Serum Endocan Levels in Patients with Age-related Macular Degeneration

Yaşa Bağlı Maküla Dejenerasyonu Olan Hastalarda Serum Endocan Seviyeleri

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ABSTRACT

Aim: Our aim was to compare the serum endocan levels of wet and dry type age-related macular degeneration (AMD) patients with control subjects.

Materials and Methods: This cross-sectional case control study was conducted on a total of 89 subjects consisting of 26 wet type AMD and 30 dry type AMD cases and 33 control subjects. Serum endocan levels were measured by an enzyme-linked immunosorbent assay kit in all participants.

Results: There was no significant difference among the groups for mean age, gender distribution or body mass index ($p>0.05$). Serum endocan levels were 15.7±6 ng/mL, 16±5.7 ng/mL and 18.1±5.3 ng/mL in wet and dry type AMD patients and control subjects, respectively, with no significant difference among the groups ($p=0.192$).

Conclusion: We did not find a significant difference in serum endocan levels between wet and dry type AMD patients and control subjects. However, further studies evaluating vitreous endocan levels are needed to investigate the potential pathophysiological role of endocan in AMD pathogenesis.

Key words: Age-related macular degeneration, endocan, inflammation, neovascularization, vascular endothelial growth factor

ÖZ

Amaç: Amacımız yaş ve kuru tip yaşa bağlı maküla dejenerasyonu (YBMD) olan hastalarda serum endocan seviyelerini kontrollerle karşılaştırmaktır.

Gereç ve Yöntem: Bu kesitsel vaka kontrol çalışması, 26 yaş tip YBMD, 30 kuru tip YBMD ve 33 kontrolden oluşan, toplam 89 gönüllü üzerinde gerçekleştirilmiştir. Serum endocan seviyeleri tüm olgularda bir ELISA kit ile değerlendirilmiştir.

Bulgular: Ortalama yaş, cinsiyet dağılımı ve vücut kitle indeksi açısından gruplar arasında anlamlı bir farklılık yoktu ($p>0.05$). Serum endocan seviyeleri yaş ve kuru tip YBMD hastalarında ve kontrollerde sırasıyla 15.7±6 ng/mL, 16±5.7 ng/mL ve 18.1±5.3 ng/mL idi ve gruplar arasında anlamlı farklılık yoktu ($p=0.192$).

Sonuç: Yaş ve kuru tip YBMD hastaları ve kontroller arasında serum endocan seviyeleri açısından anlamlı bir fark bulmadık. Ancak, YBMD patogenezindeki endocan'ın patofizyolojik rolünü araştırmak için vitreus endocan seviyelerini değerlendiren ileri çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Endocan, inflamasyon, neovaskülarizasyon, vasküler endotelyal büyüme faktörü, yaşa bağlı maküla dejenerasyonu

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INTRODUCTION

Age-related macular degeneration (AMD) is a leading cause of visual loss. The elderly population is constantly growing, increasing the incidence of the disorder.[1] AMD pathogenesis is still unclear despite many studies, although it is known to be a multifactorial disease with metabolic, genetic and environmental factors playing a role in the etiology. It can be divided in two subgroups as dry type and wet type according to the clinical and pathological features. Wet type can be treated with effective treatment options such as anti vascular endothelial growth factor (VEGF) but there is still no effective treatment for geographical atrophy.[2] AMD therefore continues to be a significant health problem.

Endocan, previously called endothelial cell specific molecule-1 (ESM-1), is a soluble proteoglycan from the vascular endothelium.[3] Lassalle et al.[4] were the first to report this molecule in 1996, after cloning it from a human endothelial cell DNA library. It was initially named as ESM-1 because its confined distribution to endothelial cells. Later, it was renamed as endocan in 2001.[5] Endocan is expressed by endothelial cells especially in the lung and renal vasculature. Its expression occurs preferentially more in endothelial tip cells that regulate vascular growth and angiogenesis than stalk cells that are no active in vascular growth.[6] It was also detected in epithelial cells of the gastrointestinal cancers.[7] We also reported endocan overexpression in both epithelial and endothelial cells of pterygium.[8] It is believed to have an active role in inflammation, endothelial dysfunction and vascular problems. It may also have a role in healing, neovascularization and tumorigenesis.[6] It can be determined in the circulation as an angiogenesis and endothelial cell activation marker.[3]

We hypothesized that serum endocan levels could increase in AMD and especially the wet type due to the localized inflammation and neovascularization. We therefore compared the serum endocan levels of wet and dry type AMD patients with control subjects in this study.

MATERIALS AND METHODS

This cross-sectional case control study was conducted at the Ahi Evran University Training and Research Hospital's Ophthalmology Clinic on a total of 89 subjects consisting of 26 wet type AMD and 30 dry type AMD cases and 33 control subjects. The control group included randomly selected age-matched volunteers who had no AMD disorder. Consent was obtained from the local ethics committee and the participants also provided written voluntary informed consent. The study was conducted according to the Helsinki declaration principles.

A detailed medical history was obtained and full ophthalmic examination including fluorescein angiography and optical coherence tomography was performed in all patients. The International ARM Study Group Classification was used to diagnose and classify AMD.[9] Exclusion criteria for both AMD patients and control subjects were known malignancy, ocular, local or systemic infection or inflammation, any systemic disease other than hypertension such as diabetes mellitus, inflammatory diseases and heart disease, thyroid, renal or hepatic dysfunction, intravitreal drug injection within the last 3 months, and the use of any topical or systemic medication that could interfere with serum endocan measurement or influence serum angiogenic/antiangiogenic factors such as corticosteroids and non steroidal anti inflammatory drugs.

Blood sample collection

Blood was obtained from all subjects and the serum quickly was separated from the cells by centrifuging at 3000g for 10 min. The samples were then kept at -80 °C until the time of study.

Endocan measurements

We used an enzyme-linked immunosorbent assay kit (CUSABIO, Wuhan, P.R. China) in accordance with the manufacturers' instructions to measure serum endoccan levels. Serum samples were diluted four fold in sample diluent buffer and applied to wells in duplicate. The endocan ELISA kit had an assay range of 0.3–10 ng/mL. We read the results at 450 nm with the SPECTRO star Nano microplate reader (BMG Labtech). The data were processed with the MARS software (BMG Labtech) and results expressed in ng/mL. Standard curves were generated using a four-parameter curve fitting equation, and endocan levels were calculated according to this curve, with values given as ng/ml. The resulting value was multiplied by the dilution factor of the sample to correct for the final concentration.

Statistical analysis

We used SPSS software version 22.0 for data analysis. Analysis of variance (One-way ANOVA) was used to detect any difference between the continuous variables of the three groups. The chi-square test was used to assess differences among groups for categorical variables. A p value smaller than 0.05 was accepted as statistically significant.

RESULTS

There were 21 naive wet type AMD cases and 5 previosuly treated wet type AMD cases with anti-VEGF drugs. There were 10 early, 14 intermediate and 6 late AMD cases in dry type AMD group. The mean ages for the wet type AMD, dry type AMD and control groups were 76.7±5.2, 73±8.5 and 72.7±6.7 years, respectively. There was no significant difference among the groups for mean age, gender distribution or body mass index (p=0.057, p=0.100 and p=0.405, respec-

tively). Serum endocan levels were 15.7±6 ng/mL, 16±5.7 ng/mL and 18.1±5.3 ng/mL in the three groups, respectively, with no significant difference among the groups (p=0.192). Table 1 presents the demographic features and serum endocan levels of the groups.

DISCUSSION

Age-related macular degeneration is commonly classified into wet and dry types. The pathophysiology is not fully understood but it believed that the processes of lipofuscinogenesis, drusogenesis, local inflammation and neovascularization contribute to its development.[2] The dry type characteristics are progressive retinal pigment epithelium degeneration and photoreceptor loss, resulting in impaired vision.[10,11] The wet type is characterized by an imbalance between the proangiogenic (VEGF, fibroblast growth factor, transforming growth factor-beta) and antiangiogenic (Pigment epithelium derived factor) factors, causing excessive VEGF production.[2,12] VEGF and other proangiogenic factors lead to endothelial cell activation, resulting in endothelial cell proliferation and migration and finally neovascularization.[12,13] VEGF-A is the substance most relevant for intraocular angiogenesis.[13]

Endocan is synthesized by vascular endothelial cells in humans and can be found in the bloodstream. It is a soluble chondroitin/dermatan sulfate proteoglycan.[3] The regulatory mechanisms of endocan production are not clear but many cytokines and growth factors play a role. VEGF-A, VEGF-C, IL-1, TNF-alpha, transforming growth factor-beta 1, and fibroblast growth factor-2 lead to upregulation while interferon-gama causes downregulation.[3,6,14,15] Endocan expression is increased by VEGF-A and VEGF-C while endocan itself has a stimulatory effect on the mitogenic and promigratory activity of these molecules, creating an autocrine, positive feedback loop that acts to increase the stimulation caused by VEGF.[16] Endocan plays a role in many different biological processes that are essential for the regulation of cell adhesion, migration, proliferation and neovascularization. Increased endocan tissue expression or serum levels indicate endothelial cell activation and neovascularization, which in turn are associated with inflammation and tumor progression.[6,17]

Endocan overexpression has been reported in many conditions such as inflammation, sepsis, cancer and cardiovascular disorders in experimental and clinical studies.[3,15,17-23] Systemic inflammation can cause endothelial damage and plays a significant role in septic conditions.[24] Scherpereel et al.[18] have reported that the endocan blood levels in patients suffering from sepsis are correlated with the patient's condition and outcome. Endocan could therefore become a novel endothelial dysfunction marker in these patients.[18,19] Similarly, high endocan levels have been found in patients suffering from Behçet's disease, again supporting its use as a marker in this condition.[25] Angiogenesis ensures that the actively proliferating tumor cells receive oxygen and nutrients and is therefore vital for tumor progression. Hypoxia-inducible factor signaling is activated and VEGF secretion increases in angiogenesis. Endocan mediates the vascular growth promoting action of VEGF.[12] Grigoriu et al.[26] found significantly more endocan mRNA expression in lung tumors together with a positive correlation with survival and time to tumor progression. Shin et al.[16] suggested endocan as a novel mediator of lymphangiogenesis and stated that it could be used to target the inhibition of pathologic lymphatic vessel growth and activation induced by VEGF-A or VEGF-C. The use of endocan in blood and tissues as a biomarker for various cancers and inflammation has currently shown promising results. It is known that many cytokines are included in endocan's regulation such as VEGF. Increased expression of VEGF have been shown in pterygium. Therefore, we also evaluated endocan expression in pterygium because its role in inflammation and relationship with VEGF. We indicated overexpression of endocan in pterygium tissues.[8]

We had thought that serum endocan levels could be higher in the wet type AMD group than in the control subjects due to the inflammation and neovascularization. However,

Table 1. *The demographic features and serum endocan levels in patients with wet and dry AMD and the control subjects*

	Wet type AMD (n=28)	Dry type AMD (n=30)	Control Subjects (n=33)	P
Age (year)	76.7±5.2	73±8.5	72.7±6.7	P=0.057
Gender (F/M)	15/10	18/12	12/21	P=0.100
BMI (kg/m^2)	27.4±4.3	28.9±5	27.5±4.3	P=0.405
HT (present/absent)	12/14	14/16	13/20	P=0.811
Endocan level (ng/mL)	15.7±6	16±5.7	18.1±5.3	P=0.192

AMD, age-related macular degeneration; BMI, body mass index; HT, hypertension; F, female; M, male

we found no significant difference between the dry and wet AMD patients and the control subjects for serum endocan levels. The reason may be due to the negligible localized choroidal and retinal ischemia, inflammation and angiogenesis when compared with the bloodstream or whole vascular structures in the body and thus a lack of influence on serum endocan levels. On the other hand, it have been reported that dysregulated systemic parainflammation may contribute AMD pathogenesis.[27] These findings suggest that endocan is not a part of dysregulated systemic parainflammation in AMD patients.

A potential limitation was the inability to evaluate serum and vitreous VEGF levels and vitreous endocan levels at the same time. Abu El-Asrar et al.[28] have reported high vitreous endocan levels that might be associated with angiogenesis in proliferative diabetic retinopathy patients.

Based on all the information, it seems that antibodies against endocan may also be useful as anti-angiogenic agents and find use as an alternative to anti-VEGF substances in various disorders. Endocan therefore provides a valid target for AMD treatment. We did not find a significant difference in endocan levels between wet and dry type AMD patients and control subjects. However, further studies evaluating vitreous endocan and VEGF levels together are needed to investigate the potential pathophysiological role of endocan in AMD pathogenesis.

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