivariate analysis, by forming a logistic regression model (excluding variables such as total protein and cholesterol), was found that independent predictors were ALT ($\beta = 0,38$; $p = 0,004$), albumin level ($\beta = -0,2$; $p = 0,009$) and total bilirubin ($\beta = 0,32$; $p = 0,0001$). The factorial logistics of model was reliable with a coefficient of determination $R^2 = 52\%$.

Table 4

Evaluation of the reliability of correlations between CC/GC genotypes of the TLR4 gene and indicators of liver function tests in patients with CHC (multifactor analysis with formation of a logistic regression model)

	β	Standard deviation of β	t	p
Intercept *			1,08267	0,281054
ALT	0,384525	0,133447	2,88149	0,004666
AST	-0,122213	0,123973	-0,98581	0,326148
GGT	-0,051852	0,107939	-0,48038	0,631801
ALP	0,145772	0,093870	1,55292	0,122992
Albumin	-0,201151	0,076006	-2,64651	0,009187
Total bilirubin	0,329260	0,074035	4,44736	0,000019

Note: * - Intercept is a mathematical constant that does not require analysis and evaluation.

CONCLUSIONS:

1. Individuals, carrying the CC and GC genotypes of rs11536889 + 3725G/C of the TLR4 gene, have a significantly more severe course of CHC than individuals carrying the GG genotype, according to analysis of the main indicators of cytolytic, cholestatic and hepatocellular insufficiency syndromes.

2. In multivariate correlation analysis, a strong significant correlation was established between the main biochemical markers of liver damage and the presence of the minor allele C of rs11536889 + 3725G/C of the TLR4 gene.

3. It was found that the independent predictors of the activity of the liver inflammatory process, as levels of ALT, albumin and total bilirubin, were associated with the C allele of rs11536889 + 3725G/C of the TLR4 gene.

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