Can Serum Endocan Level Predict Stage of Diabetic Retinopathy?

Erdinc BOZKURT[1], Abdullah GUMUS[2], Yaran KOBAN[3]

ABSTRACT

Purpose: We aimed to evaluate the relationship between serum endocan levels and diabetic retinopathy (DR) severity in diabetic patients.

Material and Method: The study included 72 patients with diabetes mellitus (DM) and 28 volunteer control patients without any systemic disease. Patients with no systemic disease were the 1st group (n=28), diabetic patient without DR were the 2nd group (n=21), patients with non-proliferative DR were 3rd group (n=24) and patients with proliferative DR were 4th group (n=27). BMI, serum endocan levels, HbA1c, urea, creatinine levels and macular thickness were measured.

Results: Serum endocan levels were 170.05±85.67, 333.91±13.41, 340.42±105.07, 472.83±147.40 ng/L, respectively. BMI was 23.11±1.37 in the 1st group, 28.05±4.62 in the 2nd group, 29.00±5.69 in the 3rd group, and 28.85±5.88 in the 4th group. HbA1c levels were 4.83±0.55 in 1st group, 6.60±1.15 in 2nd group, 8.49±1.24 in 3rd group, and 8.89±1.30 in 4th group. Macular thickness measured with OCT was 216.96±11.64, 253.29±23.62, 274.88±61.11, 352.93±120.08µm in the right eye and 218.96±11.93, 240.65±17.57, 283±62.89, 334.78±127.71µm in the left eye, respectively.

Conclusion: In our study, there was a relationship between serum endocan level and DR stage. Serum endocan level can reveal important information about the severity and stage of DR such as serum creatinine and HbA1c level. There is a potential need for further research to demonstrate whether endocan is involved in DR pathogenesis.

Keywords: Endocan, Diabetic retinopathy, HbA1c, Optic coherence tomography, BMI.

INTRODUCTION

Diabetic retinopathy (DR) is the primary retinal vascular complication of diabetes mellitus (DM), causing lack of vision and blindness in the absence of treatment.[1-2] DR, a progressive disease, can be examined in two stages according to its severity: proliferative and non-proliferative. Nonproliferative diabetic retinopathy shows; microaneurysm, cotton wool spot, hard exudate, intraretinal microvascular abnormalities and venous pilling. Proliferative diabetic retinopathy presents in the optic disc or anywhere in the retina in the form of neovascularization, preretinal and vitreous hemorrhage.[3] According to the Global DR study, the frequency of DR, PDR and vision-reducing DR (severe retinopathy and macular edema) at any stage in 2010 were 93 million (34.6%), 17 million (7.0%) and 28 million (10.2%), respectively.[2]

Endocan or endothelial cell-specific molecule 1 (ESM-1) is a 50-kDa dermatan sulfate-based proteoglycan which is thought to reflect endothelial activation released from the vascular endothelium.[4] Endocan has been asserted to play an important role in the regulation of angiogenesis and inflammatory process and is thought to be used as a marker of vascular dysfunction.[5-6]. The control of the secretion of endocan is mediated by cytokines and growth factors. It is known that vascular endothelial growth factor (VEGF), IL-8, TNF-α, IL-1β, e-selectin increase the secretion of endocan.[7] Endocan is simultaneously a target and a modulator of VEGF signaling. On one hand, it increases VEGF-A expression and the interaction between VEGF-A and its receptor, VEGFR2, increasing vascular permeability.[8] On the other hand, VEGF signaling directly induces endocan expression.[9] This bidirectional interaction between endocan and VEGF cascades is crucial during angiogenesis, inflammation, and permeability in both physiological and pathological states. Modulation of this

1- Assist. Prof., Kafkas University Faculty of Medicine, Department of Ophthalmology, Kars, Turkey

2- Research Asistant, Kafkas University Faculty of Medicine, Department of Microbiology, Kars, Turkey

3- Assoc. Prof., Ersoy Hospital, Department of Ophthalmology, Istanbul, Turkey

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Correspondence Adress:
Erdinc BOZKURT
Kafkas University Faculty of Medicine, Department of Ophthalmology, Kars, Turkey
Phone: +90 506 615 7788
E-mail: drerdincbozkurt@hotmail.com

interaction may be considered a valid diagnostic target.[10] It is very meaningful that VEGF, which plays a major role in the pathogenesis of DR, increases the endocan level.

Any serum biomarker or reagent predicting retinopathy status of diabetic patients is not yet known. Our aim in this study is to determine whether serum endocan level is associated with diabetic retinopathy progression.

MATERIAL AND METHOD

The study included 72 patients with diabetes mellitus (DM) who were admitted to Kafkas University Faculty of Medicine, Department of Ophthalmology and 28 volunteer control patients without any systemic disease. The 1st group was 28 patients with no systemic disease, 2nd group was 21 patients with diabetes diagnosed with retinopathy, 3rd group was 24 patients with non-proliferative diabetic retinopathy and 4th group was 27 patients with proliferative diabetic retinopathy. Body mass index (BMI) was calculated for all patients (BMI = Weight (kg) / Length (m) ² formula was used), serum endocan levels glycosylated hemoglobin (HbA1c) and urea, creatine levels were examined. Optical coherence tomography (OCT) (Optovue RTvue RT-100) was used to evaluate macular thickness.

During the study, the ethical standards set out in the Helsinki Declaration were complied with. Local ethics committee approval was obtained (Protocol number: 2018-6-26 / 124). All subjects included in the study were informed about the study.

Inclusion criteria: age of 50-75, newly or previously diagnosed as DM type 2 according to Criteria of American Diabetes Association[11].

Exclusion criteria: Uncontrolled hypertension (systolic and diastolic blood pressure higher than 160 mmHg or 100 mmHg respectively), intraocular surgery or laser photocoagulation in the last six months, uveal or retinal pathologies other than DR, glaucomatous eyes, eyes with rubeosis iridis, patients who had a history of intravitreal steroid injection or an anti-VEGF agent, patients with a history of cardiovascular disease, chronic kidney disease, chronic inflammatory disease, and malignancy were not included in the study.

Measurement of serum Endocan

Blood samples of all patients were taken between 09: 00-11: 00 in the morning after 12 hours of starvation. Venous blood samples were centrifuged for 5 min. at 4000/ min without waiting. Serum samples were transferred to eppendorf tubes for biochemical analysis and stored at -80°C.

To analyze the serum endocan, one tube (4 mL) of blood sample was collected in the morning. The blood tube centrifuged to separate serum from plasma. Serum samples kept in eppendorf tubes in -80°C until the day of endocan analysis.

The serum endocan levels (ng/mL) were determined as previously reported using an enzyme-linked immunosorbent commercial assay Elabscience sandwich ELISA kit (Elabscience Biotechnology Co., Ltd, WuHan, China) based upon the company's protocol. Measurements were carried out using enzyme-linked immunosorbent assay plate reader Bio-Tek Synergy HT (Biotek Instruments, Winooski, Vermont). All the samples were measured in duplicate.

Statistical Analysis

Statistical analysis was performed with SPSS (Statistical Package for Scientific Studies, SPSS Inc., Chicago, USA) 21.0 statistical package program. While evaluating the study data, descriptive statistics; mean, standard deviation, median, frequency, ratio, minimum, maximum were used. Kolmogorow smirnow test was used for normality distribution of independent data. One-way Anova and Chi-square tests were used to compare quantitative data. Using variables found to be significant in the One-way Anova , linear logistic regression analysis was conducted to identify the independent determinants, which are risk factors for DR. Evaluations were performed at 95% confidence interval and statistical significance was accepted as $p < 0.05$.

RESULTS

72 patients with Type 2 DM aged 50-87 years and 28 volunteer control patients were included in the study. The 1st group consisted of 28 patients without any systemic disease (13 male, 15 female,). The 2nd group consisted of 21 patients with diabetes but without retinopathy (8 male, 13 female). The 3rd group consisted of 24 patients with non-proliferative diabetic retinopathy (13 male, 11 female). The 4th group consisted of 27 patients with proliferative diabetic retinopathy (16 male, 11 female). No significant difference was observed between the groups in terms of gender ($p = 0.486$). The mean age of the groups was 60.00±4.11, 60.00±12.29, 62.38±8.5, 64.93±7.47 respectively ($p=0.107$).

BMI was 23.11±1.37 in the 1st group, 28.05±4.62 in the 2nd group, 29.00±5.69 in the 3rd group, and 28.85±5.88 in the 4th group. The BMI of the patients in the 1st group was significantly lower than the other groups. HbA1c levels were 4.83±0.55 in 1st group, 6.60±1.15 in 2nd group, 8.49±1.24 in 3rd group, and 8.89±1.30 in 4th group. There

was a statistically significant difference between HbA1c levels of all groups except for the 3rd and 4th groups. Urea levels were 40.14±14.35, 38.57±13.21, 66.00±96.21, 64.04±35.58 mmol/L respectively (p=0.112). Creatine levels were 0.72±0.14, 0.78±0.26, 0.91±0.23, 1.03±0.24 µmol/L respectively (p<0.05).

Macular thickness measured with OCT was 216.96±11.64, 253.29±23.62, 274.88±61.11, 352.93±120.08µm in the right eye and 218.96±11.93, 240.65±17.57, 283±62.89, 334.78±127.71µm in the left eye, respectively. In 4th group macular thickness was significantly higher in both eyes than in the other groups (p <0.05). According to groups the serum endocan levels were 170.05±85.67, 333.91±13.41, 340.42±105.07, 472.83±147.40 ng / L, respectively. There was a statistically significant difference between all groups except for the 2nd and 3rd groups (Table 1) (Figure 1). HbA1c, serum endocan levels increase with the stage of DR (Figure 2). HbA1c, serum endocan and creatinine levels increase with the stage of DR, and these parameters were found to be independent predictors showing the stage of DR in regression analysis. (Table 2)

DISCUSSION

Type 2 DM is a chronic metabolic disease with a continuous increase in prevalence worldwide.[12] In diabetic patients, advanced glucosylation end products (AGEs) are formed due to the increase in insulin level and decrease in insulin sensitivity. AGEs cause the release

Table 1. *Between groups comparisons of demographic data, biochemical values and endocan levels.*

Variable	Group 1 Mean±SD	Group 2 Mean±SD	Group 3 Mean±SD	Group 4 Mean±SD	p values
Age	60.00±4.11,	60.00±12.29	62.38±8.5	64.93±7.47	p=0.107
Gender (M/F)	28(13/15)	21(8/13)	24(13/11)	27(16/11)	p=0.486
BMI (Kg/m²)	23.11±1.37	28.05±4.62	29.00±5.69	28.85±5.88	P=0.096
Urea (Mmol/L)	40.14±14.35	38.57±13.21	66.00±96.21	64.04±35.58	p=0.112
Creatinine (µmol/L)	0.72±0.14	0.78±0.26	0.91±0.23	1.03±0.24	p<0.05
HbA1c (%)	4.83±0.55	6.60±1.15	8.49±1.24	8.89±1.30	p<0.05
Endocan (Ng/L)	170.05±85.67	333.91±13.41	340.42±105	472.83±147	p<0.05
Right Macular Thickness (µm)	216.96 ±11.6	253.29±23.6	274.88±61.1	352.93±120	p<0.05
Left Macular Thickness (µm)	218.96 ±11.9	240.65±17.5	283±62.89	334.78±127	p<0.05

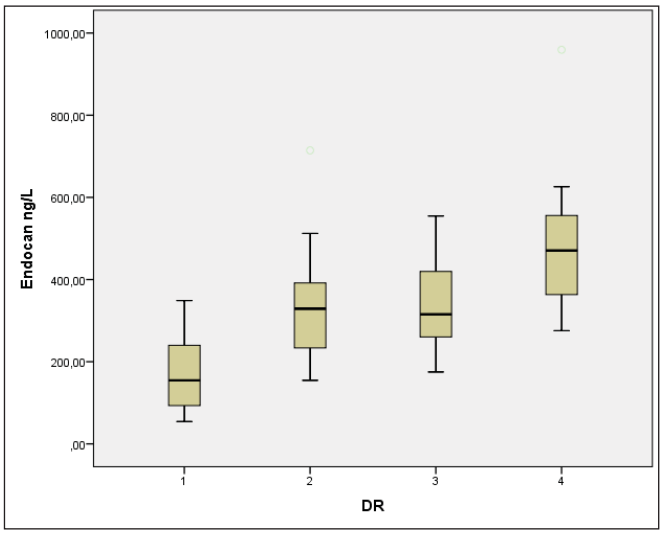

Figure 1. *Endocan (Endothelial cell-specific molecule-1) levels according to diabetic retinopathy stage (One Way Anova; p<0.05).*

DR: Diabetic Rethinopathy

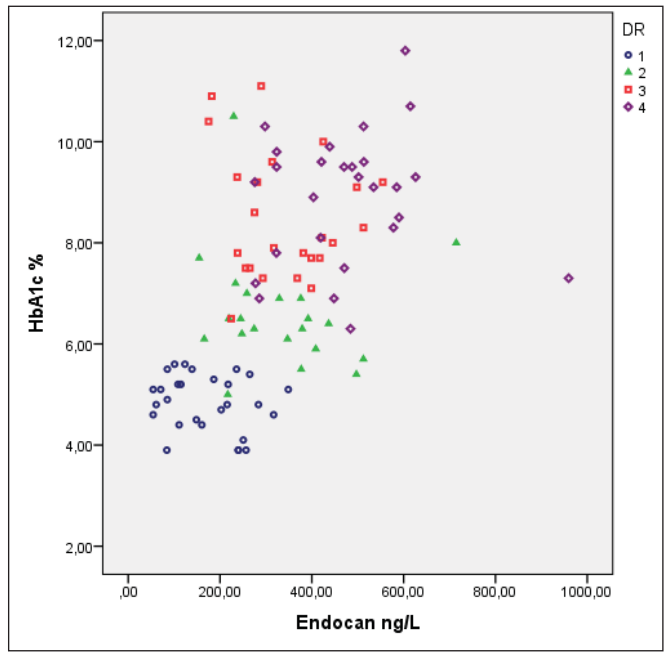

Figure 2. *HbA1c levels according to endocan levels of groups (One Way Anova; p<0.05).*

Table 2. *Independent predictors of DR with Lineer logistic regression analysis p-value, OR with 95% CI*			
Variable	**p-value**	**Odd ratio (OR)**	**95% C.I.**
Age (year)	0.329	0.007	0.007-0.021
Endocan (ng/L)	**<0.05**	0.002	0.001-0.003
HbA1c (%)	**<0.05**	0.358	0.291-0.426
BMI (Kg/m²)	0.363	0.01	0.012-0.032
Creatinine (µmol/L)	**<0.05**	1.126	0.641-1.611
Urea (Mmol/L)	0.122	0.002	0.000-0.004

of proinflammatory molecules and free radicals that contribute to the pathogenesis of diabetic retinopathy.[13] These proinflammatory molecules and free radicals cause chronic low-grade inflammation and vascular endothelial dysfunction.[14] This chronic inflammation and vascular endothelial dysfunction also cause vascular dilatation deterioration and increase in adhesion molecules through nitric oxide, endothelin and prostaglandins. This condition is involved in the pathogenesis of many diseases such as atherosclerosis and hypertension, and is primarily responsible for the microvascular and macrovascular complications of diabetes such as diabetic retinopathy, neuropathy and nephropathy.[15-17]

It has been suggested that endocan, which is released from the vascular endothelium and is thought to reflect endothelial activation, has an important role in the regulation of angiogenesis and inflammatory process.[4] Inflammatory molecules are also known to affect endocan levels. Growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and proinflammatory molecules such as TNF-alpha, IL-1 increase the endocan level.[6]

It has been found that impaired kidney function and chronic kidney failure are associated with increased DR severity.[18] It was emphasized that serum creatinine level is associated with the presence and severity of DR, and it is important to achieve stable kidney functions in order not to increase retinopathy in diabetic patients.[19] Our study also supports these studies and it was observed that there was a significant relationship between DR stage and serum creatinine level. There are many studies showing the relationship between endocan levels and other complications of diabetes such as diabetic nephropathy. In these studies, it has been stated that endocan level has an important role in the pathogenesis of vascular endothelial dysfunction.[20-21] Çıkrıkçıoğlu et al.[20] reported that serum endocane levels of patients with diabetic nephropathy were significantly higher than the control group, and serum endocane levels may be a marker for the management of diabetic nephropathy progression.

Chronic subclinical inflammation is one of the most important causes of microvascular complications such as retinopathy in diabetic patients.[22] Shalwala et al.[23] reported that patients with proliferative DRP and patients with non-proliferative DRP had a similar level of endocan levels in vitreous specimens, and were significantly higher than patients without diabetes alone. Serum endocan levels were not significant between the groups**.** Arman et al.[24] reported that serum endocan levels of patients with unregulated type 2 diabetes were significantly higher than those of the control group, and that both endocan levels and HbA1c levels decreased with lifestyle changes. Asrar et al.[25] investigated the relationship between endocan levels and disease activity and angiogenic markers in the vitreous samples of proliferative DR patients and found that the level of endocan in the vitreous of diabetic patients was significantly higher. They also showed a positive correlation between endocan levels and disease activity and VEGF levels. In our study, we found that serum endocan levels of diabetic patients were significantly higher than the control group, similar to Arman et al.'s study. Although there was no significant difference between group 3 and group 4, the endocane level of the patients in group 4 was higher than the other groups including the third group. This makes us think that serum endocane levels may be increased according to DR's stage, and that endocane may be a marker for DR progression.

In our study, we found a positive correlation between the stage of DR and HbA1c levels. In many studies, it was stated that the strongest risk factor for DR progression was high HbA1c level.[26-27] Glycemic control has shown to have an effect on the presence and severity of DR.[28] The study by Andreasson et al has demonstrated a relationship between HbA1c level and DR stage. Keeping the HbA1c level close to normal has been noted increasing DR development time.[29] Our study supports these studies, we concluded that HbA1c level, which is an indicator of impaired glycemic regulation, is elevated with the stage of DR, and is very important in the progression of DR. However, there was no significant relationship between BMI and DR stage. We attributed this situation to the fact that BMI is a continuously variable parameter. Hwang et al.[30] reported that there is no relationship between DR stage

and BMI (inverse relationship).

Diabetic macular edema is the most important cause of visual impairment in diabetic patients. The pathogenesis of diabetic macular edema is multifactorial and the most important contributor is inflammation that causes deterioration of the inner and outer blood retinal barrier.[31] It is known that endocan is involved in the regulation of angiogenesis and inflammatory processes and can be used as a determinant of vascular dysfunction.[5-6] This suggests that endocan may play a role in the regulation of inflammation in diabetic macular edema. No study has been found in the literature comparing serum endocan levels with diabetic macular edema. In our study, we found that serum endocan level was correlated with diabetic macular edema and there was a positive correlation between DME and endocane levels. Further studies are needed at the experimental and molecular level to determine the role of endocan in the pathogenesis of diabetic macular edema.

CONCLUSION

In our study, there was a relationship between serum endocan level and DR stage. Serum endocan level can reveal important information about the severity and stage of DR such as serum creatinine and HbA1c level. There is a potential need for further research to demonstrate whether endocan is involved in DR pathogenesis.

Declaration of competing interest

All the authors declared no conflicts of interest in association with the present study.

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Ethics committee approval

Ethics committee approval was received for this study from the ethics committee of Kafkas University, Faculty of Medicine, Clinical Research Ethics Committee (2018-6-26/124).

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