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ASSESSMENT OF THE CONTRIBUTION OF BIALLELIC POLYMORPHISM OF THE EDN1 GENE (LYS198ASN) TO THE DEVELOPMENT OF CHRONIC TONSILLITIS

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Abstract. *This study aims to evaluate the role of the biallelic polymorphism of the EDN1 gene (Lys198Asn) in the development of chronic tonsillitis (CT). The study included 94 patients aged 3 to 55 years with chronic tonsillitis, as well as 90 conditionally healthy individuals examined in the Khorezm region between 2021 and 2025. Patients were divided into groups according to the clinical form of the disease (simple form, toxic-allergic form I and II). Molecular genetic analysis was performed using real-time polymerase chain reaction (PCR).*

The results demonstrated that the minor Asn allele of the EDN1 gene was significantly more frequent in patients with chronic tonsillitis compared to the control group, increasing the risk of disease development by 1.7 times. At the same time, a decrease in the frequency of the protective Lys allele and the Lys/Lys genotype was observed. The strongest association was identified in patients with the simple form and toxic-allergic form I, where the risk of disease increased by 1.8 and 1.9 times, respectively. No statistically significant association was found with toxic-allergic form II.

In conclusion, the EDN1 (Lys198Asn) gene polymorphism is an important genetic marker associated with an increased risk of chronic tonsillitis, particularly its simple and toxic-allergic form I. These findings contribute to a deeper understanding of disease pathogenesis and may support the development of personalized treatment approaches.

Key words: *chronic tonsillitis, genetic polymorphism, EDN1 (Lys198Asn), allele, genotype, risk of development.*

Introduction.

Chronic tonsillitis (CT) is a chronic inflammation of the palatine tonsils of the lymphoid pharyngeal ring, and is one of the most common diseases of the pharynx in people regardless of age [2,13].

The problem of CT remains highly relevant in medicine to this day and extends far beyond otolaryngology. According to the latest data, CT occurs in 12–43.8% of cases in children and 5–15.8% in adults. [5, 14] HT is a disease with a hereditary predisposition [6, 15]. The dominant factors in the development of CT include chronic foci of infection in the oral cavity. In addition, various unfavorable social and environmental factors also contribute to the development of inflammation in the palatine tonsils [7, 11, 16]. The literature does not sufficiently cover the issues of the location and nature of the hereditary predisposition to CT in children born to mothers with this disease. CT is a common disease among throat diseases during pregnancy in women [3]. Along with this, special importance in understanding the mechanism of various pathologies, including CT, is given to the study and clarification of the role of molecular genetic factors [1,8,10,12].

The study of a number of molecular genetic polymorphisms makes it possible to understand the mechanism of development of this disease [6]. According to many authors, there are many genetic markers of predisposition to CT [4,9,17]. Meanwhile, the contribution of the EDN1 gene polymorphism (Lys198Asn) to the increased risk of developing CT has not been sufficiently studied, which determines the necessity and relevance of this study.

Objective: To study the role of biallelic polymorphism of the EDN1 gene (Lys198Asn) in the development of chronic tonsillitis.

Material and methods.

The study was based on the voluntary participation of 94 patients with chronic tonsillitis aged 3 to 55 (median age 29.27 ± 1.7 years). Patients were randomly selected upon admission for diagnosis and treatment at the Khorezm Regional Multidisciplinary Medical Center and the private clinic "Khorezm LOR Shifo" (P. Makhmud Street, Urgench, Uzbekistan) from 2021 to 2025. The diagnosis was established taking into account diagnostic standards based on diagnostic criteria for the nature of clinical manifestations and instrumental data.

Depending on the form of chronic tonsillitis (CT), the selected patients (n=94) were divided into the following groups:

- 1st main group (n=94) – patients with chronic tonsillitis;
- 2nd group (n=39) – patients with a simple form of CT;
- 3rd group (n=32) – patients with toxic-allergic form I (TAF I),
– 4th group (n = 23) - patients with toxic-allergic form II (TAF II),
- 5th control group (CG) (n=90) – included conditionally healthy individuals without ENT diseases.

The material for the molecular genetic study was lymphocytes obtained from peripheral blood using a standard method.

The molecular genetic study included genotyping of the EDN1 (Lys198Asn) gene SNP using a real-time PCR system and software (Applied Biosystems, USA). Fragments of the EDN1 (Lys198Asn) gene SNP were amplified on a programmable thermal cycler from Applied. Biosystems » (USA) with preliminary denaturation at 95 ^{0} C (7 min), amplification for 40 cycles, subsequent denaturation at 95 ^{0} C (1 min), primer annealing at 59 ^{0} C (1 min), elongation at 72 ^{0} C (1 min) and final synthesis at 72 ^{0} C (3 min).

Mathematical analysis and statistical processing of the obtained research results were carried out using a statistical software package using methods for calculating the $\chi^{2\ criteria.}$ and the odds ratio (OR). The distribution of genotype variants of the studied EDN1 gene (Lys198Asn) was matched according to the Hardy-Weinberg equilibrium (HWE) and analyzed using the GenePop (Genetics of Population) program for analyzing genetic data.

Using the OpenEpi 2009, Version 2.9 statistical software package, the 95% CI (confidence interval) and P-values were calculated in addition to $^{the}\chi 2$ and OR. Comparisons of the observation groups were made using the student's t-test, which yields a probability (P) estimate of the significance of differences between the compared groups, with a significance level of $P < 0.05$.

Results and discussion.

Studying the distribution of genotypes of the polymorphic gene EDN 1 (Lys 198 Asn) in the main group of patients with CT and CG, it was found that the distribution of the observed frequencies of genotypic variants Lys / Lys, Lys / Asn and Asn / Asn had a statistically insignificant difference in relation to their expected frequencies ($\chi^2 < 3.84$; $P = 0.899$), i.e. the distribution of genotypes did not deviate from the Hardy-Weinberg equilibrium (HWE, $P > 0.05$).

The results of the analysis of the distribution of the EDN 1 gene polymorphism (Lys 198 Asn) in the studied groups showed the highest frequency of occurrence of the minor Asn allele, as well as the Lys / Asn and Asn / Asn genotypes among patients with CT, with a predominance of their frequencies in the group of patients with a simple form of CT and TAF I. The predominance of the frequencies of unfavorable allele variants and genotypes indicates their possible involvement in the implementation of CT. At the same time, to reliably verify this, comparative statistical analyses of the differences between the studied groups were conducted (Table 1).

Table 1.

Analysis of the structure of the EDN 1 gene polymorphism (Lys 198 Asn) among patients with chronic tonsillitis depending on the form and healthy

Group	Frequency of alleles and genotypes									
	Lys		Asn		Lys / Lys		Lys / Asn		Asn/Asn	
	n	%	N	%	n	%	n	%	n	%
1st MG of patients with chronic tonsillitis (n = 94)	141	75.0	47	25.0	53	56.4	35	37.2	6	6.4
2nd group with a simple form of CT, (n = 39)	58	74.4	20	25.6	22	56.4	14	35.9	3	7.7
3rd group with TAF I, (n = 32)	47	73.4	17	26.6	17	53.1	13	40.6	2	6.3
4th group with TAF II, (n = 23)	36	78.3	10	21.7	14	60.9	8	34.8	1	4.3
5th CG - healthy, (n=90)	151	83.9	29	16.1	63	70.0	25	27.8	2	2.2

In the 1st MG of patients with CT, compared with the CG, the minor allele Asn was statistically significantly more frequent by 1.7 times ($\chi^2 = 4.4$; P = 0.05; O R = 1.7; 95% CI: 1.04 - 2.9), while the frequency of the wild allele Lys was statistically significantly lower ($\chi^2 = 4.4$; P = 0.05; O R = 0.6; 95% CI: 0.34 - 0.96). Moreover, along with a reliable decrease in the wild allele, a pronounced tendency towards a decrease in the frequency of the wild genotype Lys / Lys was also observed ($\chi^2 = 3.7$; P = 0.1; O R = 0.6; 95% CI: 0.3 - 1.01).

However, in terms of the frequencies of unfavorable genotypic variants Lys / Asn ($\chi 2 = {}^{1.9;}$ P=0.2; OR = 1.5; 95% CI: 0.83–2.87) and Asn / Asn ($\chi 2 = {}^{1.9;}$ P=0.2; OR = 3.0; 95% CI: 0.63–14.23), despite their higher frequency in the group of patients compared to the control group, the differences were not statistically significant (Table 2).

Table 2.

Evaluation of differences in the distribution of the polymorphic gene EDN 1 (Lys 198 Asn) between the main and control groups

Alleles and genotypes	Frequency				χ^2	R	RR	95% CI	OR	95% CI
	MG (n=94)		CG (n=90)							
	n	%	n	%						
Lys	141	75.0	151	83.9	4.4	0.05	0.9	0.59 - 1.36	0.6	0.34 - 0.96
Asn	47	25.0	29	16.1	4.4	0.05	1.1	0.61 - 2.04	1.7	1.04 - 2.9
Lys/Lys	53	56.4	63	70.0	3.7	0.10	0.8	0.47 - 1.39	0.6	0.3 - 1.01
Lys/Asn	35	37.2	25	27.8	1.9	0.20	1.3	0.77 - 2.33	1.5	0.83 - 2.87
Asn/Asn	6	6.4	2	2.2	1.9	0.20	2.9	1.25 - 6.63	3.0	0.63 - 14.23

The statistically significant differences in the frequencies of the attenuated allele Asn, as well as the wild Lys allele and the Lys / Lys genotype of the polymorphic gene EDN 1 (Lys 198 Asn) between the MG and CG show that the attenuated allele increases the reliable risk of developing CT by 1.7 times ($\chi^2 = 4.4$; P = 0.05) due to a decrease in the activity of the protective allele Lys ($\chi^2 = 4.4$; P = 0.05) and the genotype Lys / Lys ($\chi^2 = 3.7$; P = 0.1). Thus, the obtained results prove the role of the polymorphic gene EDN 1 (Lys 198 Asn) in increasing the chance of developing CT.

In the 2nd group of patients with a simple form of CT, compared with the control group, the most noticeable differences were found between the allelic variants of the polymorphic gene EDN 1 (Lys 198 Asn), characterized among patients by a pronounced tendency to an increase in the unfavorable effect of the minor allele A sn by 1.8 times ($\chi^2 = 3.2$; P = 0.1; O R = 1.8; 95% CI: 0.95-

3.41) and a simultaneous pronounced tendency to the protective activity of the wild allele Lys (χ^2 = 3.2; P = 0.1; O R = 0.6; 95% CI: 0.29 – 1.06).

At the same time, the differences in the groups between the frequencies of the wild genotype Lys / Lys (χ^2 = 2.2; P = 0.2; O R = 0.6; 95% CI: 0.26 - 1.2), heterozygote Lys / Asn (χ^2 = 0.9; P = 0.4; O R = 1.5; 95% CI: 0.66 - 3.24) and minor homozygote Asn / Asn (χ^2 = 2.2; P = 0.2; O R = 3.7; 95% CI: 0.65 - 20.53) did not reach a statistically significant level of values.

Thus, studying the distribution features of the genetic polymorphism EDN 1 (Lys 198 Asn), the presence of a pronounced tendency to increase the unfavorable effect of the minor allele A sn (χ^2 = 3.2; P = 0.1) and decrease the protective activity of the main wild allele Lys (χ^2 = 3.2; P = 0.1) contributes to an increase in the risk of developing a simple form of CT by 1.8 times.

In the 3rd group of patients with CT and TAF I, compared with the control group, a pronounced tendency towards an increase in the unfavorable effect of the minor allele A sn by 1.9 times (χ^2 = 3.4; P = 0.1; O R = 1.9; 95% CI: 0.96 – 3.7) was found in the structure of the polymorphic gene EDN 1 (Lys 198 Asn), which was simultaneously accompanied by a pronounced tendency towards a decrease in the protective activity of the wild Lys allele (χ^2 = 3.4; P = 0.1; O R = 0.5; 95% CI: 0.27 - 1.04) and the Lys / Lys genotype (χ^2 = 3.0; P = 0.1; O R = 0.5; 95% CI: 0.21 - 1.1). Just as in the 2nd group of patients, in the 3rd group there was no statistically significant difference in the distribution of the Lys / Asn heterozygote (χ2 =1.8; P=0.2; O R =1.8; 95% CI: 0.77 - 4.11) and the minor Asn / Asn homozygote (χ2 = 1.2; P=0.3; O R =2.9; 95% CI: 0.43 - 20.0).

The presence of a pronounced tendency to an increase in the frequency of the minor allele A sn (χ^2 = 3.4; P = 0.1), as well as to a decrease in the frequencies of the major allele Lys (χ^2 = 3.4; P = 0.1) and the genotype Lys / Lys (χ^2 = 3.4; P = 0.1) in the structure of the genetic polymorphism EDN 1 (LYS 198 ASN) in the group of patients with CT with TAF I compared to healthy individuals shows the possible participation of the studied genetic marker in increasing the chance of developing this form of CT by 1.9 times.

However, in the 4th group of patients with CT and TAF II, compared with the control group, no statistically significant differences were found between the frequencies of the Lys (χ^2 = 0.8; P = 0.4; O R = 0.7; 95% CI: 0.31 - 1.54) and A sn (χ^2 = 0.8; P = 0.4; O R = 1.4; 95% CI: 0.31 - 1.54) alleles, as well as the genotypes Lys / Lys (χ^2 = 0.7; P = 0.5; O R = 0.7; 95% CI: 0.26 - 1.72), Lys / Asn (χ^2 = 0.4 P = 0.6; O R = 1.4; 95% CI: 0.52 - 3.66) and Asn / Asn (χ^2 = 0.3; P = 0.6; O R = 2.0; 95% CI: 0.18 - 22.06).

The absence of statistically significant differences in the structure of the polymorphic gene EDN 1 (Lys 198 Asn) between the groups of CT with TAF II and the control shows the absence of a contribution of the studied marker to the formation of this form of chronic tonsillitis.

EDN 1 (Lys 198 Asn) between different forms of CT patients also appeared interesting. Thus, analyzing the level of significance of differences between patients with a simple form of CT and CT with TAF I due to minor differences between the frequencies of alleles and genotypes, no statistically significant differences were revealed: alleles Lys (χ^2<3.84; P = 0.95; O R = 1.0; 95% CI: 0.49 - 2.23) and A sn (χ^2<3.84; P = 0.95; O R = 1.0; 95% CI: 0.45 - 2.02), genotypes Lys / Lys (χ^2 = 0.1; P = 0.8; O R = 1.1; 95% CI: 0.45 - 2.92), Lys / Asn (χ^2 = 0.2; P = 0.7; O R = 0.8; 95% CI: 0.31 - 2.14) and Asn / Asn (χ^2 = 0.1; P = 0.9; O R = 1.3; 95% CI: 0.2-7.95.

A similar analysis of the significance level of differences between patients with a simple form of CT and CT with TAF II also, due to insignificant differences, did not allow us to identify statistically significant differences for the Lys alleles (χ^2 = 0.2; P = 0.7; O R = 0.8; 95% CI: 0.34 - 1.91) and A sn (χ^2 = 0.2; P = 0.7; O R = 1.2; 95% CI: 0.52 - 2.95), as well as the Lys / Lys genotypes (χ^2 = 0.1; P = 0.8; O R = 0.8; 95% CI: 0.29 - 2.38), Lys / Asn (χ^2<3.84; P = 0.95; O R = 1.1; 95% CI: 0.36 - 3.09) and Asn / Asn (χ^2 =0.3; P = 0.7; O R =1.8; 95% CI: 0.18 - 18.18).

Differences in the distribution of the polymorphic gene EDN 1 (Lys 198 Asn) between patients with CT with TAF I and CT with TAF II were also statistically insignificant for the alleles Lys (χ^2 =

0.3; P = 0.6; O R = 0.8; 95% CI: 0.31 - 1.87) and A sn (χ^2 = 0.3; P = 0.6; O R = 1.3; 95% CI: 0.53 - 3.18), as well as the genotypes Lys / Lys (χ^2 = 0.3; P = 0.6; O R = 0.7; 95% CI: 0.25 - 2.16), Lys / Asn (χ^2 = 0.2; P = 0.7; O R = 1.3; 95% CI: 0.42 - 3.89) and Asn / Asn (χ^2 = 0.1; P = 0.8; O R = 1.5; 95% CI: 0.13 - 16.99).

Conclusion.

Thus, summing up the discussion of the results of the study of the distribution features and functional significance of the allelic genetic polymorphism EDN 1 (Lys 198 Asn), we can emphasize its role as a genetic factor with a risk effect that statistically significantly increases the risk of developing CT among carriers of the minor Asn allele by 1.7 times (χ^2 = 4.4; P = 0.05), an increase in the activity of which was associated with a significant decrease in the protective effect of the Lys allele ($\chi^{2 = 4.4; P = 0.05)}$ and the Lys / Lys genotype (χ^2 = 3.7; P = 0.1).

With regard to the role of the genetic polymorphism EDN 1 (Lys 198 Asn) in increasing the chance of developing various forms of CT, a pronounced tendency was revealed towards an increase in the risk of developing a simple form of CT (χ^2 = 3.2; P = 0.1) and CT with TAF I (χ^2 = 3.4; P = 0.1) among carriers of the minor allele Asn by 1.8 and 1.9 times, respectively.

Meanwhile, the associative link between the genetic polymorphism EDN 1 (Lys 198 Asn) and the development of CT TAF II has not been established.

In connection with the presented results, it can be concluded that the genetic polymorphism EDN 1 (Lys 198 Asn) is a genetic marker involved in increasing the risk of developing HT, namely its forms such as simple and toxic-allergic form I. (TAF I).

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