ASSOCIATION OF SERUM ANNEXIN A3 (ANXA3) LEVELS WITH AGE-RELATED MACULAR DEGENERATION

Yaşa Bağlı Makula Dejenerasyonu İle Serum Anneksin A3 (Anxa3) Düzeylerinin İlişkisi

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ABSTRACT

Objective: Age-related macular degeneration (AMD) is the most common cause of advanced visual loss in developed societies. Annexin A3 (ANXA3) contributes to tumor development by affecting cell signaling pathways, cell profiling, invasion, metastasis and cell apoptosis. The aim of this study was to compare serum ANXA3 levels in wet type and dry type macular degeneration patients with the control group and to investigate whether there is a significant difference between the groups.

Material and Methods: A total of 78 patients (39 patients with wet AMD, 39 patients with dry AMD diagnosed at the same time period and 39 healthy controls without any previous diagnosis of macular degeneration) were enrolled in this study. ANXA3 levels were determined by enzyme-linked immunosorbent assay (ELISA).

Results: Serum ANXA3 levels were found to be significantly higher in wet AMD than in the control group (p=0.005). In addition, the difference between serum ANXA3 levels of wet and dry AMD patients was found to be significant. It was observed that there was a significant increase in wet type AMD patients (p=0.004). However, no significant difference was found in ANXA3 levels in dry type AMD compared to the control group (p=0.444).

Conclusion: These findings suggest that ANXA3 protein may play an important role in age-related macular degeneration.

Keywords: *Age-Related Macular Degeneration, ANXA3, ELISA*

ÖZET

Amaç: Yaşa bağlı maküler dejenerasyon (YBMD) gelişmiş toplumlarda ileri derecede görme kaybının en sık nedenidir. Anneksin A3 (ANXA3), hücre sinyal yolakları, hücre profilerasyonu, invazyon, metastaz ve hücre apoptozuna etki ederek tümör gelişimine katkı sağlamaktadır. Bu çalışmanın amacı, yaş tip ve kuru tip makula dejenerasyonu hastalarında serum ANXA3 düzeylerini kontrol grubu ile karşılaştırmak ve gruplar arasında anlamlı bir fark olup olmadığını araştırmaktır.

Gereç ve Yöntemler: Bu çalışma yaş tip YBMD tanısı alan 39 hasta, aynı zaman diliminde kuru tip YBMD tanısı alan 39 olmak üzere toplam 78 hasta ve daha önce herhangi bir maküler dejenerasyon tanısı almamış sağlıklı 39 kontrol grubundan oluşturuldu. ANXA3 düzeyleri enzim bağlantılı immünosorbent tahlil (ELISA) yöntemi ile tespit edildi.

Bulgular: Serum ANXA3 düzeylerinin yaş tip YBMD hastalığında kontrol grubuna göre anlamlı olarak yüksek düzeyde olduğu tespit edildi (p=0,005). Ayrıca yaş tip ve kuru tip YBMD hastalarının serum ANXA3 düzeyleri arasındaki farklılık anlamlı bulundu. Yaş tip YBMD hastalarında anlamlı ölçüde yükseklik olduğu görüldü (p=0,004). Ancak kontrol grubuna göre kuru tip YBMD hastalığında ANXA3 seviyelerinde anlamlı bir farklılık saptanmadı (p=0,444).

Sonuç: Bu bulgular sonucunda ANXA3 proteininin yaşa bağlı maküler dejenerasyon hastalığında önemli rol oynayabileceği düşünülmektedir.

Anahtar Kelimeler: *Yaşa Bağlı Maküler Dejenerasyon, ANXA3, ELİSA*

INTRODUCTION

Age-related macular degeneration (AMD) affects millions of people worldwide and is one of the leading causes of blindness (1). There are 2 main types of the disease, neovascular and non-neovascular, which can be further classified according to their specific features. The main reason for this distinction is whether or not new blood vessels form. If new vascularization is present, it is referred to as wet AMD, neovascular AMD, exudative AMD or disciform AMD. Wet AMD accounts for 20% of all patients. If no new vessel formation is present, it is called non-exudative or dry AMD or Geographic atrophy (GA). Dry AMD accounts for 80% of all patients and generally carries a more favorable prognosis. Wet AMD accounts for the overwhelming majority (75%) of visual loss (2,3). Neovascular AMD ("wet" AMD) affects the remaining 15% to 20% and accounts for approximately 80% of severe vision loss (4).

Annexins (AnxA) are a large family of proteins widely distributed in various organisms, tissues and cell types (5). AnxA binds calcium and phospholipids to form calcium-dependent ion voltage channels (6). It has been suggested that calcium binding underlies the effect of AnxA in processes such as anticoagulation, endocytosis and exocytosis, signal transduction, cellular proliferation and apoptosis (7,8). In mammals, 12 different annexins named A1-A11 and A13 have been identified (9). Changes in tissue or cellular expression of AnxA have been associated with various conditions such as asthma (10), atherosclerosis (11), autoimmune diseases (12), cancers (13), Parkinson's disease (14) and Alzheimer's disease (15). It has been shown that AnxA may also play a role in many ocular diseases and may be a target for treatment (16). The roles of some AnxAs have been investigated in the visual system, leaving room to explore the promising therapeutic potential these proteins may have in ocular diseases. Others AnxAs have been studied in infectious and autoimmune diseases (17-18). Considering that eye diseases can have many etiologies, further research on the role of AnxAs in the eye could advance the field of ophthalmology because the eye is an organ that can be exposed to many microorganisms and can be affected by certain autoimmune diseases. Therefore, a better understanding of the function of AnxAs in the eye could lead to at least partial elucidation of disease mechanisms in the visual system.

There is no study investigating the relationship between serum ANXA3 levels in patients with age-related macular degeneration (dry and wet types). The aim of this study was to compare serum ANXA3 levels in wet and dry macular degeneration patients with the control group and to investigate whether there is a significant difference between the groups.

MATERIAL AND METHOD

Patients and Controls

In this study, a total of 78 patients, including 39 patients who were first diagnosed with wet AMD and 39 patients who were diagnosed with dry AMD in the same period of time, who presented to Sivas Cumhuriyet University Faculty of Medicine Ophthalmology Outpatient Clinic with complaints of decreased vision and curved vision, were included in the study. Patients were informed about the diseases in their eyes. The treatments applied worldwide and the treatments they would receive in our hospital were explained in detail. The consent document was read to the patients and their consent was obtained by making explanations about the exclusion criteria.

The control group consisted of 39 healthy volunteers who had not been diagnosed with macular degeneration before and who were similar in age and gender to the patients in the same period.

Patients who had previously initiated anti-vasculer endothelial growth factor (VEGF) therapy and used other anti-VEGF agents during the treatment period, patients who could not be started on bevacizumab due to cardiovascular and cerebrovascular reasons, patients who could not complete 3 intravitreal bevacizumab injections during the treatment period, patients who had previous eye surgery for any reason, patients with additional retinal pathology such as diabetic retinopathy, branch vein occlusions, Patients with anterior chamber inflammation for any reason or whose vision was affected as a result of this inflammation, patients diagnosed with glaucoma, patients with ±3D refractive error, patients with media opacities such as corneal opacity, vitreous hemorrhage, and patients with systemic diseases such as diabetes mellitus, hypertension, and malignancy that may

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affect serum ANXA3 levels were excluded from the study. In the control group, patients with any retinal abnormality, glaucoma, previous intravitreal anti-VEGF disease, systemic disease, previous ocular surgery, and corneal opacities, cataracts, vitreous haze that would prevent Optic coherence tomography (OCT) were excluded from the control group. Systemic and ocular findings of the patients were recorded in the data. All patients underwent a complete ophthalmologic examination before enrollment in the study. Visual acuity was recorded using Snellen's threshold. Biomicroscopic examination was recorded by measuring intraocular pressure and refraction values with Tonoref 2 (Nidek, Gamagori, Japan). Following pupil dilatation, fundus examination was performed using a 90 diopter lens. Fundus fluorescein angiography (CLARUS 700™, Carl Zeiss Meditec Inc., Californea, USA, FFA), optical coherence tomography (OCT RS- 25 3000 Advance, NIDEK CO., LTD., JAPAN, OCT) and Solix Fullrange optical coherence tomography-angiography (Optovue Inc, Freemont CA, USA, OCT-A) imaging modalities were performed in all patients at the initial examination and wet AMD and dry AMD were diagnosed. Our study was performed using human serum samples obtained from the blood of patients and controls collected within the scope of the project with ethical decision number 2021-11/03.

Blood samples were collected in 5 ml vacuum tubes containing citrate. The samples were centrifuged at 3000 rpm (1400xg) for 10 minutes at room temperature to obtain serum. These serum samples were placed in sterile eppendorf tubes and stored at -80°C.

ANXA3 (ELK Biotechnology, Denver, CO 80202 USA, Cat: ELK3218) levels were determined using a commercially available human enzyme-linked immunosorbent assay (ELISA) kit. The range of detection was 0.16-10 ng/mL with <8% intra-assay and <10% inter-assay variation coefficient. The sensitivity of this kit is 0.065 ng/mL.

The statistical analysis of the data in our study was performed by loading into the SPSS Software (Version 26.0) program. The normality of the obtained data was evaluated with the Kolmogorov-Smirnov test. Levene's test was used for homogeneity of variances. Mann-Whitney U test, Kruskal-Wallis test and chi-square test was used to compare data that did not show normal distribution. Tukey post-hoc test of variances was recorded to determine the differences between groups after ANOVA. Statistical significance level was considered as $p<0.05$.

An application was made to the Sivas Cumhuriyet University Clinical Research Ethics Committee (Sivas, Türkiye), and the necessary ethics committee approval was obtained (Number: 2022-12/54). The study was carried out in compliance with the ethical principles of the Declaration of Helsinki.

RESULTS

The control group was compatible with the wet AMD group and the dry AMD group in terms of age and gender ($p>0.05$). Active leakage areas were observed on Fundus fluorescein angiography (FFA) in 39 (100.0) patients with wet AMD and active membrane was observed on Optical coherence tomography angiography (OCT-A) in 32 (82.0) patients (Table 1).

In the control group, mean serum ANXA3 levels were 3.94 ng/mL (minimum: 1.43 ng/mL, maximum: 10.84 ng/mL). In the wet type group, mean serum ANXA3 levels were 5.39 ng/mL (minimum: 1.46 ng/mL, maximum: 11.95 ng/mL). In the dry type group, mean serum ANXA3 levels were 3.51 ng/mL (minimum: 1.19 ng/mL, maximum: 8.52 ng/mL).

In this study, serum ANXA3 levels were significantly different in wet type AMD compared to the control group ($p=0.005$). In addition, the difference between serum ANXA3 levels of wet type and dry type AMD patients was significant ($p=0.004$). However, no significant difference was found in ANXA3 levels in dry AMD compared to the control group ($p=0.444$). These statistical results between groups are shown in table 2.

DISCUSSION

In our study, serum ANXA3 levels were investigated in dry AMD and wet AMD patients. ANXA3 levels were found to be higher in wet type AMD patients compared to dry AMD group and normal population. These results showed that high serum ANXA3 levels may play a role in the pathogenesis of AMD, especially wet type AMD.

Age-related macular degeneration is a chronic macular degenerative disorder that causes age-related central vision loss in individuals. It is a multifactorial disease resulting from irregularities in the angiogenic, inflammatory, lipid and extracellular matrix pathways

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Table 1. Demographic characteristics of wet AMD, dry AMD and control group

	CONTROL n (%)	WET AMD n (%)	DRY AMD n (%)	*p*
Number of individuals	39	39	39	
Gender				
Female	21 (53,8)	21 (53,8)	21 (53,8)	
Male	18 (46,2)	18 (46,2)	18 (46,2)	
Average age				
Female	72.00 ± 7.77	72.42 ± 7.78	71.76 ± 7.74	
Male	72.88 ± 6.79	73.11 ± 6.38	74.00 ± 6.59	
Cigarette story	19 (48,7)	18 (46,1)	22 (56,4)	*p>0.05*
Eye				
Right	21	21	20	*p>0.05*
Left	22	22	23	
Leakage in fundus fluorescein angiography	-	39 (100,0)	-	
Active membrane in optical coherence tomography		32 (82,0)		

AMD: Age-related macular degeneration

Table 2. Comparison of ANXA3 levels in wet AMD, dry AMD and control groups

ANXA3	n	Mean ± SD	*p value*
CONTROL – DRY TYPE	39	3.94 ± 2.50 3.51 ± 2.21	*p=0.444**
CONTROL – WET TYPE	39	3.94 ± 2.50 5.39 ± 2.96	***p=0.005****
DRY TYPE – WET TYPE	39	3.51 ± 2.21 5.39 ± 2.96	***p=0.004****

*p<0.05

(1). Studies have reported that reduced risk of neovascular AMD is associated with higher carotenoid serum levels (19). In a study conducted with 100 patients diagnosed with AMD and 100 healthy individuals, a significant difference was found in interleukin 17 serum levels compared to the control group (20). ANXA3 is a Ca2±dependent phospholipid and membrane-binding protein (21,22). It has important functions in membrane transport and other calmodulin-dependent activities on the membrane surface and is also involved in the regulation of inflammatory responses, cell differentiation and interactions of cytoskeletal proteins. It has been reported that ANXA3 expression may be associated with many human diseases.

ANXA3 contributes to tumor development by affecting cell signaling pathways, cell profiling, invasion, metastasis and cell apoptosis (23-27) reported that ANXA3 is important in gastric cancer cell proliferation by using fluorescent two-dimensional differential gel electrophoresis and liquid chromatography-mass spectrometry methods in a study conducted in gastric cancer. In another study by Xie et al (28), ANXA3 expression was investigated in rectal tumor tissues and ANXA3 expression was found to be high in rectal cancer tissues. As a result of this study, overexpression of ANXA3 was reported to be associated with poor prognosis of patients. In a study conducted in serum samples of patients with cholelocele cancer, ANXA3 serum levels were found to be significantly higher than healthy controls (29). In a study conducted in hepatocellular

carcinoma (HCC) patient sera, ANXA3 levels were found to be at the highest level and it was determined that ANXA3 could differentiate patients with HCC from individuals at risk of Hepatocellular carcinoma (HCC) (30). In our study, serum ANXA3 levels were found to be significantly higher in patients with dry AMD compared to the control group. Our study supports the use of serum ANXA3 levels as a biomarker for AMD. Histopathologic studies have reported that dry atrophic AMD is characterized by loss of the retinal pigment epithelium (RPE) component of the blood-retinal barrier in the macula in the absence of clinical exudation (31,32). In the dry lesion, pigment masses accumulate at the edge of the atrophy zone (33). Holz et al. in patients with AMD suggested that increased fundus autofluorescence induced by lipofuscin (LF) in eyes with geographic atrophy (GA) may reflect variable reactive changes in RPE cells (34). VEGF expression is controlled by many cellular factors such as growth factors and hormones. VEGF has a specific and strong angiogenic effect and contributes to tumor development by regulating tumor neovascularization. There are studies reporting that ANXA3 may affect vascular signaling by directly or indirectly regulating VEGF expression. In a study to investigate whether VEGF production is involved in tube formation in ANXA3-stimulated human umbilical vein endothelial cells (HUVECs), it was found that cells overexpressing ANXA3 produced significantly more VEGF than control cells. As a result of this study, it is thought that increased VEGF levels may be associated with ANXA3-induced angiogenic activity involved in migration and tube formation (35).

Our study resulted in increased serum levels of ANXA3 in accordance with the mentioned studies and this result suggests that vascularization may occur in ischemic ocular retinopathy and may be protective in the course of the disease. In this study, the difference between serum ANXA3 levels in wet and dry AMD patients was also significant. Therefore, serum ANXA3 levels may be meaningful as a biomarker to differentiate wet and dry AMD in patients diagnosed with the disease.

Study Limitations: The most important limitations of our study were that the study population was small, ANXA3 molecule levels were affected by many factors, and that only ELISA analysis was performed and not confirmed by genetic tests.

CONCLUSIONS

As a result of this study, we think that ANXA3 protein can be used as a diagnostic and therapeutic option for AMD. The lack of such a study in the literature makes our study valuable. Larger centered studies will be more useful in terms of the accuracy of the results. In larger studies to be conducted with the ANXA3 molecule, studies including genetic analysis must be carried out in order to accept the results as definitive.

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