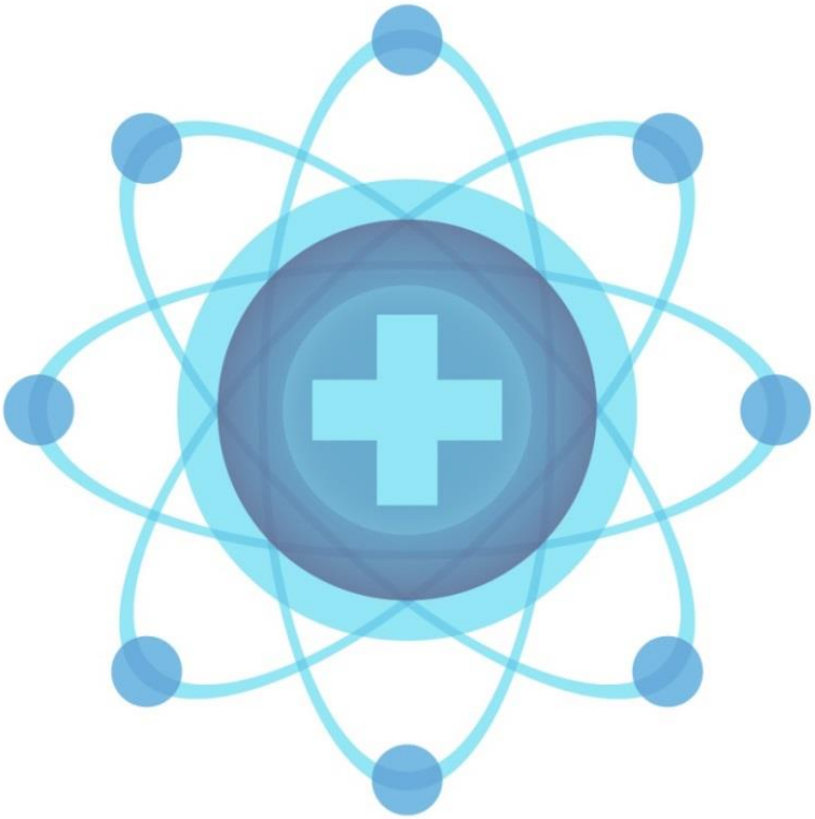

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Evaluation of genotypic allelic variants of *IL6* and *IL10* genes in the pathogenesis of gastric ulcer according to SNP-PCR detection (pilot report)

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Purpose of the study. To study the prevalence and role of allelic variants of the *IL6* and *IL10* genes in the pathogenesis of gastric ulcer (GU) in patients of the Gastroenterological Department of the Tashkent Medical Academy (TMA).

Material and research methods. The allelic variant (rs1800795, *IL6* (C174G)) of the *IL6* gene and (rs1800896, *IL10* (G1082A)) *IL10* were detected in patients with GU (n=55) and healthy individuals (n=88) by SNP-PCR.

Results. The results obtained on the distribution features and assessment of the degree of significance of the detected differences in the frequencies of occurrence of the allele variant and genotype for the *IL10* polymorphic gene (rs1800896) between GU compared with healthy ones were characterized by the absence of statistically significant differences in the carriage of the minor allele A (χ^2<3.84; P>0.05), major genotype G/G (χ^2<3.84; P>0.05) and heterozygous genotype G/A (χ^2<3.84; P>0.05).

The obtained results on distribution features and assessment of the degree of significance of the detected differences in the frequencies of occurrence of variants of alleles and genotypes for the polymorphic *IL10* gene (rs1800896) between GU compared with healthy ones were characterized by the absence of statistically significant differences between the carriage of the minor allele A (χ^2<3.84; P>0.05), major genotype G/G (χ^2<3.84; P>0.05) and heterozygous genotype G/A (χ2<3.84; P>0.05).

Conclusions: Based on the results of the study, it can be argued that the C174G (rs1800795) polymorphic loci of the *IL6* gene play a significant role in the mechanisms of GU formation in Uzbekistan.

Keywords: detection, SNP-PCR method, allelic variants of genes, *IL6*, *IL10*, gastric ulcer, risk of formation.

Introduction.

Peptic ulcer of the stomach is still considered one of the etiologically multifactorial diseases, rich in pathogenetic nature of topical problems. Currently, the analysis of the distribution of polymorphic alleles and genotypically unfavorable variants of pro- inflammatory and anti-inflammatory cytokines in patients with gastroduodenal pathology is considered one of the most advanced areas of modern gastroenterology.

According to the literature data, interleukin proteins, endogenous polypeptide mediators of intercellular interaction, IL6 and IL10, 1].

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Synthesis of these interleukin proteins (IL6 and IL10) is due to specific *IL6* and *IL10* genes located in chromosomal regions 7p15.3 and 1q32.1, respectively. The genes encode the amino acid sequence of interleukin proteins: IL6 and IL10 [2].

Several allelic variants (polymorphisms) of the IL6 gene are known in the promoter region: –174 G → C (replacement of a guanine nucleotide with a cytosine one (rs1800795)), –634 C → G, –572 G → C, and –597 G → A. nucleotide replacement produces alternative variants of the mediator (cytokine) of intercellular interaction - IL6. Cytokine isoforms resulting from alternative splicing include: mRNA IL6Delta2, IL-6Delta4, IL6Delta2.4, IL6R [3]. Studies have shown that carriage of the G allele of *IL6* –174 is associated with higher production of IL6 than the C allele [4].

In addition, six allelic variants (polymorphisms) of the *IL10 gene have been described* at positions –1082, –819, –652, –592, –127, –41 relative to the transcriptional site [5]. An *IL10* allele variant (G1082A, rs1800896) identified in the *IL10* promoter region showed that the G allele at position –1082 is associated with higher IL10 production than the A allele at the same position [6, 7].

It is known that the "bright side" of the IL 10 protein is its tolerogenicity. The protein inhibits the production of pro- inflammatory cytokines and suppresses the inflammatory response [8]. Its genetic allelic variants may play an important role in the pathogenesis of gastric cancer and atrophic gastritis (“dark side”) [8, 9, 10].

Thus, the study and identification by SNP-PCR of allelic variants of the *IL6* and *IL10 genes* associated with a “predisposition” to GU are crucial for establishing the pathogenesis of the disease and identifying promising biomarkers and therapeutic targets.

Material and methods.

Before the start of the scientific work, the incidence of GU in the gastroenterology department of the TMA clinic was determined, which was 4.2%, the average age of patients was 51.6±17.1 years.

The whole complex of molecular genetic studies on the study of the allelic variant of the *IL6* gene (rs1800795) and *IL10* (rs1800896) was performed in n=55 patients with GU (main group). From healthy donors (n=88) without inflammatory and ulcerative lesions of the stomach, a control (comparable) group was formed. All patients of the main group were observed at the clinic of the Tashkent Medical Academy in the period from 2019 to 2021.

Molecular genetic studies were carried out in the laboratory of "Molecular Medicine and Cell Technologies" of the Republican Specialized Scientific and Practical Medical Center for Hematology of the Center for Hematology.

IL6 (rs1800795) and *IL10 (*rs1800896) genes was carried out by SNP-PCR on a programmable thermal cycler from «Applied Biosystems» 2720 (USA), using the test systems of the «Литех» company (Russia), according to the manufacturer's instructions. DNA isolation was carried out from venous blood leukocytes in accordance with the standard DNA isolation protocol. Statistical processing of the results was carried out using the statistical software package «OpenEpi 2009, Version 9.3».

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The results of their discussion.

In our studies, the frequency distribution of genotype variants of the studied polymorphic *IL6* gene (C174G) fully corresponded to the Hardy-Weinberg equilibrium (p>0.05). Assessing the proportions of the frequency distribution of allelic and genotypic variants for the polymorphic *IL6* gene (C174G) in the control (healthy) group (n = 88), the occurrence of major (C) and minor alleles (G) was found in 82.4% (n = 145) and 17.6% (n =31) cases, respectively.

Table 1

The frequency of occurrence of alleles and genotypes for the polymorphism of the *IL6* gene (C174G) with GU and healthy

No.	Group	Allele frequency				Genotype frequency					
		C		G		C/C		C/G		G/G	
		n	%	n	%	n	%	n	%	n	%
1.	GU, n=55	82	74,6	28	25,4	31	56,3	20	36,4	4	7,3
2.	Comparable control group, n=88	145	82,4	31	17,6	61	69,3	23	26,1	4	4,6

As can be seen from Table 1, in the main group of patients with GU (n = 55), compared with healthy controls, a pronounced decrease in the frequency of the major allele C to 74.6% (n = 82) and an increase in the proportion of the minor allele G to 25.4% (n =28). At the same time, among this group of patients, the frequency of the major C/C genotype decreased quite sharply to 56.3% (n = 31) with a noticeable increase in cases of carriage of the heterozygous C/G and minor G/G genotype to 36.4% (n = 20) and 7.3% (n = 4).

A decrease in the frequencies of the major allele (C) and genotype (C/C) among patients with GU may indicate a decrease in their protective activity against the formation of GU, which is also emphasized by an increase in the activity of the minor allele (G) and genotypes (C/G and G/G), possibly having a predictive value at the onset of this disease.

To reliably confirm the significance of the revealed differences in the proportions of the distribution of allelic and genotypic variants for the polymorphic *IL 6* gene (C174G) between individuals of the studied groups and to determine their role in the pathogenetic mechanisms of GU, a deeper statistical analysis of their intergroup comparison was carried out (Table 2).

Table 2

Statistical analysis of the difference in the carriage of polymorphic loci of the *IL 6* gene (C174G) between patients with GU and healthy people

Study group	Alleles and genotypes	Statistical difference compared to control					
		RR	95%CI:	OR	95%CI:	χ^2	p (confidence)
GU (n=55)	C	0,9	0,48 - 1,69	0,6	0,35 - 1,11	2,5	0,2
	G	1,1	0,66 - 1,84	1,6	0,9 - 2,84		
	C/C	0,8	0,37 - 1,81	0,6	0,28 - 1,15	2,5	0,2
	C/G	1,4	0,61 - 3,15	1,6	0,78 - 3,33	1,7	0,2
	G/G	1,6	0,39 - 6,64	1,6	0,4 - 6,79	0,5	0,5

Significant results were obtained when comparing the frequencies of alleles and genotypes for the polymorphic gene *IL6* (C174G) between patients with GU (n=55) and healthy (n=88), which made it possible to detect among patients a pronounced tendency to increase the activity of the minor allele G by 1.6 times (χ^2=2.5; P=0.2; OR=1.6; 95% CI: 0.9-2.84) with a decrease in the activity of the major genotype C/C (χ^2=2.5; P=0.2; OR=0.6; 95% CI: 0.28-1.15), with a simultaneous less pronounced tendency to increase the activity of the heterozygous C/G genotype by 1.6 times (χ^2 =1.7; P=0.2; OR =1.6; 95% CI: 0.78-3.33) and a non-significant increase in the frequency of the homozygous G/G genotype by 1.6 times (χ^2= 0.5; P=0.5; OR=1.6; 95% CI: 0.4-6.79) (Table 2).

Thus, a comparative analysis between patients with GU and healthy people made it possible to detect differences in the frequencies of the studied alleles and genotypes for the polymorphic gene *IL 6* (C174G), which were distinguished by the presence of a pronounced tendency to increase the risk of onset of the disease among carriers of the minor allele G 2.5 times (χ^2 =2.5; P=0.2).

Therefore, based on the results of the study, we can state that the C174G polymorphic loci of the *IL6* gene have a certain role in the mechanisms of formation of GU in the Gastroenterological Department of TMA.

Starting to study the features of the occurrence of polymorphic loci of the *IL10* gene (G1082A) in the group of healthy people (n = 88), the carriage of the major and minor allele was determined G and A in 77.8% (n =137) and 22.2% (n =39) cases, respectively. Among the same category of examined genotypes G/G, G/A and A/A

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were found in 57.9% (n = 51), 39.8% (n = 35) and 2.3% (n = 2) cases, respectively (see Table 3) .

Table 3

The frequency of occurrence of alleles and genotypes according to the polymorphism of the *IL 10* gene (G1082A) with GU and healthy

No	Group	Allele frequency				Genotype frequency					
		G		A		G/G		G/A		A/A	
		n	%	n	%	n	%	n	%	n	%
1	GU, n=55	84	76,4	26	23,6	33	60,0	18	32,7	4	7,3
2	Comparable control group, n=88	137	77,8	39	22,2	51	57,9	35	39,8	2	2,3

Analyzing the features of the occurrence of polymorphic loci of the *IL10* gene (G1082A) in the group of patients with GU (n=55), there was an even closer approximation of the levels of occurrence of allelic variants G (76.4% vs. 77.8%) and A (23.6% vs. 22.2%), as well as G/G genotype variants (60.0% vs.) and G/A (32.7% versus 39.8%) to those among healthy people. At the same time, the share of carriage of the minor A/A genotype among patients with GU, compared with healthy ones, still remained noticeably high (7.3% versus 2.3%) of genotypes (Table 3).

The identified feature may indicate the participation of the minor genotypic variant A/A at the polymorphic locus of the *IL10 gene* (G1082A) in increasing the likelihood of developing inflammatory and ulcerative diseases of the stomach.

To confirm this assumption, it was appropriate and necessary to further conduct a comparative analysis of differences in the frequencies of alleles and genotypes between GU and healthy people with the determination of their statistical significance (Table 4).

Table 4

Statistical difference in the carriage of polymorphic loci of the *IL 10 gene* (G1082A) between GU and healthy people

Study group	Alleles and genotypes	Statistical difference compared to control (n =88)					
		RR	95%CI:	OR	95%CI:	χ^2	p (confidence)
GU (n=55)	G	1,0	0,5 - 1,92	0,9	0,52 - 1,62	0,1	0,8
	A	1,0	0,66 - 1,58	1,1	0,62 - 1,91		
	G / G	1,0	0,45 - 2,38	1,1	0,55 - 2,16	0,1	0,9
	G /A	0,8	0,34 - 1,99	0,7	0,36 - 1,49	0,7	0,4
	A/A	3,2	0,98 - 10,5	3,4	0,65 - 17,43	2,1	0,2

The results of the study conducted between the groups of patients with GU and healthy people were characterized by the absence of statistically significant differences in the percentage of carriage of the minor allele A (23.6% vs. 22.2%; χ^2=0.1; P=0.8; OR=1.1), major homozygous genotype G/G (60.0% vs 57.9%; χ^2 =0.1; P=0.9; OR=1.1; 95%CI: 0.55 - 2.16) and heterozygous genotype G/A (32.7% vs 39.8%; χ^2=0.7; P=0.5; OR=0.7; 95%CI: 0.36 - 1.49). However, in the proportion of carriers of the homozygous minor A/A genotype (7.3% vs. 2.3%; χ^2=2.1; P=0.2; OR=3.4; 95% CI: 0.65 - 17.43), a very pronounced tendency to increase by 3.4 times among patients with GU, which also confirms the participation of this genotype in the polymorphic *IL10* gene (G1082A) in the mechanisms of initiation of gastric ulcer (see Table 4).

Therefore, an increase in the activity of the A/A mutant genotype in the polymorphic *IL10* gene (G1082A) may increase the likelihood of developing GU in Uzbekistan.

Conclusion.

Thus, although the cohort of the compared groups differs greatly in age, we can preliminarily state that, according to the results of detection using SNP-PCR, genotypic allelic variants of the *IL6* and *IL10* genes have certain significance in the pathogenesis of gastric ulcer in Uzbekistan. Further research requires sequencing (WGS) of the entire genome of patients, including both protein-coding regions (exomes) and non-coding "silent" regions of human DNA.

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