Investigation of the effect of TNF-α on damage to retinal pigment epithelial cells in age-related macular degeneration

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Oxidative stress alters cellular homeostasis and elicits a cellular response that depends on the severity and type of damage: some cells activate defense mechanisms designed to ensure survival; the other, provided that the defense mechanisms are inhibited, triggers alternative signaling pathways that lead to apoptosis, necrosis, pyroptosis, autophagy, and so on. However, the exact cause of such damage and induction of oxidative stress, including the associated oxidative effects around pigment epithelial cells in the context of the onset and progression of age-related macular degeneration - one of the world's most common eye diseases with blindness, remains unclear. Therefore, in the course of the study we turned to key biogenetic points of regulation of inflammation and apoptosis, in particular TNF. The aim of the work is to shed light on the role of TNF as a genetic determinant that can initiate and influence the course of age-related macular degeneration. For this purpose, the main pathognomonic markers of the morphological structure of the macula were determined in 291 persons with age-related macular degeneration and in 105 persons without ophthalmic pathology, using optical coherence tomography to confirm or exclude the diagnosis of the disease. To detect polymorphism of the TNF gene, we used the method of real-time PCR diagnostics on the BioRad CFX 96 amplifier using LiTech reagents. Statistical processing of the results was performed using Hardy-Weinberg equilibrium, Kruskal-Wallis method, logistic regression analysis and construction of the ROC curve to determine the AUC range and sensitivity and specificity values. The study revealed a significant difference in the distribution of mutant genotypes between patients with both forms of AMD and the control group. There was also a statistically significant effect of mutant allele A on the development of both "dry" (OR = 3.40; 95.0 %; CI = 1.90-6.07, p<0.001) and "wet" form of AMD (OR = 4.78; 95.0 % CI 2.65-8.64, p<0.001), and in the analysis of mutant genotypes it was found that the GA genotype increases the chances of "dry" and "wet" forms of the disease by 3.13 and 4.74 times, respectively, while AA - 5 times, regardless of the form of the disease. confirms the influence of TNF gene polymorphism on the occurrence and progression of age-related macular degeneration. In the analysis of ROC-curves and AUC regions, it was found that all mutant genotypes have a significant effect on the occurrence of age-related macular degeneration (p<0.05). However, the obtained values of sensitivity and specificity, especially in the AA genotype in both "dry" (17.9 % and 95.8 %, respectively) and "wet" (18.2 % and 95.8 %, respectively) forms of age-related macular degeneration indicate a low chance of error-free confirmation of the diagnosis. a disease that may be associated with multifactorial disease and requires further research.

Keywords: age-related macular degeneration, TNF-α, ROS, NOS, gene.

Introduction

Age-related macular degeneration (AMD) is a common irreversible eye disease characterized by visual impairment in the elderly and is a major cause of blindness/vision loss in developed countries [3, 10]. The vast majority of modern classifiers agree with the division of AMD nosological units into "dry" form and "wet" form [4]. According to the European Consortium of Epidemiology of Eye Diseases, there is an increase in morbidity in the European population. Mathematical models indicate that if this rate of eye pathology persists, by 2040 the number of
people in Europe with early-onset age-related macular degeneration will range from 14.9 to 21.5 million, and with late-onset from 3.9 to 4.8 million [3]. The World Health Organization’s World Report on Vision (2019) highlights the urgency of the problem - at least 2.2 billion people worldwide have impaired vision, where AMD ranks third in morbidity with 196 million people suffering from various forms of it, while yielding only to biopsy and presbyopia [21].

Numerous factors determine the risk of developing age-related macular degeneration, including both genetic and environmental factors. Oxidative stress and aging have a powerful effect, which resonate in terms of the pathogenesis of age-related macular degeneration [5]. Retinal pigment epithelial cells are the main target of pathological changes in age-related macular degeneration - they involve phagocytosis of photoreceptor outer segments (POS) and removal of cellular waste through physiological conditions. Age-related cumulative oxidative stimuli mediated by reactive oxygen species (ROS) and reactive nitrogen species (RNS) cause degeneration of pigment epithelial cells and incomplete POS lysis, leading to accumulation of cellular wastes [24]. The ubiquitin proteasome and the lysosomal/autophagic pathway are the two main proteolytic systems for removing damaged proteins and organelles, and their disruption due to deregulation of signaling pathways leads to the accumulation of lethal mutations or trigger apoptosis [11]. Cytokine tumor necrosis factor α (TNF-α) is one of the main regulators of the immune system that can affect apoptosis, cell proliferation and differentiation, local and systemic inflammation, immune response through its binding site - TNF receptor 1 type (TNFR1). It is known that TNFR1 can cause oxidative stress directly by activating enzymes that produce ROS and active forms of nitrogen RNS, involving the fundamental signaling pathways NFE2L2, PGC-1, p62, AMPK & PI3K/Akt/mTOR [8, 19, 24]. In addition, the activity of mTNF-α to reactogenic formulation is regulated by the matrix metalloproteinases MMP and the associated ADAM17 complex - metalloproteinases of this family themselves can cause remodeling of the extracellular matrix, particularly in the Bruch membrane [10]. Ways of regulation are really branched, but regularly return to the fight against pathophysiological oxidants [9]. In turn, the TNF-R1 type 1 receptor activates a complex caspase cascade in the inflammatory cluster of signaling pathways, resulting in changes in NF-κB and p53 activity, thus retaining the ability to modulate proapoptotic effects at the systemic level via TNF-α. On the other hand, the TNF-R2 type 2 receptor simultaneously interacts with NF-κB and ROS-sensitive AP-1, indicating a significant role of oxidative stress in the development of deregulation of apoptosis, endothelial cell degeneration, where TNF-R2 is most expressed. Similarly, the heterodimeric redox transcription factor AP-1 is activated, which responds to the presence of reactive oxygen species around [14]. We see this as age-related macular degeneration, as TNF-α attracts the most likely triggers of pathology - cell cycle disruption, extracellular matrix rearrangement, and oxidative stress of cells in photosensitive cells under the influence of aging [15].

Therefore, the aim of the study was to shed light on the role of TNF as a genetic determinant capable of initiating and influencing the course of age-related macular degeneration.

Materials and methods

The study group included 291 middle-aged and elderly people with AMD (89 - with "dry" form, 97 - with "wet" form), the control group - 105 people without ophthalmic pathology in the anamnesis of the relevant age range.

The work adhered to the ethical principles of the Declaration of Helsinki of the World Medical Association (World Medical Association Declaration of Helsinki, 1964) [22]. Each subject was provided with all the details of medical procedures, given the opportunity to discuss any issues with health professionals, and then signed a detailed form of informed consent for the study. The scientific work was approved by the Commission on Biomedical Ethics of VMNU of the Ministry of Health of Ukraine in accordance with Protocol №6 of 17.09.20.

Optical coherence tomography of the macular area of the retina was used to determine the presence or absence of pathognomonic markers of the morphological structure of the macula that was characteristic of AMD. It was performed using a SOCT Copernicus optical coherence tomograph "Optopol" with the possibility of angiography (Poland) in 3D-Scan mode (sequential scanning of the entire retinal segment). To study the anatomical and topographic relationship between the layers and the thickness of the retina in the macular area and the analysis of the thickness of the macula in different departments, the parameter - ILM-RPE (internal limiting membrane-retinal pigmented epithelium; internal boundary membrane-retinal pigment epithelium) was used. When drusen were found, the AMD form was verified as "dry," while cystic edema of the neuroepithelium, transudative detachment of the pigment epithelium, chorioretinal vascular proliferation, and subtrenal fibrosis were verified, AMD form was verified as "wet."

DNA was isolated from the buccal scraper using the Chelex® 100 kit from Bio-Rad according to the manufacturer’s instructions. To detect the rs1800629 polymorphism of the TNF gene, real-time polymerase chain reaction (PCR) was used employing a set of reagents according to the manufacturer’s instructions (Litech, Russia). Amplification was performed on a Bio-Rad CFX96 thermal cycler (BioRad, USA).

Statistical processing of the results was performed using Statistica 10 (StatSoft, Inc., USA) and SPSS 23.0. To determine the frequencies of distribution of genotypes and alleles in the studied groups was determined using Hardy-Weinberg equilibrium. The Kruskal-Wallis ANOVA by ranks
and Friedman ANOVA and Kendall Coeff criteria were used to compare categorical variables (genotypes) and to determine a significant difference in the distribution of genotypes between groups. In order to compare the distribution of values of qualitative features used H-criterion. The degree of association of mutant genotypes with the development of AMD was determined by logistic regression using odds ratios (OR) and 95 % confidence interval (±95 % CI; Confidence limit for means Interval - CI). To assess the adequacy of logistics models and the predictive ability of the diagnostic test used the method of analysis of curves of operational characteristics (ROC - Receiver Operating Characteristic curve analysis). For this purpose, the area under the ROC curve (AUC - Area under the ROC curve) was calculated. The model was considered adequate with a statistically significant difference in AUC from 0.5. Special formulas using 2x2 tables were also used to calculate the specificity and sensitivity of mutant genotypes.

**Results**

In the study, we analyzed the genotype distribution of the rs1800629 polymorphism of the TNF gene in both patients with "dry" and "wet" forms of age-related macular degeneration, as well as in patients of the control group. In particular, the influence of mutant genotypes and alleles on the development of the disease was determined.

After genetic testing as a result of statistical calculations by the Kruskal-Wallis method, high statistical significance of differences in the distribution of genotypes of rs1800629 polymorphism of the TNF gene in patients with "dry" and "wet" forms of age-related macular degeneration and control group (H = 29.57) at p<0.001, which was also found when comparing the genotype distribution of patients in the control group with "dry" form of AMD (H = 16.28) and "wet" (H = 26.42), respectively, at p<0.001 (Table 1).

By evaluating the data, it was found that the wild-type (GG) polymorphism rs1800629 of the TNF gene predominated among the control group (23.4 %), while in patients with "dry" (11.0 %) and "wet" (9.3 %) forms it was not widely represented. Mutant genotypes were mostly observed in patients with AMD, which previously indicates a possible association between pathology and SNP. Thus, the heterozygous variant was most important among people with "wet" (22.0 %) and "dry" (17.2 %) forms of the disease, while in the control group the subjects were distributed 1.9 and 1.5 times less than patients. Regarding the mutant AA genotype, the highest number of people was among the "dry" form of age-related macular degeneration (2.4 %), followed by the "wet" form (2.1 %), and the lowest - the control group (1.0 %).

The outlined results, together with the lack of statistical significance in the difference in the distribution of genotypes of patients between "dry" and "wet" forms (H = 0.77, p>0.05), may indicate that polymorphism rs1800629 TNF gene affects both disease development and its further progression.

The frequency of distribution of genotypes and alleles of the rs1800629 polymorphism of the TNF gene among patients with "dry" and "wet" forms of AMD and the control group was studied using Hardy-Weinberg analysis (Fig. 1).

As can be seen from the results of the analysis in Figure 1, the genotype distribution of the rs1800629 polymorphism of the TNF gene had significant differences between patients with AMD and the control group. The frequency of homozygous genotype for the main allele in the control group (GG) was 1.59 times lower compared to the "dry" form (0.410) and 1.76 times lower compared to the "wet" form (0.370) compared to the control group (0.654). In contrast to the results of the wild-type distribution studied with the heterozygous variant, it was the lowest among the control group (0.309), which was 1.49 times less than patients with "dry" form (0.461) and 1.5 times less than patients with "wet" form (0.477). The mutant genotype of the rs1800629 polymorphism of the TNF gene was represented by a small number of subjects (see Table 1), so the frequency of distribution changed insignificantly: it was greatest in the case of "wet" form of AMD (0.154), which was 4.3 times more than the control group (0.036), and in the "dry" form was 0.130. When estimating the frequency of allele distribution (Fig. 1) it was found that the mutant allele A was most observed in the "wet" form of AMD (0.39), in the "dry" - slightly less (0.360), while in the control group found 2 times and 1.88 times less, respectively (0.190).

Regarding the wild-type allele (G), it was most common among the control group (0.810), while no significant difference was observed between patients - 0.640 in the

| Table 1. Distribution of rs1800629 TNF gene polymorphism genotypes in patients with "dry" and "wet" forms of age-related macular degeneration and control group. |
|---|---|---|---|---|
| **"Dry" form** | **GA (n, %)** | **AA (n, %)** | **H-criterion, p** |
| GG (n, %) | 32 (11.0 %) | 50 (17.2 %) | 7 (2.4 %) | 0.77* |
| "Wet" form | 27 (9.3 %) | 64 (22.0 %) | 6 (2.1 %) | 16.28** |
| Control group | 68 (23.4 %) | 34 (11.7 %) | 3 (1.0 %) | - |
| Total (100 %) | 127 (43.6 %) | 148 (50.9 %) | 16 (5.5 %) | 29.57^ |

**Notes:** * - the level of significance of the H-criterion in the comparison of the "dry" form with the "wet" form, p>0.05. ** - the level of significance of the H-criterion compared to the control, p<0.001. ^ - the level of significance of the H-criterion in comparison with the studied groups, p<0.001.
case of "dry" and 0.610 - "wet". As a result, it can be argued that mutant variants of the rs1800629 polymorphism of the TNF gene are associated with the development and progression of age-related macular degeneration, namely the AA genotype and the A allele may be associated with the "wet" form.

To determine the degree of association of genotypes of the rs1800629 polymorphism of the TNF gene with the development of "dry" and "wet" forms of AMD, a logistic regression method was performed, the results of which are presented in Table 2.

Logistic regression analysis revealed a direct statistically significant association between the presence of a heterozygous variant of the rs1800629 polymorphism of the TNF gene and the occurrence of age-related macular degeneration regardless of form at p<0.001. A high level of statistical significance was also found in the case of association of allele A with age-related macular degeneration of both "dry" and "wet" forms (p<0.001). A statistically significant association was found in the study of the correlation between mutant genotypes and disease with both forms at p<0.05 (see Table 2).

Table 2 shows that allele A increases the risk of "dry" form of AMD by 3.4 times (OR = 3.40; 95 % CI 1.90-6.07), and in the case of "wet" form the risk is even higher - 4.8 times (OR = 4.78; 95.0 % CI 2.65-8.64). When assessing the degree of association of mutant genotypes of the rs1800629 polymorphism of the TNF gene with the development of the disease, it was found that homozygous variants of the minor allele almost 5 times increase the risk of "dry" (OR = 4.96; 95.0 % CI 1.18-20.77) and "wet" (OR = 5.04; 95.0 % CI 1.15-21.98) forms of age-related macular degeneration. The chances of developing "wet" AMD in individuals with GA genotype are 4.7 times higher compared to controls (OR = 4.74; 95.0 % CI 2.57-8.76), and for the "dry" form are 3.1 times (OR = 3.13; 95.0 % CI 1.70-5.74).

Therefore, due to the obtained results, it is possible to assert a significant effect of the rs1800629 polymorphism of the TNF gene on the development of age-related macular degeneration. Mutant genotypes show high statistical significance in heterozygous variants, and genotype AA can be considered a risk factor for age-related macular degeneration, regardless of form. Allele A is particularly closely associated with the "wet" form, which may indicate its important role in the progression of the pathology.

To further determine the probability of developing age-related macular degeneration, the Receiver Operating Characteristic Curve Analysis (ROC) method was used to determine the area under the ROC curve (AUC).

The prognostic significance of the rs1800629 TNF gene polymorphism was estimated using Figure 2 and the calculation of the area under the ROC curve. Thus, the GA genotype shows high statistical significance for the development of age-related macular degeneration.

### Table 2. Results of logistic regression analysis of rs1800629 TNF gene polymorphism in patients with "dry" and "wet" forms of AMD.

<table>
<thead>
<tr>
<th>Form of nosology</th>
<th>Genotypes / allelic variants</th>
<th>N subjects with pathology</th>
<th>N subjects from the control group</th>
<th>OR</th>
<th>p</th>
<th>95 % CI</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Dry&quot; form</td>
<td>GG</td>
<td>32</td>
<td>68</td>
<td>3.125</td>
<td>&lt;0.001</td>
<td>1.699 - 5.74</td>
<td>14.144</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>50</td>
<td>34</td>
<td>4.958</td>
<td>&lt;0.05</td>
<td>1.18 - 20.77</td>
<td>5.4562</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>7</td>
<td>3</td>
<td>3.4</td>
<td>&lt;0.001</td>
<td>1.9 - 6.07</td>
<td>18.138</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>114</td>
<td>170</td>
<td>3.4</td>
<td>&lt;0.001</td>
<td>1.9 - 6.07</td>
<td>18.138</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>64</td>
<td>40</td>
<td>4.78</td>
<td>&lt;0.001</td>
<td>2.65 - 8.64</td>
<td>29.53</td>
</tr>
<tr>
<td>&quot;Wet&quot; form</td>
<td>GG</td>
<td>27</td>
<td>68</td>
<td>4.74</td>
<td>&lt;0.001</td>
<td>2.566 - 8.76</td>
<td>26.995</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>64</td>
<td>34</td>
<td>5.037</td>
<td>&lt;0.05</td>
<td>1.15 - 21.98</td>
<td>5.0976</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>6</td>
<td>3</td>
<td>4.78</td>
<td>&lt;0.001</td>
<td>2.65 - 8.64</td>
<td>29.53</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>118</td>
<td>170</td>
<td>5.037</td>
<td>&lt;0.05</td>
<td>1.15 - 21.98</td>
<td>5.0976</td>
</tr>
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<td></td>
<td>A</td>
<td>76</td>
<td>40</td>
<td>4.78</td>
<td>&lt;0.001</td>
<td>2.65 - 8.64</td>
<td>29.53</td>
</tr>
</tbody>
</table>

Notes: OR - odds ratio; 95 % CI - 95 % confidence interval; χ² - chi-square criterion; p - level of significance χ².
development of both "dry" (AUC = 0.761±0.044 at 95.0 % CI 0.67-0.85; p<0.001) and "wet" (AUC = 0.761±0.045 at 95.0 % CI 0.67-0.85; p<0.001) forms of AMD. In the study of homozygous genotypes for the minor allele, a reliable association was not established (p>0.05), which can be attributed to multifactorial pathology or the distribution of genotypes, which had a small number of representatives of the AA genotype among the control group and among patients (see table. 1).

Patients' results were presented in the form of categorical data, which did not allow to adequately assess the cut-off threshold formed by the ROC curve, so the specificity and sensitivity of the diagnostic test were analyzed using separate formulas using tables 2x2 (Table 3).

As can be seen from Table 3, in both forms of AMD the susceptibility of genotype AA, which according to logistic regression had the best prognostic value, but was inaccurate in estimating the ROC curve and AUC, was the worst and was 17.9 % in "dry" disease and 18.2 % - with "wet", while the specificity of this genotype for the minor allele was the highest in both forms of age-related macular degeneration and amounted to 95.8 %. Such ambiguous results of sensitivity and specificity can be attributed to the fact that age-related macular degeneration is a multifactorial disease, making it difficult to claim a single effect of this gene on the development and progression of pathology. Regarding the heterozygous variant of the rs1800629 polymorphism of the TNF gene, the sensitivity for it in the "dry" form was 61.0 %, and in the "wet" form - 70.3 %, while the specificity of its detection in both forms of the disease was 66.7 %.

Thus, the results of the statistical methods indicate the high significance of the prognostic influence of the rs1800629 polymorphism of the TNF gene on the development of both forms of AVG, especially "wet", in the case of mutant genotypes. Indicators of specificity and sensitivity of SNP genotypes indicate a complex predisposition to the development of pathology with the involvement of other genetic factors, which in the study of their combination can significantly increase the sensitivity and specificity of the diagnostic test.

**Discussion**

The results of our study indicate a high statistical significance of the prognostic effect of the rs1800629 polymorphism of the TNF gene on the development of both forms of age-related macular degeneration, especially "wet" (p<0.05). When determining the distribution of genotypes, it was found that mutant genotypes are widely represented among patients from the cohorts of "dry" and "wet" forms of AMD, compared with the control group: 17.2 % and 22.0 % in the case of GA gene variant, respectively, and 2.4 % and 2.1 % for genotype AA, respectively. A significant difference in the distribution of mutant allelic variants between the two forms of AMD and control was confirmed by the Kruskal-Wallis method.

In the frequency of distribution of genotypes and alleles by Hardy-Weinberg equilibrium, the mutant variant AA and

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**Table 3. Sensitivity and specificity of rs1800629 TNF gene polymorphism in the development of "dry" and "wet" AMD.**

<table>
<thead>
<tr>
<th></th>
<th>&quot;Dry&quot; form</th>
<th></th>
<th>&quot;Wet&quot; form</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GA</td>
<td>AA</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>- 61.0 %</td>
<td>17.9 %</td>
<td>- 70.3 %</td>
</tr>
<tr>
<td>Specificity</td>
<td>- 66.7 %</td>
<td>95.8 %</td>
<td>- 66.7 %</td>
</tr>
</tbody>
</table>
allele A prevailed among people with "wet" form of the disease. Thus, the AA genotype was 4.3 times more common than the control, while the A allele was 2.0 times more common than the control. According to the results of logistic regression, it was found that the chances of developing "dry" form of AMD in patients with GA genotype, compared with the control group, were 3.1 times higher, while in the presence of allelic variant AA - 5.0 times. The same OR values were recorded when assessing the prognostic effect of this genotype on the development of the "wet" form of AMD, which indicates the effect of this allelic variant on both the occurrence and progression of the pathology. The analysis of the ROC curve and the determination of the area under it revealed a high statistical significance of the GA genotype for the development of both "dry" and "wet" forms of AMD, while no significant association was found in the study of homozygous genotype for minor allele. The specificity of determining the mutant genotype in the case of "wet" form of AMD was 95.8 %. The best sensitivity index was determined for the heterozygous genotype in the case of the "wet" form - 70.3 %.

In our work we tried to shed light on the most important links in the development of age-related macular degeneration, which can be modulated by modern pharmacogenetic methods. Numerous studies indicate the role of TNF-α in the pathogenesis of proliferative diabetic retinopathy and the wet form of age-related macular degeneration - such conclusions lead to information about the increase in the concentration of protein necrosis factor-alpha in the aqueous fluid of the eye among patients with such pathologies [20]. The gene expressing this biological agent contains one of the most studied polymorphisms that is likely to affect the expression of TNF-α, located in the promoter region of the gene in front of the transcription initiator, at 308 nucleotides, and known as G-308A (rs1800629) [1]. All allelic variations of this polymorphism have different ability to pathogenic activation, in particular: the wild genotype GG has a complex effect, without changing the risk of associated pathological processes. Heterozygotes (AG) and homozygotes by mutant allele (GG) increase the risk of compatible nosologies by 2.0 and 2.5 times, respectively. Sometimes in sources the rs1800629 allele (A) is referred to as 308.2 or TNF2, and the more common allele (G) is referred to as 308.1 or TNF1 [7, 17].

Aging, inflammation, and dysregulation of the complement system affect the retinal pigment epithelium, and evidence has been found to involve tumor necrosis factor alpha and complement component 3 (C3) as one of the key factors in the development of age-related macular degeneration [6]. The primary effects of TNF are related to the cytoplasmic domain TNF-R1, which sequentially recruits death domains in a number of key signaling proteins: primarily the death domain associated with the TNF-α receptor (tumor necrosis factor receptor type 1-associated via death domain protein, TRADD); Fas associated death domain (FADD); a starter of caspase-8 apoptosis (also known as FADD-like ICE, Fas-associated death domain-like IL-1β-converting enzyme, or FLICE), causing degeneration of numerous proteins [14]. The effects of TNF-α are not limited to this, the protein exerts stimulatory and inhibitory effects on signaling pathways regulating apoptosis, proliferation and differentiation, immune recognition and inflammatory response, for example: interaction with antiapoptotic receptor DCR1 (decoy receptor 1, also known as TRAIL receptor 3 (TRAILR3)), the levels of which are significantly lower in patients with AMD and almost indistinguishable in patients with dry and wet forms [2]. Issues of cross-regulation with VEGF, another potential determinant of AMD, remain important. The results suggest that VEGF secretion under inflammatory conditions depends on cell polarization, and TNF-α-induced VEGF suppression can lead to choroidal atrophy in polarized physiological cells of the retinal pigment epithelium. TNF-α-induced upward regulation of VEGF can cause neovascularization, which is considered a component of late age-related macular degeneration [16]. This is confirmed by the fact of successful application of biological agents of targeted action to VEGF and TNF-α in the context of slowing the progression of age-related macular degeneration [18]. Another point of application is considered to be a family of antioxidant systems that protect the cell from oxidative stress - their regulation also involves tumor necrosis factor, triggering not only downward effects, but also receiving feedback on their own expression. It actively interacts through cellular and molecular markers of inflammation and oxidative stress (eg, IL-1β, TGF-α, ABCG1, ABCA1, reduced glutathione), and almost all of them alter the metabolism of reactive oxygen species in retinal pigment epithelial cells, limiting oxidative stress for physiological functioning [13]. Additional evidence of an association between TNF and ROS is the ability of caspase inhibitors and fat-soluble free radical scavengers (eg, tocopherol) to reduce AMD-induced cytotoxicity, particularly in the treatment of TNF blockers [12].

In a study that looked at the potential cytoprotective and anti-inflammatory effects of carbon monoxide-releasing molecules, they markedly inhibited TNF. Moreover, CORMs have been shown to exert their inhibitory effects by blocking nuclear translocation of nuclear factor κB/p65 and IκB degradation in retinal pigment epithelial cells under the influence of TNF-α - this also echoes the information above [23].

**Conclusions**

1. The results of our study indicate the high statistical significance of the prognostic effect of the rs1800629 polymorphism of the TNF gene on the development of both forms of age-related macular degeneration, especially the wet nosological form.

2. Mutant variant AA and allele A of the rs1800629 polymorphism prevailed among individuals with a wet form of the disease, which suggests the association of this allele.
with the development of pathology. Thus, the AA genotype was 4.3 times more common in people with age-related macular degeneration compared to the control, while the A allele was 2.0 times more common than the control. According to the results of logistic regression, it was found that the chances of developing dry AMD in patients with GA genotype, against the control group, were 3.1 times higher, while in the presence of allelic variant AA - 5.0 times, which is confirmed by literature data.

References


захисних механізмів, запускає альтернативні сигнальні шляхи, які призводять до апоптозу, некрозу, піроптозу, аутофагії тощо. Однак, точна причина такого пошкодження та індуції окисного стресу, включаючи пов'язані з цим окислювальні ефекти навколо клітин піементного епітелію в контексті виникнення та прогресування вікової макулярної дегенерації - одного з найпоширених у світі захворювань ока із наслідками у вигляді сліпоти, залишаються недостатньо зрозумілими. Тому в процесі дослідження ми звернулися до ключових біогенетичних точок регуляції запалення та апоптозу, зокрема TNF.

Мета роботи - освітлення ролі TNF як генетичної детермінанти, здатної ініціювати та впливати на перебіг вікової макулярної дегенерації. Для цього у 291 особи, які хворіли на вікову макулярну дегенерацію, та у 105 осіб без офтальмологічної патології були визначені основні патогномонічні маркери морфологічної структури макули, використовуючи оптичну когерентну томографію для підтвердження або виключення діагнозу захворювання. Для виявлення поліморфізму гена TNF використовували метод ПЛР-діагностики в режими реального часу на ампліфікаторі BioRad CFX 96, використовуючи реактиви ЛиТех. Статистичну обробку результатів проводили за допомогою рівноваги Харді-Вайнберга, методу Краскела-Уоллеса, логістичного регресійного аналізу та побудови ROC-кривої з визначенням області AUC і значень чутливості та специфічності. У результаті дослідження виявлено достовірну різницю у розподілі мутантних генотипів між пацієнтами з обома формами ВМД та контрольною групою. Також встановлено статистично високозначущий вплив мутантного апеля А на розвиток як "сухої" (OR = 3,40; 95,0 %; CI = 1,90-6,07, p<0,001), так і "вологої" форм ВМД (OR = 4,78; 95,0 % CI 2,65-8,64, p<0,001), а при аналізі мутантних генотипів виявлено, що генотип AA підвищує шанси виникнення "сухої" та "вологої" форм захворювання в 3,13 і 4,74 разів відповідно, тоді як AA - у 5 разів, незалежно від форми хвороби, що підтверджує вплив поліморфізму гена TNF на виникнення та прогресування вікової макулярної дегенерації. При аналізі ROC-кривих та областей AUC встановлено, що всі мутантні генотипи мають достовірний вплив на виникнення вікової макулярної дегенерації (p<0,05). Проте, отримані значення чутливості та специфічності, особливо при генотипі AA як при "сухій" (17,9 % і 95,8 % відповідно) так і при "волої" (18,2 % і 95,8 % відповідно) формах вікової макулярної дегенерації свідчать про низький шанс безпомилкового підтвердження діагнозу захворювання, що може бути пов'язано з мультифакторіальністю захворювання і потребує подальших досліджень.

Ключові слова: вікова макулярна дегенерація, TNF-α, ROS, NOS, ген.