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Original article

Endothelial dysfunction and hypertension in obstructive sleep apnea – Is it due to intermittent hypoxia?

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ABSTRACT

Background: Obstructive sleep apnea (OSA) is a prevalent disorder causing hypertension. Endothelial dysfunction appears to underlie development of hypertension. It is not known whether hypoxia during sleep is necessarily the prerequisite process for endothelial dysfunction and hypertension in OSA. We therefore examined the relationship between endothelial-dependent vasodilatory capacity, hypoxia and circulating angiogenesis inhibitors in OSA.

Methods and results: We studies 95 subjects with and without OSA and hypertension. Endothelial-dependent vasodilation was assessed using brachial artery flow-mediated vasodilation method (FMD). Plasma angiogenesis inhibitors, endoglin (sEng) and fms-like tyrosine kinase-1 (sFlt-1), were measured using ELISA. The apnea–hypopnea indexes were 41 ± 5 and 48 ± 4 events/hr in normotensive OSA (N-OSA) and hypertensive OSA (H-OSA), respectively, indicating severe OSA. The sleep time spent with $SaO_2 < 90\%$ ($T < 90\%$) were 34 ± 8 and 40 ± 9 min, respectively. FMD was markedly impaired in H-OSA (8.0% ± 0.5) compared to N-OSA (13.5% ± 0.5, $P < 0.0001$), H-non-OSA (10.5% ± 0.8, $P < 0.01$), and N-non-OSA (16.1% ± 1.0, $P < 0.0001$). There was no correlation between $T < 90\%$ and FMD. Both OSA groups had elevated levels of sFlt-1 (62.4 ± 5.9 and 63.9 ± 4.7 pg/ml) compared to N-non-OSA (32.1 ± 6.5, $P = 0.0008$ and $P = 0.0004$, respectively) and H-non-OSA (41.2 ± 7.0, $P < 0.05$ and $P = 0.03$, respectively). In contrast, sEng was only elevated in H-OSA (4.20 ± 0.17 ng/ml) compared with N-OSA (3.64 ± 0.14, $P = 0.01$) and N-non-OSA (3.48 ± 0.20, $P = 0.01$). There was a modest but statistically significant inverse correlation between sEng and FMD in only H-OSA group ($r = -0.38$, $P < 0.05$).

Conclusion: These data show that patients with OSA and hypertension have marked impairment of FMD, independent of hypoxia exposure, which is associated with increased sEng.

Key Messages

Obstructive sleep apnea is a highly prevalent disorder with associated high morbidity and mortality. Obstructive sleep apnea is now considered as one of the causes of systemic hypertension. No all patients with obstructive sleep apnea develop hypertension. There appears to be divergent responses to apnea-associated hypoxia. In this article a group of patients with severe obstructive sleep apnea and hypoxia exposure during sleep had normal blood pressure and relatively preserved endothelial-dependent vasodilatory capacity. A comparable group of patients with similar apnea severity and hypoxia exposure had marked impairment in endothelial-dependent vasodilatory capacity and hypertension. This group had significantly elevated circulating levels of soluble endoglin, an angiogenesis inhibitor, with known effect on endothelial function and development of hypertension. It is conceivable that inflammatory state of obstructive sleep apnea provokes release of angiogenesis inhibitors causing downstream perturbation of endothelial function.

1. Introduction

Obstructive sleep apnea (OSA) is a highly prevalent sleep disorder that affects 15–24% of the adults and is associated with increased morbidity and mortality.[1] Individuals with OSA are particularly at increased risk for premature atherosclerosis, coronary artery disease, stroke and hypertension.[2–5] Systemic hypertension affects up to two-thirds of patients with OSA.[6,7] Some investigators have proposed that endothelial dysfunction is mechanistically implicated in a sustained increase in blood pressure[8,9] and is an early process in the development of atherosclerosis.[10,11] Patients with OSA have been shown to have endothelial

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dysfunction[12–15] and evidence for premature atherosclerosis.[16] Current evidence suggests that inflammatory processes, oxidative stress and endothelial dysfunction may play roles in the pathogenesis of hypertension and vascular complications in OSA.[15]

We have previously shown elevated concentrations of circulating soluble endoglin (sEng) and soluble fms-like tyrosine kinase-1 (sFlt-1) in hypertensive OSA compared to normotensive OSA.[17] These angiogenesis inhibitors are released under inflammatory state.[18,19] These circulating proteins have been shown to have pathogenetic roles in hypertension in preeclampsia,[20] in human chronic kidney disease,[21] coronary artery disease[22] and in animal models of hypertension.[23,24] However, none of the studies have examined the relationship between endothelial dysfunction and hypertension in relationship to hypoxia exposure in OSA patients. Further, it is not known whether endothelial dysfunction is related to angiogenesis inhibitors.

The specific aims of the present study were to examine the relationship between endothelial-dependent vasoregulatory capacity in OSA patients with and without hypertension, hypoxia exposure and angiogenesis inhibitors.

2. Methods

2.1. Subjects

Patients were recruited consecutively from among those screened for sleep-disordered breathing at Yale Center for Sleep Medicine. Patients with newly diagnosed and untreated OSA and those without OSA (apnea-hypopnea index, AHI $<$ 5 events/hr) as control group were enrolled. The subjects are a subset of a cohort that has been published previously.[25] We studied hypertensive and normotensive OSA as well as hypertensive and normotensive non-OSA patients. Hypertension was defined by blood pressure $\geq$ 140 mm Hg systolic and/or $\geq$90 mm Hg diastolic, which had been previously documented by using appropriate sized cuff and measurements that had been made at least in three different occasions according to the standard criteria.[26] Subjects were excluded if they had known peripheral vascular disease, liver disease, hemolytic anemia, inflammatory disease, active infection, or if they were pregnant, on therapy for OSA, on chronic steroid treatment, or younger than 18 years of age. Each subject was informed of the experimental procedures and signed the consent form for this study that had been approved by the Human Investigation Committee of the Yale University School of Medicine.

2.2. Sleep study

Nocturnal polysomnography was performed as previously described.[17] Respiratory events were scored according to the American Academy of Sleep Medicine. Hypopnea was scored when there was at least 30% decrease in airflow signal with a $\geq$4% decrease in oxygen saturation. The percentage of total sleep time associated with oxyhemoglobin saturation of $<$90% was calculated as a measure of hypoxemia duration.

2.3. Endothelial function

Conduit vessels respond to alterations in blood flow by increasing vessel diameter via an endothelial-dependent mechanism. Endothelial function was assessed by a standard flow-mediated vasodilation (FMD) method using Doppler ultrasound of the brachial artery between 10 am and 3 pm.[25] In order to best visualize the brachial artery, the arm was comfortably immobilized in the extended position, and the brachial artery was scanned in the longitudinal section 3–5 cm above the antecubital fossa. Gain and depth settings were optimized to identify the lumen-vessel wall interface. After optimal transducer positioning, the skin was marked for reference for later measurements and the arm was kept in the same position throughout the study. After baseline measurements of the brachial artery were recorded, the cuff was placed on forearm and inflated to 200 mm Hg for 5 min to create forearm ischemia. Subsequently, the cuff was deflated and the arterial diameter was measured every 3–5 s after deflation up to 5 min. FMD was expressed as the percentage of change in the brachial artery diameter from baseline to following peak reactive hyperemia.

The artery diameters were measured independently by the two investigators (one blinded to grouping of subjects) using a digital caliper and were verified by an automated border recognition software. Peak vasodilation was calculated as the percent change in the brachial artery diameter from baseline to peak reactive hyperemia. The inter-observer and intra- observer variability in diameter measurements were less than 5%.

2.4. Blood sample

Venous blood sample was obtained 1 h after the subjects had been seated and rested for 60 min between 10 am and 2 pm. Plasma and serum were separated with centrifugation at 1200 g for 10 min at 4 °C, aliquoted and stored at −80 °C for further analysis.

2.5. Measurement of plasma sEng and sFlt-1

Circulating levels of sEng and sFlt-1 in plasma were measured using enzyme-linked immunosorbent assay using commercially available reagents and recombinant standards (R&D Systems, Minneapolis, MN, USA). All samples were assayed in duplicate. Standards and control samples were run simultaneously for validation. The minimum detection limits for sEng and sFlt-1 were 0.007 ng/ml and 3.5 pg/ml, respectively. Inter- and intra-assay coefficient of variations for both assays were $<$10%. The assay kit measures total plasma sFlt-1.

2.6. Data analysis

The primary outcome was endothelial-dependent vasodilation as measured by FMD. The required sample size to detect a significant change in FMD (delta = 4, SD = 2.7) was 14 per group (alpha = 0.05, power = 80%). However, we over sampled the OSA groups to account for the differential susceptibility to hypertension and responses to OSA and hypoxia in this population. Data are expressed as means ± SE. Data were analyzed using ANOVA for simultaneous comparisons of the groups (Graphpad Prism, La Jolla, CA). Spearman correlation was used to analyze the relationship between FMD and sEng and sFlt-1. A multivariable linear regression analysis was used to identify independent clinical variables associated with FMD. The following variables were considered for inclusion in the comprehensive model: age, BMI, smoking, diabetes, dyslipidemia, hypertension and OSA. Inclusion in the final model was determined by a backward-stepwise technique evaluating all potential univariate variables ($P < 0.20$) to create a multivariable model containing variables with $P < 0.05$ (SAS Institute Inc, Carey, NC). P values were 2-sided with a level of significance of $P < 0.05$.

3. Results

3.1. Subjects characteristics

As shown in Table 1 both OSA groups had moderately severe OSA with significant oxygen desaturations during sleep. The control