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THE VALUE OF POLYMORPHISM + 3725G / C TLR4 GENE AS A MARKER OF LIVER FIBROSIS PROGRESSION IN PATIENTS WITH CHRONIC HEPATITIS C

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Abstract

As a rule, chronic hepatitis C is manifested by the development of successive stages of liver fibrosis with the formation of eventually cirrhosis and liver cancer. However, in some patients the fibrosis progresses slowly, while in others there is a rapid formation of cirrhosis, which causes high mortality among such patients. According to published data, despite a fairly large number of studies regarding liver cirrhosis in chronic hepatitis C, factors contributing to liver fibrogenesis have not been definitively determined. The available data are quite contradictory. In this article, we review studies have assessed the TLR4 + 3725G/C gene polymorphism as a factor that may influence the rate of fibrotic progression. The research will build new approach for avoiding fast fibrogenesis.

Key words: HCV; chronic hepatitis C; liver fibrosis; cirrhosis; TLR4 gene; viral load.

BACKGROUND

Viral hepatitis C continues to attract the attention of the international community. The number of people with the viral hepatitis C is still steadily increasing despite the availability of effective treatments. According to WHO, there are more than 170 million people infected

with the hepatitis C virus (HCV) in the world, and this number is increasing annually by an average of 3-4 million people [6, 9].

During long period of time, most amount of infected patients do not have any symptoms of the disease, leading to the development of chronic hepatitis C in 70-80% of HCV-infected people. Liver cirrhosis develops in about 20% of patients with chronic hepatitis C and is usually found 10-20 years after infection. According to some authors, the mortality rate for 5-7 years and after diagnosis of cirrhosis of the liver is 40-80% [3, 5]. The main way of the progression of chronic liver damage is the development of successive stages of liver fibrosis with the formation of cirrhosis and liver cancer, which in many respects predetermines the poor prognosis and short survival time of this category of patients. To a greater extent, chronic liver damage is mediated by immune mechanisms rather than by the direct cytopathic action of the virus itself [8]. TLR4 receptors play a key role in innate immunity by activating inflammatory responses[1, 10]. Activation of TLR4 signals in hepatocytes leads to increased activity of proinflammatory cytokines and chemokines, as well as an increase in inflammatory cells in the liver [4].

Currently, the role of a number of factors contributing to the progression of the disease and the development of serious complications, among which are the factors of the virus (genotype and quasi-types of the virus, the level of viremia); host factors (age and duration of infection, gender, HBV and/or HIV co-infection, metabolic factors, genetic factors) and environmental factors (bad habits, toxic effects, immunosuppression)[3, 5].

Determination of the concentration of viral HCV RNA in the serum is an extremely important marker for clinical hepatology, as large-scale cohort studies indicate the role of high levels of viral load of HCV as a prognostic marker of adverse disease. hepatocellular carcinoma (HCC). The quantitative determination of HCV RNA, along with biochemical parameters, is a major marker for monitoring antiviral therapy and evaluating its effectiveness, as well as predicting treatment success [2].

The study of pathogenesis, improvement of diagnostics and he effectiveness of therapy of chronic hepatitis C are quite actual problems of modern hepatology.

In recent years, particular attention has been paid to the study of genetic factors in chronic hepatitis C, which, on the one hand, can determine the individual features of the disease, and on the other - to serve as a marker for the assessment of disease prognosis and response to therapy.

AIM

To determine the prevalence of the carrier of the allelic polymorphism +3725 G/C of the TLR4 gene and evaluate its effect on the progression of liver fibrosis in patients with chronic hepatitis C.

MATERIALS AND METHODS

We examined 131 patients with chronic hepatitis C, including 59 women and 72 men. The average age of patients was 43.8 ± 8.4 years. All patients were residents of the Polissya region of Ukraine, and were in outpatient and inpatient treatment at the Rivne Regional Hepatology Center. The diagnosis of chronic hepatitis C was confirmed by qualitative and quantitative determination of HCV-RNA, HCV genotyping. The control group consisted of 42 healthy individuals, representative by age and gender to the main group.

Fibrosis was assessed by using the FibroMax biochemical test and evaluated according to the Metavir scale. According to the degree of fibrosis, patients were divided into three groups: patients without fibrosis F0 (46 people), patients with moderate fibrosis F1-F2 (44 people), and patients with severe fibrosis and cirrhosis F3-F4 (41 people). The groups were comparable by sex, age and disease duration [7].

Also, all patients were divided into two groups by viral load level: patient with high viral load ≥600000 IU/ml (56 individuals) and patients with low viral load <600000 IU/ml (75 ones) [2].

The technique of analysis of the +3725 G/C polymorphism of the TLR4 gene was proposed by Ph.D. Kucherenko A.M., which is a modification of the method developed in Hishida et. al., 2009, which is a site-specific polymerase chain reaction (PCR) with two pairs of confronting primers. The study was conducted in the laboratory of human genomics at the Institute of Molecular Biology and Genetics of Nationa Academy of Science of Ukraine [4].

Statistical processing of the obtained results was carried out using the program "STATISTICA 8,0" (owned by National Pirogov Memorial Medical University, Vinnytsya, licensed number AXXR910A374605FA). The method of determining the reliability using mean errors (m) was used and the required reliability of the obtained values was established at the level of 95.5% confidence with a possible deviation from the obtained results of not more than ± 5%, which is a standard requirement for clinical trials and proving their accuracy.

RESULTS

In the analysis of the polymorphism +3725 G/C of the TLR-4 gene among healthy patients carriers of the genotype GG are predominant - 35 (83.33%) individuals, with the genotype GC - 7 (16.77%) ones, genotype CC was not detected in any of the control subjects. Among the group of patients with chronic hepatitis C, we found that the overwhelming number of patients have the genotype GG - 101 (71.10%) patients, and the smallest - the genotype CC - 3 (2.29%), genotype GC have 27 (20.61%) patients. (Table 1).

Table 1

Frequency of detection of the +3725 G/C polymorphism of the TLR-4 gene in healthy individuals and patients with chronic hepatitis C

+3725 G/C of TLR-4 gene	Control group n=42	Patients with chronic hepatitis C n=131	p
GG	35 (83,33%)	101 (71,10%)	p=0,73*
GC	7 (16,77%)	27 (20,61%)	
CC	0 (0%)	3 (2,29%)	

Note: * Paired t-test

Our analysis has found that among patients with chronic hepatitis C, 3.4 times more monozygotic carriers of the allele G of the TLR-4 gene than those of the allele C were observed. We did not find a significant difference in the prevalence of the +3725 G/C polymorphism of the TLR-4 gene among the comparison groups (p = 0.73).

Also, we did not find a significant difference between the frequency of detection of the +3725 G/C polymorphism of the TLR-4 gene by sex and age among patients with chronic hepatitis C.

When comparing genotypes by allelic polymorphism +3725 G/C of TLR4 gene in patients with chronic hepatitis C, we found that patients with high virologic load (≥600000 IU/ml) are 2.4 times more likely to be among carriers of the C allele (GC and CC genotypes). than with low virological load, in turn, among monozygotic carriers of the +3725 G allele (genotype GG), patients with low virological load than with high are more often found (Table 2).

Table 2

Dependence of viral load in patients with chronic hepatitis C on polymorphism +3725 G/C of TLR-4 gene

Locus		Patients with high virological load		Patients with low virological load		Odds ratio		
	Genotype	n	f	n	f	p	OR	CI 95%:
+3725 G/C *TLR4* gene	*GG*	34	0.607	67	0.893	0.0001	0.18	0.07 - 0.46
	GC	19	0.339	8	0.106		5.42	2.18 - 13.44
	CC	3	0.053	0	0.000			
	Total	56		75		–		
	Allele G	83	0.775	142	0.946	–		
	Allele C	25	0.225	8	0.053	–		

Note: n - is the absolute number, f - is the frequency, CI - is the confidence interval

The average viral load in patients with the G/G genotype was $1.5*10^5$, in patients with the genotype GC - $1.2*10^6$, in patients with the genotype CC – $1.01*10^7$. The appearance of the C allele +3725 G/C of the TLR4 gene increased the viral load by 1 log (Fig. 2).

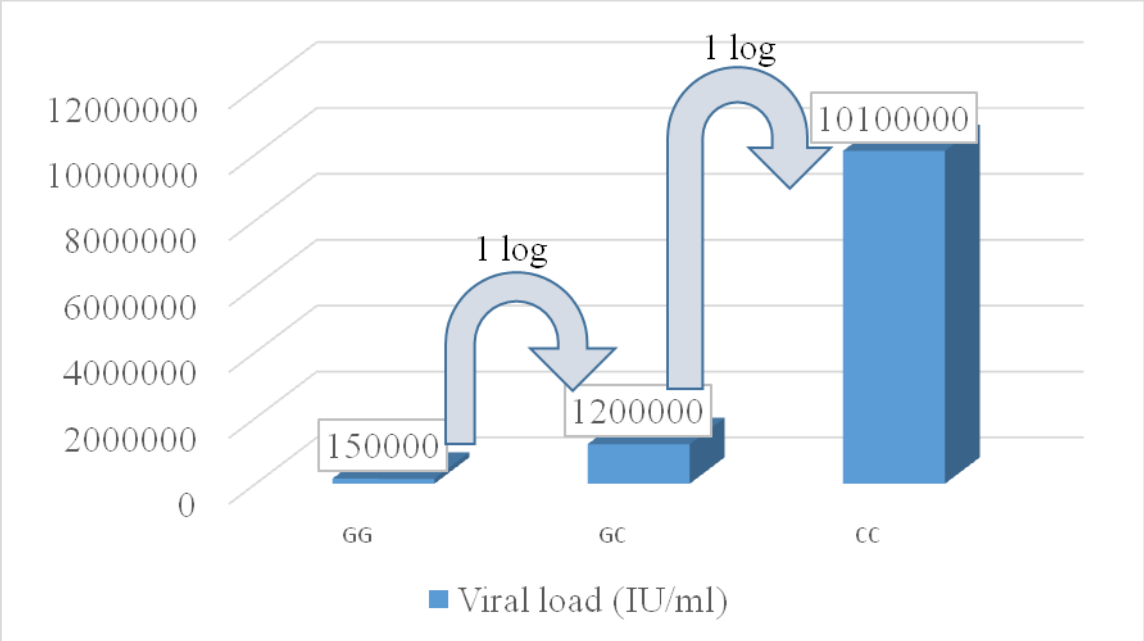

Fig. 1 - Viral load in patients with chronic hepatitis C with different genotypes +3725 G/C of the TLR4 gene.

Based on the odds ratio, individuals with the TLR4 gene allele +3725 C were found to be >5 times more likely to have a high virological load (OR = 5.42; CI 95%: 2.18 to 13.44) (Table 2).

We found that all patients with monozygotic carriers of the C allele (CC genotype) had a marked degree of fibrosis (F4). In the analysis of the dependence of the severity of fibrotic changes in the liver in patients with chronic hepatitis C with different variants of the +3725 G/C polymorphism TLR4 gene revealed a strong correlation (r (Spearman) = 0.97; p <0,0001) expressed fibrosis (F 3-4) with the C allele in both mono- and heterozygous variants of TLR4 genotypes (Fig. 2).

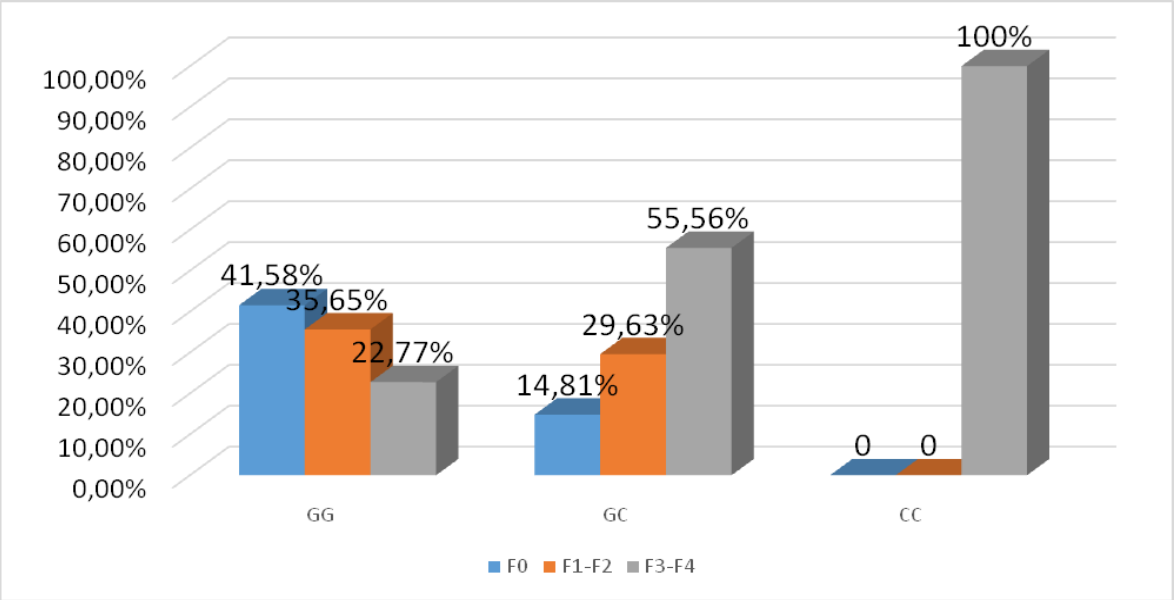

Fig. 2 - The severity of fibrotic changes in patients with chronic hepatitis C with polymorphism +3725 G/C TLR4 gene

Note: r (Spearman) = 0.97; p <0.0001 - correlation between the degree of fibrosis and polymorphism +3725 G/C of TLR4 gene

Also, by comparing the distribution of genotypes by the allelic variant +3725 G/C of the TLR4 gene in patients with different degrees of fibrosis using Fisher's exact two-sided test, it was found that the carrier frequency of +3725 C alleles (genotypes GC and CC) was reliable (p=0.0003) higher in the group of patients with more severe degree of fibrosis (0.44) compared to the group of patients with mild degree of fibrosis (0.027) (Table 3).

Table 3

Dependence of the degree of liver fibrosis in patients with chronic hepatitis C on polymorphism +3725 G/C of the TLR-4 gene

Locus		F 0		F 1-2		F 3-4		Odds ratio		
	Genotype	n	f	n	f	n	f	p	OR	CI 95%
+3725 G/C *TLR4* gene	*GG*	42	0,91	36	0,82	23	0,56	0.0003	0.2 ***	0.08 – 0.48
	GC	4	0,09	8	0,18	15	0,37		4.96	2.08 - 11.79
	CC	0	0,00	0	0,00	3	0,07			
	Total	46		44		41		-		
	*χ^2=5,991; p = 0,027									
	Allele G	84	0,95	80	0,91	61	0,74	-		
	Allele C	4	0,05	8	0,09	21	0,26	-		
	**p=0,029									

Notes:

* Non-parametric Kruskal-Wallis test

** Paired t-test

***to determine OR, we combined patients with no fibrosis and with moderate fibrosis (F 1-2) into one group

Based on the odds ratio, individuals with the TLR4 gene allele +3725 C have a 4-fold higher risk of developing severe fibrosis (OR = 4.96; CI 95%: 2.08 - 11.79).

CONCLUSIONS

1. In the cohort of patients with chronic hepatitis C, there is a prevalence of carriers of the GG +3725 G/C genotype TLR4.

2. Among the carriers of the C3737 G/C allele of the TLR4 gene, the proportion of patients with high viral load was 38.2%, which was significantly ($p<0.05$) more than twice the number of patients with low viral load.

3. We have found that individuals carrying the +3725 C allele of the TLR4 gene (GC and CC genotypes) have more than 5 times bigger risk of higher viral load (OR = 5.42; CI 95%: 2.18 to 13.44) than monozygotic carriers +3725 G allele (GG genotype).

4. The allele C +3725 G / C of the TLR4 gene in mono- and heterozygous variants of the genotypes is associated with the onset of severe fibrosis (F 3-4) in patients with CHC (r (Spearman) = 0.97; p <0.0001).

5. Individual carriers of the allele +3725 C of the TLR4 gene (GC and CC genotypes) were found to have a 4-fold higher risk of developing severe fibrosis (OR = 4.96; CI 95%: 2.08 - 11.79) than monozygotic allele carriers of +3725 G (GG genotype).

REFERENCES

1. Chaiwiang, N., & Poyomtip, T. (2019). The associations between Toll-like receptor 4 gene polymorphisms and hepatitis C virus infection: a systematic review and meta-analysis. Bioscience reports, 39(2).

2. Collister, M., Ellison, C., Li, Q., Minuk, G. Y., Rempel, J. D., & Kung, S. K. (2019). The Influence of Hepatitis C Viral Loads on Natural Killer Cell Function. Gastroenterology research, 12(1), 8.

3. Erman, A., Krahn, M. D., Hansen, T., Wong, J., Bielecki, J. M., Feld, J. J., ... & Thein, H. H. (2019). Estimation of fibrosis progression rates for chronic hepatitis C: a systematic review and meta-analysis update. BMJ open, 9(11).

4. Kucherenko, A. M., Moroz, L. V., Bevz, T. I., Bulavenko, V. I., Antypkin, Y. G., Berezenko, V. S., ... & Livshits, L. A. (2019). Investigation of rs11536889+ 3725G/C polymorphism of the TLR4 gene in patients with autoimmune and chronic viral hepatitis C. Cytology and Genetics, 53(4), 300-306.

5. Lu, M., Li, J., Rupp, L. B., Zhou, Y., Holmberg, S. D., Moorman, A. C., ... & Schmidt, M. A. (2018). Changing trends in complications of chronic hepatitis C. Liver International, 38(2), 239-247.

6. Mohamed, A. A., Elbedewy, T. A., El-Serafy, M., El-Toukhy, N., Ahmed, W., & El Din, Z. A. (2015). Hepatitis C virus: A global view. World journal of hepatology, 7(26), 2676.

7. Moroz, L. V., Bondaruk, I. Y., Popovich, A. A., & Kulias, S. N. (2019). Comparison of the diagnostic efficiency of various noninvasive methods for diagnosing liver fibrosis in patients with chronic viral hepatitis C. Journal of Education, Health and Sport, 9(6), 546-555.

8. Patra, T., Ray, R. B., & Ray, R. (2019). Strategies to circumvent host innate immune response by hepatitis C virus. Cells, 8(3), 274.

9. Petruzziello, A., Marigliano, S., Loquercio, G., Cozzolino, A., & Cacciapuoti, C. (2016). Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. World journal of gastroenterology, 22(34), 7824.

10. Yang, Y., Tu, Z. K., Liu, X. K., & Zhang, P. (2018). Mononuclear phagocyte system in hepatitis C virus infection. World journal of gastroenterology, 24(44), 4962.