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MOLECULAR GENETIC MECHANISMS OF HCV INFECTION CHRONICITY

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Abstract : Research objective: to analyze the distribution of alleles and genotypes frequencies and to assess the predictive significance of the polymorphism of the TNF-α, CTLA-4, CYP2E1, CYP2C9 *2, CYP2C9 *3, CYP3A4 and CYP1A2 genes of cytochrome P450 in Uzbek patients with chronic hepatitis C and liver cirrhosis.

Material and methods. The study included the analysis of a core group, consisting of 107 patients with chronic hepatitis C (CVHC) and the control of 81 conditionally healthy individuals. All surveyed patients were of Uzbek nationality, with a verified diagnosis of chronic hepatitis C and liver cirrhosis. DNA extraction from lymphocyte nuclei was carried out according to the Sambrook J. et al method. SNP-genotyping of gene polymorphisms was carried out by standard PCR on soft thermal cyclers "Corbett Research" (Australia) and "Applied Biosystems" (USA).

Results. The research showed that the predictive efficacy of each of the genetic polymorphisms in the core group was relatively low (AUC value was from 0.51 to 0.57). Besides, the revealed features of the prevalence of allele frequencies and the genotypes distribution of the studied cytochrome P450 isoenzymes were a significant factor in determination of the CVHC pathogenesis and the disease course. The results indicated a link between the expression of CYP2E1, CYP2C9, CYP1A2 and disease progression.

Conclusion. According to the marker incidence, CTLA-4 -49A/G gene polymorphism has a good predictive efficiency in relation to the development and course of CVHC.

Key words: chronic hepatitis C, gene polymorphism, genotypes, cytochrome P450 isozymes, predictive value.

INTRODUCTION

To date the focus of many scientists is drawn towards the issues of chronic hepatitis due to their high morbidity and development of severe complications [1]. As it known, about 170 million people suffer from chronic hepatitis C, and about 3-4 million new cases are

registered annually [2,3]. The problem of chronic hepatitis is still relevant in modern science and practical medicine due to both high morbidity, and insufficient efficacy of modern methods of diagnosis and prevention [4, 5]. Currently, sufficient data is presented on the role of the genetic characteristics of the organism, both in the metabolism of drugs and in the development of pathology [6]. Therewith, factors should be emphasized such as complexity of predicting the nature of disease course, and revealing the predisposition or resistance to the development of chronic liver damage. After all, knowledge of the genetic basis of the pathological process allows to determine the genetic characteristics of the disease in each individual patient. In clinical practice, significant importance is being increasingly attached to genes polymorphism responsible for the synthesis of enzymes that catalyze metabolic processes, particularly, the cytochrome-P450 system [7]. CYP3A4, CYP2E1, CYP2C9 and CYP1A2 were the most significant in terms of their contribution to drug metabolism and isoenzyme content in the liver [8].

However, the mechanisms of this issue are still insufficiently studied, affording ground for further in-depth study and analysis of this aspect. For our region this issue is also quite relevant [9].

RESEARCH OBJECTIVE

The aim of this paper is to analyze the distribution of allele and genotype frequencies and to assess the predictive significance of the 308G> A polymorphism of the TNF-α gene, 49A> G of the CTLA-4 gene, 9896C> G of the CYP2E1 gene, 430C> T of the CYP2C9 *2 gene, 1075A> C of the CYP2C9 gene *3, 392A> G of the CYP3A4 gene and 164A> C of the CYP1A2 gene of cytochrome P450 in Uzbek patients with chronic hepatitis C and liver cirrhosis.

MATERIAL AND METHODS

During the study we analyzed results of a core group, consisting of 107 patients with chronic hepatitis C (CVHC) and the control group. Considering the degree of disease activity, the core group was divided into subgroups: the first - patients with moderately active CVHC (n=33), the second - highly active CVHC (n=37), and the third – patients with liver cirrhosis (n=37). All patients were of Uzbek nationality, with a verified diagnosis of chronic hepatitis C and liver cirrhosis. The control group consisted of 81 conditionally healthy residents of the various regions of the Republic of Uzbekistan.

Molecular genetic studies were performed in the Department of Molecular Medicine and Cell Technologies of the Research Institute of Hematology and Blood Transfusion, under the Ministry of Health of the Republic of Uzbekistan. DNA extraction from lymphocyte nuclei was carried out in accordance with the Sambrook J. et al method, with some modifications. SNP-genotyping of gene polymorphisms was carried out by standard polymerase chain reaction (PCR) on soft thermal cyclers "Corbett Research" (Australia) and "Applied Biosystems" (USA) using test systems according to the manufacturer's instructions.

In order to determine the efficacy of the studied genetic markers, we determined the sensitivity (Se), specificity (Sp), and the predictive value of the marker - AUC (area under curve). Sensitivity (Se) is the ability of a research method to show the correct result, defined as the proportion of true positive results among all tests performed. Specificity (Sp) is the ability of the research method not to show a false-positive result in the absence of the disease, defined as the proportion of true-negative results among healthy individuals in the study group. Based on the data on sensitivity and specificity, the predictive value of the marker is identified, i.e. disease probability, subject to a known test result. The value characterizing the predictive efficiency of the marker is the AUC indicator. According to the prognostic value scale, the marker is considered a random classifier, if AUC is 0.5; poor classifier, if AUC is 0.5-0.6; fair classifier, if AUC is 0.6-0.7; good classifier if, AUC is 0.7-

0.8 and excellent classifier if AUC> 0.8.

RESEARCH RESULTS AND DISCUSSION

Analysis of the distribution of allele frequencies of CYP2E1 gene polymorphism of cytochrome-P450 in the comparative study of the core and control groups showed that the mutant G allele was found in the core group statistically significantly more often compared to the control (15.4% versus 6.8%; X^2 = 6.6; P = 0.01; OR = 2.503; 95% CI 1.22 -5.12). The obtained data affords to suggest the presence of the association between the mutant allele of the CYP2E1 gene polymorphism and the development of CVHC. In the studied subgroups of patients with CVHC with moderately active (16.7%) and highly active courses (18.9%), we noted a significantly higher frequency of the mutant G allele, compared to the control. In the subgroup of patients with liver cirrhosis, the mutant allele frequency was the lowest among the subgroups of patients. However, the difference with the control group was statistically significant (X^2 = 1.1; P = 0.3; OR = 1.7; 95% CI 0.64, 4.326).

Some literature data show that the early stages of chronic viral hepatitis C are associated with the induction of CYP2E1 to a lesser degree than the stages of disease progression [10,11]. However, our study showed that the presence of the CYP2E1 variant, determined by the G allele and increasing the level of CYP2E1 enzyme production, is characteristic for the early stages of CVHC in a greater degree than for liver cirrhosis. The increase in CYP2E1 expression determines a high metabolic rate of toxins and xenobiotics, and, consequently, the increased formation of LPO products, which have a damaging effect on hepatocytes. Besides, they are involved in the pathogenesis of fibrosis and further development of irreversible liver pathology. Thus, our results indicate a link between CYP2E1 expression and disease progression.

When studying the alleles frequencies of CYP2C9 *2 polymorphism, it was revealed that in both groups of patients (core and control), the wild-type allele was predominant. At the same time, in patients with CVHC, the incidence of the wild-type C allele of CYP2C9 *2 polymorphism was 86.9%, and the mutant T allele - 13.1%. The identified values of the allele frequency in the core group of patients practically did not differ from the studied population. Some differences in indicators were revealed when studying patient population in accordance with the stage and activity of the pathological process. Similar values of the mutant allele frequency indicator were observed in the control group and in the subgroup of patients with liver cirrhosis (12.2%; χ^2 = 0.002; P = 0.97; OR = 1.02; 95% CI -0.4393-2.35). Besides, we noted an increased frequency of the T allele in the subgroup of patients with moderately active CVHC (19.7%; χ^2 = 2.05; P = 0.15; OR = 0.57; 95% CI -0.2669-1.235), and the lowest frequency of the mutant allele in the second group of patients. The increased frequency of the T allele in the group of patients with moderately active CVHC indicated its possible association with low-active disease course. This assumption is also proved by the reduced frequency of the mutant allele in the subgroup of patients with highly active CVHC, which may indicate a protective role of this allele in relation to the activation of the inflammatory process in CVHC. Thus, there is a clear relationship between the accumulation of the mutant allele with a more favorable course of CVHC.

The study of incidence of alleles of CYP2C9 *3 gene polymorphism showed that both in the control group and in subgroups of patients, the wild-type C allele was predominant: in subgroup 1 - 86.4%, in subgroup 2 - 94.6%, in subgroup 3 - 89.2%. The indicators were close in value (p> 0.05). So, in the control, the incidence of the wild-type allele of the studied polymorphism was 93.8% and in patients with CVHC - 90.2%. Comparative analysis showed that in the core group of CVHC patients the mutant C allele of the CYP2C9 *3 gene was more frequent (9.8%) in comparison with the control (6.2%), but the difference was not significant (χ^2 = 1, 61; P = 0.20; OR = 0.60; 95% CI- 0.2765-1.322). When analyzing the frequency index of the mutant allele in accordance with the stage of the disease and the

activity of the pathological process, we noted some scatter of values. The highest frequency of the C allele among all studied subgroups was found in patients with moderately active CVHC (13.6%). The value of this indicator in the group of patients with liver cirrhosis was lower than in the first group (10.8%; χ^2 = 0.26; P = 0.61; OR = 1.303; 95% CI -0.4716-3.598), and the minimum frequency of the mutant allele was registered in the group of patients with active CVHC course (5.4). The increased frequency of the C allele of the CYP2C9 *3 gene polymorphism in the group of patients with moderately active CVHS indicates its possible association with the low-active disease course. This assumption is also confirmed by the reduced frequency of the mutant allele in the subgroup of patients with highly active CVHC, which may indicate a protective role of this allele in relation to the activation of the inflammatory process in CVHC. Thus, there is a clear link between the accumulation of the mutant allele of the CYP2C91075A>C gene polymorphism with a more favorable course of CVHC.

Comparative analysis of the prevalence of allele frequencies and the distribution of genotypes of the CYP3A4 gene in the group of CVHC patients and the control revealed the following features of this polymorphic site. The mutant G allele was found in the core group of CVHC patients 2.4 times more often than in the control (6.1% and 2.5%, respectively; X^2 = 2.78; P = 0.09; OR = 0.39; 95% CI -0.1252-1.224).). It was noted that in the group of patients with CVHC there was frequency increase not only of the G allele, but also the heterozygous genotype A/G (12.1% versus 4.9% in the control; X^2=2.91; P= 0.09; OR = 0.37; 95% CI -0.1177-1.199). Nevertheless, despite the tendency towards the mutant allele accumulation in the group of patients, the absence of significant difference in the values of this indicator in these groups does not allow us to assume the participation of the CYP3A4 gene polymorphism in the development of CVHC. In the studied subgroups of patients with CVHC, the highest incidence of the mutant G allele was observed in patients with moderately active CVHC (9.1%). With further activation of the process, we observed frequency decrease in the G allele.

The comparative analysis of the prevalence of allele frequencies and the distribution of genotypes of CYP1A2 gene polymorphism revealed that the mutant C allele was found in the core group of CVHC patients 2.3 times more often than in the control (14.0% versus 6.2%) This factor affords to suggest a link between the mutant allele of CYP1A2 gene polymorphism with CVHC development. The incidence of the mutant C allele was higher than the control value in all subgroups of patients with CVHC. Herewith, the maximum value was observed in patients with liver cirrhosis (16.2%; X^2 = 6.062; P = 0.014; OR = 0.34; 95% CI - 0.1396-0.8274). In the group of patients with moderately active process, the mutant C allele frequency was also characterized by a high rate (15.2%; X^2 = 4.724; P = 0.03; OR = 0.37; 95% CI - 0.1456-0.9323). In patients with highly active CVHC, the C allele frequency was 9.5% without a significant difference with the indicators of the first and third subgroups (X^2 = 0.21; P = 0.65; OR = 0.82; 95% CI - 0.3606 -1.887).

The link between the accumulation of certain alleles and genotypes in patients with an unfavorable course of CVHC indicates that these genetic variants of polymorphism may contribute to the CVHC progression and the development of severe liver damage. On the other hand, genetic variants of polymorphisms, found most frequent in patients with a more favorable disease course, may be markers of a weak-form liver damage. The variants of the studied polymorphisms associated with the weak-form liver damage were assessed as genetic markers with a protective value in relation to the severity of CVHC course and the development of liver cirrhosis.

Indicators of the predictive efficiency in the studied polymorphisms of the TNF-α, CTLA-4, CYP2E1, CYP2C9, CYP3A4 and CYP1A2 genes were calculated for patients with CVHC at different stages of the disease.

Studies showed that the least sensitive polymorphisms were CYP3A4 (Se=0.12) and

CYP2C9 *3 (Se=0.20), and the most sensitive - CTLA-4 (Se=0.75). The sensitivity of the latter was 6.2 times higher than in CYP3A4; 3.8 times higher than CYP2C9 *3; 3.1 times higher than the sensitivity of TNF-α; 2.9 times higher than CYP2C9 *2 and CYP1A2; 2.8 times higher than CYP2E1. The high sensitivity of the CTLA-4 polymorphism indicates the possibility of using this marker to detect a moderately active form of CVHC in its carriers, especially at the first stage of diagnosis. However, it should be taken into consideration that the use of the highly sensitive marker can show many false-positive results, which requires additional costs for further examination, especially since this polymorphism had the least specificity among all the polymorphic genes studied by us.

High values of specificity in the core group of CVHC patients (in the range of 0.86-0.95) revealed CYP3A4, TNF-α, CYP2E1, CYP1A2 and CYP2C9 *3 polymorphisms. High specificity suggests the possibility of using these markers to determine the risk of CVHC development. Furthermore, the high values of this predictive indicator for the studied polymorphisms afford to speak of the reliability of these genetic markers as disease confirmators, proving the diagnosis and not regarding a healthy person to a sick. However, when using the studied polymorphisms as specificity indicators, we should consider the possibility of a significant number of missed diseases.

Our calculation of the integral indicator on the predictive efficacy of AUC afforded to assess the balance between the indicators of sensitivity and specificity of the markers studied.

The research revealed that the predictive efficacy of each genetic polymorphisms in the core group of patients was relatively low: the AUC value ranged from 0.51 to 0.57. In general, for the core group of patients with CVHC, the indicator of predictive efficacy had mean value, and differed from the AUC values in various subgroups of patients. This fact may indicate the significance of the studied genetic markers as predictors not only for CVHC development, but also for the disease course and severity of liver damage in patients.

CONCLUSION

Thus, the conducted study established that the genetic factors contributing to the development of moderately active CVHC are A allele of the polymorphic variant of the TNF-α gene - in carriers of both heterozygous and homozygous genotypes; G allele and G/G genotype of the polymorphic CYP2E1 gene; C allele and A/C genotype of the polymorphic CYP2C9 *3 gene; G allele and A/G genotype of the polymorphic CYP3A4 gene; C allele and T/C genotype of the polymorphic CYP1A2 gene.

According to the marker incidence, it was established that CTLA-4 -49A/G gene polymorphism has a good predictive efficiency in relation to the development and course of CVHC. Summarizing the above, we can conclude that the revealed features of the prevalence of allele frequencies and the genotypes distribution of the studied cytochrome-P450 isoenzymes is a significant factor, which determines the pathogenesis of CVHC and the disease course.

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