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Other Mediators At Age-Related Macular Degeneration

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Abstract

Objective: *In some patients, even if anti-VEGF therapy is repeatedly administered at certain intervals, no response is obtained. Therefore, a search for new treatment methods has arisen. The aim of this study was to investigate the relevance of mediators such as Erythropoietin, Interleukin 17, and* Angiotensin2*, which are all involved in proliferation, apoptosis, oxidative stress, and inflammation in age-related macular degeneration.*

Metods: *EPO, IL-17, and Angiotensin 2 levels were evaluated by examining the blood samples of patients who did not have any systemic disease or chronic eye disease except Age-related Macular Degeneration (AMD).*

Results: *The patients were divided into 3 groups. Groups 1,2, and 3 were determined as the control group, dry type group, and neovascular type group respectively. For the purpose of this study, erythropoietin, interleukin 17,* angiotensin2 *were examined in the blood samples of patients. As a result of the analyses, a statistically significant difference was detected between the groups in terms of EPO, IL-17, and Angiotensin 2.*

Conclusion: *If the relationship between many factors related to age-related macular degeneration can be clearly defined, the perspective on treatment may change, especially in treatment-resistant patients.*

Keywords: *Age-related Macular Degeneration, Erythropoietin, Interleukin 17,* Angiotensin 2

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Introduction

AMD accounts for 6-9% of legal blindness (1). The disease is divided into two types: Dry type (DT) and neovascular type (NVT). In both the DT and NVT, the main causes are the deterioration in retinal pigment epithelium (RPE), which is triggered by apoptosis, oxidative stress, carbonyl stress, and inflammation. It is estimated that approximately 288 million people will have the disease by 2040, and 15 million of these patients will have the NVT in 2050. Despite the high morbidity of the disease, treatment options and preventive factors are limited. (1-6) In fact, although a complete cure cannot be achieved, anti-inflammatory drug treatment, anti-vascular endothelial growth factor (anti-VEGF) intravitreal injection, and nutritional therapy are commonly used for treatment. (4, 5) In the NVT, for which the prognosis is the worst, anti-VEGF preparations are administered intravitreally. Treatment is scheduled monthly or includes regimens that can be repeated as needed. Even with such a strict treatment protocol, response is observed only in one third of the patients. (2)

The use of mediators that play an important role in inflammation and apoptosis in inflammatory diseases such as AMD is a controversial issue. Mediators such as EPO, IL-17, Ang-2 have been studied in many inflammatory diseases and found to be relevant. (7-10) If the relationship of these mediators with AMD can be clearly identified, it can be tested in patients unresponsive to anti-VEGF therapy.

The aim of our study was to determine the relationship between the AMD and consisting of Erythropoietin (EPO), Interleukin 17 (IL-17), Angiotensin2 (Ang-2), previously studied as regards inflammatory diseases.

Subjects and Methods

Late-stage, symptomatic patients diagnosed with AMD who applied to the university retina outpatient clinic were examined in this prospective study. (11) The patients with systemic diseases (diabetes, hypertension, thyroid, rheumatological disorders and cancer, etc.) and concomitant chronic eye diseases (uveitis, glaucoma, vaso-occlusive disorders), and those who had received intraocular injections, as well as those with a BMI within the obesity range were excluded from the study. Of all the patients, 14 of them (Group 1) with DT AMD and 18 (Group 2) of them with the NVT AMD were included in the study. For the control group, 14 participants with the same inclusion criteria were included in the study (Group 3).

Blood samples were collected from patients and health checks doneto measure the EPO, IL-17, Ang-2levels at 9 am after overnight fasting in all subjects.

All subjects rested for 15 min before the blood collection process. The samples were delivered to the laboratory within 20 min. and centrifuged in 2000 rpm for 10 min at 4°C. The sera were stored at −80°C until the bio-chemical assay was performed. For serum Human Salusin-β, EPO, IL-17, Ang-2 measurements, a commercial kit (Sunred Bio, Baoshan, Shanghai) was used. Serum salusin-β, EPO, IL-17, Ang-2 levels were assayed by enzyme-linked immunosorbent test (ELISA), following the manufacturer's instructions. The minimum detectable level (sensitivity) was less than 0.157 ng/mL and the assay range for EPO, IL-17,

Ang-2 was 0.2–60 ng/mL. Intra- and inter-assay CVs were less than 10% and 12%, respectively. All samples were measured spectrophotometrically using ELx-800TM Absorbance Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA) at 450 nm. The biochemist blindly assayed samples. The results are presented as ng/ml.

The study was conducted in full accordance with the Declaration of Helsinki and approved by the Ethics Committee of the university (Date: 30.03.2017, Number: 16). All the study subjects were provided with an informed consent.

The data obtained from the patients were transferred to SPSS. “Shapiro-Wilk Test” was used to detect whether the data ofEPO, IL-17, Ang-2” (ng/ml) in the control group, NVT and DT groups separately showed a normal distribution. Comparison analyses were calculated by Kruskal-Wallis Test and correlation analyses were calculated with Spearman-Brown correlation coefficient.

Results

This study included a total of 46 participants, 14 of whom were healthy, whereas32 of them had AMD. The age range of the patients in the groups were 76.35 ± 8.13 years, 77.32±7.15 years, and 77.27±7.80 years for the DT group, NVT group and the control group, respectively. There were 8 male and 6 female participants in the DT, 8 male and 10 female participants in the NVT, and 7 male and 7 female participants in the control group. No statistically significant difference was found between the groups in terms of age and gender ($p>0.05$).However, there was a statistically significant difference between the groups in terms of the EPO, IL-17 and Ang-2.The DT group had the highest values of EPO and the control group had the highest value of IL-17. The highest Ang-2 value was found in the NVT group. The mediators examined in the patients are shown in Table 1.

Table 1. Molecular levels in blood

Mediators	Group	N	Value (ng/ml)	P
EPO	Control	14	234.57±57.98	0.0001
	Dry type	14	1109.07±691.32	
	Neovascular type	18	689.09±730.97	
IL-17	Control	14	304.56±278.77	0.0001
	Dry type	14	89.71±30.81	
	Neovascular type	18	80.1±18.97	
Ang-2	Control	14	7272.62±1531.08	0.001
	Dry type	14	10013.27±1842.66	
	Neovascular type	18	11634.31±36667.86	

N, number of the participants; Erythropoietin, EPO; Interleukin 17, IL-17; *Angiotensin2,* Ang-2

EPO is a glycoprotein hormone, which plays a critical role as antiapoptotic, antioxidant, angiogenic, anti-inflammatory, neuroprotective and stem cell protector in proliferation, differentiation, and apoptosis mechanisms. (12) Its main mechanism of action is the hematopoietic system, but its secretion is increased secondary to hypoxia in many organs. In the eye, both its receptor and EPO have been shown in photoreceptor cells, ganglion cells, inner nuclear layer, retinal pigment cells and choroid.(13,14) EPO levels are higher in the

RPE than in plasma.(13)It is claimed in some studies that an EPO level is elevated in many eye diseases to the extent that it creates a protective effect, while some studies argue the opposite.(7) One study, for example, showed that EPO could affect the progression of diabetic retinopathy positively, while another reported that it negatively affected the prognosis.(7, 15, 16) In fact, the stages of diabetic retinopathy are different in each study. While it was observed to be protective in the early stage, it aggravated the worsening in the late stage. Further studies are needed to show whether EPO levels change as to disease and its stages. In the current study, the lowest EPO level was found in the control group, while the highest level was found in the DT AMD group. Whether elevated EPO levels are a protective mechanism from NVT AMD should be investigated with new studies.

IL-17 is a cytokine, which is produced by helper 17 cells. (17) It has been studied in serum and cell cultures of patients with the AMD. Three different values have been observed: a value with no statistical difference from to the control group, and that of a greater level and a lower level.IL-17 level was found to be lower especially in treatment-resistant groups. (18-23) In the current study, IL-17 levels were the lowest in the group with the NVT. It is quite interesting that IL-17 levels were variable. Observing low levels in treatment-resistant patients is especially a controversial issue. Whether or not IL-17 levels show a genetic pattern is one of the subjects to be investigated. In such a case, the perspective on treatment may change completely. For this, it may be appropriate to compare the results of a multicentre study.

Ang-2 is a hormone that is important for blood pressure regulation. Ang-2 and renin angiotensin system are known to reside in the RPE.(24) Another widely-known issue is that Ang-2 increases the production of extracellular matrix while disrupting the RPE barrier.(25-27) In this connection, some studies have shown the relationship between angiotensin activity and the choroidal neovascular membrane.(28)Another study argued that the activity of the angiotensin 1 receptor is associated with the activation of VEGF, which is a risk factor for AMD.(29)This was supported by the current study, in which the highest Ang-2 level was found in NVT AMD.

AMD degeneration formation and treatment mechanisms have not been fully elucidated. There is currently no effective treatment method. While vitamin supplements are recommended for patients with the DT, intravitreal VEGF is actively used for those with the NVT. The response is good in some patients, but the others turn out to be resistant to treatment, leading to the search for new treatment models. In the current study, blood levels of various agents involved in proliferation, apoptosis, oxidative stress, and inflammation related to the eye were examined. The levels of these agents had been studied for different diseases before, but the values were observed differently in each study. No clear conclusions could be drawn from the studies because of the differences in patients' samples, countries, diseases, stages of the diseases, and ages. In many studies, as is the case in the current study, the number of participants seems to be small. This is the most important limiting factor in the current study and the others. However, the common feature of the studies is that these molecules affect the pathogenesis of the disease in direct or indirect ways. In order to determine the effects of these molecules, future studies are needed with multifocal, similar,

and larger groups. It has not been clarified whether these molecules are protective or aggravating. However, the increase in the number of such studies may change the perspective for AMD.

Conclusion

As a result, other mediators besides the VEGF may play an active role in AMD. Understanding the roles of the molecules will make positive contributions to treatment efficacy.

Conflicts of Interest

Turgut B, None; Erdogan H, None; Ilhan N, None; Ersan I, None

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