Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2020; 59 (4): 310-315

Higher serum Endocan levels are involved in the pathophysiology of chronic venous insufficiency

Kronik venöz yetmezlik patofizyolojisinde yüksek serum Endokan seviyeleri yer alır

Mustafa Doğduş[1] Aydın Koç[2]

[1] Department of Cardiology, Usak University, Training and Research Hospital, Usak, Turkey

[2] Department of Cardiovascular Surgery, Usak University, Training and Research Hospital, Usak, Turkey

Abstract

Aim: Chronic venous insufficiency (CVI) is a common but neglected pathology of the cardiovascular system with high diagnosis and treatment costs and negative effects on patients' quality of life. Endocan is a dermatan sulfate proteoglycan and secreted by activated vascular endothelium. We hypothesized that higher Endocan levels may be associated with the pathophysiology of CVI. Thus, in the current study, we aimed to assess the relationship between serum Endocan levels and CVI.

Materials and Methods: Forty-four patients with CVI and 50 age- and gender- matched subjects were enrolled into the study. The baseline clinical characteristics of the patients were obtained and serum Endocan levels were calculated.

Results: The mean Endocan level and mean triglyceride (TG) level were significantly higher in the CVI (+) group compared to the CVI (-) group ($p<0.001$ and $p=0.001$, respectively). In multivariate logistic regression analysis; Endocan ($p<0.001$, Odds ratio (OR) = 3.48, 95% Confidence interval (C.I.) = 1.54–8.16), and TG ($p=0.009$, OR = 1.85, 95% C.I. = 1.36–3.55) were found to be independent predictors of CVI. ROC analysis was performed to find out the ideal Endocan cut-off value for predicting CVI. An Endocan value of >2.58 ng/mL has 92.4% sensitivity, 76.6% specificity for the prediction of the CVI (AUC 0.841, ($p<0.001$)).

Conclusions: In the present study, we evaluated the relationship between serum Endocan levels and CVI. Our findings suggest that increased Endocan levels may be involved in the pathogenesis of CVI.

Keywords: Endocan, chronic venous insufficiency, endothelial dysfunction.

Öz

Amaç: *Kronik venöz yetmezlik (KVY), kardiyovasküler sistemin yüksek tanı ve tedavi maliyetleri ile hastaların yaşam kalitesi üzerinde olumsuz etkileri olan, yaygın fakat ihmal edilmiş bir patolojisidir. Endokan bir dermatan sülfat proteoglikandır ve aktif damar endoteli tarafından salgılanır. Yüksek Endokan seviyeleri ile KVY patofizyolojisi arasında ilişkili olabileceğini varsaydık. Bu nedenle bu çalışmada serum Endokan düzeyleri ile KVY arasındaki ilişkiyi değerlendirmeyi amaçladık.*

Gereç ve Yöntem: *Kronik venöz yetmezliği olan kırk dört hasta ile yaş ve cinsiyet uyumlu 50 olgu çalışmaya dahil edildi. Hastaların başlangıç klinik özellikleri kaydedildi ve serum Endokan düzeyleri hesaplandı.*

Bulgular: *Ortalama Endokan düzeyi ile ortalama trigliserit (TG) düzeyi, KVY (+) grubunda KVY (-) grubuna göre anlamlı olarak yüksekti (sırasıyla $p<0,001$ ve $p = 0,001$). Çok değişkenli lojistik regresyon analizinde; Endokan ($p<0,001$, Oran oranı (OR) = 3,48, %95 Güven aralığı (CI) = 1,54–8,16) ve TG ($p = 0,009$, OR = 1,85, %95 CI = 1,36-3,55)' nin bağımsız KVY prediktörleri oldukları bulundu.*

Corresponding author: Mustafa Doğduş
Department of Cardiology, Usak University, Training and Research Hospital, Usak, Turkey
E-mail: *mdogdus@hotmail.com*
Application date: 06.07.2020 Accepted: 28.08.2020

KVY tahmininde ideal Endokan kesme değerini bulmak için ROC analizi yapıldı. > 2,58 ng/ml'lik bir Endokan değeri, KVY tahmini için %92,4 duyarlılığa, %76,6 özgüllüğe sahiptir (AUC 0,841, p <0,001).

***Sonuçlar**: Bu çalışmada, serum Endocan seviyeleri ile KVY arasındaki ilişkiyi değerlendirdik. Bulgularımız, artmış Endokan düzeylerinin KVY patogenezinde rol oynayabileceğini göstermektedir.*

***Anahtar Sözcükler:** Endokan, kronik venöz yetmezlik, endotel disfonksiyonu.*

Introduction

Chronic venous disease (CVD) is a common but neglected pathology of the cardiovascular system with high diagnosis and treatment costs and negative effects on patients' quality of life (1, 2). "Venous hypertension (V-HT)" plays a pivotal role in this pathophysiological process. V-HT is associated with venous reflux due to incompetent venous valves, which ultimately reduces venous return, leading to blood pooling, hypoxia and inflammation. The inflammatory mechanisms accompanied by the liberation of cytokines, proteases, and reactive oxygen products contribute to endothelial damage and further pathological remodeling of the vein wall (3). Endothelial cells undergoing injury release a variety of soluble particles known as biochemical markers of endothelial dysfunction (4). The spectrum of CVD clinical presentations has been defined according to the Clinical, Etiological, Anatomical, and Pathophysiological (CEAP) classification system, for which the clinical description ranges from C0 to C6 (5). When more advanced signs of CVD (C3-C6) are present, the term "chronic venous insufficiency (CVI)" is used.

Endocan (endothelial cell-specific molecule-1) is a dermatan sulfate proteoglycan and secreted by activated vascular endothelium (6, 7). It was known that Endocan has an active role in the pathogenesis of cardiovascular diseases, endothelial dysfunction, and inflammatory processes (8, 9). Throughout the last decades, it has been thought that inflammation and endothelial dysfunction have potential roles in the pathogenesis of CVI. Also, increased serum Endocan levels have been shown to be related to endothelial dysfunction and inflammation. Therefore, we hypothesized that higher Endocan levels may be associated with the pathophysiology of CVI. In the current study, we aimed to assess the relationship between serum Endocan levels and CVI.

Materials and Methods

Study population

The present study was a single center, cross-sectional observational study. 44 patients with the stage of C3-C6 CVD [CVI (+) group] and 50 age- and gender- matched subjects [CVI (-) group] were enrolled into the study between October 2018 and December 2019. CVD was evaluated according to CEAP classification. CVI was defined as CVD patients with C3-C6 stage. Exclusion criteria were the history of acute coronary syndrome during at past 3 months and coronary artery bypass grafting, severe systemic inflammatory disease, connective tissue disorders, cardiac pacemaker, severe heart failure, cardiomyopathy, valvular heart diseases, LVEF < 55%, uncontrolled hypertension, chronic renal and hepatic failure, malignancy and use of cardiotoxic agent, thyroid dysfunction, congenital heart disease, and secondary CVD. The study was approved by the local ethics committee. Informed consent form was obtained from all of the patients included in the study.

Demographic, clinical, and echocardiographic assessment of the study population

The baseline clinical characteristics of the study population were recorded. Information regarding risk factors, including age, gender, diabetes mellitus, hypertension, hyperlipidemia, and smoking status was obtained. Body mass index (BMI) was calculated as body weight (kg) divided by height squared (m2). Cigarette smoking was defined as smoking ≥ 1 packet of cigarettes a day. Venous blood samples were collected for blood count, routine biochemistry parameters, and lipid profiles. The serum levels of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) of all the subjects were analyzed. TC (reference range 65-200 mg/dL), TG (reference range 50-200 mg/dL) and HDL-C (reference range 35-60 mg/dL) concentrations were measured by biochemical analyses using commercial kits. LDL-C

(reference range 60-130 mg/dL) was determined using the Friedewald equation.

Echocardiographic image obtaining was performed in accordance with the American Society of Echocardiography (ASE) criteria from the parasternal long-axis, parasternal short-axis and apical four chamber sections in the left lateral position, and subcostal section in the supine position with one-lead ECG monitoring (10). All patients underwent 2-dimensional transthoracic echocardiographic (HD11 XE Ultrasound system, Philips, Canada) evaluation equipped with a 1.5- 4.0 MHz transducer. Standard 2-dimensional, M-Mode, pulsed-Doppler measures were done according to the updated recommendations for cardiac chamber quantification by echocardiography in adults. The left ventricular ejection fraction (LVEF) was measured using modified Simpson's method.

Analysis of serum Endocan levels

The peripheral venous blood (4 ml) was collected into plain blood collection tubes without any additives. The patient's serum samples were separated by centrifugation at 1500g for 10 min and stored at -80°C until analysis. Serum Endocan levels were calculated using an enzyme-linked immunosorbent assay (ELISA) kit with high sensitivity and specificity for detection of Endocan (Human Endothelial Cell-specific Molecule-1 ELISA Kit; Shanghai YB-Technology, Shanghai, China). All blood samples were routinely tested by ELISA in duplicate, and the results were averaged. Preliminary data obtained in our laboratory showed that the intra-assay and inter-assay coefficients of variation for Endocan were 4.9% and 5.3%, respectively.

Statistical analysis

For variable analysis, SPSS 25.0 (IBM Corp., Armonk, NY, USA) program was used. Normally distributed continuous data were expressed as mean ± standard deviation. Continuous variables that are not normally distributed were expressed as median, and categorical variables were expressed as n and percentages. The normal distribution of the data was evaluated by Kolmogorov-Smirnov test and Shapiro-Wilk test and the variance homogeneity was evaluated by the Levene test. Data with a normal distribution were compared using Student's t-test and data with a non-normal distribution were compared using the Mann-Whitney U-test. To compare categorical variables, Pearson chi-square and Fisher Exact tests were tested using exact results. Receiver operating characteristics (ROC) curve was performed to determine the cut-off value of Endocan to predict the CVI. Multivariate logistic regression analysis was performed to identify the independent predictors of CVI. Variables were examined at 95% confidence level. A p-value < 0.05 was considered as statistically significant.

Results

A total of 94 patients were enrolled in the current study [44 patients in CVI (+) group and 50 patients in CVI (-) group]. The baseline clinical characteristics of the study population are demonstrated in Table-1. The mean age of the patients was 64.1 ± 10.4 years, and 53 (56.4%) of the patients were female. The number of smokers and the frequency of HLP were higher in the CVI (+) group than in the CVI (-) group (p=0.003 and p=0.026, respectively) (Table-1).

The mean Endocan level (3.08 ± 0.33 vs. 2.11 ± 0.29 ng/mL), mean TG level (170.5 ± 44.3 vs. 133.4 ± 42.9 mg/dL), mean PDW value (14.3 ± 4.8 vs. 11.7 ± 4.6 K/uL), and mean MPV value (11.9 ± 2.1 vs. 10.8 ± 1.9 fL) were significantly higher in the CVI (+) group compared to the CVI (-) group (p<0.001, p=0.001, p=0.035, and p=0.041, respectively). There were not any significant differences between groups in terms of age, gender, LVEF, BMI, TC, HDL-C, LDL-C, and history of HT and DM (Table-1).

In multivariate logistic regression analysis; Endocan (p<0.001, Odds ratio (OR) = 3.48, 95% Confidence interval (C.I.) = 1.54–8.16), and TG (p=0.009, OR = 1.85, 95% C.I. = 1.36–3.55) were found to be independent predictors of CVI (Table-2).

ROC analysis was performed to find out the ideal Endocan cut-off value for predicting CVI. An Endocan value of > 2.58 ng/mL has 92.4% sensitivity, 76.6% specificity for the prediction of the CVI (AUC 0.841, p<0.001) (Figure-1).

Discussion

In the present study, we demonstrated that serum Endocan level is an independent predictor of the CVI. We can say that Endocan has an active role in the pathogenesis of CVI.

CVI is associated with disability and significant adverse impact on quality of life. In the worldwide, CVI may affect more than 60% of the adult population (11). The etiology and

pathophysiology of CVI have been intensively studied in the past decades. Various epidemiological studies showed that women tend to suffer from CVI more frequently than men (12, 13). Similar to the literature, in our study, women (56.8%) were more common among patients with CVI.

Table-1. The baseline clinical characteristics of the patients.

	CVI (-) group (n=50)	**CVI (+) group (n=44)**	**P Value**
Age, years	63.2 ± 10.4	64.8 ± 10.9	0.492
Female gender, n (%)	28 (56)	25 (56.8)	0.677
LVEF (%)	60.8 ± 4.8	59.5 ± 4.1	0.128
Smoking, n (%)	16 (32)	24 (54.5)	0.003
BMI (kg/m^2)	28.3 ± 3.9	27.9 ± 3.8	0.486
Hypertension, n (%)	26 (52)	24 (54.5)	0.162
Diabetes Mellitus, n (%)	12 (24)	12 (27.2)	0.104
Hyperlipidemia, n (%)	14 (28)	18 (40.9)	0.026
Endocan (ng/mL)	2.11 ± 0.29	3.08 ± 0.33	<0.001
Fasting Glucose (mg/dl)	110.8 ± 21.7	119.5 ± 24.4	0.093
Creatinine (mg/dl)	1.06 ± 0.3	1.12 ± 0.2	0.213
TC (mg/dL)	147.2 ± 30.3	151.6 ± 31.8	0.112
TG (mg/dL)	133.4 ± 42.9	170.5 ± 44.3	0.001
HDL-C (mg/dL)	36.5 ± 8.2	35.8 ± 8.4	0.449
LDL-C (mg/dL)	115.8 ± 36.4	127.3 ± 37.5	0.064
Hemoglobin (g/dL)	14.2 ± 2.2	14.1 ± 2.5	0.789
Platelet (x1000) (K/uL)	272 (121 / 395)	295 (119 / 427)	0.101
MPV (fL)	10.8 ± 1.9	11.9 ± 2.1	0.041
PDW (K/uL)	11.7 ± 4.6	14.3 ± 4.8	0.035

CVI: chronic venous insufficiency, BMI: body mass index, LVEF: left ventricular ejection fraction, TC: total cholesterol, TG: triglyceride, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, MPV: mean platelet volume, PDW: platelet distribution width

Table-2. The independent predictors of CVI in multivariate analysis.

Variable	P	Odss Ratio (%95 C.I.)
Endocan	<0.001	3.48 (1.54 – 8.16)
TG	0.009	1.85 (1.36 – 3.55)
Smoking	0.087	1.26 (1.13 – 1.92)
PDW	0.094	1.05 (0.92 – 1.22)
MPV	0.135	0.93 (0.85 – 1.17)
Hyperlipidemia	0.244	0.89 (0.76 – 1.11)
Diabetes Mellitus	0.437	0.85 (0.72 – 1.02)

CVI: chronic venous insufficiency, TG: triglyceride, PDW: platelet distribution width, MPV: mean platelet volume, C.I.: Confidence interval

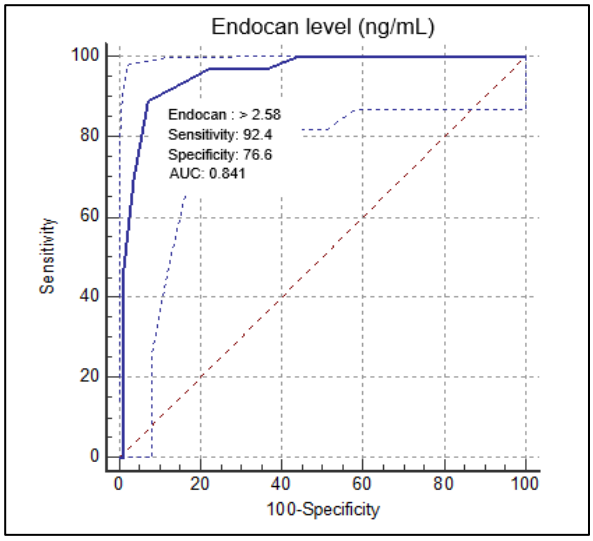

Figure-1. ROC curve of serum Endocan level for predicting CVI.

Subjects with V-HT, which may occur consequent to predisposing factors such as advanced age, family history, obesity, and environmental/occupational factors are at high risk of developing CVI (14, 15). V-HT is the main pathophysiological mechanism that potentiate disease progression. It probably generates a mechanical stress in the vein wall, which may initiate early activation of inflammatory cascade (16-18). Therefore, CVI is considered as a blood pressure-driven inflammatory disease, where inflammatory factors play a significant role.

Although endothelial dysfunction is considered to be implicated in the pathogenesis of CVI, the number of studies assessing the endothelial status in patients with venous disease is limited. Budzyń et al. measured the concentration of selected markers of endothelial dysfunction: von Willebrand factor (vWf), soluble P selectin (sP-selectin), soluble thrombomodulin (sTM) and soluble VE cadherin (sVE-cadherin) in CVI women who constitute the most numerous group of patients suffering from venous disease (19). They showed the presence of endothelial dysfunction in patients with CVI and thought that it may be associated with inflammation and enhanced oxidative stress. In another study, Bryan et al. evaluated whether higher P-selectin is associated with CVI (20). They demonstrated that higher circulating P-selectin was associated with severe CVI. Increased serum Endocan levels have been shown to be related to endothelial dysfunction, oxidative stress, and inflammation. Endocan causes endothelial dysfunction by promoting adhesion molecules and migration of leukocytes across the damaged endothelium. In the current study, we evaluated the association between serum Endocan levels and CVI. We found that the mean Endocan level was significantly higher in the CVI (+) group compared to the CVI (-) group, and we demonstrated that elevated serum Endocan level is an independent predictor of CVI. According to these results, we can say that Endocan has an active role in the pathophysiology of CVI and might be used as a biomarker.

Auzky et al. evaluated the association between symptoms of CVD in the lower extremities and cardiovascular risk factors (21). A significantly higher prevalence of the following were observed in women with any or severe CVD symptoms: coronary artery disease, history of diabetes mellitus, increased body mass index, waist circumference, serum TG, serum C-reactive protein and lower serum HDL cholesterol. Similar to their study, in our study, the frequency of smoking and TG values were higher in patients with CVI. This relationship may be attributed to the roles of TG and smoking in chronic inflammation, oxidative stress, endothelial dysfunction, and atherosclerosis.

Limitations

The current study has several limitations. Small patient population (94 patients) is the main limitation of our study. Because of the cross-sectional design of our study was unable to distinguish causality between serum Endocan levels and CVI clearly. We could not evaluate another biomarker of endothelial dysfunction, platelet activation or inflammation. Hence, more large-scale, multicenter studies with follow-up are needed to determine the effect of Endocan in clinical practice.

Conclusion

In the current study, we evaluated the relationship between serum Endocan levels and CVI. Our findings suggest that increased Endocan levels may be involved in the pathogenesis of CVI. The multivariate logistic regression models revealed that Endocan was found to be independent factor predicting CVI. More comprehensive prospective studies investigating the pathophysiology of CVI are needed.

Conflict of interests

There is no conflict of interests regarding the publication of this paper for the authors.

References

1. Bergan JJ, Schmid-Schonbein GW, Smith PD, Nicolaides AN, Boisseau MR, Eklof B. Chronic venous disease. N Engl J Med 2006; 355: 488-98.
2. Eberhardt RT, Raffetto JD. Chronic venous insufficiency. Circulation 2014; 130: 333-46.
3. Raffetto JD, Khalil RA. Mechanisms of varicose vein formation: valve dysfunction and wall dilation. Phlebology 2008; 23: 85-98.
4. Constans J, Conri C. Circulating markers of endothelial function in cardiovascular disease. Clin Chim Acta 2006; 368: 33-47.
5. Eklof B, Rutherford RB, Bergan JJ, et al. Revision of the CEAP classification for chronic venous disorders: Consensus statement. J Vasc Surg 2004; 40: 1248-52.
6. Sarrazin S, Adam E, Lyon M, et al. Endocan or endothelial cell specific molecule-1 (ESM-1): a potential novel endothelial cell marker and a new target for cancer therapy. Biochim Biophys Acta Rev Cancer 2006; 1765: 25-37.
7. Bechard D, Scherpereel A, Hammad H, et al. Human endothelial-cell specific molecule-1 binds directly to the integrin CD11a/CD18 (LFA-1) and blocks binding to intercellular adhesion molecule-11. J Immunol 2001; 167: 3099-106.
8. Aparci M, Isilak Z, Uz O, Yalcin M, Kucuk U. Endocan and endothelial dysfunction. Angiology 2015; 66: 488–9.
9. Zhang SM, Zuo L, Zhou Q, et al. Expression and distribution of endocan in human tissues. Biotech Histochem 2012; 87: 172–8.
10. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging 2015; 16 (3): 233-70.
11. Rabe E, Guex JJ, Puskas A, Scuderi A, Fernandez Quesada F. Epidemiology of chronic venous disorders in geographically diverse populations: results from the Vein Consult Program. Int Angiol 2012; 31: 105-15.
12. Nicolaides AN, Allegra C, Bergan J, et al. American venous forum. Management of chronic venous disorders of the lower limbs: guidelines according to scientific evidence. Int Angiol 2008; 27: 1-59.
13. Canonico S, Gallo C, Paolliso G, et al. Prevalence of varicose veins in an Italian elderly population. Angiology 1998; 49: 129-35.
14. Fowkes FG, Evans CJ, Lee AJ. Prevalence and risk factors of chronic venous insufficiency. Angiology 2001; 52: 5-15.
15. Jawien A. The influence of environmental factors in chronic venous insufficiency. Angiology 2003; 54: 19-31.
16. Georgescu A, Alexandru N, Popov D, et al. Chronic venous insufficiency is associated with elevated level of circulating microparticles. J Thromb Haemost 2009; 7: 1566-75.
17. Pocock ES, Alsaigh T, Mazor R, Schmid-SchoÈnbein GW. Cellular and molecular basis of venous insufficiency. Vasc Cell 2014; 6: 24.
18. Hadi HA, Carr CS, Al Suwaidi J. Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. Vasc Health Risk Manag 2005; 1: 183-98.
19. Budzyń M, Iskra M, Turkiewicz W, Krasiński Z, Gryszczyńska B, Kasprzak MP. Plasma concentration of selected biochemical markers of endothelial dysfunction in women with various severity of chronic venous insufficiency (CVI)-A pilot study. PLoS One 2018; 13 (1): e0191902.
20. Bryan LJ, Callas PW, Criqui MH, Cushman M. Higher soluble P-selectin is associated with chronic venous insufficiency: The San Diego Population Study. Thromb Res 2012; 130 (5): 716-9.
21. Auzky O, Lanska V, Pitha J, Roztocil K. Association between symptoms of chronic venous disease in the lower extremities and cardiovascular risk factors in middle-aged women. Int Angiol 2011; 30 (4): 335-41.