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TLR4 AND TLR7 GENETIC POLYMORPHISM IN PATIENTS WITH HIV/HCV-COINFECTION: PREVALENCE AND GENDER-RELATED FEATURES

POLIMORFIZM TLR4 ORAZ TLR7 U PACJENTÓW Z KO-INFEKCJĄ HIV/HCV: WYSTĘPOWANIE I CHARAKTERYSTYKA W ZALEŻNOŚCI OD PŁCI

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ABSTRACT

Introduction: HIV-infection and chronic hepatitis C is of great concern of current infectology. The paper is aimed at the study of the prevalence of the of TLR4 gene Gly and TLR7 gene Leu polymorphic alleles among patients with HIV/HCV-coinfection in general and with regard to gender, as well as to determine their role in the development of the infection.

Materials and methods: To achieve the objective of the research a cohort and case-control study has been carried out. The total of 535 people has been examined, including: HIV/HCV-coinfected – 104, HIV-monoinfected – 90, patients with chronic hepatitis C – 166 and almost healthy people (population control group) – 175 subjects.

Results and conclusion: The study found that the prevalence of the TLR4 gene Gly polymorphic allele among patients with HIV/HCV-coinfection, HIV-monoinfection and chronic hepatitis C accounted for 23.1 %, 14.4 % and 14.5 %, respectively, which is significantly higher than the similar index in controls – 3.3 %. The presence of the TLR4 gene Gly polymorphic allele in the genome increases the risk of HIV/HCV-coinfection development, in case of infection, by 9 (OR=8.70, p=0.000), HIV-monoinfection and chronic hepatitis C by 5 times (OR=4.89, p=0,016 and OR=4.9, p=0.011, respectively). TLR7 gene Leu polymorphic allele is recorded with the frequency of 19.9-26.0 % in patients with HIV/HCV-coinfection, HIV-monoinfection and chronic hepatitis C as compared to controls (25.9 %). In female patients with HIV/HCV-coinfection, HIV-monoinfection, chronic hepatitis C and healthy individuals the TLR7 gene Leu polymorphic allele is recorded by 2.1-3.6 times more frequently than in male patients.

KEY WORDS: HIV-infection, chronic hepatitis C, coinfection, TLR4 gene, TLR7 gene

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INTRODUCTION

HIV-infection and chronic hepatitis C is of great concern of current infectology [1-2]. The HIV/HCV combined co-infection contributes to the progression of liver damage and increases the risk of the development of antiretroviral therapy side effects [3]. Despite the availability of effective treatment, the chronic hepatitis C remains to be one of the major, except the opportunistic diseases, causes of mortality among HIV-infected individuals [4]. Recently, the correlation between the processes of immunopathogenesis and the development of the liver fibrosis, metabolic disorders in HIV/HCV-coinfected patients have been actively studied with the emphasis on the inherent immunity, namely, the TLR genes [2, 4]. The TLR polymorphism is crucial in the development of the immune response, since the TLR polymorphism-associated dysfunction leads to disturbance of identification of the infectious agents and the dysfunction of the system of the inherent immunity, thereby causing the susceptibility to a wide range of the diseases and peculiarities of their progress [5-6]. From the point of view of the HIV/HCV-coinfection investigation, the TLR4 and TLR7 genes are of scientific interest, since the TLR4 interacts with the protein layer, structural and nonstructural viral proteins, and viral RNA is the TLR7 ligand [4-5, 7-8].

Several publications on the study of prevalence of TLR polymorphic genotypes, TLR4 Asp299Gly and TLR7 Gln-11Leu, in particular, among patients with HIV and chronic hepatitis C have been found. Thus, according to T. Kyrychenko et al. (2013), the TLR4 gene Asp299Gly polymorphism has been recorded in 12,0 % of HIV-infected people, and it presence burdens the progress of the disease [9]; O. de Souza Pires-Neto et al. (2015) [10] and A. A. Al-Qahtani et al. (2014) [11] report that in patients with chronic hepatitis C the frequency of this genotype accounts for 6.9-9.1 %, whereas, according to C. Guarner-Argente et al. (2010) it reaches 40.0 % [12]. The prevalence of the TLR7 gene Gln11Leu polymorphism among HIV-infected people accounts for 23.4 % as described in the findings by D. Y. Oh et al. (2009) [13], and, according to other authors, in chronic hepatitis C it constitutes 15.2-41.7 % and defined gender peculiarities of the prevalence [14-17]. It is reported that the presence of a polymorphic allele Leu TLR7 in the genome is associated with the low rate of liver fibrosis progression in chronic hepatitis C [18]. Meanwhile, limited number of publications on the study of the prevalence of the abovementioned polymorphisms among HIV/HCV-coinfected people has been found: Yurko K. (2016) describes the frequency of recording of the TLR4 Asp299Gly genotype at the level of 17.0 % and its

effect on the metabolic processes in this category of patients [2], and data on the prevalence of the TLR7 gene Gln11Leu polymorphism are not presented at all.

Consequently, current scientific data show that occurrence of the abovementioned polymorphisms in HIV/HCV-coinfected people can influence on the clinical course of the disease, and their study will be promising in terms of individualization of the diagnostic and therapeutic approach for the infection, making the investigation to be relevant.

THE AIM

The paper is aimed at the study of the prevalence of the of TLR4 gene Gly and TLR7 gene Leu polymorphic alleles among patients with HIV/HCV-coinfection in general and with regard to gender, as well as to determine their role in the development of the infection.

MATERIAL AND METHODS

To achieve the objective of the research a cohort and case-control study has been carried out. The total of 535 people has been examined, including:

- HIV/HCV-coinfected: 104 subjects; males – 77 (74.0 %), females – 27 (26.0 %) aged 17 to 51 years; average – 34.0±0.6 (Main Group);
- HIV-monoinfected: 90 subjects; males – 58 (64.4 %), females – 32 (35.6 %) aged 21 to 55 years; average – 34.9±0.8 (comparison Group I);
- patients with chronic hepatitis C: 166 subjects; males – 111 (66.9 %), females – 55 (33.1 %) aged 20 to 63 years; average – 40.3±0.7 (comparison Group I);
- healthy people: for TLR4 gene: 90 subjects; males – 50 (55.6 %), females – 40 (44.4 %) aged 18 to 33 years; average – 19.5±0.2 and for TLR7 gene: 85 subjects; males – 47 (55.3 %), females – 38 (44.7 %) aged 20 to 67 years; average – 30.1±1.4 (population control group).

Chronic hepatitis C and HIV have been diagnosed in compliance with the International Classification of Diseases, 10th Edition, and verified by the detection of specific serological and molecular biological markers of the infections (for chronic hepatitis C: detection of the HCV IgG antibodies in the blood by the ELISA method and HCV-RNA by the RT-PCR method) with genetic typing; for HIV-infection: detection of the antibodies to HIV in the blood by the ELISA method). Similarly, the occurrence of chronic hepatitis C in HIV-infected people has been confirmed in patients with HCV-monoinfection. Clinical and laboratory examination of the patients has been carried out at Poltava Regional Infectious Hospital, Poltava Regional Center for HIV/AIDS prevention and private laboratories.

All patients have been provided with genetic tests. TLR4 Asp299Gly polymorphic area has been genetically typed by the PCR, using the specific oligonucleotide primers, on the «Tertsik» amplifier («NPO DNA-Technology», LTD, Russia) and TLR7 Gln11Leu polymorphic area by the method of allele-specific RT-PCR on the «DT Light» amplifier («NPO DNA-Technology», LTD, Russia) at the Research Institute for Genetic and Immunological Grounds of Pathology and Pharmacogenetics of the HSEEU «Ukrainian Medical Stomatological Academy».

Statistical analysis of the findings has been carried out using the «SPSS 23.0» (USA) program. The allele frequencies have been compared by the analysis of contingency tables using the Fisher's exact test (FET) and the χ^2 test depending on the baseline conditions of the analysis. The risk of the pathology development has been estimated by the calculation of the odds ratio (OR) and its 95 % confidence interval (CI) using the method of univariate logistic regression, where 95 % CI characterizes the degree of data validity, and the p-value indicates the probability of deviation from of null hypothesis. OR=1 was regarded as the null hypothesis, OR>1 – high risk, OR<1 – low risk for pathology development. The differences were considered reliable in $p<0.05$; the tendency to reliability was considered in $p<0.1$.

RESULTS AND DISCUSSION

The study has been established that carriers of both normal (Asp299Asp TLR4, among women – Gln11Gln, among men – Gln11/- TLR7) and polymorphically modified (Asp299Gly TLR4, among women – Gln11Leu and Leu11Leu, among men – Leu11/- TLR7) genotypes of investigated genes have been recorded among patients of main and comparison groups. The overall prevalence of genotypes and alleles of the TLR4 gene is shown in table I.

Data of the table I show that homozygous polymorphic TLR4 gene Gly299Gly genotype has not been found among the examined groups, which is consistent with the publication data as for its low frequency in the population [2, 9-10, 18-20].

The frequency of TLR7 genotype registration, taking into account gender specificities due to its X-chromosomal localization [13-14], as well as the general prevalence of the alleles of this gene, are presented in table II.

Taking into account the lack of carriers of the polymorphic homozygous Gly299Gly genotype of the TLR4 gene as well as the low overall frequency of the registration of the Leu11Leu and Leu11/- TLR7 genotypes, limiting the possibility of statistical aggregation, when comparing the signs, the subjects examined in groups were united on the basis of the carrier of the polymorphic alleles of the subjects genes.

The comparative analysis of the distribution of TLR4 genes polymorphic alleles, made between patients and healthy people has established statistically significant differences regarding the TLR4 gene Gly polymorphic allele, which gives the grounds to consider its presence as the risk factor for HIV-infection and chronic hepatitis C development (table III).

Data of the Table III show that among patients with HIV/HCV-coinfection the frequency of the TLR4 gene Gly allele accounts for 23.1 %, that 7 times higher than the same index in the population controls – 3.3 % (p=0.000). The OR calculation has established that carryability of this allele increases the risk of coinfection by 9 times (OR=8.70 [95 % 2.52-30.0],

Table I. The frequency of record of TLR4 gene alleles and genotypes among patients with HIV/HCV-coinfection, HIV-monoinfection, chronic hepatitis C and healthy people

Groups	TLR4				
	Genotype, abs. number (%)			Allele, abs. number (%)	
	Asp299Asp	Asp299Gly	Gly299Gly	Asp	Gly
HIV/HCV (n=104)	80 (76.9)	24 (23.1)	-	184 (88.5)	24 (11.5)
HIV (n=90)	77 (85.6)	13 (14.4)	-	167 (92.8)	13 (7.2)
Chronic hepatitis C (n=166)	142 (85.5)	24 (14.5)	-	308 (92.7)	24 (7.3)
Healthy people (n=85)	87 (96.7)	3 (3.3)	-	177 (98.3)	3 (1.7)

Table II. The frequency of record of TLR7 gene alleles and genotypes among patients with HIV/HCV-coinfection, HIV-monoinfection, chronic hepatitis C and healthy people

Groups	TLR7						
	Genotype, abs. number (%)					Allele, abs. number (%)	
	Females			Males			
	Gln11Gln	Gln11Leu	Leu11Leu	Gln11/-	Leu11/-	Gln	Leu
HIV/HCV (n=104)	12 (44.4)	14 (51.9)	1 (3.7)	65 (84.4)	12 (15.6)	103 (78.6)	28 (21.4)
HIV (n=90)	15 (46.9)	12 (37.5)	5 (15.6)	49 (84.5)	9 (15.5)	91 (74.6)	31 (25.4)
Chronic hepatitis C (n=166)	38 (69.1)	15 (27.3)	2 (3.6)	95 (85.6)	16 (14.4)	186 (84.1)	35 (15.9)
Healthy people (n=85)	23 (60.5)	12 (31.6)	3 (7.9)	40 (85.1)	7 (14.9)	98 (79.7)	25 (20.3)

Table III. The frequency of record of TLR4 gene alleles among patients with HIV/HCV -coinfection, HIV-monoinfection, chronic hepatitis C and healthy people

Polymorphic allele carrier	Groups, abs. number (%)				p
	HIV/HCV	HIV	Chronic hepatitis C	Healthy people	
Absence of allele Gly	80 (76.9)	77 (85.6)	142 (85.5)	87 (96.7)	p_1=0.000 p_2=0.015 p_3=0.005
Presence of allele Gly	24 (23.1)	13 (14.4)	24 (14.5)	3 (3.3)	

Note. p – significance value obtained using the FET while comparing the groups of patients and healthy people: p_1 – HIV/HCV-coinfected/healthy, p_2 – HIV-monoinfected/healthy, p_3 – chronic hepatitis C/healthy.

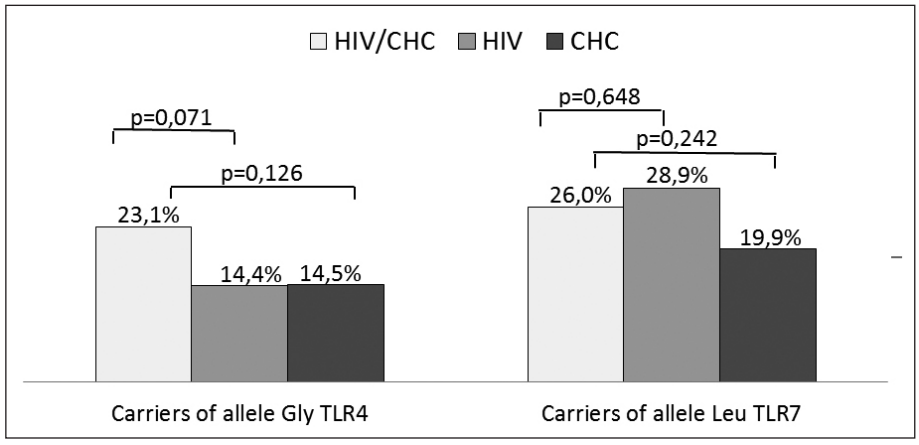
p=0.000). The findings were confirmed during the study of the prevalence of the polymorphic allele of this gene among patients with HIV-monoinfection and chronic hepatitis C, where it has been recorded with similar, significantly higher frequency in contrast to healthy individuals - 14.4 % (p=0.000) and 14.5 % (p=0.005), respectively). The OR calculation shows that in carriers of TLR4 gene Gly allele the risk of HIV and chronic hepatitis C development, in case of infection, increases by 5 times (OR=4.89 [95 % 1.35-17.82], p=0,016 and OR=4.9 [95 % CI 1.43-16.76], p=0.011, respectively).

No difference in the frequency of the TLR7 gene Leu allele among patients with HIV/HCV-coinfection, HIV-monoinfection, chronic hepatitis C and healthy people of population control has been found - 26.0 %, 28.9 %, 19.9 % and 25.9 %, respectively to groups (p>0.05), the OR calculation was statistically insignificant, too, indicating about the absence of its impact on the development of the abovementioned infections.

The next stage of the study included the comparative analysis of the frequencies of the TLR4 and TLR7 genes polymorphic alleles among the groups of patients (Figure 1).

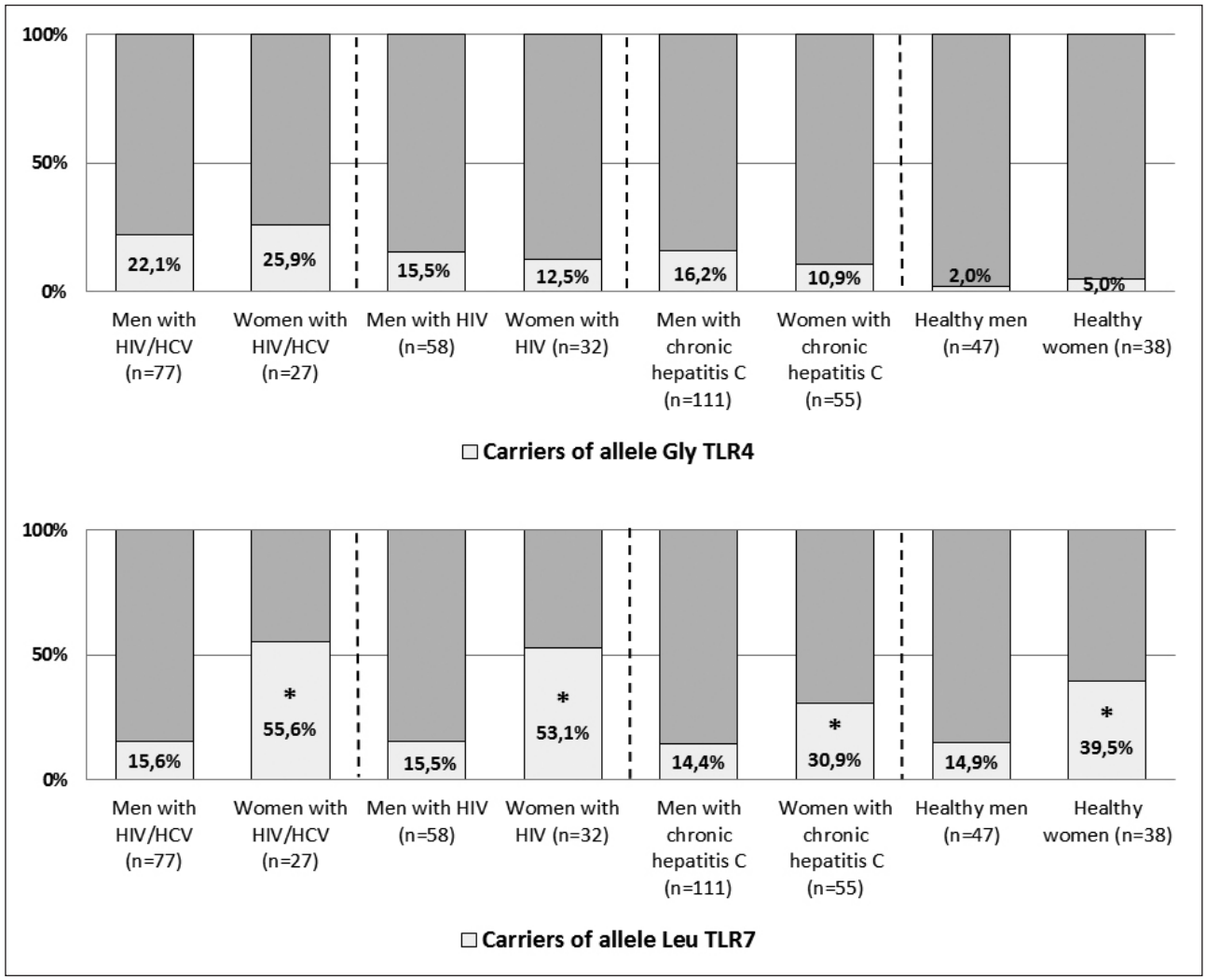
The Figure 1 shows that among HIV/HCV-coinfected patients the TLR4 gene Gly allele has been recorded more frequently than in chronic hepatitis C and HIV-monoinfection; however, the tendency to reliability of difference has been noted only in chronic hepatitis C (χ^2=3.25, p=0.071). No differences in the TLR7 Leu allele frequency has been found during the comparison between the groups.

Subsequently, it was appropriate to carry out the analysis of the prevalence of the TLR4 and TLR7 genes polymorphic alleles among patients and healthy people with regard to its gender, which revealed absence of gender differences in the frequency of records of the TLR4 gene Gly polymorphic allele and significant prevalence of the TLR7 gene Leu allele among female patients both with HIV/HCV-coinfection, HIV-monoinfection, chronic hepatitis C and healthy women (Figure 2).



Note. p – significance value obtained using the χ^2 test.

Figure 1. The frequencies of the TLR4 and TLR7 genes polymorphic alleles in patients with HIV/HCV-coinfection, HIV-monoinfection and chronic hepatitis C



Note. * – p<0.05 (significance value obtained using the χ^2 test and FET depending on the baseline conditions of the analysis).

Figure 2. Gender distribution of the TLR4 and TLR7 polymorphic alleles in patients with HIV/HCV-coinfection, HIV-monoinfection, chronic hepatitis C and healthy people

The Figure 2 shows that among women with HIV/HCV-coinfection the TLR7 gene Leu polymorphic allele has been recorded by 3.6 (χ^2=16.61, p=0.000); with HIV-monoinfection by 3.4 (p=0.000); with chronic hepatitis C by 2.1 (χ^2=6.28, p=0.012) and in controls by 2.7 (p=0.013) times more frequently than in men which is connected to X-chromosome localization of the gene.

CONCLUSIONS

1. The prevalence of the TLR4 gene Gly polymorphic allele among patients with HIV/HCV-coinfection, HIV-monoinfection and chronic hepatitis C accounted for 23.1 %, 14.4 % and 14.5 %, respectively, which is significantly higher than the similar index in controls – 3.3 % (p=0.000, p=0.015 and p=0.005, respectively).
2. The presence of the TLR4 gene Gly polymorphic allele in the genome increases the risk of HIV/HCV-coinfection development, in case of infection, by 9 (OR=8.70, p=0.000), HIV-monoinfection and chronic hepatitis C by 5 (OR=4.89, p=0.015 and OR=4.90, p=0.011, respectively) times.
3. TLR7 gene Leu polymorphic allele is recorded with the frequency of 19.9-26.0 % in patients with HIV/HCV-coinfection, HIV-monoinfection and chronic hepatitis C as compared to controls (25.9 %, p>0.05).
4. In female patients with HIV/HCV-coinfection, HIV-monoinfection, chronic hepatitis C and healthy individuals the TLR7 gene Leu polymorphic allele is recorded by 2.1-3.6 times more frequently than in male patients (p=0.000, p=0.000, p=0.012 and p=0.013, respectively).

REFERENCES

1. Mamedova ES, Holubovska OA, Proniuk Kh.O. Suchasnyi pohliad na perebih ta likuvannia ko-infektsii VIL i VHS [Current view on course and treatment of HIV and HCV co-infection]. Tuberkuloz, lehenevi khvoroby, VIL-infektsiia. 2014;1(16):77–82.
2. Iurko KV. Poshyrenist polimorfizmu hena TLR4 u khvorykh na ko-infektsiiu VIL/KhHS. Science rise. 2015;11/3(16):86–89.
3. EASL Clinical Practice Guidelines: Management of hepatitis C virus infection. J Hepatol. 2014;60:392-420.
4. Crane M, Visvanathan M, Lewin SR. HIV infection and TLR signalling in the liver. Gastroenterol Res Pract. 2012. doi: 10.1155/2012/473925
5. Netea MG, Wijmenga C, O'Neill LAJ. Genetic variation in Toll-like receptors and disease susceptibility. Nat Immunol. 2012;13(6):535–542.
6. Skevaki C, Pararas M, Kostelidou K, Tsakris A, Routsias JG. Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious diseases. Clin Exp Immunol. 2015;180(2):165-177.
7. Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity. 2011;34(5):637–650.
8. Xu Y, Zhong J. Innate immunity against hepatitis C virus. Curr Opin Immunol. 2016;42:98–104.
9. Kirichenko TS, Koval TI, Kajdashev IP, Dubinskaya GM. Kliniko-immunologicheskaya harakteristika VICh-infekcii u bolnyh s polimorfizmom Asp299Gly gena Toll-podobnogo receptora [Clinical and immunological characteristics of HIV-infection in patients with Asp299Gly polymorphism of the Toll-like receptor 4 gene]. Georgian medical news. 2013;11(224):30–35.
10. de Souza Pires-Neto O, de Sá KSG, Santana BB. et al. Lack of association between polymorphisms of the TLR4 gene and infection with the hepatitis B and C viruses. Mediators of Inflammation. 2015. doi: 10.1155/2015/150673.
11. Al-Qahtani AA, Al-Anazi MR, Al-Zoghaibi F. et al. Toll-like receptor 4 polymorphism with hepatitis C virus infection in Saudi Arabian patients. Biomed Res Int. 2014. doi: 10.1155/2014/357062.
12. Guarner-Argente C, Sanchez E, Vidal S. et al. Toll-like receptor 4 D299G polymorphism and incidence of infections in cirrhotic patients. Aliment Pharmacol Ther. 2010;31(11):1192–1199.
13. Oh DY, Baumann K, Hamouda O. et al. A frequent functional toll-like receptor 7 polymorphism is associated with accelerated HIV-1 disease progression. AIDS. 2009;23(3): 297–307.
14. Schott E, Witt H, Neumann K. et al. A toll-like receptor 7 single nucleotide polymorphism protects from advanced inflammation and fibrosis in male patients with chronic HCV-infection. J Hepatol. 2007;47(2):203–211.
15. Ascar E, Ramadori G, Mihm S. Toll-like receptor 7 rs179008/Gln11Leu gene variants in chronic hepatitis C virus infection. J Med Virol. 2010;82(11):1809–1983.
16. Taghavi SA, Damangir H, Kamali Sarvestani E, Eshraghian A. Relation between C.32 A>T polymorphism in TLR7 and response to treatment in chronic HCV-infection. Armaghane-e-Danesh. 2009;14(2):105–116.
17. Elsedawy YS, Khattab MA, El Hady SA, El Sayed AA, Albreed AM, Hefny ZM. Single nucleotide polymorphisms of Toll-like receptor 7 in hepatitis C virus infection and hepatocellular carcinoma patients. Egyptian Journal of Medical Microbiology. 2016; 25(4):73–80.
18. Dubynska G, Sizova L, Koval T, Kovalyova E, Kaydashev I. Clinical and genetic predictors and prognostic model of rapidly progressive hepatic fibrosis in chronic hepatitis C. Georgian Medical News. 2016;7–8(256–257):37–45.
19. Sizova L, Koval T, Kaidashev I, Ilchenko V, Dubinskaya G. Rol geneticheskogo polimorfizma TOLL-LIKE receptorov 4 i 7 v razvitii hronicheskogo gepatita S i gendernye osobennosti ih raspredeleniya [The role of genetic polymorphisms Toll-like receptor 4 and 7 in the chronic hepatitis C and gender features of their distribution]. Georgian Medical News. 2016;1(250):51–56.
20. Dubinskaya GM, Prijmenko NO, Kajdashev IP, Pohil'ko VI, Chub KF. Rol' polimorfizma genov TLR-2, TLR-3, TLR-4 pri grippe [The role of TLR-2, TLR-3, TLR-4 genes polymorphism of grippe]. Georgian medical news. 2014;7-8 (232-233):51–55.

Authors' contributions:
According to the order of the Authorship.

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