



HHS Public Access

Author manuscript

Angiology. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

Angiology. 2016 April ; 67(4): 364–374. doi:10.1177/0003319715590558.

Effect of CPAP on New Endothelial Dysfunction Marker, Endocan, in People With Obstructive Sleep Apnea

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Abstract

Obstructive sleep apnea (OSA) is associated with increased cardiovascular (CV) morbidity and mortality. Endocan is a surrogate endothelial dysfunction marker that may be associated with CV risk factors. In this study, we tested whether serum endocan is a biomarker for OSA. Serum endocan levels were measured at baseline in 40 patients with OSA and 40 healthy controls and after 3 months of continuous positive airway pressure (CPAP) treatment in the patients with OSA. All participants were evaluated by full polysomnography. Flow-mediated dilatation (FMD) and carotid intima media thickness (cIMT) were measured in all participants. Endocan levels were significantly higher in patients with OSA than in healthy controls. After adjusting confounders, endocan was a good predictor of OSA. Endocan levels correlated with OSA severity (measured by the apnea–hypopnea index [AHI]). After 3 months of CPAP treatment, endocan levels significantly decreased. Endocan levels were significantly and independently correlated with cIMT and FMD after multiple adjustments. The cIMT and FMD also had significant and independent correlation with AHI. Endocan might be a useful marker for the predisposition of patients with OSA to premature vascular disease.

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Authors' Note

All authors were involved in conception and design, acquisition of data, or analysis and interpretation of data; drafting and revising the article; final approval of the version to be published; and all authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

The study was approved by local ethical committee of Namik Kemal University.

Keywords

biomarkers; carotid intima media thickness; endocan; flow-mediated dilatation; obstructive sleep apnea

Introduction

Obstructive sleep apnea (OSA), a frequent and treatable, yet often overlooked condition, has been implicated in the initiation and progression cardiovascular (CV) disease.¹⁻³ Accordingly, a reliable biomarker of OSA would be useful, particularly to recognize patients at elevated risk of future CV events.

The mechanisms underlying the association between CV disease and OSA are not completely understood; however, endothelial dysfunction, an early indicator of atherosclerosis, has been proposed as a key mechanism linking OSA with CV risk.⁴⁻⁶ Even in patients with OSA without overt CV disease, endothelial dysfunction may influence the progression of vascular conditions such as hypertension, coronary artery disease (CAD), and ischemic stroke.⁷

The association between OSA and endothelial dysfunction is thought to be caused by recurrent hypoxia and reoxygenation (intermittent hypoxia) that occurs during recurrent sleep apnea and hypopnea.⁸ Mechanisms by which chronic intermittent hypoxia may negatively affect endothelial function include increased reactive oxygen species and oxidative stress,⁹ reduced endothelial nitric oxide bioavailability,¹⁰ sympathetic over activity,¹¹ and vascular inflammation.¹² Impaired endothelial function is thought to be a predictor of progression of atherosclerosis.⁷

An early step in vascular inflammation progressing to atherogenesis is the attachment of circulating leukocytes to the endothelium and their movement across endothelial cells into inflammatory sites. Recruitment and accumulation of leukocytes to the endothelium are mediated by an upregulation of adhesion molecules such as intracellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), which are expressed on the endothelial membrane in response to several cytokines.^{13,14} The serum levels of these soluble adhesion molecules have been shown to be increased in patients with OSA.¹⁵

A novel biomarker endocan, earlier termed endothelial cell specific molecule 1, is a proteoglycan secreted by vascular endothelium and can be detected in the blood.¹⁶ Endocan can take part in molecular interactions with a wide range of biologically active moieties, which are essential for the regulation of biological processes such as cell adhesion, migration, and proliferation. The binding of circulating endocan to leukocyte ligand for VCAM-1—very late antigen 4 (VLA-4)—and to leukocyte ligand for ICAM-1—lymphocyte function-associated antigen 1—seems to be important in leukocyte adhesion and interaction with activated endothelium.^{17,18} Endocan may play a role in endothelium-dependent pathological disorders and may be a surrogate endothelial dysfunction marker.¹⁹

We assessed the effect of OSA on serum endocan levels compared with matched controls and whether these levels are related to the severity of OSA and improve following 3 months of continuous positive airway pressure (CPAP) treatment. We also tested whether endocan levels were significantly correlated with endothelial dysfunction, which was measured by flow mediated dilatation (FMD) and carotid intima media thickness (cIMT).

Methods

Study Population

We performed a single center, prospective study on consecutive participants attending our sleep laboratory for the first time between September 2014 and March 2015. All the patients had confirmed clinical and polysomnographic diagnosis of OSA. Based on the American Academy of Sleep Medicine (AASM) guideline,²⁰ patients with moderate-to-severe OSA (apnea-hypopnea index (AHI) >15 events/h) were included.

The control group consisted of participants who were referred to our sleep laboratory with a suspicion of OSA. However, we confirmed by full polysomnography (PSG; AHI <5/h) that they did not have OSA. The controls were matched according to age, body mass index (BMI), sex, and smoking status. They were subject to the same exclusion criteria as the patients with OSA.

Exclusion Criteria

Already CPAP-treated patients and patients who had conditions that could potentially affect circulating endocan levels were excluded. Accordingly, we excluded patients who had underlying cancer, chronic inflammatory disease, any systemic infection, uncontrolled hypertension, and diabetes mellitus (DM), a known acute coronary syndrome, valvular heart disease, a known thyroid, renal or hepatic dysfunction, and medication interfering with the study protocol such as glucocorticoids or nonsteroidal anti-inflammatory drugs.

Study Design

Data were collected in all participants during recruitment; these data included clinical assessment, full PSG, assessment of FMD, measurement of cIMT, fasting venous blood collection for endocan and other biochemistry markers, and Epworth Sleepiness Scale (ESS). Endocan and other putative markers of inflammation were reevaluated in patients with OSA after 3 months of CPAP therapy and compared with baseline values.

Clinical Assessment and Questionnaires

Physical examination and the administration of questionnaires were performed the day before the sleep study. Current hypertension was defined as systolic blood pressure (BP) ≥ 140 mm Hg or diastolic BP ≥ 90 mm Hg.²¹ The BMI was calculated as weight (kg) divided by the square of height (m). History of hypertension was defined as the use of antihypertensive medication, excluding the use of β -blockers for reasons other than hypertension. Coronary artery disease was defined as previous myocardial infarction, percutaneous coronary intervention, or coronary artery bypass grafting. Angina was not included due to the lack of objective assessment. Smoking status is reported as self-reported

current versus previous/never smoking. All participants completed the ESS questionnaire before the PSG recording.²² Excessive daytime sleepiness was defined as having an ESS score >10.

Sleep Study

All participants underwent full PSG (Embla N 700 sleep system; Natus Medical Incorporated, Pleasanton, California). At least 6-hour PSG data were recorded. The PSG recordings included 6-channel electroencephalography, 2-channel electrooculography, 2-channel submental electromyography, oxygen saturation by an oximeter finger probe, respiratory movements via chest and abdominal belts, airflow both via nasal pressure sensor and oronasal thermistor, electrocardiography, and leg movements via both tibial anterolateral electrodes. Sleep stages were scored in 30-second periods by a certified registered sleep physician according to the criteria of AASM.²⁰ Apneas were defined as decrements in airflow 90% from baseline for 10 seconds using an oronasal thermal sensor. Hypopneas were defined as a 30% decrease in flow lasting at least 10 seconds using a nasal cannula pressure transducer and associated with a 3% or greater oxyhemoglobin desaturation or associated with an arousal. The number of apneas and hypopneas per hour of sleep was calculated to obtain the AHI. The oxygen desaturation index (ODI) was defined as the total numbers of episodes of oxyhemoglobin desaturation 3% from the immediate baseline, 10 seconds but <3 minutes, divided by the total sleep time. The OSA severity was assessed as mild, moderate, and severe according to the AHI values of 5 to 14, 15 to 29, and >30, respectively.²⁰

Titration and Usage of CPAP

Manual titration was started from an initial pressure level of 4 cm H₂O and was increased based on the presence of apneas, hypopneas, snoring, or respiratory effort-related arousals. The pressure was increased in steps of 1 to 2 cm H₂O increments under polysomnographic control by an experienced sleep laboratory technician at intervals of at least 10 minutes. Titration was considered successful when all significant respiratory events stopped, and the patient had spent at least 15 minutes of sleep at the final CPAP level. Before discharge from the hospital, each patient was instructed to use the CPAP therapy regularly each night for at least 6 hours to ensure a therapeutic effect. However, patients were considered compliant with CPAP if they used it for 4 hours/night. Patients were encouraged to consult a sleep technician, should they require any further information with the therapy, equipment, or mask fitting. After 3 months of treatment, CPAP adherence (hours of use) was evaluated by downloaded data from the CPAP device.

Assessment of cIMT

For cIMT measurement, high-resolution B-mode ultrasound (GE Vivid S5; General Electric VingMed Systems, Horten, Norway) equipped with a 12L-RS broadband linear array transducer was used. All participants were positioned in the supine position. The transducer was placed parallel to the far and near walls of the common carotid artery (CCA) so that the lumen diameter of CCA was maximized in the longitudinal plane. The cIMT was measured at a region 1 cm proximal to the bifurcation from both right and left CCA; the mean of both values was calculated. The measurement was acquired from 4 contiguous sites at 1 mm

intervals, and the mean of the 4 measurements was calculated. To evaluate cIMT, lumen–intima and media–adventitia borders were detected as double lines on both walls. Still images were achieved during sonography, and all measurements were evaluated manually. Carotid plaques were eliminated from measurements; any thickening >1.5 mm with narrowing of lumen >50% or 0.5 mm was classified as plaque. All participants were blindly examined by 1 experienced operator (the intraoperator variability was 3.9%).

Flow-Mediated Dilatation

The technique for the measurement of FMD performed was consistent with published guidelines.²³ Briefly, participants fasted for 12 hours and avoided exercise for 6 hours. The participants remained at rest in the recumbent position for at least 15 minutes before the test. Flow-mediated dilatation (FMD) was evaluated in the right arm by high-resolution B-mode ultrasound using a GE Vivid S5 (General Electric VingMed Systems, Horten, Norway) with a 12 MHz linear array transducer. The brachial artery was imaged approximately 2 to 4 cm above the antecubital fossa in the longitudinal plane, where the best image was achieved, and the end diastolic diameter was acquired. The end-diastolic diameter of brachial artery was measured from a single 2-dimensional frames (the average of 3 measurements was used in the analysis). Reactive hyperemia was accomplished by inflating a BP cuff of 50 mm Hg greater than the systolic BP for 5 minutes.²³ Then the cuff was deflated creating shear stress, which acts as the stimulus to induce dilation. Brachial artery diameter was remeasured at maximal dilatation 60 seconds after cuff deflation. Thus, the diameter of the brachial artery was measured at baseline and 60 seconds after cuff deflation. Ultrasound images were recorded on super-VHS videotape and 1 experienced operator blinded to patient data calculated the FMD (the intraoperator variability was 1.7%). The formula used for FMD calculation was [maximum diameter after cuff deflation – baseline diameter]/baseline diameter × 100.

Biochemical Analysis

Blood samples were collected prior to the sleep study and after 3 months of CPAP treatment in those patients with OSA. Investigations were performed in the morning under fasting conditions. Blood samples were taken from an antecubital vein into 3.8% sodium citrate in a proportion of 9:1 (v/v). Platelet poor serum samples were prepared conventionally, aliquoted, and stored at –70°C until the assay. Serum endocan (Aviscera Bioscience, Santa Clara, California) concentrations were analyzed using sandwich enzyme-linked immunosorbent assay according to the manufacturer’s instructions. Intra-assay coefficient of variation of endocan assay ranged from 6% to 8%, whereas interassay coefficient of variation ranged from 10% to 12%. The lower limit of detection for endocan was 0.005 ng/dL. Measurements were carried out using enzyme-linked immunosorbent assay plate reader Bio-Tek Synergy HT (Bio-tek Instruments, Winooski, Vermont). All the samples were measured in duplicate. The normal range of serum endocan was 0.03 to 2.0 ng/dL.

Ethical Committee

The study complied with the Declaration of Helsinki and was approved by the ethics committee of Namik Kemal University Hospital. All participants provided written informed consent.

Sample Size Calculation

The study was designed to show a decrease in endocan level after 3 months of CPAP therapy. For the primary outcome, a difference in endocan level of 0.40 ng/dL between baseline and after CPAP treatment was estimated. A standard deviation (SD) in the difference of 0.22 ng/dL was assumed according to previous findings.¹⁹ To show this difference in a paired sample test design with statistical power of 95% allowing for a type I (α) error of 0.05, 40 patients were required to account for a 10% dropout rate.

Statistical Analysis

Normally distributed continuous variables are expressed as mean \pm SD, and continuous variables with nonnormal distribution are presented as median values and interquartile range. Analyses of normality in the continuous variables were performed using the Shapiro-Wilk test, histograms, and Q-Q plots. Categorical variables were expressed as numbers and percentage. The chi-square test was used to compare proportions in different groups. Student *t* test or Mann-Whitney *U* test was used to compare the 2 independent groups according to distribution state. If tests of normality were met, one-way analysis of variance was used to compare more than 2 groups with a post hoc Tukey's honest significant difference test, and the Kruskal-Wallis test was used, when tests of normality failed. In cases where the Kruskal-Wallis test yielded a statistical significance, post hoc analysis was performed to identify the groups, which showed differences, by Bonferroni-corrected Mann-Whitney *U* test; the cutoff level of α error was reduced to $0.05/(\text{number of tests; Bonferroni correction})$.

Correlations between levels of circulating endocan and AHI, cIMT, and FMD were determined by Pearson correlation. Endocan and cIMT values were natural log-transformed because they were not normally distributed. Univariate associations between continuous baseline characteristics and the presence of OSA were assessed with logistic regression analysis. The Wald test was used to obtain logistic regression analysis parameters. In all multivariate models, backward stepwise selection was used to derive the final model, and significance levels of 0.2 were chosen to include the variable. Variables that correlated significantly with endocan levels in univariate analysis (Pearson correlation) were included in a backward stepwise multiple linear regression analysis. In these models, we forced hypertension, dyslipidemia, and C-reactive protein (CRP) as covariates to adjust for their potential effects on endocan. The CRP was natural log-transformed. The area under the curve (AUC) and receiver operating characteristics (ROCs) for endocan were analyzed to differentiate OSA from the controls. All statistical analyses were performed using SPSS software (version 21.0; IBM Corporation, Armonk, New York). A 2 sided $P < .05$ was considered significant.

Results

A total of 40 patients with OSA and 40 controls were recruited; 14 patients had moderate OSA and 26 had severe OSA. There were no significant intergroup differences in relation to age, sex, BMI, and smoking status. The differences in the percentage of diabetes, dyslipidemia, and hypertension presentation between the groups did not reach statistical

significance. As expected, patients with OSA had a higher ODI and arousal index and tended to have a higher ESS score.

No significant intergroup differences were seen in terms of fasting concentrations of glucose, high-density lipoprotein cholesterol, and triglycerides or the putative inflammatory markers (eg fibrinogen and CRP).

Serum endocan levels were significantly different across the groups ($P < .001$). There were significant differences among the groups in the cIMT ($P < .001$) and FMD ($P < .001$). Patient characteristics, PSG, laboratory, and USG data are shown in Table 1.

Relationship Between Endocan Levels and AHI, cIMT, and FMD

In Pearson correlation, there was a significant positive correlation between serum endocan levels and AHI ($r = 0.714$, $P < .001$) and cIMT ($r = 0.603$, $P < .001$) as shown in Figures 1 and 2, respectively. However, there was a significant inverse correlation between endocan levels and FMD ($r = -0.529$, $P < .001$) as shown in Figure 3.

Variable Associated With Serum Endocan Levels

Simple univariate linear regression analysis of data from all study participants showed that serum endocan levels were higher in relation to having OSA, DM, and hypertension and were directly proportional to FMD, cIMT, AHI, and arousal index. Table 2 shows the results of regression analyses to identify variables that could predict serum endocan level. These variables were then used in a multivariate linear regression analysis, with the exception of arousal index and ODI, which were removed from the model as they showed a very high degree of collinearity with AHI. Additionally, since AHI, cIMT, and FMD had very strong collinearity, 3 different models were used for each variable. Multiple linear regression with serum endocan as the dependent variable showed that serum endocan levels had a significant and independent correlation with the AHI or the cIMT or the FMD and DM (Table 2). Model I to model III explained; 54%, 42%, and 36% of circulating endocan levels orderly (data were not shown in the table).

Effects of CPAP Treatment on Serum Endocan Levels and Other Putative Inflammatory Markers

All patients who completed the study were CPAP compliant (5.60 ± 1.59 h/night); 95th percentile CPAP level was 11.2 ± 2.5 cm H₂O in patients with OSA. After 3 months of CPAP treatment, median endocan levels were significantly decreased compared to baseline values (3.25 ± 2.24 vs 5.01 ± 3.17 ng/dL; $P < .001$; Figure 4). However, endocan levels after CPAP were still higher than in the control group (3.25 ± 2.24 vs 2.48 ± 1.64 ng/dL; $P < .019$; Figure 5).

Other putative inflammatory markers, CRP ($P = .589$) and fibrinogen ($P = .895$) levels, were not changed by CPAP treatment (data not shown in the table).

Power of Endocan in Distinguishing Patients With OSA From Controls by ROC Curve and by Logistic Regression

As shown in Figure 6, ROC curve analysis with a very high AUC value (0.87, $P < .001$, 95% CI: 0.795–0.945) suggested that the optimal diagnostic endocan cutoff value that maximally increased the sensitivity and specificity in the estimation of OSA was 3.61 ng/dL (sensitivity, 82.5%; specificity, 77.5; positive predictive value, 78.6%; and negative predictive value, 81.6%).

After adjustment for hypertension, diabetes, dyslipidemia, and CRP, multivariate logistic regression analysis with OSA as the dependent variable showed that endocan was a good predictor of OSA (odds ratio [OR] = 3.080, $P < .001$, 95% confidence interval (CI): 1.767–5.370) as shown in Table 3.

Evaluation of cIMT and FMD in OSA by Logistic Regression Analysis

Multivariate logistic regression analysis after adjusting for hypertension, diabetes, dyslipidemia, and CRP showed that cIMT (OR = 1.588, $P < .001$, 95% CI: 11.245–2.024) and FMD (OR = 0.224, $P < .001$, 95% CI: 0.117–0.429) had significant and independent relationship with OSA as shown in Table 3.

Discussion

Our key findings were that serum endocan levels were significantly higher in patients with OSA than in the controls. Patients with OSA had a greater cIMT and lower FMD than the controls. In addition, endocan levels correlated with cIMT, FMD, and severity of OSA. Moreover, multivariate modeling of endocan level determinants revealed that AHI, cIMT, FMD, and DM were significantly and independently associated with endocan levels. Furthermore, the high endocan levels decreased after 3 months of CPAP treatment. Thus, circulating endocan levels may be a useful biomarker to identify patients with OSA and they could be useful in evaluating treatment response in these patients. To the best of our knowledge, this is the first well-controlled study that has investigated serum endocan in OSA.

The OSA is associated with increased CV morbidity and mortality; furthermore, the severity of OSA correlates with morbidity.^{24,25} The majority of patients with OSA who need to be treated cannot be identified.²⁶ An explanation is that the diagnostic methods for OSA, such as PSG, are burdensome to perform. Accordingly, a credible biomarker for OSA would be beneficial in recognizing patients who have OSA with a degree of severity that places them at risk for CV disease.

Endocan is a soluble proteoglycan of 50 kDa secreted by vascular endothelium cells, bronchi, and lung submucosal glands.^{27,28} Endocan has been implicated in the development of vascular tissue in health and disease, and its expression is associated with angiogenic switch in stem cells and endothelial–mesenchymal transition process like arterial wall remodeling.²⁹ Current research suggests that endocan might play a key role in endothelium-dependent pathological disorders that makes it a new candidate immune-inflammatory marker for CV diseases and their clinical consequences.³⁰ Accordingly, increased levels of

endocan were reported in deep vein thrombosis,³¹ chronic kidney disease with CV complications,^{14,13} Behçet disease,³² psoriasis vulgaris,³³ and in patients with untreated essential hypertension.¹⁹ Additionally, Wang et al and Kose et al showed an independent correlation between endocan level and the presence and severity of CAD.^{34,35} To verify whether endocan is a potential biomarker of OSA and related with CV disease as a complication of OSA, we recruited patients with moderate and severe OSA, and endocan levels were measured on admission and after 3 months of CPAP treatment as well as compared with a control group. In our study, higher endocan levels were detected in patients with OSA than in controls. In multivariate logistic regression analysis, endocan was an independent and significant indicator of OSA. Furthermore, endocan levels correlated with disease severity, as measured by AHI. We also derived a cutoff value with a high sensitivity and specificity for endocan to distinguish patients with OSA from healthy controls by ROC curve analysis. Taken together, the data suggest that endocan might be a distinguishing biomarker of OSA and can be used to assess disease severity.

Endothelial dysfunction has been implicated as one of the earliest detectable and possibly reversible abnormalities during atherosclerosis and the development of CV disease. Moderate to severe OSA has been associated with endothelial dysfunction, which can improve after CPAP.³⁶ Kohler and coworkers showed impaired endothelial function, even in minimally patients with symptomatic OSA.³⁷ The intermittent hypoxia observed in patients with OSA has been suggested to represent a form of oxidative stress that results in heightened production of oxygen species. Oxidative injury is implicated in the pathogenesis of endothelial dysfunction, atherosclerosis, and CV disease.⁷

Endothelial function within the macrovasculature can be assessed by several methods. It can be measured by invasive catheterization, which is not practical in large numbers of relatively healthy participants.³⁸ However, the FMD test, the approved noninvasive tool used to evaluate endothelial function, and cIMT, a widely accepted surrogate of subclinical atherosclerosis, have been extensively used for their strength to prognosticate future CV events in epidemiologic and clinical studies.³⁹ Recent studies suggest an association exists between OSA and cIMT, FMD.^{37,40–43} Similarly, in our study, multivariate logistic regression analysis showed that OSA was significantly and independently associated with cIMT and FMD. Previous studies showed that age, BMI, and the presence of hypertension, diabetes, and hyperlipidemia can affect cIMT and FMD.^{44,45} In our study, we strictly matched patients with OSA and controls, thus we eliminated important covariates. Here we also showed that endocan levels were associated with increased cIMT and decreased FMD values even after multiple adjustments in linear regression analysis, advocating that in patients with OSA endocan could be a powerful biomarker of vascular disturbances.

The link between OSA and inflammatory markers such as CRP and fibrinogen is still unclear. Observational studies have shown conflicting results concerning the effect of OSA and CPAP treatment on CRP and fibrinogen levels.^{46–49} A prospective study did not yield a reduction in CRP levels after long-term CPAP therapy in patients with OSA.⁵⁰ In a recent randomized well-designed study, CPAP therapy incorporating weight loss did not have a notable cumulative effect on CRP levels, as compared with a weight loss intervention alone.⁵¹ In our study, there was no difference in CRP and fibrinogen levels between patients

with OSA and controls. Furthermore, CPAP did not have any effect on CRP and fibrinogen levels in the OSA group. Our strict matching of groups by age and BMI may account for the lack of difference between patients with OSA and controls. We speculate that obesity, and not OSA, is associated with elevated serum levels of CRP and fibrinogen. However, our study was not powered to show a decrease in CRP and fibrinogen levels.

The impact of CPAP, the first-line therapy of OSA, on CV or inflammatory markers is still debated.⁵² The authors of a recent review summarized the current state of CPAP in OSA, quoting “Although the effects of CPAP on various biomarkers have been investigated in hundreds of open clinical studies, the real effects of CPAP on cardiometabolic biomarkers are conflicting mainly owing to different study designs and the presence of major confounders.” (p.1)⁵³ However, in our study, we eliminated confounders by strictly matching the control and OSA groups and demonstrated a significant reduction in endocan levels after 3 months of CPAP therapy with excellent adherence. We believe that the beneficial effect of CPAP on endocan levels is robust and that changes occur rapidly, making endocan a useful biomarker of OSA.

Limitations

Several limitations should be considered. First, our sample size was relatively small. However, our control group was meticulously matched, and the differences in serum endocan levels between controls, moderate, and severe patients with OSA were considerable. Thus, the results could be considered reliable. Second, ultrasonographic evaluation of FMD and cIMT has been criticized for being technically demanding and operator dependent. So, intraoperator and interobserver variability is important; in our study, we assessed intraobserver variability, but we could not perform interoperator variability. However, advances in the methodology (use of stereotactic probe holder and video recording for accurate edge detection diameter) have greatly diminished inter- and intraoperator variability. Third, we excluded patients who were receiving treatment with glucocorticoids from this analysis because glucocorticoids may alter endocan levels. Thus, further work will be required to assess the generalizability of our findings. Fourth, it is uncertain whether the effect of CPAP on the endocan system would continue over the long period. A long-term prospective study is required to elucidate this issue. Fifth, we could not carry out a comparison between CPAP users and sham CPAP users. A future study that compares CPAP users and sham CPAP users may be warranted. However, such study designs are complicated due to ethical and logistic reasons. Finally, most studies have used an exogenous nitric oxide donor, such as a high dose nitroglycerin to determine the maximum obtainable vasodilator response in FMD assessment. Because of ethical concerns regarding giving high dose nitroglycerin to healthy controls, this vasodilator response was not evaluated.

Conclusions

The study findings suggest that patients with moderate-to-severe OSA had decreased endothelial function and increased atherosclerosis propensity when compared with matched control participants without OSA.

1. Endocan may have a functional role in endothelium-dependent pathology that may provide more pertinent information on initiation and progression of atherosclerosis than nonspecific markers like CRP and fibrinogen.
2. Serum endocan might be a cost-effective and useful marker for the predisposition of patients with OSA to premature vascular disease.
3. Serum endocan may be employed as a molecular signature for monitoring therapeutic response to CPAP.
4. Further larger studies are required to determine whether endocan can be a surrogate endothelial dysfunction marker in OSA.

Acknowledgments

The study was performed at Namik Kemal University, Department of Pulmonary, Sleep Medicine and Department of Cardiology.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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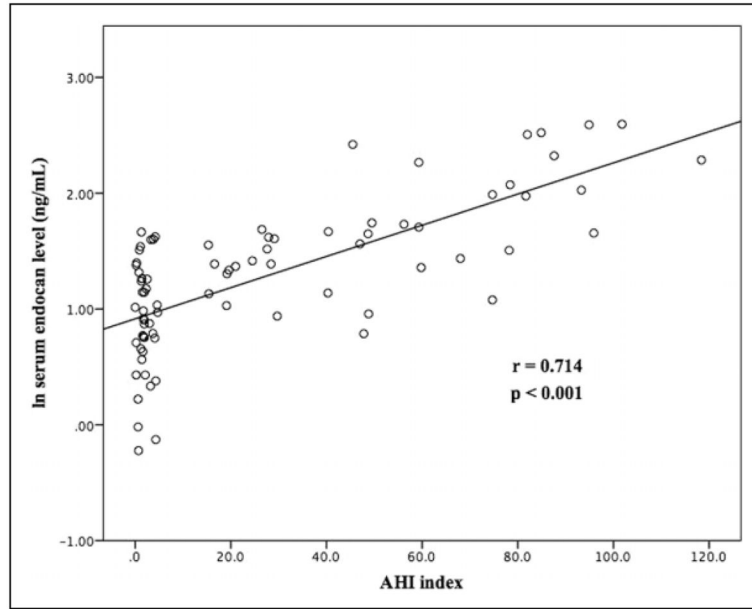


Figure 1. Correlation between serum endocan and apnea–hypopnea index (AHI). Serum endocan was correlated with AHI using Pearson correlation in the total cohort (n = 80; $r = 0.714$ and $P < .001$).

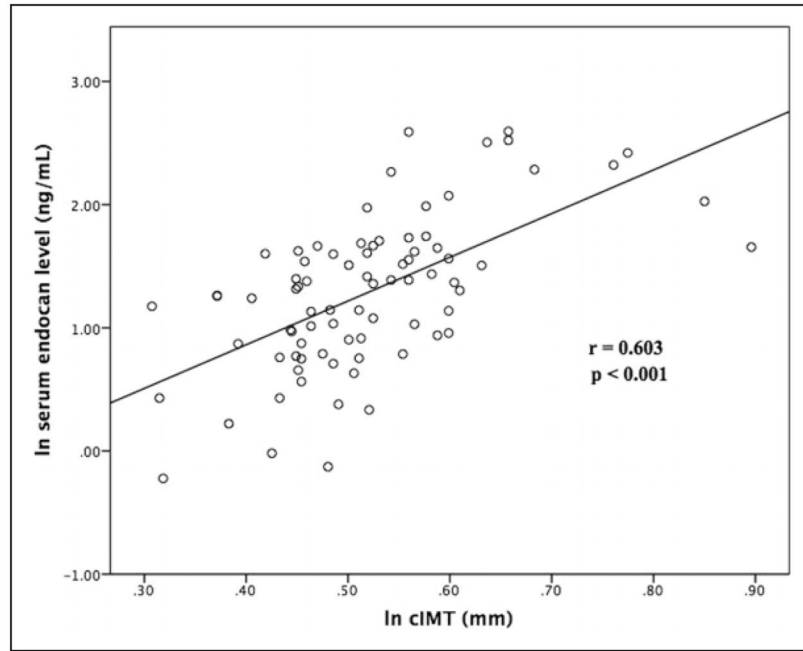


Figure 2. Correlation between serum endocan and carotid intima media thickness (cIMT). Serum endocan was correlated with cIMT using Pearson correlation in the total cohort ($n = 80$; $r = 0.603$ and $P < .001$).

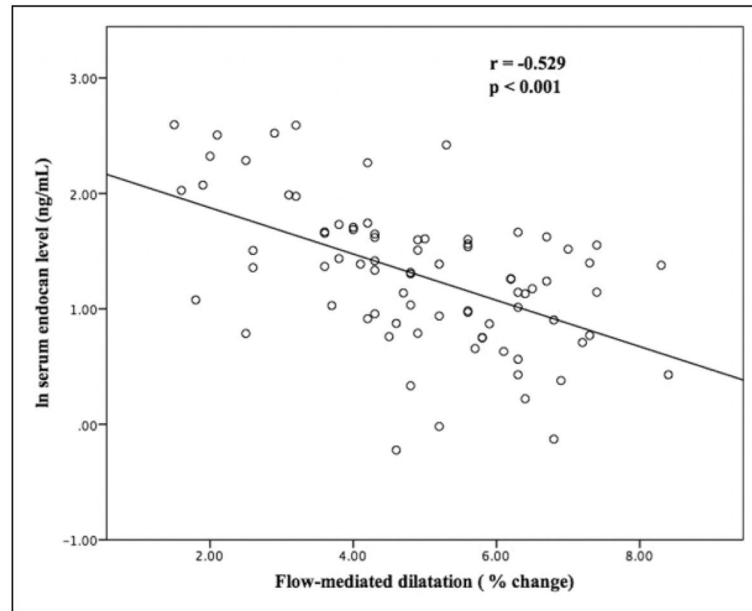


Figure 3. Correlation between serum endocan and flow-mediated dilatation (FMD). Serum endocan was inversely correlated with FMD using Pearson correlation in the total cohort (n = 80; $r = -0.529$ and $P < .001$).

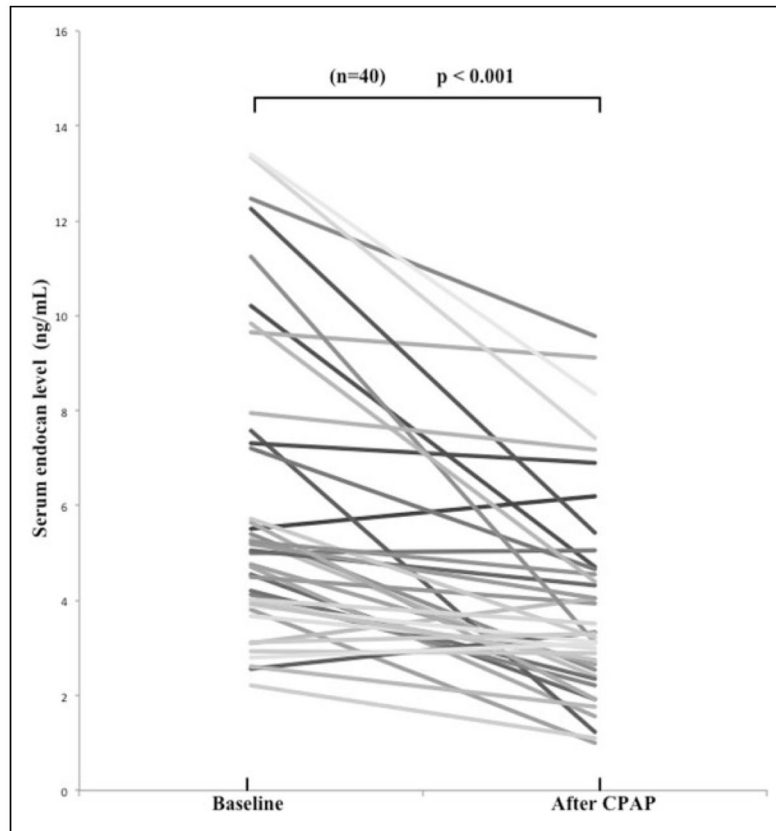


Figure 4. Serum endocan level at baseline and after continuous positive airway pressure (CPAP). Median circulating serum concentration of endocan at baseline was 5.01 ± 3.17 ng/dL compared to 3.25 ± 2.24 ng/dL after CPAP ($n = 40$, $P < .001$).

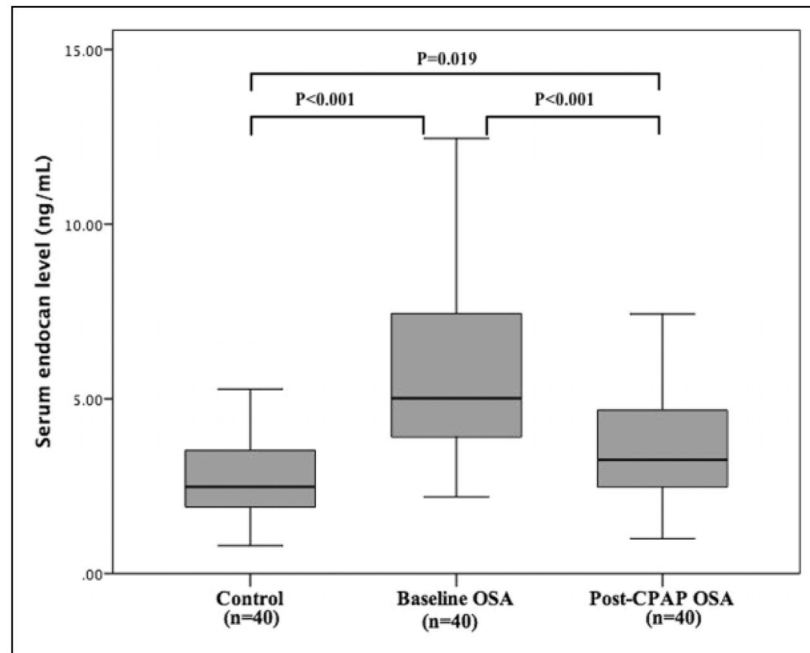


Figure 5. Serum endocan levels in controls, at baseline, and after continuous positive airway pressure (CPAP). Serum endocan levels significantly decreased after CPAP treatment ($P < .001$). However, median endocan level after CPAP was higher than control group (3.25 ± 2.24 vs 2.48 ± 1.64 ng/dL, $P < .019$). The box encompasses the 25% to 75% quartiles, and the median is represented by the horizontal line within the box. The whiskers extend to the highest and lowest values within the higher and lower limits, respectively.

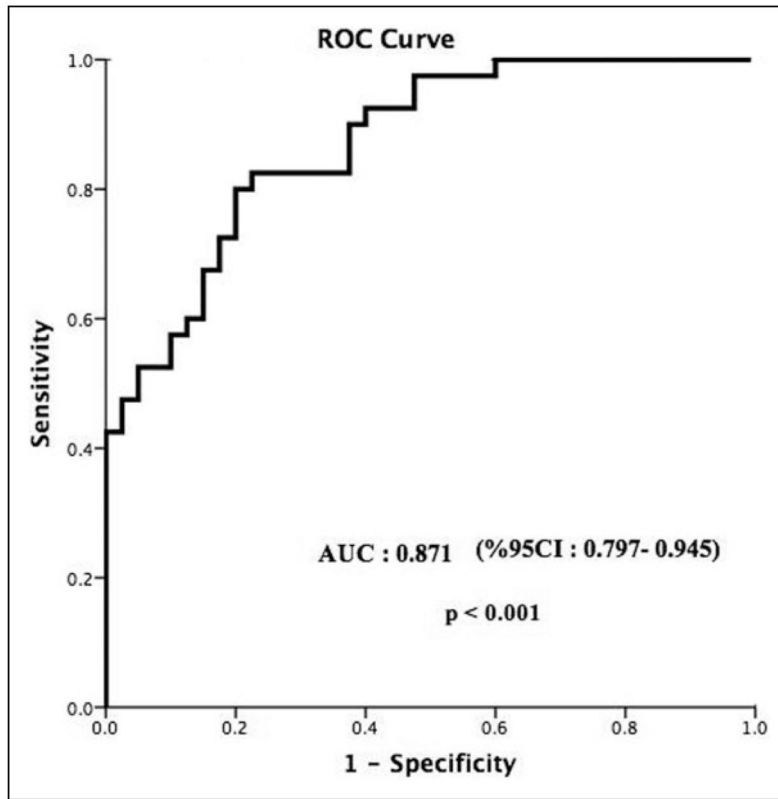


Figure 6. The receiver operating characteristic (ROC) curve of endocan, predicting obstructive sleep apnea (OSA).

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Table 1

Patient Characteristics, PSG, Laboratory, and Ultrasonography Data According to Severity of OSA.

	Control	Moderate OSA	Severe OSA	P Value
Participants, n	40	14	26	
Age, years, mean, SD	51.5 (6.7)	55.4 (9.7)	54.6 (10.8)	.225
Sex, male	27 (67.5)	7 (50)	18 (69.2)	.428
BMI, kg m ⁻² , mean, SD	32.9 (4.7)	34.2 (5.5)	35.2 (6.6)	.225
Ever smoker	27 (67.5)	7 (50)	18 (69.2)	.428
Current smoker	15 (37.5)	1 (7.1)	9 (34.6)	.098
Pack years	21.5 (25)	15 (10)	30 (47)	.114
ESS	4 (4) ^{a,b}	14 (19) ^a	18 (12) ^b	<.001
Comorbidity				
Hypertension	17 (42.5)	5 (35.7)	16 (61.5)	.198
Dyslipidemia	12 (30)	3 (21.4)	11 (42.3)	.361
Diabetes mellitus	6 (15)	2 (14.3)	8 (30.8)	.247
PSG data				
TST, min	402.5 (67.9)	390.3 (79.9)	404.1 (69.1)	.314
Sleep efficiency, %	87 (15)	81.5 (14)	79 (10)	.112
Arousal index, h ⁻¹	1.7 (2.2) ^{a,b,c}	22.3 (9.4) ^{a,b,c}	68.5 (36) ^{a,b,c}	<.001
AHI events, h ⁻¹ , mean,SD	1.9 (1.4) ^{a,b,c}	22.9 (5.4) ^{a,b,c}	69.9 (21.4) ^{a,b,c}	<.001
3% ODI events, h ⁻¹	2.3 (2.6) ^{a,b,c}	25.3 (11.2) ^{a,b,c}	76.2 (33.2) ^{a,b,c}	<.001
Minimum SpO ₂ , %	90 (6) ^{a,b}	81.5 (6) ^a	67.5 (20.3) ^b	<.001
SpO ₂ <90% %TST	0.15 (2) ^{a,b}	4.9 (18) ^a	13.6 (40.7) ^b	<.001
Blood				
Glucose	94 (12)	93 (11)	96.5 (21)	.523
TC, mg/dL	190 (61)	190 (45)	200 (43)	.792
HDL-C, mg/dL	45 (16)	46 (10)	41 (15)	.117
TG, mg/dL	154 (84)	140 (66)	186 (118)	.529
CRP, mg/dL	2.8 (4.4)	4.4 (6.1)	3.3 (7.6)	.310
Fibrinogen, mg/dL	300.5 (97)	364.5 (115)	310 (141)	.087
Endocan, ng/dL	2.48 (1.64) ^{a,b,c}	4.01 (1.25) ^{a,b,c}	5.68 (5.49) ^{a,b,c}	<.001
Ultrasound				
cIMT, mm	0.58 (0.09) ^{a,b,c}	0.74 (0.09) ^{a,b,c}	0.81 (0.20) ^{a,b,c}	<.001
FMD, % change, mean, SD	6.15 (1.73) ^{a,b,c}	4.55 (1.43) ^{a,b,c}	3.20 (1.80) ^{a,b,c}	<.001

Abbreviations: AHI, apnea/hypopnea index; BMI, body mass index; cIMT, carotid intima media thickness. CRP, C-reactive protein; ESS, Epworth Sleepiness Scale; FMD, flow-mediated dilatation; HDL-C, high-density lipoprotein cholesterol; ODI, oxygen desaturation index; OSA, obstructive sleep apnea; TC, total cholesterol; TG, triglycerides; TST, total sleep time; SpO₂, arterial oxygen saturation measured by pulse oximetry; PSG, polysomnography.

Notes: (1)# Where the *P* value is significant, values within a row with the same superscript letter are significantly different, (2) Data are presented as n, median (interquartile range), or n (%), unless otherwise stated.

Table 2
 Linear Regression Analysis of all 80 Participants (Participants + Patients) With Endocan Levels as the Dependent Variable.

Analysis	β	SE	t Test	P Value	95% CI for β
Univariate					
OSA	0.765	0.109	7.034	<.001	0.549–0.982
AHI events, h ⁻¹	0.013	0.001	9.015	<.001	0.010–0.016
Arousal index events, h ⁻¹	0.014	0.002	9.015	<.001	0.011–0.017
FMD	-0.200	0.036	-5.505	<.001	-0.273 to -0.128
ln cIMT	3.548	0.531	6.684	<.001	2.491–4.605
DM (Yes = 1)	0.386	0.168	2.292	.025	0.051–0.721
Hypertension (Yes = 1)	0.325	0.134	2.424	.018	0.058–0.593
Dyslipidemia (Yes = 1)	0.287	0.145	1.985	.051	-0.001–0.576
Age	0.010	0.008	1.251	.215	-0.006–0.025
BMIx	0.139	0.138	1.009	.316	-0.136–0.415
Sex (male = 1)	-0.130	0.145	-0.897	.373	-0.419–0.159
Ever smoker (yes = 1)	-0.008	0.146	-0.054	.957	-0.298–0.282
Cigarette/pack year	0.002	0.003	0.847	.401	-0.003–0.007
ln Fibrinogen	-0.006	0.295	-0.022	.983	-0.594–0.581
ln CRP	0.088	0.066	1.330	.187	-0.044–0.220
Multivariate					
Model I					
AHI	0.013	0.002	7.955	<.001	0.009–0.016
Model II					
ln cIMT	3.275	0.552	5.930	<.001	2.174–4.375
Model III					
FMD	-0.180	0.036	-4.942	<.001	-0.253 to -0.107
DM	0.322	0.143	2.244	.028	0.036–0.608

Abbreviations: AHI, apnea/hypopnea index; BMI, body mass index; CI, confidence interval; cIMT, carotid intima media thickness; CRP, C-reactive protein; DM, diabetes mellitus; ESS, Epworth Sleepiness Scale; FMD, flow-mediated dilatation; ln, natural log-transformed; OSA, obstructive sleep apnea; SpO₂, arterial oxygen saturation measured by pulse oximetry; SE, standard deviation.

Table 3
Logistic Regression Analysis of Variables in all 80 Participants With OSA as the Dependent Variable.

Analysis	β	SE	Wald	P Value	OR (95% CI for β)
Univariate					
Endocan	1.035	0.249	17.359	<.001	2.816 (1.730–4.584)
cIMT	0.399	0.103	15.118	<.001	1.490 (1.219–1.822)
FMD	-1.453	0.318	20.910	<.001	0.234 (0.125–0.436)
Multivariate					
Endocan	1.125	0.284	15.737	<.001	3.080 (1.767–5.370)
cIMT	0.462	0.124	13.930	<.001	1.588 (1.245–2.024)
FMD	-1.497	0.333	20.265	<.001	0.224 (0.117–0.429)

Abbreviations: cIMT, carotid intima media thickness; FMD, flow-mediated dilatation; OSA, obstructive sleep apnea; SE, standard deviation.