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FEATURES OF THE OCCURRENCE OF SINGLE-NUCLEOTIDE POLYMORPHISM IL10 (C592A) AMONG PATIENTS WITH PURULENT-INFLAMMATORY DISEASES OF THE MIDDLE EAR AND ASSESSMENT OF ITS CONTRIBUTION TO THE MECHANISMS OF DISEASE FORMATION

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Abstract: The features of the single-nucleotide polymorphism of the IL10 (C592A) gene in purulent-inflammatory diseases of the middle ear were investigated. The polymorphism of the IL10 (C592A) gene was evaluated by analyzing DNA samples by setting up a polymerase chain reaction (PCR) in Real Time mode.

The results of the study on the characteristics of the polymorphism of the IL10 (C592A) gene showed that it was revealed that the minor A allele can contribute to the initiation of inflammatory processes that realize the onset of purulent-inflammatory diseases of the middle ear, increasing the risk of their development as a whole by 1.6 times ($\chi2 = 2.5$; $P = 0.2$). Moreover, the association became apparent. Minor allele A ($\chi2 = 4.3$; $P = 0.05$) with an increased risk of onset of mild course of purulent-inflammatory diseases of the middle ear. In addition, there was a tendency to increase the risk of formation of a mild course of the disease on the part of all three genotypes (C / C - $\chi2 = 3.2$, $P = 0.1$; A / G - $\chi 2 = 1.3$, $P = 0.3$; OR = 1.7; A / A - $\chi2 = 1.9$, $P = 0.2$) of the polymorphic gene IL10 (C592A). Meanwhile, there are no significant associative relationships between the carriage of minor alleles A ($\chi2 = 0.5$; $P = 1.0$), as well as genotypes C / A ($\chi2 = 0.2$; $P = 0.7$) and A / A ($\chi2 = 0.2$; $P = 0.7$) of the polymorphic il10 (C592A) gene and the development of purulent-inflammatory diseases of the middle ear of medium and severe course.

Keywords: purulent-inflammatory diseases of the middle ear, polymorphism of the IL10 gene (C592A),,, allele, frequency, genotype, share of carriage.

Relevance. Among all diseases of the ENT organs, purulent-inflammatory diseases of the middle ear are very widespread [3], in which, in addition to external environmental infectious factors, polymorphic genes of pro-inflammatory cytokines can have a special contribution to the basis of the pathogenetic mechanism [1,2,4]. However, due to the multifactorial pathogenesis of purulent-inflammatory diseases of the middle ear, assessing the contribution of genetic polymorphisms to their development is a very difficult task.

Among the genes of pro-inflammatory cytokines involved in the initiation and maintenance of inflammation in the body, an important representative is the cytokine

gene IL10, located on the first chromosome and containing five exons [9,10]. Il 10 production is mainly carried out by monocytes in small quantities under normal conditions, but under the influence of pathogenic microflora, its expression increases significantly, during which it also increases lymphocytic cells are also connected [7].

The contribution of the polymorphic IL10 (C592A) gene to the development of inflammatory diseases has been studied as a result of experimental and clinical studies [5,11]. Meanwhile, the role of the multifunctional gene of the pro-inflammatory cytokine IL10 (C592A) in the initiation of pathological processes in inflammatory diseases of the middle ear as reported in the literature is very ambiguous [6,8].

With this in mind, it seemed very interesting for us to study the features of the occurrence of allelic and genotypic variants of the IL10 (C592A) polymorphism among patients with purulent-inflammatory diseases of the middle ear and healthy to study its association with an increased risk of their formation.

Material and methods. The study was conducted with the participation of 87 patients with purulent-inflammatory diseases of the middle ear (I general group, in the age range from 15 to 67 years) who were treated in a specialized hospital of the Urgench branch of the Tashkent Medical Academy and in the private hospital KHRAZM ENT SHIFO in the period from 2016 to 2022 All patients (n = 87), depending on the severity of the course of the disease, are divided into two groups: II (n = 36) group of patients with mild course and III (n = 51) group of patients with moderate and severe course of purulent-inflammatory diseases of the middle ear. The control (IV group) comparison group included 71 conditionally healthy individuals without inflammatory diseases, comparable in age and sex to the general group of patients.

Molecular genetic studies were conducted in the laboratory of molecular genetics, cytogenetics and FISH of the Republican Specialized Scientific and Practical Medical Center of Hematology (Republic of Uzbekistan, Tashkent). In accordance with the generally accepted technique, DNA was isolated from blood leukocytes. At the same time, an analysis was carried out using the Applied Biosystems 2720 system (USA) (SNP-PCR Real time).) polymorphic gene IL10 (C592A) using test systems "Litekh" (Russia). Mathematical analysis of the results was carried out using the program "OpenEpi 2009, Version 9.3".

Results and discussion. In the distribution of frequencies of genotypic variants of the studied single-nucleotide polymorphic gene IL10 (C592A) among patients with purulent-inflammatory diseases of the middle ear and healthy individuals, discrepancies from the canonical distribution in PCV ($\chi 2<0.84$; $p>0.05$) were not detected.

The molecular genetic study of the single-nucleotide polymorphism of the IL10 (C592A) gene in the I general group of patients with purulent-inflammatory diseases of the middle ear and the IV group of comparative healthy control made it possible to detect the presence of differences in the distribution of its allelic and genotypic variants. So, if in the group of comparative healthy control of the frequency of the main C and minor A alleles were detected in 83.1% (n = 118) and 16.9% (n = 24)

cases, respectively, at the same time in the I general group of patients, the proportion of carriage of the main C allele decreased to 75.9% (n = 132) with a simultaneous increase in the proportion of the minor A allele to 24.1% (n = 24).

Similar dynamics was recorded in the distribution of all three possible variants of genotypes C / C, C / A and A / A. In particular, among healthy individuals of group IV, the main wild genotype C / C was found in 70.4% (n = 50) of the surveyed, and, in regularity with this, heterozygous C / A and mutant A / A genotypes were found in smaller cases, which amounted to 25.4% (n = 18) and 4.2% (n However, in the first group of patients, the cases of carriage of the main genotype C / C decreased to 59.8% (n = 52) with an increase in the proportions of heterozygous C / A and mutant A / A genotypes to 32.2% (n = 28) and 8.0% (n = 7), respectively,.

Differences in the distribution of allele frequencies and genotypes in the polymorphic IL10 (C592A) gene compared to their values in the healthy control group were also observed in the II and III groups of patients with purulent-inflammatory diseases of the middle ear with mild and moderate and severe course. For example, in groups II and III of patients, the frequencies of the basic C allele decreased to 70.8% (n = 51) and 79.4% (n = 81), respectively, and the frequencies of minor alleles naturally increased to 29.2% (n = 21) and 20.6% (n = 21).

In addition, compared with the healthy group in the II and III groups of patients, the proportion of the main wild genotype C / C decreased to 52.8% (n = 19) and 64.7% (n = 33) of carrier cases, and the frequencies of heterozygous C / A and mutant A / A genotypes, on the contrary, increased to 36.1% (n = 13) and 11.1% (n =4) respectively in group II, as well as up to 29.4% (n = 15) and 5.9% (n = 3), respectively, in group III of patients (Table 1.).

Table 1

Distribution of allelic and genotypic variants of single nucleotide polymorphism IL10 (C592A) among patients with purulent-inflammatory diseases of the middle ear and healthy individuals

№	Group	Frequency of allelic variants				Frequency of genotypic variants					
		C		A		C/C		C/A		A/A	
		n	%	n	%	n	%	n	%	n	%
I	Core group, (n= 87)	132	75.9	42	24.1	52	59.8	28	32.2	7	8.0
II	Group with a slight current, (n = 36)	51	70.8	21	29.2	19	52.8	13	36.1	4	11.1
III	Group with moderate course, (n = 51)	81	79.4	21	20.6	33	64.7	15	29.4	3	5.9
IV	Control group, (n = 71)	118	83.1	24	16.9	50	70.4	18	25.4	3	4.2

Thus, investigating the features of the distribution of allele frequencies and genotypes by the polymorphic il10 gene (C592A) among the studied groups of patients with purulent-inflammatory diseases of the middle ear, we found facts of a decrease in the proportion of carriage of the main alleles (C: for group I - 75.9% vs. 83.1%; for group II - 70.8% against 83.1% and for group III - 79.4% against 83.1%, respectively) and genotype (C/C: for group I – 59.8% vs. 70.4%; for group II – 52.8% vs. 70.4% and for group III – 64.7% vs. 70.4%, respectively) compared with healthy controls, and, in regularity with this, on the contrary, an increase in the frequencies of the minor allele (A: for group I – 24.1% vs. 16.9%; for group II – 29.2% vs. 16.9% and for III group - 20.6% against 16.9%, respectively) and genotypes (C / A: for group I - 32.2% against 25.4%; for group II - 36.1% against 25.4% and for group III - 29.4% against 25.4%, respectively and A / A: for group I - 8.0% against 4.2%; for group II - 11.% against 4.2% and for group III - 5.9% against 4.2%, respectively).

Based on the results obtained, it is obvious that the most pronounced discrepancies in the frequencies of alleles and genotypes according to the studied genetic polymorphism are visualized among patients of group II with a mild course of purulent-inflammatory diseases of the middle ear with their lowest severity among patients iii groups with moderate and severe course of purulent-inflammatory diseases of the middle ear with a lung. These data show the presence of a greater contribution of the single-nucleotide polymorphic gene IL10 (C592A) to the mechanisms of formation of a mild course of purulent-inflammatory diseases of the middle ear.

Meanwhile, to substantiate these assumptions, we then conducted a statistical analysis comparing the differences found between the studied groups of patients and healthy.

The results of a comparative assessment in the features of the distribution of allelic and genotypic variants of the IL10 (C592A) gene polymorphism between the general I group of patients and the IV group of healthy patients were characterized by a clear tendency to increase the minor allele A by 1.6 times (24.1% versus 16.9%; $\chi 2 = 2.5$; $p = 0.2$; OR = 1.6; 95% CI: 0.9-2.73) among patients. In addition, a decrease in the protective effect of the main genotype variant in relation to the formation of inflammatory processes initiating the development of purulent-inflammatory diseases of the middle ear was evidenced by a tendency to decrease among patients of the wild genotype C / C (59.8% versus 70.4%; $\chi 2 = 1.9$; $p = 0.2$; OR = 0.6; 95% CI: 0.32-1.21).

For heterozygous C/A and mutant A/A genotype variants among patients compared with healthy ones, an increase in their frequencies was found by 1.4 times (32.2% vs. 25.4%; $\chi 2 = 0.9$; $p = 0.4$; OR = 1.4; 95% CI: 0.7-2.81) and 2.0 times (8.0% vs. 4.2%; $\chi 2 = 1.0$; $p = 0.4$; OR = 2.0; 95% CI: 0.5-7.79), but these differences did not reach statistically significant values (Table 2).

Table 2

Differences in the distribution of frequencies of allelic and genotypic variants of single-nucleotide polymorphism IL10 (C592A) among patients with purulent-inflammatory diseases of the middle ear and healthy individuals

Alleles and genotypes	Number of alleles and genotypes examined				χ^2	R	RR	95%CI	OR	95%CI
	I group		IV group							
	n	%	n	%						
C	132	75.9	118	83.1	2.5	0.2	0.9	0.6-1.4	0.6	0.37 - 1.12
A	42	24.1	24	16.9	2.5	0.2	1.1	0.56-2.15	1.6	0.9 - 2.73
C/C	52	59.8	50	70.4	1.9	0.2	0.8	0.49-1.46	0.6	0.32 - 1.21
C/A	28	32.2	18	25.4	0.9	0.4	1.3	0.72-2.24	1.4	0.7 - 2.81
A/A	7	8.0	3	4.2	1.0	0.4	1.9	0.82-4.44	2.0	0.5 - 7.79

Thus, the results of a statistical analysis conducted in a comparative aspect between the general group of patients with purulent-inflammatory diseases of the middle ear and healthy ones show the possible significance in the implementation of the disease of only the minor allele A of the polymorphism of the IL10 (C592A) gene in respect of which a clear trend was traced to an increase of 1.6 times ($\chi2$ = 2.5; P = 0.2) of the risk of formation purulent-inflammatory diseases of the middle ear.

Assessing the features of the distribution of frequencies of similar alleles and genotypes by polymorphism of the IL10 (C592A) gene in groupsII of patients with a mild course of purulent-inflammatory diseasesof the middle ear in relation to healthy ones, more significant differences were found in the frequencies of the minor allele A, which among patients is statistically significant increased by 2.0 times (29.2% vs. 16.9%; $\chi2$=4.3; P=0.05; OR=2.0; 95%CI: 1.04-3.93), which is direct evidence of the association of this allele with a high risk of onset of purulent-inflammatory diseasesof the middle ear.

In addition, in the increased risk of the onset of purulent-inflammatory diseases of the middle ear, a pronounced tendency was found to reduce the protective effect of the main genotype C / C (52.8% versus 70.4%; $\chi2$=3.2; P=0.1; AboutR=0.5; 95%CI: 0.21-1.07) and a less pronounced tendency to increase the activity of the heterozygous genotype A / G by 1.7 times (36.1% vs. 25.4%; $\chi2$=1.3; P=0.3; AboutR=1.7; 95%CI: 0.7-3.94) and mutant genotype A/A 2.8 times (11.1% vs 4.2%; $\chi2$=1.9; P=0.2; AboutR=2.8; 95%CI: 0.63-12.7) compared to similar among healthy people (Table 3).

Table 3

Differences in the distribution of frequencies of allelic and genotypic variants of single-nucleotide polymorphism IL10 (C592A) among patients with mild purulent-inflammatory diseases of the middle ear and healthy individuals

Alleles	Number of alleles and	χ^2	R	RR	95%CI	OR	95%CI

and genotypes	genotypes examined									
	II group		IV group							
	n	%	n	%						
C	51	70.8	118	83.1	4.3	0.05	0.9	0.4-1.82	0.5	0.25 - 0.96
A	**21**	**29.2**	**24**	**16.9**	**4.3**	**0.05**	**1.2**	**0.66-2.07**	**2.0**	**1.04 - 3.93**
C/C	19	52.8	50	70.4	3.2	0.1	0.7	0.27-2.08	0.5	0.21 - 1.07
C/A	13	36.1	18	25.4	1.3	0.3	1.4	0.5 - 4.08	1.7	0.7 - 3.94
A/A	4	11.1	3	4.2	1.9	0.2	2.6	0.66-10.41	2.8	0.63 - 12.7

Consequently, the results obtained indicate a statistically significant association of the minor allele A ($\chi2 = 4.3$; $P = 0.05$) with an increased risk of mild onset of purulent-inflammatory diseases of the middle ear, as well as the presence of a tendency to their development on the part of all three genotypes (C / C - $\chi2 = 3.2$, $P = 0.1$; A / G - $\chi2 = 1.3$, $P = 0.3$; OR = 1.7; A / A - $\chi2 = 1.9$, $P = 0.2$) due to a decrease in the activity of the wild genotype and an increase in the activity of the heterozygous, as well as mutant genotypes by the polymorphism of the IL10 (C592A) gene.

The opposite results were obtained in a comparative analysis of differences in the carriage of alleles and genotypes of the polymorphic il10 gene (C592A) in the III group of patients with moderate and severe course of the disease, in which, compared with healthy people, despite the increase in the frequency of the minor allele A by 1.3 times (20.6% vs. 16.9%; $\chi2=0.5$; P=1.0; AboutR=1.3; 95%CI: 0.67-2.44), as well as a 1.2-fold increase in heterozygous C/A genotype frequencies (29.4% vs. 25.4%; $\chi2=0.2$; P=0.7; AboutR=1.2; 95%CI: 0.55-2.74) and mutant genotype 1.4 times (5.9% vs 4.2%; $\chi2=0.2$; P=0.7; AboutR=1.4; 95%CI: 0.28-7.27) not even a tendency to increase the risk of onset of purulent-inflammatory diseases of the middle ear with a moderate and severe course has not been traced.

The results obtained prove the absence of connections between carriers of minor alleles A ($\chi2 = 0.5$; $P = 1.0$), as well as genotypes C / A ($\chi2 = 0.2$; $P = 0.7$) and A / A ($\chi2 = 0.2$; $P = 0.7$) of the polymorphic il10 (C592A) gene and the development of purulent-inflammatory diseases of the middle ear of medium and severe course.

The final stages of this study was a comparative analysis of the degree of differences in the frequencies of distribution of alleles and genotypes of the polymorphic gene IL10 (C592A) between the II and III groups of patients, i.e. depending on the severity of the course of purulent-inflammatory diseases of the middle ear.

The results of the analysis were characterized by the presence of a not pronounced tendency to increase the risk of onset of mild disease by 1.6 times among carriers of the minor A allele (29.2% versus 20.6%; $\chi2=1.7$; P=0.2; AboutR=1.6; 95%CI: 0.79-3.19). Meanwhile, the increase in the frequency of carriage of heterozygous C / A and mutant A / A genotypes in 1.4 (36.1% versus 29.4%; $\chi2=0.4$; P=0.6; AboutR=1.4; 95%CI: 0.55-3.36) and 2.0 times (11.1% vs 5.9%; $\chi2=0.8$; P=0.4; AboutR=2.0; 95%CI: 0.43-9.31) had no statistically significant

association with an increased risk of developing a mild form of purulent-inflammatory diseases of the middle ear.

Consequently, the results obtained show the possible role of the minor allele A ($\chi2 = 1.7$; $P = 0.2$) in increasing the risk of mild onset of purulent-inflammatory diseases of the middle ear.

Conclusion. Analyzing the results of studying the distribution features in the frequency of occurrence of alleles and genotypes of the IL10 gene polymorphism (C592A) among the studied groups of patients with purulent-inflammatory diseases of the middle ear and healthy people, it was found that the minor allele A may contribute to the initiation of inflammatory processes that implement the onset of purulent inflammatory diseases of the middle ear, increasing the risk of their development in general by 1.6 times ($\chi2=2.5$; $P=0.2$). Moreover, the association of the minor allele A ($\chi2=4.3$; $P=0.05$) with an increased risk of the onset of a mild course of purulent-inflammatory diseases of the middle ear became obvious. In addition, a trend towards an increased risk of developing a mild course of the disease was found for all three genotypes (C/C - $\chi2=3.2$, $P=0.1$; A/G - $\chi2=1.3$, $P=0.3$; OR=1.7; A/A - $\chi2=1.9$, $P=0.2$) polymorphic gene IL10 (C592A).

Meanwhile, reliable associative relationships between the carriage of minor alleles A ($\chi2 = 0.5$; $P = 1.0$), as well as genotypes C / A ($\chi2 = 0.2$; $P = 0.7$) and A / A ($\chi2 = 0.2$; $P = 0.7$) of the polymorphic il10 (C592A) gene and the development of purulent-inflammatory diseases of the middle ear of medium and severe course have not been established.

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