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GENETIC PREDICTORS FOR THE FORMATION OF CHRONIC PURULENT OTITIS MEDIA

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Abstract:
84 patients with chronic suppurative otitis media (41 patients with mesotympanitis, 43 with epitympanitis) were examined. To verify the diagnosis, all subjects underwent standard general clinical, radiological, audiometric studies, molecular genetic analysis of DNA by PCR and immunological examination by ELISA. The obtained data were statistically processed using the STATISTICA 6.0 software package (StatSoftInc., USA). The critical level of significance when testing statistical hypotheses was taken to be less than 0.05. A statistically significant predominance of the homozygous C/C genotype and the heterozygous -T/C genotype for the high-producing allele C* of the IL-1β gene, respectively, in polymorphic loci 31 and 511 and homozygous genotypes A/A and C/C for low-producing alleles A* and C, respectively, was established. * of the IL-10 gene, respectively, in polymorphic loci -1082 and -819 in the group of patients with a carious-destructive course of chronic suppurative otitis media. High levels of IL-1β ($p<0.05$) were registered against the background of low levels of IL-10 ($p<0.05$) in blood serum in individuals with epitympanitis with high-producing variants of pro-inflammatory cytokine genes and low-producing alleles of anti-inflammatory cytokines. Lymphocyte-platelet depression was found in all patients with chronic suppurative otitis media.
Keywords: interleukins, single nucleotide polymorphism, chronic suppurative otitis media, lymphocytic-platelet adhesion.

Relevance: CHSO is usually a complication of persistent acute otitis media, but the risk factors for CHSO vary across settings. Frequent upper respiratory tract infections and poor socioeconomic conditions are often associated with the development of CHSO. Chronic inflammation of the middle ear, despite significant progress in prevention, diagnosis and treatment, remains one of the most common and dangerous childhood diseases. This is due to many medical and social reasons, as well as such adverse effects as hearing loss and the risk of intracranial complications caused by exacerbations of a chronic process in the middle ear.

At the same time, chronic otitis media with cholesteatoma is detected in 24-63% of patients, regardless of the location of the perforation of the tympanic membrane. Bone resorption that accompanies such cases is found in 78.8% or more of cases, which is the cause of otogenic complications [2]. In this regard, CHSO is not only a medical, but also a socio-economic problem, since more than half of all patients are people of working age.

In the pathogenesis of CHSO, both local and general defense mechanisms are involved in the immune response. The level of immune reactions, especially the mucous membrane of the middle ear, is directly related to the problem of the

formation of chronic inflammation and, to a certain extent, to the organization of the nature of the inflammatory response. The main role in ensuring intercellular cooperation in the implementation of positive and negative immunoregulation is assigned to cytokines. The functioning of the cytokine network depends on individual differences in the production of expressed interleukins due to the genetic characteristics of the individual [3–5]. In connection with the foregoing, a comprehensive study of the pathogenetic patterns of the course of CHSO, as well as the search for genetic and immunological predictors of the severity of the disease, seems relevant..

Purpose of the study. To study the frequency of occurrence of genetic polymorphism IL-1β (C3953T, T511C, T31C), IL-10 (G1082A, C592A, C819T) and its association with the concentration of IL-1β, IL-10 in blood serum in patients with chronic suppurative otitis media.

Materials and methods: We examined 84 patients with chronic suppurative otitis media who were hospitalized in the ENT department of the TMA clinic, Urgench branch. Division by gender: men 58 (69%), women 26 (30.9%), division by localization of the inflammatory process: epitympanitis - 9 (10.7%), mesotympanitis - 53 (63%), epimesotympanitis - 22 (26 .1%) of patients. The duration of the disease ranged from 4 to 11 years. All subjects were divided into 2 groups. The first group consisted of 41 patients suffering from the tubotympanic form of chronic suppurative otitis media. The second group was represented by 43 patients with epitympano-antral form of chronic otitis media, in which complaints, anamnestic data, otomicroscopy, radiographs and further surgical treatment confirmed carious-destructive processes in the middle ear.

All subjects underwent standard general clinical, radiological, audiometric studies. Genomic DNA of 84 patients was subjected to molecular genetic analysis, isolated from whole blood leukocytes using the "DNA Express-Blood" reagent, followed by amplification (DT-9I6, Maxygene, Germany) and real-time detection of products. The results of the analysis made it possible to draw three types of conclusions: homozygous genotype for the "wild" allele, heterozygous genotype for the mutant allele, homozygous genotype for the mutant allele.

For the study, when testing the DNA of patients, those cytokines that are functionally significant for the development of destructive processes in the middle ear were included. Of particular interest were the following gene polymorphisms of pro- and anti-inflammatory cytokines IL-1β (C3953T, T511C, T31C), IL-10 (G1082A, C592A, C819T). The calculation of the total number of leukocytes was carried out by the standard method in the Goryaev chamber. Subpopulations of lymphocytes were determined by immunohistochemistry using monoclonal antibodies of MedBiospektr LLP (Moscow). Determination of the indicator of lymphocytic-platelet adhesion, related to functional tests for assessing immunocompetent cells, was carried out according to the method proposed by Yu.A. Witkovsky et al. [6].

To determine the concentration of cytokines (IL-1b, IL-6, IL-10, TNFa), reagent kits of OOO Vector-Best were used.

Statistical processing of the obtained data was carried out using electronic programs Microsoft Excel 2012, STATISTICA 6.0 (StatSoftInc., USA), with the

determination of the statistical significance of differences at p<0.05.). To assess associations of polymorphic gene variants with a pathological phenotype, the odds ratio (OR) was calculated. The value OR=1 indicated the absence of an association, OR>1 - is observed with a positive association of the "risk factor" and OR<1 - a negative association of the allele, genotype with the disease. The discussion of OR values was carried out at a significance level of no more than 5%. An assessment of the relationship of qualitative features was calculated on the principle of mutual contingency using the Yule coefficient (Q-coefficient) and the contingency coefficient (Phi, F).

Results: A molecular genetic study revealed all the desired mutations in the homo- and heterozygous state of polymorphic DNA loci of the genes of the following cytokines IL-1β (C3953T, T511C, T31C) and IL-10 (G1082A, C592A, C819T) in sick and healthy people. Deviation from the Hardy-Weinberg equilibrium due to the difference between the observed and expected heterozygous genotype or homozygous genotype for the mutant allele was detected only for IL-1β (T31C) and IL-1β (T511C) (Table 1).

Table 1

Hardy-Weinberg test for studied genetic polymorphisms in chronic suppurative otitis media (χ2, df=1)

Indicators	**Genotypes**	**Observed Frequencies**	**HWE**	**χ2**	**p**
IL-1β (T31C)	T/T T/C C/C	0,217 0,431 0,351	0,188 0,491 0,321	4,17	0,04
IL-1β (T511C)	T/T T/C C/C	0,188 0,491 0,321	0,323 0,491 0,186	9,9	0,001

Note: polymorphisms with a frequency deviation from the Hardy-Weinberg equilibrium are indicated.

An analysis of the promoter regions of the cytokine genes that have a regulatory effect on the functions of the immune system showed that there are genetically determined prerequisites in the development of CHSO. Residents of the Khorezm region suffering from chronic inflammation of the middle ear are characterized by the presence of homozygous C/C genotype of the IL-1β gene at position 3953 (Q=0.6), the presence of the homozygous C/C genotype of the IL-1β gene at position 31 (Q= 0.6), homozygous A/A genotype of the IL-10 gene at position 1082 (Q=0.7; F=0.5) and homozygous T/T genotype of the IL-10 gene at position 819 (Q=0.5) . At the same time, the risks of developing a chronic inflammatory process in the middle ear will be significantly lower with the existing C / C genotype of the IL-1β (T511C) gene (OR = 0.24; 95% CI (0.15 - 0.37)), T genotype /T of the IL-1β gene (T31C) (OR = 0.29; 95% CI (0.19–0.43)) and the G/G

genotype of the IL-10 gene (G1082A) (OR = 0.27; 95% CI (0.18 -0.41)). A review of the current literature indicates that personal differences in the production of inflammatory mediators, which underlie the activity of the inflammatory process, are determined by gene polymorphism [7]. The presence of a positive relationship between the state of a certain genetic polymorphism and the manifestation of pathology allows us to speak about the relationship of this variant with a specific pathology [8].

Functional polymorphic variants of genes encoding IL-1β and IL-10 proteins can affect not only the predisposition to the development of the disease, but also the nature of its course. So, for persons with a carious-destructive form of CHSO, the carriage of the homozygous C/C genotype of the IL-1β gene in the polymorphic locus 3953 (OR = 3.264; 95% CI (2.059 - 5.174); p<0.01), homozygous genotype C/ From the IL-1β gene at polymorphic locus 31 (OR = 7.822; 95% CI (4.654 – 13.148)). In addition, the presence of the heterozygous T/C genotype of the IL-1β gene at position 511 is also significant for the formation of an aggressive pathological process in the middle ear (OR = 6.7; 95% CI (4.095–10.972)). At the same time, the available homozygous genotypes for low-producing alleles A* and C* of the IL-10 gene at positions 1082 (OR = 8.254; 95% CI (4.906 –13.886); p<0.01) and 819 (OR = 3.136; 95% CI (1.708 - 5.756); p<0.01), respectively, also contribute to the aggravation of the destructive process in the middle ear.

At the same time, the identified carriage of the homozygous C/C genotype of the IL-1β gene in position 3953 (OR = 3.146; 95% CI (1.975 - 5.012); p<0.01), the homozygous C/C genotype and the heterozygous T/ genotype C by the high-producing allele C* of the IL-1β gene, respectively, in polymorphic loci 511 and 31 (OR = 1.913; 95% CI (1.181–3.099); p<0.01), (OR = 2.209; 95%, p<0, 01), as well as an increase in the frequency of occurrence of heterozygous genotypes G/A and C/T of the IL-10 gene, respectively, in polymorphic loci -1082 and -819 ((OR = 3.271; 95% CI: (2.074 - 5.157); p<0, 01) and (OR = 1.632; 95%, p<0.01), respectively) determined the development of chronic inflammation in the middle ear of the mucosal type, clinically manifested by symptoms of the tubotympanic form of chronic suppurative otitis media.

The main role in determining the variants of the inflammatory response and the development of specific immunological reactions during the introduction of pathogens in CHSO, due to the initial stages of the development of the inflammatory reaction, is assigned to cytokines. Quantitative changes in the production of inflammatory mediators are associated with the replacement of a single chemical unit in DNA nucleotide sequences, the so-called single nucleotide polymorphism. The level of expressed cytokines affects the functional activity of cells involved in the reactions of innate and acquired immunity during the implementation of the inflammatory response [7]. the development of the phenomenon of leukocyte depression, which often occurs with a sluggish disease. This was confirmed by the extremely low value of the lymphocytic-platelet index in patients with mesotympanitis relative to healthy individuals. In cholesteatoma-destructive processes in the middle ear, the lymphocytic-platelet index exceeded the control values. Activated lymphocytes strongly adhere to platelets and, due to the retraction

of the latter, move further through the damaged wall deep into the injured area, where an immune response develops [15-17]. Consequently, low LTA values in patients with epitympano-antral form of CHSO were the result of increased migration of lymphocytes from the vascular bed to the lesion.

The regression of inflammatory reactions is associated with an increase in the pathological focus and in the blood serum of anti-inflammatory cytokines and IL-10 produced by Th2. An increase in the level of and IL-10, according to the literature, is accompanied by a decrease in LTA and, consequently, the cessation of migration of immunocytes to the site of damage [6, 18]. The effect of IL-10 makes it possible to regulate, and in the end, to break the migration flow of lymphocytes at the site of the development of the inflammatory reaction. At the same time, IL-10, by slowing down blood clotting and stimulating fibrinolysis, eliminates tissue hypercoagulation and ischemia [19-21]. However, it was in individuals with a severe destructive course of chronic otitis media that the level of the expressed cytokine in the blood serum was found to be at a low concentration.

Therefore, the general immunopathogenetic link of CHSO is the axis: predictor genotypes of IL-1β, IL-10 genes → associated level of cytokines of the same name → lymphocytic-platelet adhesion. The concentration of encoded activating (IL-1β) or inhibitory (IL-10) cytokines, which predetermine the contact interactions of lymphocytes and platelets, depends on the polymorphism of cytokine genes. In summary, the presented facts significantly affect the immune response in middle ear pathology.

Conclusion. The obtained data suggest that the common immunopathogenetic link of chronic suppurative otitis media is the axis: genes IL-1β, IL-10 - corresponding cytokines - lymphocytic-platelet adhesion. Carriage of the -31CC and -511TC genotypes of the IL-1β gene promoter increases the likelihood of developing a destructive form of the disease.

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