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Association of A313g polymorphism of GSTP1 gene with biochemical, pro- and antioxidant blood indicators in chronic hepatitis patients

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Abstract
Investigation of A313G polymorphism of GSTP1 gene in chronic nonviral hepatitis patients was performed. Prevalence of G-allele of A313G polymorphism in GSTP1 gene did not differ significantly in chronic hepatitis patients compared with healthy individuals (OR = 1,36, CI = 0,70 – 2,66, p<0,05). Chronic hepatitis patients were characterized by elevated plasma content of total bilirubin and its fractions, triacylglycerols, uric acid, higher activity of hepatic transaminases, alkaline phosphatase and gamma-glutamyl transferase compared with proper parameters in healthy individuals regardless of allelic distribution of GSTP1 gene. Alanineaminotransferase activity was at 44,0% (p = 0,046) higher in chronic hepatitis patients G-allele carriers compared with proper indicator in patients with AA-genotype, pointing out a higher activity of cytolysis. Analysis of pro- and antioxidant indicators reviled that chronic hepatitis patients both AA-genotype and G-allele carriers of GSTP1 gene had lower restored glutathione level, higher glutathione peroxidase activity and higher reactive products of thiobarbituric acid content in the blood, compared to such indicators in the control group. Chronic hepatitis patients G-allele carriers also noted significantly lower catalase activity compared with healthy persons, which was not typical for patients with A-allele. Significantly lower restored glutathione blood content at 16,2% (p = 0,04) was found in patients with G-allele compared with patients AA-genotype carriers.

Keywords: A313G polymorphism of GSTP1 gene, chronic hepatitis, alanineaminotransferase, restored glutathione.

1. Introduction
Genes encoding the synthesis of glutathione-S-transferase (GST) are one of the key genes which are involved in chronic diffuse liver diseases pathogenesis, including chronic hepatitis [1, 2]. Single (A to G) substitution at nucleotide position 313 in GSTP1 gene is found in homozygous variant in 4 to 12% of the population, plays an important role in different diseases development [3]. K. Wu *et al*. investigated that GSTP1 313 G/G polymorphism serves as a risk factor for bladder cancer [4]. I.A. Goncharova *et al*. showed that patients with liver cirrhosis AA genotype carriers have in 2, 5 times higher survival rate compared with the patients with the GG and AG genotypes of GSTP1 gene [5]. On the other hand, Wu S. *et al*., investigated that patients with tuberculosis A-allele carriers of GSTP1 gene (A313G) have a higher risk for anti-tuberculosis drug-induced hepatotoxicity development [6]. In one of our previous studies we have shown that presence of G-allele was associated with higher risk of nonalcoholic fatty liver disease (NAFLD) development in Ukrainian population. Moreover NAFLD patients G-allele carriers were characterized by increased total bilirubin blood concentration and higher alanine aminotransferase activity [7]. M. Hashemi have also showed that GSTP1 genetic polymorphisms were associated with NAFLD occurrence in the Iranian population [8]. The role of GSTP1 gene polymorphism (A313G) in the development and course of chronic hepatitis remains unclear and should be clarified.

2. Material and methods: A313G polymorphism of GSTP1 gene was studied in 57 patients with nonviral chronic hepatitis and 45 healthy individuals (control group). Blood samples were obtained in the morning before taking meal from antecubital vein in the first day of hospitalization until the appointment of the treatment. 5% solution of disodium salt of ethylene diamine tetraacetate was used as an anticoagulant. The study was performed in compliance with the Council of Europe Convention on Human Rights and Biomedicine and the Recommendations of the Committee on Bioethics of the Ministry of Public Health of Ukraine, and was approved by the Biomedical Ethics Commission of the Higher State Education

Establishment of Ukraine "Bukovinian State Medical University" (Chernivtsi, Ukraine). Written informed consent was obtained from all the participants.

All of the patients and healthy individuals underwent general complex clinical, laboratory and instrumental diagnostic investigations. Biochemical studies were performed on the blood biochemical analyzer "Accent-200" ("Cormay SA", Poland). The range of indicators of biochemical blood analysis included: total bilirubin and its fractions, cholesterol, triglycerides, uric acid, total protein and albumin, urea, creatinine, plasma enzyme activity (aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), alkaline phosphatase (AP)).

Key processes of free radical oxidation were determined by means of spectrophotometric method containing reactive products of thiobarbituric acid (TBA-reactive products) in the blood. Antioxidant systems were studied by measuring restored glutathione content, catalase and glutathione peroxidase (GP) activities in the blood.

Investigation of A313G polymorphism of GSTP1 gene was performed in the State Institution "Reference Center for Molecular Diagnostics of the Ministry of Public Health of Ukraine" (Kyiv, Ukraine). Genomic DNA for molecular genetic studies was isolated from peripheral blood with the help of commercial test kits "innuPREP Blood DNA Mini Kit" (Analytik Jena, Germany) using centrifugal filters. To determine the polymorphic variants of GSTP1 (A313G) rs 1695 modified protocols of oligonucleotide primers were used [9] by the method of polymerase chain reaction and subsequent analysis of restriction fragment length polymorphism. Investigation of gene domain was amplified using specific primers («Metabion», Germany), mentioned in Table 1.

The products of amplified DNA fragments of GSTP1 gene underwent hydrolytic cleavage using restriction endonucleases and BstUI Alw261 (BsmAI) («Thermo Scientific», USA). Amplified fragments were analyzed by 2% agarose gel, and restriction fragment GSTP1 (A313G) – 3% agarose gel (agarose company «Cleaver Scientific», UK), with the addition of ethidium bromide, molecular weight marker GeneRuler 50 bp DNA Ladder («Thermo Scientific», USA) and subsequent visualization by the computer program Vitran.

Table 1: Primer sequences, restriction enzyme and allele calling for GSTP1 SNPs

Gene (polymorphism)	Primer sequences (5'-3')	Allele calling (size of fragments, b.p.)
GSTP1 (A313G)	GTAGTTTGCCCAAGGTCAAG- forward AGCCACCTGAGGGGTAAG - reverse	432 b.p.
Internal control of amplification	GCCCTCTGCTAACAAGTCCTAC GCCCTAAAAAGAAAATCGCCAATC	350 b.p.

Hydrolytic cleavage of normal allele by restryctase was performed by one restriction site appearance 5'...GTCTCN1↓ ...3', resulting molecular weight fragments formation 328 and 104 b.p. (AA-genotype). When nucleotides adenine replace to guanine second restriction site appeared, resulting molecular weight fragments formation 222 and 104 b.p. (GG-genotype).

The quality of DNA isolation and conditions for multiplexed PCR setting was controlled by fragment of the albumin gene amplification with a molecular weight of 350 b.p. In case of separate fragment absence in the sample during the multiplexed polymerase chain reaction electrophoretic division, results for this sample were not considered.

To determine the type of data distribution, comparing the arithmetic mean, median and mode, and Wilcoxon-Shapiro test were used. To determine the statistical differences between two independent groups Mann-Whitney test was applied. Hardy-Weinberg equilibrium was calculated by a chi-square test. Odds-ratio was determined. P values < 0,05 were considered statistically significant.

3. Results and Discussion: A313G polymorphism of GSTP1 gene in 57 patients with chronic nonviral hepatitis and 45 healthy individuals (control group) was investigated. The distribution of genotypes of GSTP1 gene polymorphism in chronic hepatitis patients is shown in Table 2. Among these patients AA genotype was diagnosed in 32 persons (56,1%), AG – 21 (36,9%), GG – 4 (7,0%); A-allele of GSTP1 gene was observed in 85 cases (74,6%) among 114 selected alleles, G-allele – in 29 cases (25,4%) respectively.

In the group of healthy individuals 28 (62,2%) homozygous carriers of Ala-allele were found; 16 persons (35,6%) from this group were heterozygotes, 1 person (2,2%) – homozygous carrier of G-allele. A-allele of GSTP1 gene was observed in 72 cases (80,0%) among 90 selected alleles, G-allele – in 18 cases (20,0%) respectively. By OR calculation we did not found any statistically proofed difference between distribution of A313G allele polymorphism of GSTP1 gene in chronic hepatitis patients and practically healthy individuals (OR = 1, 36, CI = 0, 70-2, 66, p < 0, 05).

Table 2: GSTP1 gene polymorphism (A313G) distribution in chronic nonviral hepatitis patients and healthy individuals

GSTP1 gene genotype	Chronic hepatitis patients (n = 57)		Healthy individuals (n = 45)	
	Absolute quantity, n	Percentage	Absolute quantity, n	Percentage
AA	32	56,1%	28	62,2%
AG	21	36,9%	16	35,6%
GG	4	7,0%	1	2,2%

Association of biochemical blood parameters in chronic hepatitis patients with GSTP1 gene polymorphic variants are mentioned in Table 3. The analysis of possible differences in parameters which reflect synthesizing, detoxification, excretory functions of the liver and markers of cytolytic and cholestatic syndromes between different polymorphic variants of A313G polymorphism GSTP1 gene was performed.

Chronic hepatitis patients were characterized by elevated plasma content of total bilirubin and its fractions, triacylglycerols, uric acid, higher activity of hepatic transaminases, AP and GGT compared with those parameters in healthy individuals regardless of allelic distribution of

GSTP1 gene. Moreover, ALT plasma activity was at 44,0% (p = 0,046) higher in chronic hepatitis patients G-allele carriers compared with proper parameter in patients with AA-genotype, indicating a higher activity of cytolysis.

Table 3: Biochemical blood parameters in chronic nonviral hepatitis patients according to A313G polymorphism of GSTP1 gene

Plasma level	Healthy volunteers, n = 45	Chronic hepatitis patients, n = 57	
		AA-genotype carriers, n = 32	G-allele carriers, n = 25
Glucose, mmol/L (N= 3,9-6,0 mmol/L)	4,7 ± 0,08	5,2 ± 0,21	5,3 ± 0,23
Total bilirubin, mkmol/L (N=5,0-20,5)	11,1 ± 0,79	23,9 ± 3,81 p_1 = 0,005	20,7 ± 3,50, p_1 = 0,001
Direct bilirubin, mkmol/L (N=0,5-5,0)	3,1 ± 0,31	8,7 ± 2,04, p_1 = 0,004	7,6 ± 1,65, p_1 = 0,002
Cholesterol, mmol/L (N=3,1-5,2)	4,6 ± 0,15	4,9 ± 0,21	5,0 ± 0,29
Triglycerides, mmol/L (N=0,4-1,8)	1,0 ± 0,07	1,4 ± 0,12, p_1 = 0,01	1,3 ± 0,11, p_1 = 0,03
Uric acid, mkmol/L, (N=200-450)	243,3 ± 9,89	309,1 ± 15,56, p_1 = 0,0001	296,8 ± 23,05,p_1 = 0,03
Albumin, g/L (N=35-50)	45,0 ± 0,41	41,6 ± 0,96, p_1 = 0,006	44,2 ± 0,90
Total protein, g/L (N=65-85)	69,3 ± 0,62	68,6 ± 0,82	70,8 ± 1,41
Urea, mmol/L (N=2,4-8,3)	4,2 ± 0,23	4,8 ± 0,40	4,7 ± 0,46
Creatinine, mkmol/L (N=40-110)	82,6 ± 1,80	80,2 ± 2,93	85,4 ± 3,70
Aspartate aminotransferase, units of action/L (N<37)	22,6 ± 1,37	45,3 ± 7,18 p_1 = 0,0008	60,0 ± 5,78 p_1 = 0,005
Alanine aminotransferase, units of action/l (N<32)	18,5 ± 1,46	50,0 ± 9,29 p_1 < 0,0001	72,0 ± 11,74 p_1 < 0,0001 p_2 = 0,046
Lactate dehydrogenase, units of action/L (N=210-420)	387,0 ± 13,59	421,5 ± 24,94	458,0 ± 35,47
Alkaline phosphatase, units of action/L (N=42-141)	80,3 ± 3,20	113,7 ± 10,80, p_1 = 0,007	123,6 ± 14,62, p_1 = 0,01
Gamma-glutamyl transferase, units of action/L (N=10-50)	21,9 ± 1,62	97,4 ± 22,75 p_1 < 0,0001	103,3 ± 27,63 p_1 < 0,0001

p_1 – significance of differences compared with the indicators in the group of healthy people; p_2 – significance of differences compared with rates in chronic hepatitis patients AA-genotype carriers.

Analysis of pro- and antioxidant indicators reviled that chronic hepatitis patients both AA-genotype and G-allele carriers of GSTP1 gene had lower restored glutathione level, higher glutathione peroxidase activity and elevated TBA-reactive products content in the blood, compared to proper indicators in the control group (Table. 4).

Table 4: Pro- and antioxidant systems indicators in chronic nonviral hepatitis patients according to A313G polymorphism of GSTP1 gene

Plasma level	Healthy volunteers, n = 45	Chronic hepatitis patients, n = 57	
		AA-genotype carriers, n = 32	G-allele carriers, n = 25
Restored glutathione, mmol/l	1,18 ± 0,03	0,86 ± 0,05 p_1 < 0,0001	0,74 ± 0,06 p_1 < 0,0001, p_2 = 0,04
Glutathione peroxidase, nmol/min*mg of hemoglobin	115,4 ± 3,21	136,7 ± 5,74 p_1 = 0,02	141,0 ± 6,18 p_1 = 0,009
Catalase, mkmol/min*l	15,1 ± 0,46	14,2 ± 0,63	13,7 ± 0,51 p_1 = 0,04
Thiobarbituric acid reactive substances, mkmol/l	14,2 ± 0,48	16,4 ± 0,82 p_1 = 0,03	17,1 ± 0,90 p_1 = 0,01

p_1 – significance of differences compared with the indicators in the group of healthy people; p_2 – significance of differences compared with rates in chronic hepatitis patients AA-genotype carriers.

Chronic hepatitis patients G-allele carriers also noted significantly lower catalase activity compared with healthy persons, which was not typical for patients with A-allele. Moreover, significantly lower restored glutathione blood content at 16,2% (p = 0,04) was found in patients with G-allele compared with patients AA-genotype carriers.

4. Conclusions: Prevalence of G-allele of A313G polymorphism in GSTP1 gene did not differ significantly in chronic hepatitis patients compared with healthy individuals in Ukrainian population, but presence of G-allele was associated with higher activity of cytolytic syndrome. Chronic hepatitis patients with G-allele were also found significantly lower restored glutathione blood content in comparison with patients AA-genotype carriers.

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