Is Serum Endocan Levels Important in Branch Retinal Vein Occlusion?

Retinal Ven Dal Tıkanıklığında Serum Endokan Seviyeleri Önemli mi?

Ali KURT[1], Raşit KILIÇ[1], Rukiye NAR[2], Özkan KOCAMIŞ[1], Osman Ahmet POLAT[1]

ABSTRACT

Purpose: We aimed to compare serum endocan levels in branch retinal vein occlusion (BRVO) patients and control subjects.

Material and Methods: This study was conducted on 33 BRVO patients and 32 control subjects as a cross-sectional case control study. Serum endocan levels were measured using an enzyme-linked immunosorbent assay.

Results: The mean age, gender distribution and body mass index were similar in the two groups with no significant difference (p>0.05). Serum endocan levels were 34.11±11.33 ng/mL in the BRVO patients and 32.34±8.86 ng/mL in the control group (p=0.487).

Conclusions: Increased serum endocan levels were found in BRVO patients comparing controls subjects, however, the difference was not statistically significant. Further studies evaluating vitreous and/or humor aqueous endocan levels are needed to clarify the pathophysiological role of endocan in BRVO pathogenesis.

Key Words: Endocan, ischemia, neovascularization, branch retinal vein occlusion, vascular endothelial growth factor.

ÖZ

Amaç: Retinal ven dal tıkanıklığı (RVDT) hastaları ve kontrol grubu bireylerin serum endokan seviyelerini karşılaştırmayı amaçladık.

Gereç ve Yöntemler: Bu çalışma 33 RVDT hastası ve 32 kontrol olgusu içeren kesitsel bir vaka kontrol çalışması olarak planlanmıştır. Serum endokan seviyeleri, bir enzime bağlı immünosorbent assay (ELİSA) kullanılarak ölçüldü.

Bulgular: İki grup arasında yaş ortalamaları, cinsiyet dağılımı ve vücut kitle indeksi açısından anlamlı fark yoktu (p>0.05). RVDT hastalarında serum endokan düzeylerinin ortalaması 34.11 ± 11.33 ng / mL olarak saptanırken kontrol grubunda 32.34 ± 8.86 ng / mL idi (p = 0.487).

Sonuç: İstatistiksel düzeyde anlamlı olmasa da RVDT hastalarında serum endokan seviyesi kontrol grubuna göre daha yüksek olarak bulunmuştur. RVDT patogenezinde endokanın patofizyolojik rolünü araştırmak için vitreus ve/veya aköz hümör endokan düzeylerini de değerlendiren daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Endokan, iskemi, neovaskülarizasyon, retinal ven dal tıkanıklığı, vasküler endotelyal büyüme faktörü.

INTRODUCTION

Retinal vein occlusion is the second most common retinal vascular disorder after diabetic retinopathy.[1,2] The pathogenesis is not fully clear but it is thought to develop as a result of three systemic changes known as Virchow's triad: hemodynamic changes (venous stasis), vessel wall degenerative changes, and hypercoagulability. Risk factors include systemic metabolic disorders such as diabetes, hypertension and hyperlipidemia, systemic inflammatory conditions such as vasculitis and Behcet disease, ophthalmic

1- Yrd. Doç. Dr., Ahi Evran Üniversitesi Tıp Fakültesi, Göz Ana Bilim Dalı, Kırşehir, Türkiye

2- Yrd. Doç. Dr., Ahi Evran Üniversitesi Tıp Fakültesi, Biyokimya Ana Bilim Dalı, Kırşehir, Türkiye

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Yazışma Adresi / Correspondence Adress:
Ali KURT
Ahi Evran Üniversitesi Tıp Fakültesi, Göz Ana Bilim Dalı, Kırşehir, Türkiye

Phone: +90 531 992 8604
E-mail: dralikurt@gmail.com

risk factors such as glaucoma, congenital thrombophilic disorders, smoking, and prescription drug use.[2] It is divided into three main types as central retinal vein occlusion (CRVO), branch retinal vein occlusion (BRVO) and hemicentral retinal vein occlusion.[3] Many chemokines and cytokines play a role in the pathogenesis and the condition can lead to decreased vision due to cystoid macular edema.[4-6]

Endocan, previously named endothelial cell-specific molecule-1, is a soluble proteoglycan that is secreted by endothelial cells in response to inflammatory cytokines.[7] It is known to play vital roles in cell differentiation, migration and adhesion.[8] It has also been shown to be involved in the pathogenesis of inflammatory and vascular disorders. Endocan can be detected in the bloodstream as an endothelial marker associated both with inflammation and angiogenesis.[7]

BRVO is a sight-threatening disease where ischemia and neovascularization play a role. Our hypothesis was that the localized inflammation and retinal ischemia in BRVO could increase serum endocan levels and therefore serum endocan levels could serve as a biomarker of the severity of inflammation and retinal ischemia. To our knowledge, the serum endocan levels has not been investigated in patients with BRVO. The purpose of this study was to report the serum endocan levels in patients with BRVO.

MATERIALS and METHODS

The study was conducted at the Ahi Evran University Training and Research Hospital's Ophthalmology Clinic as a cross-sectional case control study. We enrolled 33 BRVO patients and 32 control subjects composed of randomly selected age-matched volunteers in the study. Information on the study was provided and written consent obtained from all subjects. We obtained approval from the institutional Ethics Committee and followed the Helsinki Declaration principles.

A detailed medical history was obtained from all subjects. We then performed a full ophthalmic examination including fundus fluorescein angiography (FFA) and optical coherence tomography. The BRVO diagnosis was made with the observation of multiple hemorrhages, dilated and tortuous veins, retinal and papillary edema, and dilated veins in the territory of the occluded vein on fundus examination in addition to the hypofluorescence due to capillary nonperfusion and hemorrhages, dilated and tortuous veins, and leakage when retinal and macular edema was present on FFA.[9]

We excluded patients with any inflammatory disorder, local or systemic infection, known malignancy, any systemic disease other than hypertension, a history of intravitreal drug injection within the last 3 months, and the use of any medication that could interfere with measurements of endocan or angiogenic/antiangiogenic factors in the serum.

Handling of blood samples

The sera of the blood drawn from all subjects were quickly separated using a centrifuge at 3000 g for 10 minutes and the samples stored -80 ^{o}C until they were studied.

Endocan measurements

Serum endocan levels were measured using an enzyme-linked immunosorbent assay kit (CUSABIO, Wuhan, P.R. China) with an assay range of 0.3-10 ng/mL and following the manufacturer's instructions. Serum samples were diluted five fold in sample diluent buffer and applied to wells in duplicate. The SPECTRO star Nano microplate reader (BMG Labtech) was then used to read the results at 450 nm. MARS software (BMG Labtech) was used to process the data. 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

SPSS software, version 22.0, was used for data analysis. Any difference between the continuous variables in the two groups was detected with the independent t-test while differences between the groups for categorical variables were analyzed with the chi-square test. Statistical significance was set at $p<0.05$.

RESULTS

We evaluated 33 BRVO patients and 32 control subjects in this study. The mean age was 60.94±9.24 years for the BRVO patients and 63.91±8.93 years for the control subjects. We found no significant difference between the groups for mean age (p=0.193), gender distribution (p=0.524) or body mass index (p=0.661). The serum endocan level was 34.11±11.33 ng/mL in the patient group and 32.34±8.86 ng/mL in the control group with no significant difference between the groups (p=0.487). The demographic features and serum endocan levels of the groups are presented in Table 1.

DISCUSSION

Branch retinal vein occlusion can be a sight-threatening problem when it causes macular edema and neovascularization. The increased intraluminal hydrostatic pressure and decreased tissue perfusion lead to intraretinal hemorrhages and fluid exudation, eventually resulting in tissue ischemia and even neovascularization.[9] Cystoid macular edema with retinal outer plexiform layer fluid

Table 1: *The demographic features and serum endocan levels in patients with BRVO and the control subjects*

	BRVO (n=33)	Control Subjects (n=32)	P
Age mean±SD (year)	60.94±9.24	63.91±8.93	P=0.193
Gender (M/F)	17/16	19/13	P=0.524
BMI mean±SD (kg/m²)	28.74±4.86	28.18±4.77	P=0.661
HT (present/absent)	19/14	12/20	P=0.105
Endocan level mean±SD (ng/mL)	34.11±11.33	32.34±8.86	P=0.487

BRVO, branch retinal vein occlusion; BMI, body mass index; HT, hypertension; F, female; M, male; SD, standard deviation.

collection in the intercellular spaces due a disturbed endothelial blood-retinal barrier and fluid leakage is present in most CRVO cases and also in those BRVO cases where the macula is involved.[6,10,11]

BRVO increases the aqueous and vitreous concentrations of many angiogenic (e.g. vascular endothelial growth factor (VEGF)), and inflammatory cytokines (e.g. interleukin 6 (IL-6), IL-8, IL-12, IL-15, IL-17, and IL-23).[4,5] The proangiogenic factors activate the endothelial cells, causing their proliferation and migration and resulting in neovascularization .[4] VEGF-A has been used as an important intraocular anti-angiogenic target in retinal disorders as it is the most relevant substance.[12]

Endocan, found freely in the circulation, is a soluble dermatan sulphate proteoglycan secreted by the vascular endothelium of many organs.[13,14] Its many vital biological functions include the regulation of cell proliferation, differentiation, migration, and adhesion.[8] It is also believed to play a role in pathology involving organ-specific inflammation and the endothelium.[15] How endocan production is regulated is not fully known but VEGF-A, VEGF-C, TNF-α, IL-1, FGF-2 and transforming growth factor-β1 lead to up regulation and phosphatidylinositide 3-kinases (PI3K) and interferon-γ to down regulation. VEGF increases endocan expression and endocan can in return stimulate its mitogenic and promigratory effects, thus increasing VEGF-related stimulation with a positive autocrine feedback loop.[8,14,16] This very close relationship with VEGF makes endocan one of the key players in inflammation and angioenesis and therefore the pathogenesis of various disorders.

Many studies have demonstrated that endocan is involved in inflammatory and malignant processes and that its serum levels increase together with high expression in affected tissue.[8,17-22] Angiogenesis brings both the oxygen and nutrients that actively dividing tumor cells need and is therefore essential for the tumor to progress. The hypoxia caused by the tumor activates the hypoxia-inducible factor signaling pathway and results in VEGF secretion.[8,23] Endocan is the molecule that mediates VEGF's vascular growth promoting action.[8,17] High endocan expression has been found in the vessels of cancers of the lung, colon, kidney and the pituitary, all highly vascularized tumors.[7,8,14,17] Grigoriu et al [21] found endocan mRNA expression to be significantly increased in lung tumors and to show a positive correlation with survival and time to progression of the tumor. It has also been reported that endocan is a lymphangiogenesis mediator and could find use in the inhibition of pathologic lymphatic vessel growth and activation induced by VEGF-A or VEGF-C as a potential target.[17] Systemic inflammation plays a key role in sepsis as it can cause endothelial damage. An experimental study has found endocan levels to be associated with endothelial dysfunction during systemic inflammation.[18] A relationship between serum endocan levels and the severity of sepsis has also been observed and is thought to be a significant marker of patient outcome.[19,20] Endocan has therefore been shown to play an important role in many disorders associated with inflammation and angiogenesis.

We found that serum endocan levels to be higher in the BRVO group than the control group, however, the difference was not statistically significant. The negligible and localized choroidal and retinal ischemia, inflammation and angiogenesis in BRVO compared to the whole body could be the reason. We were also unable to measure serum and vitreous endocan levels at the same time to better evaluate the relationship between endocan and BRVO. Another study has reported finding high vitreous endocan levels in patients with proliferative diabetic retinopathy and that it could be associated with angiogenesis.[24] Antibodies against endocan could therefore also be useful as anti-angiogenic agents and perhaps as an alternative to anti-VEGF substances. The current preferred treatment for macular edema is repeated intravitreal anti-VEGF injections but some patients do not respond or develop rebound edema.[25] Endocan may therefore be a future BRVO treatment target. In conclusion, increased

serum endocan levels were found in BRVO patients comparing controls subjects, however, the difference was not statistically significant. Further studies that additionally include endocan and VEGF measurements in vitreous and/or humor aqueous may enable better clarification of endocan's role in BRVO pathogenesis

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of medical faculty of Turgut Ozal University with the date of 03.11.2015 and number 99950669/243. Information on the study was provided and written consent obtained from all subjects.

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