The Association of Nocturnal Hypoxemia with Arterial Stiffness and Endothelial Dysfunction in Male Patients with Obstructive Sleep Apnea Syndrome

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Key Words

Obstructive sleep apnea syndrome • Pulse wave velocity • Flow-mediated dilatation • Hypoxemia

Abstract

Background: Obstructive sleep apnea syndrome (OSAS) is associated with cardiovascular complications, and atherosclerosis is considered to mediate this association. Arterial stiffness and endothelial dysfunction are widely accepted as early markers of atherosclerosis. **Objectives:** The purpose of this study was to investigate the influence of sleep apnea on arterial stiffness and endothelial dysfunction. **Methods:** We enrolled 83 OSAS patients including 39 with mild to moderate degree (apnea hypopnea index, AHI ≥ 5 , AHI < 30) and 44 with severe degree sleep apnea (AHI ≥ 30) and 29 normal control subjects (AHI < 5). After finishing polysomnography, carotid-femoral pulse wave velocity (cfPWV) and flow-mediated dilation (FMD) were measured using noninvasive devices. **Results:** In patients with severe degree of OSAS, cfPWV was significantly higher than mild to moderate degree of OSAS or normal control subjects. Also, the severe OSAS group showed lower FMD than the normal control group (all $p < 0.01$). The cfPWV was significantly correlated with FMD ($r = -0.26$, $p < 0.01$). Age ($\beta = 0.33$, $p < 0.01$) and percentage

of time below 90% O₂ saturation ($\beta = 0.34$, $p < 0.01$) were the significant variables to determine cfPWV (adjusted R² = 21%, $p < 0.01$) in multivariate analysis, and the lowest O₂ saturation was a significant determinant for FMD ($\beta = 0.25$, adjusted R² = 6%, $p < 0.01$). **Conclusions:** Nocturnal hypoxemia may alter arterial elasticity and endothelial function in OSAS patients, and those impairments could increase the risk of cardiovascular complications.

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Introduction

Obstructive sleep apnea syndrome (OSAS) is a disease characterized by repetitive episodes of respiratory cessation during the night resulting in nocturnal hypoxemia and frequent arousals. Patients with OSAS suffer from snoring, insufficient sleep, daytime somnolence and cognitive dysfunction. In addition, it is currently known that OSAS is associated with increased rates of cardiovascular complications [1]. Atherosclerosis could be responsible for the association between OSAS and cardiovascular diseases [2, 3].

Atherosclerosis is an inflammatory process that alters the elasticity of arterial vessel wall [4]. To assess the risk

of atherosclerosis, arterial stiffness [5] or endothelial dysfunction [6, 7] has been noninvasively measured by pulse wave velocity (PWV) [8] or flow-mediated dilatation (FMD) in the brachial artery [9], respectively. In predicting the risk of atherosclerosis of OSAS patients, arterial stiffness, compared to endothelial function, has been evaluated in only a few studies [10–12]. Arterial stiffness, a pre-atherosclerotic state, needs to be further explored in OSAS patients to understand mechanisms of cardiovascular complications of OSAS. As another marker of atherosclerosis, endothelial dysfunction has been reported in OSAS patients [13–15] and we have also demonstrated the association of endothelial dysfunction with the severity of nocturnal hypoxemia in OSAS [16]. Previously, positive correlation between increased arterial stiffness and endothelial dysfunction has been reported in patients with atherosclerotic changes [17]. It would be meaningful to investigate arterial stiffness and endothelial dysfunction in OSAS patients at the same time and find the common variables which could underlie arterial stiffness and endothelial dysfunction in OSAS.

The aims of this study were (1) to explore PWV and FMD in relation with OSAS severity, (2) to identify the determinant factors which could influence the arterial stiffness and endothelial dysfunction in OSAS, and (3) to find the correlation between PWV and FMD in OSAS patients.

Methods and Materials

Subjects

A total of 112 male subjects were included in this study. We enrolled 83 OSAS patients including 39 with mild to moderate degree (apnea hypopnea index, AHI ≥ 5 , AHI < 30) and 44 with severe degree (AHI ≥ 30) of OSAS, and 29 normal control subjects (AHI < 5). All patients with OSA were newly diagnosed and had never been treated for OSA. Among 112 subjects, 12 (10.7%) were reported in our previous study [16]. Based on the clinical interview and medical records, subjects who were suffering from inflammatory diseases, chronic obstructive pulmonary disease (COPD), or cardiovascular diseases such as coronary artery disease, myocardial infarction or congestive heart failure were excluded. Patients who were taking antihypertensives, antihyperlipidemics or hypoglycemics were also excluded [18]. The study protocol was approved by the Institutional Review Board in Seoul National University Bundang Hospital, and all the subjects provided written informed consents.

Polysomnography

Overnight polysomnography was performed by using an Embla™ N 7000 recording system (Embla, Reykjavik, Iceland) and standard electrodes and sensors. Electroencephalography electrodes were applied at C3/A2, O1/A2 and O2/A1, and 2 elec-

trooculography electrodes were applied at the sides of both eyes. Submental electromyography electrodes were applied at submental muscle and the electromyographies of both anterior tibialis muscles recorded limb movements during sleep. Strain gauges were used for recording chest and abdominal respiratory movements, and a nasal pressure cannula was used to record airflow. Arterial oxygen saturation was measured using pulse oximeters applied on index fingers. Based on the criteria of Rechtschaffen and Kales [19], we scored every epoch of 30-second nocturnal polysomnography. Apnea was defined as complete cessation of airflow for at least 10 s. Hypopnea was defined as a substantial reduction in airflow ($> 50\%$) for at least 10 s or a moderate reduction in airflow for at least 10 s associated with EEG arousals or oxygen desaturation ($\geq 4\%$) [20]. AHI was defined as the total number of apnea and hypopnea per hour of sleep, and oxygen desaturation index (ODI) was calculated as the number of oxygen desaturations ($\geq 4\%$) per hour of sleep.

Measurement of Carotid-Femoral PWV and FMD

In the morning following nocturnal polysomnography, PWV and FMD were measured by investigators blinded to the polysomnographic findings. All subjects fasted for at least 8 h prior to PWV and FMD measurements. To evaluate PWV, we measured electrocardiogram, phonocardiogram and oscillometric signals from 4 extremities and ankles, as well as tonometric signals from right common carotid and right femoral arteries [21] by using a noninvasive vascular screening device system (VP-2000; Omron-Colin, Kyoto, Japan). PWV was calculated from pulse transit time and the distance traveled by the pulse between 2 recording sites [22]. The estimated distance, D, between carotid and femoral artery, based on the Frank method [23], and time delay between the start of the sharp systolic upstroke of carotid and femoral pressure waves, Δt , were obtained. Carotid-femoral PWV (cfPWV) was calculated using the equation: cfPWV (m/sec) = D (m)/ Δt (s). Brachial artery FMD was measured using an Accuvix XO ultrasound unit (Medison, Seoul, Korea) equipped with a 6–12 MHz linear-array transducer and lower-arm occlusion technique [9]. The baseline diameter of the brachial artery was measured from the anterior to the posterior intima. A blood pressure cuff was placed on the lower part of the arm and inflated to 250 mm Hg for 5 min. The blood pressure cuff was then released and brachial artery diameters were measured 3 times at 40, 60 and 80 s after release. After recording the maximum value of 3 measurements, FMD was calculated as the ratio of change in diameter (maximum – baseline) over baseline value.

Statistical Analysis

SPSS version 14.0 for Windows (SPSS Inc., Chicago, Ill., USA) was used for statistical analysis. Results are presented as means $\pm$ SD. The Kolmogorov-Smirnov test was used to confirm normality. Differences in parametric clinical variables among 3 groups were assessed by analysis of variance with the Turkey post hoc test. The Kruskal-Wallis test was performed for nonparametric variables. Pearson or Spearman correlation coefficients were calculated to determine relationships between cfPWV or FMD and clinical or polysomnographic variables, and stepwise multiple regression analysis was used to identify factors that contributed to cfPWV and FMD. The significance criterion was defined to be $p < 0.05$ for two-tailed tests.

Table 1. Clinical characteristics and laboratory findings of the subjects

	Normal control (n = 29)	Mild to moderate de- gree of OSAS (n = 39)	Severe degree of OSAS (n = 44)	p value
Age, years	43.9 ± 6.2	44.5 ± 9.1	43.6 ± 6.4	0.87
BMI, kg/m ²	27.2 ± 2.9	26.7 ± 2.7	27.9 ± 3.4	0.17
Neck circumference, cm	39.3 ± 1.5	38.9 ± 3.1	40.2 ± 2.9	0.09
Waist-to-hip ratio	0.93 ± 0.05	0.94 ± 0.04	0.95 ± 0.05	0.12
Systolic BP, mm Hg	121.8 ± 9.4	125.0 ± 12.9	126.1 ± 13.7	0.34
Diastolic BP, mm Hg ^{b, c}	79.8 ± 8.6	79.3 ± 10.9	85.8 ± 9.4	<0.01
Smoking, N (%)	7 (24.1%)	12 (30.8%)	21 (47.7%)	0.09
Total sleep time, min	381.7 ± 42.4	365.2 ± 51.0	365.9 ± 41.8	0.26
AHI, /h	3.2 ± 1.6	17.1 ± 6.9	56.8 ± 20.0	<0.01
Sleep efficiency, %	87.4 ± 6.5	83.5 ± 9.2	85.7 ± 8.5	0.15
Respiratory arousal index, /h	2.4 ± 1.3	12.2 ± 5.5	47.8 ± 19.3	<0.01
Average O ₂ saturation, % ^{a, c}	96.3 ± 1.0	95.6 ± 1.1	92.9 ± 3.0	<0.01
Lowest O ₂ saturation, % ^{a-c}	89.9 ± 2.0	83.2 ± 4.6	73.6 ± 9.0	<0.01
Percentage of time <90% O ₂ , %	0.03 ± 0.06	1.05 ± 1.15	19.5 ± 18.7	<0.01
ODI, /h	1.5 ± 1.4	10.7 ± 6.4	48.7 ± 22.3	<0.01
cfPWV, m/s ^{b, c}	8.8 ± 1.2	9.0 ± 1.4	9.8 ± 1.6	<0.01
FMD, % ^{a, b}	8.1 ± 2.6	6.7 ± 2.0	6.1 ± 2.1	<0.01
Total cholesterol, mg/dl	201.4 ± 37.2	199.8 ± 25.8	202.6 ± 31.4	0.92
Triglyceride, mg/dl	193.4 ± 102.6	184.5 ± 105.3	192.0 ± 103.6	0.93
HDL cholesterol, mg/dl	40.4 ± 8.6	42.5 ± 9.2	46.7 ± 21.2	0.19
Glucose, mg/dl	98.6 ± 15.6	98.9 ± 25.0	103.3 ± 36.0	0.70

OSAS = Obstructive sleep apnea syndrome; BMI = body mass index; BP = blood pressure; AHI = apnea hypopnea index; ODI = oxygen desaturation index; cfPWV = carotid-femoral pulse wave velocity; FMD = flow-mediated dilatation; HDL = high-density lipoprotein. ^a p < 0.05, normal controls vs. mild to moderate; ^b p < 0.05, normal controls vs. severe; ^c p < 0.05, mild to moderate vs. severe.

Results

There was no significant difference in age, body mass index (BMI), neck circumference or waist-to-hip ratio among the 3 groups (table 1). Although there was no significant difference in systolic blood pressure among the 3 groups, diastolic blood pressure was higher in the severe OSAS group compared to the normal or mild to moderate OSAS groups (p < 0.01). The value of cfPWV was higher in the severe OSAS group (9.8 ± 1.6) compared to the normal control group (8.8 ± 1.2) or the mild to moderate OSAS group (9.0 ± 1.4; all p < 0.01). FMD was significantly lower in the severe OSAS group (6.1 ± 2.1) than in the normal control group (8.1 ± 2.6, p < 0.01). Serum levels of total cholesterol, triglyceride, high-density lipoprotein cholesterol, or glucose of the mild to moderate or severe OSAS groups were not different from those of the normal control group.

Table 2 shows the correlation coefficients of cfPWV or FMD with demographic and polysomnographic variables. cfPWV was significantly correlated with age (r =

0.30, p < 0.01) and polysomnographic findings such as AHI (r = 0.31, p < 0.01), respiratory arousal index (r = 0.28, p < 0.01), average O₂ saturation (r = -0.26, p < 0.01), lowest O₂ saturation (r = -0.26, p < 0.01), percentage of time below 90% O₂ saturation (r = 0.26, p < 0.01) and ODI (r = 0.32, p < 0.01). In addition, waist-to-hip ratio, blood pressure and serum level of glucose were significantly correlated with cfPWV (all p < 0.05). Sleep efficiency, BMI, neck circumference or serum level of lipid profile were not correlated with cfPWV. FMD was significantly correlated with AHI (r = -0.32, p < 0.01), respiratory arousal index (r = -0.22, p = 0.03), average O₂ saturation (r = 0.29, p < 0.01), lowest O₂ saturation (r = 0.30, p < 0.01), percentage of time below 90% O₂ saturation (r = -0.31, p < 0.01) and ODI (r = -0.31, p < 0.01). However, sleep efficiency or anthropometric variables such as BMI, neck circumference and waist-to-hip ratio, or serum levels of lipid profile and glucose were not significantly correlated with FMD. There was significant correlation between cfPWV and FMD (r = -0.26, p < 0.01; fig. 1).

Table 2. Correlation between cardiovascular risk factors with demographic or clinical variables

	cfPWV	FMD
Age	0.30**	-0.04
BMI	0.07	-0.04
Neck circumference	0.19	-0.13
Waist-to-hip ratio	0.20*	0.10
Systolic BP	0.19*	-0.01
Diastolic BP	0.22*	-0.15
Total sleep time	0.06	-0.06
AHI	0.31**	-0.32**
Sleep efficiency	-0.11	-0.01
Respiratory arousal index	0.28**	-0.22*
Average O ₂ saturation	-0.26**	0.29**
Lowest O ₂ saturation	-0.26**	0.30**
Percentage of time <90% O ₂	0.26**	-0.31**
ODI	0.32**	-0.31**
Total cholesterol	0.01	-0.06
Triglyceride	0.09	-0.14
HDL cholesterol	0.04	-0.03
Glucose	0.23*	-0.12

cfPWV = Carotid-femoral pulse wave velocity; FMD = flow-mediated dilatation; BMI = body mass index; BP = blood pressure; AHI = apnea hypopnea index; ODI = oxygen desaturation index; HDL = high-density lipoprotein. * p value < 0.05; ** p value < 0.01.

Stepwise multiple linear regression analysis was performed to identify significant contributing factors for cfPWV or FMD among independent variables such as age, BMI, neck circumference, waist-to-hip ratio, diastolic blood pressure, systolic blood pressure, AHI, respiratory arousal index, average O₂ saturation, lowest O₂ saturation, percentage of time below 90% O₂ saturation, ODI and serum level of glucose. Age ($\beta = 0.33$, $p < 0.01$) and percentage of time below 90% O₂ saturation ($\beta = 0.34$, $p < 0.01$) were the significant variables to determine cfPWV (adjusted $R^2 = 21\%$, $p < 0.01$), and the lowest O₂ saturation was a significant determinant for FMD ($\beta = 0.25$, adjusted $R^2 = 6\%$, $p < 0.01$).

Discussion

In this study, age and percentage of time below 90% O₂ saturation were significant determinants for cfPWV, and the lowest O₂ saturation was significantly associated with FMD. From these results, it is demonstrated that the degree of nocturnal hypoxemia may affect on arterial stiff-

ness and endothelial dysfunction in patients with OSAS. Although there was a well-designed study reporting the independent relation between arterial stiffness and OSAS [12], our study might benefit from a larger sample than the previous study.

Arterial stiffness was significantly increased in the severe OSAS group compared to the normal control or mild to moderate OSAS groups in this study. Recently, there was a study reporting increased arterial stiffness even in minimally symptomatic OSA [24]. Arterial stiffness is a useful predictive marker for cardiovascular complications [25]. As generally known, tunica intima of arterial wall is made up of one layer of endothelial cells, and tunica media is composed of vascular smooth muscle cells which are surrounded by collagen and elastic fibers. Arterial stiffness is mainly determined by structural or functional components of elastic properties, and blood pressure may have influence on the structure. Since elastic fibers are the main load-bearing components of the arterial wall, the disruption of the elastic properties could be responsible for arterial stiffness [26]. Previously, AHI, as a marker of the severity of OSAS, was shown to be the only predictor variable for PWV in OSAS patients [12]. In comparison, we observed that percentage of time below 90% O₂ saturation, rather than AHI, and age were significant determinants for PWV in OSAS patients. It was reported that continuous hypoxia may alter the structure and function of aorta by decreasing the aortic glycosaminoglycans and collagen, or modifying arterial smooth muscle cells [27, 28]. This means that nocturnal hypoxia could be responsible for the increased arterial stiffness in OSAS patients. From these supportive findings, percentage of time below 90% O₂ saturation, as a marker of nocturnal hypoxemia, rather than AHI might better explain the relationship between OSAS and arterial stiffness. Although AHI is widely accepted as a valuable index, it is controversial whether AHI is the best index to define the severity of OSAS [29]. The real meaning of AHI depends on the variability in AHI [30], the duration of apnea and hypopnea, and the association with oxygen desaturation.

It has been revealed that endothelial dysfunction might be affected by nocturnal hypoxemia in OSAS patients [16, 31, 32]. The current study also showed that lowest O₂ saturation was a significant predictor for endothelial dysfunction. Diminished bioavailability of nitric oxide (NO) induced by nocturnal hypoxemia is responsible for this reversible functional alteration of endothelium in OSAS, since the production of NO is mediated by an oxygen-dependent pathway [33]. It was reported that the se-

Fig. 1. Correlations between cfPWV and percentage of time below 90% O₂ saturation (a), between FMD and lowest O₂ desaturation (b), and between cfPWV and FMD (c).

rum level of nitrite/nitrate, stable metabolites of NO, was lower in OSAS compared to normal controls [34], and altered NO biosynthesis by recurrent nocturnal hypoxemia might lead to a prothrombotic and pro-inflammatory condition in OSAS patients [35]. While our previous study showed that ODI was a significant determinant of FMD [16], lowest O₂ saturation was found to be a significant predictor for endothelial dysfunction in the current study. However, lowest O₂ saturation was also known as a variable related with nocturnal hypoxemia [36, 37], and there was high correlation between the lowest O₂ saturation and ODI ($r = -0.87$, $p < 0.01$) in this study.

We found significant correlation between cfPWV and FMD in this study. Although FMD and PWV evaluate different aspects of atherosclerosis, the systemic progression of atherosclerosis might result in the worsening of these parameters simultaneously [14]. Recent studies have demonstrated that the bioavailability of NO and endothelial dysfunction were implicated in the regulation of arterial stiffness [38]. It is reported that glyceryl trinitrate, NO donors, reduced PWV [39]. Also, L-NG^G-monomethyl-L-arginine, an inhibitor of NO synthase, was reported to increase augmentation index which is another marker of arterial stiffness [40]. Arterial stiffness was regulated by tone of smooth muscle which is a coat of

large artery and blood pressure [26], although aging may also affect arterial stiffness [41] or endothelial dysfunction [42]. The endothelium is monolayer cells of inner surface of arterial wall, which is considered to play a pivotal role in the regulation of cardiovascular homeostasis. Impaired function of endothelium could fail to release vasoactive mediators such as NO and endothelin-1. Consequently, it seems that FMD and PWV are simultaneously influenced by NO, and the decreased level of NO bioavailability in OSAS patients may be induced by nocturnal hypoxemia. The nocturnal hypoxic states in OSAS patients could be corrected by applying the nasal continuous positive airway pressure, which resulted in improvement of impaired PWV and FMD in OSAS patients [13, 43].

We performed FMD and PWV tests in the morning to obtain more significant changes in endothelial dysfunction and arterial stiffness, contrary to another study in which PWV was measured in the afternoon (2:00 to 4:00 p.m.) [12]. Endothelial dysfunction and arterial stiffness in patients with obstructive sleep apnea were observed to be more severe in the morning than the evening [15, 44]. These phenomena seem to appear due to the influence of

repeated hypoxemia during sleep, although there may be several factors that contribute to them. We excluded female OSAS patients to avoid potential gender-associated endothelial function differences [45], which might limit applicability of the study results. The modest association between variables in the study needs to be corroborated and strengthened. In addition, among variables reflecting hypoxic stress, we were not able to decide why the percentage of time below 90% O₂ saturation determined cfPWV and lowest O₂ saturation determined FMD. Despite these limitations, we could confirm that risks for cardiovascular diseases were increased in OSAS patients by observing the increased arterial stiffness and impaired endothelial function in OSAS patients. In addition, nocturnal hypoxemia was observed to have an influence on the endothelial dysfunction and arterial stiffness in OSAS patients.

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