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EXPRESSION PROFILE AND TNF- α GENE POLYMORPHISM IN PRIMARY OPEN-ANGLE GLAUCOMA

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ПРОФИЛЬ ЭКСПРЕССИИ И ГЕННЫЙ ПОЛИМОРФИЗМ ФНО- α ПРИ ПЕРВИЧНОЙ ОТКРЫТОУГОЛЬНОЙ ГЛАУКОМЕ

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The TNF- α 308 G/A (rs1800629) gene polymorphism was studied in 56 patients with primary open-angle glaucoma (POAG), and TNF- α levels were determined in 103 lacrimal fluid samples. An increase in TNF- α – 94 [45; 165] pg/ml in patients with glaucoma compared with the control group ($p=0.001$) was revealed. The probability of developing POAG increased in residents of the homozygous for the rare allele genotype 308 A/A – 6.30 (95 % CI: 0.77–51.9, $p=0.049$), as well as in the owners of the heterozygous genotype 308 G/A – 3.60 (95 % CI: 0.96–13.6, $p=0.049$). High levels of TNF- α in the lacrimal fluid in patients with POAG are associated with the A308A genotype – 190 [153.0–220.0] pg/ml.

Keywords: primary open-angle glaucoma, lacrimal fluid, TNF- α , gene polymorphism

Проведено исследование генного полиморфизма ФНО- α 308 G/A (rs1800629) у 56 пациентов с первичной открытоугольной глаукомой и определение уровня ФНО- α в 103 образцах слезной жидкости. Выявлено увеличение содержания ФНО- α – 94 [45; 165] пкг/мл у больных с глаукомой по сравнению с группой сравнения ($p=0,001$). Вероятность развития первичной открытоугольной глаукомы возрастала у резидентов гомозиготного по редкому аллелю генотипа 308 A/A – 6,30 (95 % CI: 0,77–51,9, $p=0,049$), а также у обладателей гетерозиготного генотипа 308 G/A – 3,60 (95 % CI: 0,96–13,6, $p=0,049$). Высокие показатели ФНО- α в слезной жидкости у пациентов с первичной открытоугольной глаукомой ассоциированы с генотипом A308A – 190 [153,0–220,0] пкг/мл.

Ключевые слова: первичная открытоугольная глаукома, слезная жидкость, ФНО- α , генный полиморфизм

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CI – confidence interval
FasL – transmembrane protein expressed on cytotoxic T-lymphocytes and NK-cells
IOP – intraocular pressure
NMDA – N-methyl-D-aspartate
OR – odds analysis
POAG – primary open-angle glaucoma

RFLP analysis – restriction fragment length polymorphism analysis
RGC – retinal ganglion cell
RNFL – retinal nerve fiber layer
SNP – single nucleotide substitutions
TNF- α – tumor necrosis factor-alpha
TNFR 1, 2 – tumor necrosis factor-alpha receptor type 1 and 2

Primarily open-angle glaucoma (POAG) is a progressive disease based on damage to retinal ganglion cells (RGCs), leading to loss of peripheral vision and blindness [1, 2]. Tumor necrosis factor-alpha (TNF- α) is known to be the dominant mediator of pro-inflammatory reactions in the glaucomatous retina and optic nerve [2]. Ischemic glial cells, under conditions of increased intraocular pressure, increase the expression of TNF- α , which leads to the death of oligodendrocytes, ganglion cell apoptosis, and axonal degeneration [2]. Within the gene diagnostics [3], it has been shown that deterministic differences in the synthesis of TNF- α can affect the predisposition to the development of primary open-angle glaucoma [4]. However, the pathogenetic role of TNF- α gene polymorphism for the development of the disease and monitoring of therapy in various ethnic groups has not been fully established.

The study aimed to determine the pathogenetic significance of TNF- α gene polymorphism in POAG.

Material and Methods. We examined 56 patients with POAG of Russian nationality, including those with advanced stage II (28), advanced stage III (16), and terminal stage IV (12) aged 50 to 75 years; with IOP levels of more than 21 mm Hg; the degree of opening of the angle of the anterior chamber – 3–4; transparent optical media of the eye; indicators of RNFL more than 60 μ m and less than 85 μ m. All patients have signed free and informed consent to be included in the study. The study excluded patients with inflammatory or allergic diseases of the anterior segment of the eye, clouding of the optical environment, age-related macular degeneration, diabetic retinopathy, systemic autoimmune and oncological diseases, and eye damage. The comparison group consisted of 30 patients matched in age and sex with mild myopia or initial signs of lens opacity, with an IOP level of more than 21 mm Hg, transparent optical media, indicators of RNFL > 90 μ m, without oncological and autoimmune diseases. Topical prostaglandin preparations were not used in patients with POAG and the comparison group for two weeks before tear fluid sampling.

Genotyping of SNP – TNF- α 308 G/A (rs1800629) was performed by RFLP analysis (Restriction Fragment Length Polymorphism), using venous blood of 56 patients, using a multichannel amplifier and diagnostic test systems «SNP-express» (Litekh, Russia). The amplification products were separated by horizontal electrophoresis in 3 % agarose gel with electrophoretic detection (BioRadLaboratories, USA). The TNF- α in tear fluid was determined by enzyme-linked immunosorbent assay using Vector-Best test systems. Tear fluid samples from patients with POAG (advanced stage II – 56, advanced stage III – 30, terminal stage IV – 21). Lacrimal fluid 100 μ l was collected from the lower conjunctival fornix using a sterile micropipette and transferred into Eppendorf tubes. Samples were centrifuged at 10,000 g and stored at – 20 °C until testing.

To determine intergroup differences in quantitative traits, the Mann – Whitney and Pearson χ^2 tests were used. The degree of risk of development of events was assessed by the value of the odds ratio (OR) with the calculation of the confidence interval CI. The Attestat 10.5.1 software package (Analytera, Russia) was used for the statistical analysis of the data. Differences were considered statistically significant at $p<0.05$.

Results and Discussion. TNF- α was detected in 95.4 % of lacrimal fluid samples of patients with glaucoma and only in 85.7 % in the comparison group ($p=0.01$). A statistically significant increase in the content of TNF- α was established – 94 [45; 165] пкг/мл in patients with glaucoma in relation to the comparison group – 32 [14; 66] пкг/мл ($p=0.001$).

When determining allelic and genotypic variants of TNF- α G308A (rs1800629) in patients with POAG, the recessive allele 308A (32.1 % and 8.3 %, $p<0.001$) was expected with an increase in the risk of developing the disease by more than five times. The odds ratio was 5.21 (95 % CI: 1.92–14.1, $p=0.001$). A high risk of developing glaucoma was determined in respondents homozygous for the rare allele of the 308 A/A genotype – 6.30 (95 % CI: 0.77–51.9, $p=0.049$), as well as in the owners of the heterozygous genotype 308 G/A – 3.60 (95 % CI: 0.96–13.6, $p=0.049$) and in the combined group of patients carrying the mutant allele 308 A/A+308 G/A –

5.85 (95 % CI: 1.81–18.9, $p=0.003$). Homozygous for the dominant allele genotype 308G/G had protective properties; the prevalence in POAG was lower than in the comparison group (53.5 % and 86.7 %, respectively, $p=0.003$).

TNF- α is known to be synthesized by microglial cells activated by macrophages, astrocytes, and Mueller retinal cells under increased intraocular pressure and ischemia, resulting in apoptosis of retinal ganglion cells [2]. The decrease in the survival rate of RGC is due to an increase in the expression of membrane FasL on retinal microglial cells and tissue-infiltrating macrophages, the production of nitric oxide and NMDA in Muller cells, as well as an increase in mitochondrial dysfunction under the influence of TNF- α [1].

At the same time, it has been established that TNF- α regulates complex interactions between proapoptotic and regenerative mechanisms mediated by two receptor variants, TNFR1 and TNFR2, which makes it possible to shift the balance of endogenous TNF- α activity towards a neuroprotective response in the retinal tissue [2]. Our results show an increase in TNF- α in tear fluid consistent with previous studies [5, 6].

In the carriers of the 308A allele and the AA+GA genotypes, an increase in the level of TNF- α in the lacrimal fluid up to 165 [112.5–193.5] pg/ml was revealed compared with the GG genotype common in the population – 49 [14.0–90.0] pg/ml, $p=0.001$. The highest levels of TNF- α were detected in patients with the A308A genotype and amounted to 190 [153.0–220.0] pg/ml. Statistically significant differences were established in comparison with the owners of G308A genotypes – 132 [98.0–180.0] pg/ml, $p=0.01$ and G308G – 49 [14.0–90.0] pg/ml, $p=0.001$. The results are confirmed by the data that SNP 308G/A promotes an increase in TNF- α and the progression of the glaucomatous process [4].

Conclusion. Thus, TNF- α in the lacrimal fluid of patients with POAG is determined more frequently and in higher concentrations than in the comparison group. In patients with POAG, the 308A mutant allele and the G308A and A308A genotypes predominate. SNP 308G/A polymorphism increases TNF- α in the lacrimal fluid and increases the risk of developing POAG.

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