

Serum Levels of Vascular Endothelial Growth Factor Are Elevated in Patients with Obstructive Sleep Apnea and Severe Nighttime Hypoxia

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Obstructive sleep apnea (OSA) is associated with increased cardiovascular morbidity and mortality; however, some patients with OSA do not develop cardiovascular disease even in the presence of severe nocturnal oxygen desaturations. Vascular endothelial growth factor (VEGF) is a hypoxia-sensitive glycoprotein stimulating neoangiogenesis. We hypothesized that VEGF production is increased in OSA because of repetitive nocturnal hypoxia. Three different groups were investigated: 10 OSA patients with severe nighttime hypoxia (Group A), 10 OSA patients with moderate hypoxia (Group B), and 10 healthy volunteers (Group C). Serum levels of VEGF were measured by ELISA from peripheral venous blood samples obtained at 7 AM. Group A had significantly ($p < 0.01$) increased VEGF serum levels when compared with Group B and Group C (mean \pm SEM: 410 ± 77 pg/ml versus 224 ± 38 pg/ml and 245 ± 61 pg/ml). The degree of nocturnal oxygen desaturation in OSA significantly correlated with the VEGF concentrations ($r = 0.67$, $p < 0.01$). In conclusion, serum levels of VEGF are elevated in severely hypoxic patients with OSA and are related to the degree of nocturnal oxygen desaturation. This might constitute an adaptive mechanism to counterbalance the emergence of OSA-related cardiovascular disease.

Keywords: obstructive sleep apnea; vascular endothelial growth factor; cardiovascular disease

Obstructive sleep apnea (OSA) is linked with increased cardiovascular morbidity and mortality (1). Epidemiologic data strongly support a causal role of OSA in the development of arterial hypertension (2). Moreover, the Sleep Heart Health Study has recently shown that patients with mild sleep-disordered breathing are at enhanced risk for occlusive vascular disease, specifically, coronary artery and cerebrovascular disease (3). However, these investigators also found that the risk did not further increase in patients with more severe OSA. This is paralleled by the clinical observation that, even in the presence of severe nocturnal oxygen desaturations, not all OSA patients suffer from overt cardiovascular disease. Thus, it might be suspected that some as yet unidentified mechanisms protect individual patients with OSA from the development of cardiovascular complications.

Vascular endothelial growth factor (VEGF) is a 45-kD homodimeric glycoprotein stimulating normal and abnormal vessel growth (4, 5). VEGF is essential for neoangiogenesis during embryonic development, wound healing, and tumor

growth (6, 7). Its role in the pathophysiology of cardiovascular disease is less well established; however, preliminary data suggest a therapeutic benefit of VEGF application in patients with coronary artery and peripheral vascular disease (8, 9). Apart from a number of cytokines, hormones, and growth factors, the expression of the VEGF gene is mainly stimulated by hypoxia through mediation of hypoxia-inducible factor (HIF) (10). HIF is also responsible for the induction of a variety of further hypoxia-sensitive genes such as those encoding for erythropoietin and glycolytic enzymes. HIF-mediated gene expression might take place in patients with OSA as a result of repetitive nighttime hypoxia. For example, it is well known that erythropoietin production is upregulated in OSA (11). Based on these considerations, the hypothesis of the present study was that the VEGF system is activated in OSA.

METHODS

An expanded explanation of the METHODS can be found in the online data supplement accompanying this article.

Patients

Three different patient groups were investigated: 10 OSA patients with severe nighttime hypoxia (Group A), 10 OSA patients with moderate hypoxia (Group B), and 10 healthy volunteers (Group C). Based on the data continuously derived from overnight pulse oximetry, the patients with OSA were divided into patients with severe and moderate hypoxia. If nocturnal oxygen saturation (Sa_{O_2}) was $< 90\%$ during less than 20% of total sleep time (TST), this was scored as moderate hypoxia. On the other hand, if Sa_{O_2} ranged $< 90\%$ during more than 20% of TST, severe hypoxia was diagnosed.

On the day of admission, all patients were evaluated for the presence of vascular or metabolic disease (i.e., arterial hypertension, coronary artery disease, cerebrovascular disease, hypercholesterolemia, and diabetes mellitus) and their smoking habits. Furthermore, body plethysmography (MasterLab Pro; Jaeger, Würzburg, Germany) was performed in each subject. Finally, daytime blood gases were determined from arterialized ear lobe samples.

Patients with known cancer or collagen vascular disease were excluded from the study. The study protocol had been approved by the local ethics committee and all patients had given their informed written consent.

Polysomnography

In each subject, full-night attended polysomnography was performed employing a computerized system (SIDAS GS; IFM GmbH, Wettenberg, Germany). Nocturnal breathing was visually scored from the computer recordings. Analysis of sleep stages was performed manually in 30-s intervals according to the criteria of Rechtschaffen and Kales (12). Arousals were classified following the American Sleep Disorders Association (ASDA) criteria (13). An apnea-hypopnea index (AHI) of more than 10 per hour of sleep in the presence of sleep-related symptoms (i.e., snoring, witnessed apneas, excessive daytime sleepiness) was considered as diagnostic of OSA.

Measurement of VEGF

At 7 AM, i.e., at the end of the sleep study, blood samples were withdrawn from an antecubital vein. These samples were immediately cen-

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trifuged over 10 min at 3,000 rpm. The supernatant fluid was stored in a refrigerator at -40°C for later analysis. VEGF serum levels were determined by a commercially available ELISA test (R&D Systems, Wiesbaden, Germany). This assay is calibrated against a highly purified recombinant human VEGF₁₆₅, the major and most potent isoform of VEGF. It measures free, unbound VEGF with a high intra-assay and interassay precision and a recovery rate approximating 100% in serum. All measurements of VEGF were carried out at the same day after completion of all sleep studies, i.e., as a batch test.

Data Analysis

All data are given as mean \pm SEM. The characteristics of the three different patient groups were compared by the Wilcoxon-Mann-Whitney test. Univariate linear regression analysis was applied to relate VEGF serum levels to the degree of nocturnal oxygen desaturation. Moreover, to determine the independent association of various variables (i.e., anthropometric, pulmonary function test, and polysomnography parameters) with VEGF serum levels, stepwise multiple logistic regression analysis was performed. A p value < 0.05 was regarded as statistically significant. Calculations were performed on a personal computer using the software package NCSS (Number Cruncher Statistical System, Kaysville, UT).

RESULTS

Patient Characteristics

Additional details on patient characteristics are available in the online data supplement.

All three patient groups were age-matched and sex-matched. Between the two OSA groups, the AHI did not significantly differ; however, in the patients with severe nocturnal hypoxia more apneas than hypopneas were observed and there was a trend for a longer mean apnea duration (Table 1). Furthermore, these patients were more obese than those with only moderate hypoxia and were characterized by a lower average Po_2 while awake. Lung function parameters were within the normal range in all patients studied. The spectrum of concomitant cardiovascular and metabolic disease was similar in both OSA groups. The subjects of the control group were healthy nonsmoking volunteers. In contrast to the patients with OSA, they were not obese.

VEGF Serum Levels

The mean VEGF serum levels were markedly elevated in the OSA group with severe nocturnal hypoxia when compared with both other groups (mean \pm SEM: 410 ± 77 pg/ml versus 224 ± 38 pg/ml in Group B, [$p < 0.01$] and 245 ± 61 pg/ml in Group C [$p < 0.01$], Figure 1).

Using linear regression analysis, the degree of nocturnal oxygen desaturation in OSA significantly correlated with the VEGF concentrations ($r = 0.67$, $p < 0.01$, Figure 2). In contrast, stepwise multiple logistic regression analysis did not re-

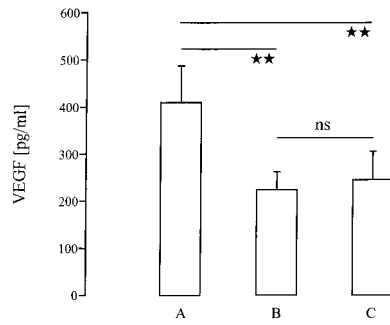


Figure 1. VEGF serum levels (mean \pm SEM) in the three different patient groups. (A: OSA patients with severe hypoxia; B: OSA patients with moderate hypoxia; C: control subjects). **p value < 0.01 . ns = nonsignificant.

veal any statistically significant relationships between VEGF concentrations and other variables (Table 2).

DISCUSSION

The main finding of the present study was that the patients with OSA with severe nocturnal hypoxia had markedly increased VEGF serum levels when compared with the patients with OSA presenting with moderate hypoxia and the control subjects. Numerous factors might influence VEGF serum levels and have to be considered when discussing these observations. The most common conditions known to be associated with elevated VEGF serum levels, such as disseminated cancer and chronic inflammatory and autoimmune disease (14–16), were not present in our patients and can thus not be responsible for the differences in VEGF concentrations.

A major point of criticism might be that the higher VEGF concentrations in the group with severe nighttime hypoxia were simply due to the lower daytime Po_2 value in these subjects. In this context, it is important to realize that Po_2 was determined from arterialized ear lobe samples. If regional blood flow is low, it is possible that the ear lobe Po_2 underestimates arterial Po_2 (17). Thus, the observation of different Po_2 levels in the two OSA groups could have been as a result of the method of blood sampling. An alternative explanation could be that the lower mean Po_2 value in Group A was linked to the higher average BMI in these patients. Although there was no measurable pulmonary function impairment in any of the subjects studied, it is possible that increased body mass index (BMI) contributes to some restrictive ventilatory deficit that is evident only in the supine position. Regardless of these considerations, multivariate analysis failed to show an independent influence of daytime Po_2 values on VEGF concentrations. Thus, we suggest that the characteristics of sleep-disordered breathing were mostly responsible for the more severe nocturnal desaturations observed in Group A. These patients had

TABLE 1. SLEEP STUDY RESULTS IN PATIENTS WITH OSA AND CONTROL SUBJECTS*

	Group A (n = 10)	Group B (n = 10)	Group C (n = 10)
AHI, n/h	44 \pm 6.8	43 \pm 6.5 [§]	5 \pm 3.0
AI, n/h	38 \pm 5.6 [†]	22 \pm 6.0 [§]	2 \pm 1.8
Mean apnea duration, s	21 \pm 1.7	19 \pm 1.1 [§]	11 \pm 1.2
Sa _{O2} mean, %	90.4 \pm 2.3	92.8 \pm 1.8	93.5 \pm 1.9
Sa _{O2} $<$ 90%, % of TST	29.3 \pm 2.3 [‡]	9.4 \pm 2.2 [§]	1.5 \pm 2.0
Lowest Sa _{O2} , %	57.4 \pm 8.5 [‡]	70.7 \pm 5.2 [§]	85.9 \pm 3.4

Definition of abbreviations: AHI = apnea-hypopnea index; AI = apnea index; TST = total sleep time.

* Data are given as mean \pm SEM for each group. Group A versus B: [†] $p < 0.05$, [‡] $p < 0.01$; Group B versus Group C: [§] $p < 0.01$; p = not significant if not indicated.

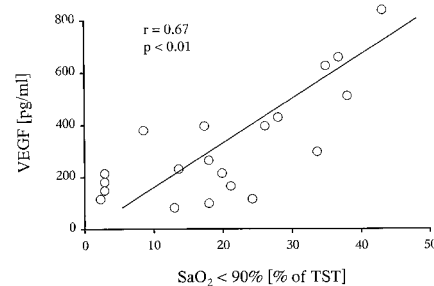


Figure 2. Univariate correlation between individual VEGF values and nocturnal oxygen desaturation (expressed as Sa_{O2} $<$ 90% in % of TST) depicted for all patients with OSA.

TABLE 2. STEPWISE MULTIPLE LOGISTIC REGRESSION ANALYSIS OF THE RELATIONSHIP BETWEEN VEGF SERUM LEVELS AND VARIOUS INDEPENDENT VARIABLES IN THE PATIENTS WITH OSA (n = 20)

Variable	r	p Value
Age, yr	-0.12	NS
BMI, kg/m ²	0.31	NS
Po ₂ , mm Hg	-0.29	NS
FEV ₁ , % pred	0.01	NS
FVC, % pred	0.01	NS
AHI, n/h	0.26	NS
Arousal index, n/h	0.15	NS
Mean apnea duration, s	0.07	NS
Sa _{O₂} < 90%, % of TST	0.60	< 0.01

Definition of abbreviations: BMI = body mass index; NS = not significant; Po₂ = partial pressure of oxygen (arterialized ear lobe); r = multiple correlation coefficient.

more apneas than hypopneas and a somewhat longer mean apnea duration, which is most likely to have led to more pronounced nighttime hypoxia.

A further aspect for debate is the selection of a 20% < 90% Sa_{O₂} cutoff value to separate patients with OSA with less and more severe nocturnal hypoxia. The major obstacle that would have emerged if we had taken lower or higher cutoff values is the fact that in typical sleep laboratory populations (as in ours) only few patients present with minimal or very severe nocturnal oxygen desaturations. Thus, an unequal number of patients would have been compared in the group of patients with less and more severe nighttime hypoxia. Nevertheless, we suggest that if we would have employed another cutoff value and would have investigated a bigger study sample, the results would not be substantially different, as indicated by the close linear relationship between Sa_{O₂} values and VEGF concentrations.

A possible methodologic limitation of the present study is that VEGF measurements were carried out in serum samples. VEGF serum concentrations have been found to be higher than those of plasma samples owing to the release of platelet-derived VEGF during coagulation. Therefore, some investigators have recommended using plasma rather than serum for the determination of VEGF (18). It has to be kept in mind, however, that this limitation was true for all subjects investigated and may thus not explain net differences between the groups. A final point of criticism might be that the assay which we employed measures only free, unbound VEGF and not total VEGF. However, we are not aware of any established technique being capable to detect the fraction of receptor-bound VEGF in addition to free VEGF.

We suggest that the most likely trigger of VEGF release in OSA is hypoxia, as the severity of hypoxia discriminated between Groups A and B, and a close linear relationship between the degree of nocturnal oxygen desaturation and VEGF concentrations was observed. A further possibility is that pulsatile stretch caused by apnea-related blood pressure oscillations stimulates VEGF secretion in OSA, as suggested by cell culture experiments performed under mechanical stress (19). It is also imaginable that the VEGF increase in OSA is secondary to alterations in other mediator systems. Free oxygen radicals and endothelin, which have been reported to be elevated in OSA, may enhance gene expression of VEGF (20–23). Finally, the inhibitory effect of nitric oxide on VEGF gene induction may be weakened through the downregulation of nitric oxide synthesis which has been found in OSA (24, 25).

On the basis of our study, we are not able to determine the exact source of VEGF release in OSA. It might be speculated

that apart from the endothelium, a further possible site of enhanced VEGF production is represented by platelets which are activated in untreated patients with OSA (26). However, *in vitro* experiments have shown that platelets do not release significant amounts of VEGF in response to hypoxia (27).

Concerning the pathophysiologic significance of our results, we hypothesize that the enhanced VEGF production in OSA constitutes an adaptive mechanism to counterbalance the emergence of OSA-related cardiovascular disease. Theoretically, increased VEGF production in OSA might contribute to new vessel formation in ischemic and atherosclerotic vascular regions. This assumption is supported by a recent study showing that in patients with coronary artery disease, the degree of hypoxic induction of VEGF correlates with the extent of collateral vessel formation (28). Thus, the results of our study might in part explain the observation of the Sleep Heart Health Study that the overall cardiovascular risk in OSA is not linearly related to apnea severity.

In conclusion, serum levels of VEGF are elevated in severely hypoxic patients with OSA and are closely correlated with the degree of nocturnal oxygen desaturations. Thus, the most likely trigger of the increased VEGF release in OSA is nighttime hypoxia. The alterations of the VEGF system in severe OSA may impact on the development of cardiovascular abnormalities in these patients.

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