DETERMINATION OF SUBCLINICAL ATHEROSCLEROSIS IN OBSTRUCTIVE SLEEP APNEA SYNDROME PATIENTS WITHOUT TRADITIONAL RISK FACTORS FOR ATHEROSCLEROSIS

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ABSTRACT

Objective: Obstructive sleep apnea-hypopnea syndrome (OSAS) is associated with high cardiovascular morbidity and mortality. Recent studies have suggested a pathophysiological link between OSAS and atherosclerosis; for which carotid intima-media thickness (CIMT) and pulse wave velocity (PWV) has been considered as an early marker. The aim of this study was to assess the presence of early signs of atherosclerosis and cardiovascular effects of OSAS depending on its severity, in patients without clinically diagnosed cardiovascular disease and any coincident risk factors for atherosclerosis.

Material and Method: Thirty one healthy subjects without any systemic disease and OSAS, and patients with OSAS without known atherosclerosis and also without any risk factors for atherosclerosis were examined in the study. According to the severity, 30 patients were in mild OSAS, 32 were in moderate OSAS and 31 patients were in severe OSAS group. Bilateral CIMT assessment and PWV analysis were performed in patients and controls.

Results: Significant differences existed between control subjects and patients with mild, moderate and severe OSAS in PWV (5.70±0.48, 6.76±0.61, 7.72±0.82, 8.94±1.72 m/sec respectively; p<0.0001) and CIMT (0.712±0.040, 0.812±0.037, 0.900±0.056, 0.971±0.74 mm respectively; p<0.0001). AHI and TST% were positively correlated with the following; the PWV (p<0.001- r=0.67 / p<0.001- r=0.70 respectively), the maximal CIMT (p<0.001- r=0.79 / p<0.001- r=0.74 respectively), The minimal SaO2 values were negatively correlated with the following; the PWV (p<0.001, r=-0.66), the maximal CIMT (p<0.001, r=-0.68).

Conclusion: OSAS patients have tendency to atherosclerosis development, and this process increases proportionally with the severity of the disease.

Key Words: Obstructive sleep apnea, pulse wave analysis, carotid intima-media thickness, atherosclerosis Nobel Med 2013; 9(3): 27-34
**INTRODUCTION**

Obstructive sleep apnea (OSAS) is characterized by repetitive apnea or hypopnea due to narrowing of the upper airways during sleep. It is a common disorder of middle-aged adults, affecting 4% of men and 2% of women.1 OSAS is an independent risk factor for cardiac mortality and morbidity. In patients without underlying cardiovascular disease, hypertension, coronary artery disease, stroke, and heart failure are shown to be associated with OSAS. These conditions are independent risk factors for cardiovascular morbidity and mortality and OSAS participates in the genesis of these conditions.13 Recent studies have indicated that OSAS is associated with multiple causal factors of endothelial damage and atherosclerosis. Systemic inflammation, oxidative stress, increased levels of soluble adhesion molecules and coagulation factors seem to be responsible for this relationship. Furthermore, all of these factors have been reported to significantly decrease after treatment with continuous positive airway pressure.4,5

Atherosclerosis is a process which is characterized by vessel wall remodeling that occurs over years. Obesity, aging, hypertension, diabetes, and hyperlipidemia have a major impact on the progression of atherosclerosis. Age, hypertension, hyperlipidemia and obesity are common risk factors for both atherosclerosis and OSAS. In recent studies there is an evolving evidence that OSAS is an independent factor for development of atherosclerosis.3

Carotid intima-media thickness (CIMT), flow-mediated dilation (FMD) and pulse wave velocity (PWV) measurement methods are used to determine subclinical atherosclerosis in OSAS.6 Endothelial dysfunction is a marker of vascular damage before development of atherosclerotic plaques. Inhibition of nitric oxide (NO) synthesis by chemical or mechanical stimulation, or inhibition of vascular response to NO are responsible mechanisms for endothelial dysfunction. Endothelial injury results in reduced arterial elasticity and increased arterial stiffness. Arterial stiffness is assessed by the degree of increase in PWV. Previous studies demonstrated that increased arterial stiffness is a marker of increased cardiovascular mortality and morbidity.15 CIMT increase develops as a result of intimal smooth muscle proliferation and accumulation of atherogenic particles. CIMT can be used in early diagnosis of atherosclerosis, risk classification and evaluation of response to treatment.9

The aim of this study was to investigate the presence of early signs of atherosclerosis in OSAS patients and correlation of these parameters with severity of the disease. In our study we excluded the patients with traditional risk factors for atherosclerosis, including hypertension, smoking, diabetes and hyperlipidemia.

We evaluated the early signs of atherosclerosis with the PWV and CIMT measurement methods in OSAS patients without any treatment for OSAS.
MATERIAL and METHOD

Patients

Patients between ages 30 and 60 years with OSAS diagnosis who were examined in department of Chest Diseases outpatient clinic between dates March 2009 and October 2010 were included in this study after conducting polysomnographies at the sleep laboratory. According to the severity which was determined by the AHI (apnea-hypopnea index), patients were examined in three groups; 30 patients in mild OSAS (AHI=5-15), 32 patients in moderate (AHI=16-30) OSAS and 31 patients in severe (AHI>30) OSAS group. As a control group, we chose 31 asymptomatic healthy individuals aging between 30 and 60 years without cardiovascular diseases who visited department of cardiology outpatient clinic for cardiovascular check-up. The healthy group used in the study included patients suitable for the study from the perspective of cardiac anatomy and functions, those with no right snoring or day-time sleepiness, who scored less than 10 in the Epworth sleepiness scale, and had low risk of OSAS in the Berlin survey form evaluation. The study was approved by the local Ethics Committee. Informed consents have taken from every individual included in the study. All patients underwent a detailed examination of the cardiovascular system. Biochemical parameters were obtained from venous blood samples drawn after a 8 hour fasting period.

Criteria for Exclusion

Exclusion criteria were as follows: (1) impaired cardiopulmonary function, defined as the occurrence of respiratory failure, pulmonary infection or congestive heart failure; (2) coronary artery disease, defined as having a typical angina pectoris, history of a prior myocardial infarction, presence of a positive stress test or positive coronary angiographic findings; (3) valvular disease, atrial fibrillation or congenital heart disease; (4) hypertension, diabetes, dyslipidemia (LDL cholesterol >160 mg/dl, total cholesterol >240 mg/dl, triglyceride >250 mg/dl), using antihypertensives, antidiabetics and lipid-lowering treatment; (5) malignancy; (6) history of prolonged use of non-steroid anti-inflammatory drugs or anticoagulants; (8) renal and liver insufficiency.

Polysomnography

Polysomnography was performed with 16 channels Embla (Medcare Inc, Iceland) with a continuous monitoring of a sleep technician. The system consists of 4 channels of EEG, 2 channels of EOG, submental EMI, oronasal air flow, thoracic and abdominal movements, pulse oximeter oxygen saturation, tibial EMG, body position detector, electrocardiogram and tracheal sound. Apnea was defined as complete stopping of airflow lasting more than 10 seconds. Hypopnea was defined as 30% or more reduction in respiratory airflow lasting more than 10 seconds and it is accompanied with a decrease of 2% in oxygen saturation. The average number of episodes of apnea and hypopnea per hour of sleep were defined as apnea hypopnea index (AHI). According to the severity, included patients were classified as mild OSAS (AHI=5-15), moderate OSAS (AHI=16-30) and severe OSAS (AHI>30). Sleep stages were scored following standard criteria with 30-s epochs and were reviewed and verified by a certified sleep physician.

Pulse Wave Velocity and Carotid Intima-Media Thickness Measurement

CIMT measurements of the individuals were performed by a physician blinded to both patient and the obtained
PWV value. Both common carotid arteries were visualized by Toshiba Powervision 7500 (Toshiba AG) ultrasound device with a 7.5 MHz linear probe. Maximum and mean thicknesses were calculated with the far edge measurement method by the CIMT measurement program MATH® standart version 2.0.1.0 (Metris AG, France) from a determined 1 cm segment of common carotid artery, 2-3 cm distal to bulbus. This method was applied for the measurement of both common carotid arteries were evaluated based on the average.

For the measurement of PWV, SphygmoCor (Artcore, Sydney, Australia) device was used. Before the procedure, blood pressures were measured in patients. Distance between the palpable point of femoral artery and sternal notch and the distance between the most distal palpable part of carotid pulse and sternal notch were recorded to system. Applanation tonometry of the device was applied through the skin to these points sequentially. Recording was done after the most appropriate waveform amplitude and shape were obtained. Simultaneous ECG records were taken from patients. Pulse transit time, in other words pulse wave velocity (PWV) were calculated automatically by the SphygmoCor device by subtracting the time between the ECG and proximal pulse from the time between the ECG and distal pulse.

Statistical Analysis

All data were analyzed with "MedCalc 11.0.4" and "SPSS 15.0 for Windows" software. Numerical
variables were defined as mean ± standard deviation, categorical variables were defined as percentiles. In comparison of three or more groups, if the variables fit the normal distribution, the one way ANOVA test, if not, the Kruskal Wallis test was used. In comparison of the categorical variables multiple comparison Chi Square test was used. In post-hoc analysis Tukey test was used after one way ANOVA test, and Mann Whitney U test was used after Kruskal Wallis test. Kolmogorov-Smirnov test was used in testing for normality of the distribution. Spearman correlation analysis performed for the determination of correlation. All hypotheses were established as two-way, and alpha critical value was accepted as 0.05.

RESULTS

Basic Characteristics of the Patients and Controls

There were no difference among groups in terms of demographic data; age, sex, body mass index, systolic and diastolic blood pressures, and in laboratory parameters; fasting blood glucose, HbA1c, serum lipid parameters (Table 1).

The mean AHI in mild OSAS patient group was 10.3±3, in moderate OSAS patient group it was 21.5±3.5, and in severe OSAS patient group it was 59.4±15.9. The difference in AHI among groups was statistically significant. Minimal oxygen saturation (min-SaO2) and the percentage of recording time spent at a SaO2 less than 90% (SaO2<90% (TST%)) were different among groups with statistical significance (Table 1).

Pulse Wave Velocity and Carotid Intima-Media Thickness

The PWV and the CIMT values of OSAS patients were higher than the normal healthy individuals (p<0.0001). The PWV and CIMT values showed a proportional increase correlated with the disease severity (p<0.0001) (Table 2).

With the increase of the PWV values, the maximal and mean CIMT values were found to be increasing (p<0.0001- r=0.79 / p<0.0001- r=0.74, respectively), and the mean CIMT (p<0.001- r=0.77 / p<0.001- r=0.72, respectively) (Figure 2). The min-SaO2 values were negatively correlated with the following; the PWV (p<0.001, r=-0.68), the maximal CIMT (p<0.001, r=0.68), and the mean CIMT (p<0.001, r=-0.65) (Figure 3).

DISCUSSION

OSAS is a systemic disorder and leads to cardiovascular complications. Recent studies showed that OSAS is not a simple respiratory abnormality during sleep, with the systemic inflammatory response it seems to be associated with cardiovascular diseases and increased atherosclerotic process. OSAS may be associated with the hypertension, diabetes mellitus and hyperlipidemia which increase the risk of atherosclerosis. In OSAS, hypoxia and intermittent reoxygenation episodes result with the oxidative stress which leads to endothelial dysfunction and increased sympathetic tone. Atherosclerosis is a progressive inflammatory process which results with fatal vascular events. Endothelial injury is the initial mechanism that triggers the atherosclerotic process. OSAS is more prevalent in patients with hypertension, diabetes mellitus, obesity, coronary artery disease, stroke and heart failure, in which the endothelial dysfunction plays a key role. Recent studies have shown the presence of the endothelial dysfunction in OSAS. It has been shown

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**Table 1: Baseline characteristics in control group and OSAS subgroups**

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=31) mean±sd</th>
<th>Mild OSAS (n=30) mean±sd</th>
<th>Moderate OSAS (n=32) mean±sd</th>
<th>Severe OSAS (n=31) mean±sd</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.1±8.4</td>
<td>46.1±8.2</td>
<td>48.3±7.6</td>
<td>47.3±7.7</td>
<td>0.71</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.9±3.9</td>
<td>28.4±3.1</td>
<td>28.7±2.7</td>
<td>29.2±3.9</td>
<td>0.77</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>119.6±8.8</td>
<td>119.1±6.7</td>
<td>121.7±6.7</td>
<td>121.6±8.9</td>
<td>0.49</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>73.7±6.0</td>
<td>73.8±5</td>
<td>74.7±5</td>
<td>75.8±5</td>
<td>0.77</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>89.4±8.2</td>
<td>89.8±8.6</td>
<td>91.7±10.5</td>
<td>92.5±9.4</td>
<td>0.49</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.5±0.4</td>
<td>5.6±0.2</td>
<td>5.6±0.4</td>
<td>5.6±0.4</td>
<td>0.78</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>191.5±23.8</td>
<td>188.7±33.1</td>
<td>190.5±43.1</td>
<td>185.6±39.8</td>
<td>0.86</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>117.5±23.5</td>
<td>113±33.9</td>
<td>119.7±35.9</td>
<td>118.6±30.6</td>
<td>0.81</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>46.8±10.8</td>
<td>44.9±13.6</td>
<td>44.2±10</td>
<td>45.2±10.4</td>
<td>0.83</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>146±67.9</td>
<td>144.8±57.9</td>
<td>148.4±81.7</td>
<td>149±50.8</td>
<td>0.99</td>
</tr>
<tr>
<td>AHI</td>
<td>10.3±3</td>
<td>21.5±3.5</td>
<td>59.4±15.9</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>SaO2 min</td>
<td>87.3±3.6</td>
<td>83.7±4.9</td>
<td>71.4±10</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>SaO2&lt;90% (TST%)</td>
<td>0.22±0.34</td>
<td>0.3±0.22</td>
<td>0.25±0.17</td>
<td>&lt;0.0001</td>
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</table>

**Table 2: Comparison of groups in terms of carotis intima media thickness and pulse wave velocity**

<table>
<thead>
<tr>
<th></th>
<th>Control group (mean±sd)</th>
<th>Mild OSAS (mean±sd)</th>
<th>Moderate OSAS (mean±sd)</th>
<th>Severe OSAS (mean±sd)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV (m/sec)</td>
<td>5.70±0.48</td>
<td>6.76±0.01</td>
<td>7.72±0.82</td>
<td>8.94±1.72</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Maximum CIMT (mm)</td>
<td>0.712±0.040</td>
<td>0.812±0.037</td>
<td>0.900±0.056</td>
<td>0.97±0.074</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean CIMT (mm)</td>
<td>0.615±0.037</td>
<td>0.806±0.040</td>
<td>0.761±0.056</td>
<td>0.833±0.075</td>
<td>&lt;0.0001</td>
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</table>
that various proinflammatory and prothrombotic factors in OSAS. Serum CRP, fibrinogen, IL-6 levels and insulin resistance were shown to be increased in OSAS patients. Nasal CPAP (continuous positive airway pressure) therapy have shown to decrease CRP, IL-6 levels and insulin resistance. Many of the studies investigating the cardiovascular morbidity and mortality in OSAS patients were cross sectional, retrospective or with a short term observation interval. Another important point of those studies was the coincidence of OSAS with the situations (obesity, hypertension and etc.) which have a role in atherosclerosis development. Because of this there is a suspicion in the causal relationship of OSAS with the cardiovascular diseases.

There were many studies investigated the relationship of OSAS with the atherosclerosis. Initially CIMT measurement method was used to determine the subclinical atherosclerosis, afterwards the PWV measurement method was used in establishment of increased arterial wall stiffness.

In our study, the relationship of OSAS with subclinical atherosclerosis were investigated in individuals aging between 30 and 60 years and without both proven atherosclerosis and the risk factors which have a role in atherosclerosis development, who were diagnosed as OSAS with the polysomnography test. The OSAS patients were examined in three different groups according to severity of the disease, and the correlation of the PWV and CIMT values with the severity of the disease was investigated.

There were studies showed that the CIMT increases in OSAS compared with normal individuals. In a study of Li Chong et al. the CIMT values found to be increased in patients with mild, moderate and severe OSAS compared with the healthy individuals. In the same study, the CIMT values of moderate and severe OSAS patients were found higher than the mild OSAS patients and there were no difference found between patients with moderate and severe OSAS in terms of CIMT. Altın et al. found that the CIMT values of severe OSAS patients were higher than the mild OSAS patients and normal healthy individuals with statistical significance. Minoguchi et al. found that the the CIMT values of OSAS patients were found higher than the obese control patients. In another study which examined 130 OSAS patients, the CIMT and PWV values were not found to be correlated with the disease severity. Tanriverdi et al. examined subclinical atherosclerosis with the flow mediated dilatation method (FMD) and CIMT, and they found that in OSAS patients the CIMT was higher and FMD was lower with a statistical significance. Also they found a correlation between the FMD and the disease severity.

In some of those studies the OSAS patients were only compared with normal healthy individuals. Only in a small number of studies the OSAS patients were classified according to the severity, but in those the patients with coincident risk factors for atherosclerosis were not excluded. In our study we excluded the patients with risk factors for atherosclerosis and we stratified the patients according to their severity. In majority of the previous reports CIMT values were found higher in OSAS patients when compared with healthy individuals, however, contradictory results exist about the relationship between this increase with the severity of the disease. In our study the CIMT was higher in OSAS patients than the healthy individuals.

**Figure 3:** Correlation between minimal oxygen saturation (Minimal SaO₂) and pulse wave velocity (PWV), maximum carotid intima-media thickness (Maximum CIMT) and mean carotid intima-media thickness (Mean CIMT).
and there was a relationship between the disease severity and the increased CIMT values. The AHI and the percentage of recording time spent at a SaO₂<90% (TST %) parameters which show the disease severity were found to be positively correlated with the increased CIMT. The min-SaO₂ was found to be negatively correlated with the CIMT.

The compliance of the arterial system has a major impact on the cardiovascular system.22,23 In many studies, the arterial stiffness had been shown to be an independent predictor of cardiovascular morbidity and mortality.23,26 The direct measurement of vascular compliance is difficult. PWV analysis method enables us to determine arterial compliance noninvasively. The PWV is negatively correlated with the arterial elasticity. In many studies, the relationship between the atherosclerosis and the changes in PWV had been shown previously.22,23,26,27 It is known that, as a reflection of arterial stiffness, the increased PWV, is known to be an indicator of atherosclerosis.26

In study of Nagahama et al., the OSAS patients without exclusion of atherosclerotic risk factors, were compared with normal healthy controls with PWV method. Ankle-brachial PWV was found to be greater in OSAS patients than controls. For the exclusion of hypertensive individuals, OSAS patients still had greater PWV than the normal individuals significantly.26 Drager et al. found that, the CIMT and PWV were significantly greater in severe OSAS patients than the mild and moderate OSAS patients, however there were no any statistically significant difference between mild and moderate OSAS patients and healthy individuals. Besides this, the PWV was found to be correlated with AHI, min-SaO₂, and TST %; whereas CIMT was only correlated with the AHI.3

Also in our study the carotid-femoral PWV was found greater in OSAS patients than controls with a statistical significance. As similar to the CIMT, the increase in PWV was related with the disease severity. Both the AHI and TST % were a positively correlated with the PWV. In addition there was a negative correlation between the PWV and min-SaO₂.

In our study we found that, the PWV and the maximum CIMT were increased in patients with OSAS. Also, by comparing the OSAS patients among themselves; we found that, both PWV and CIMT increased along with the disease severity. At the same time we found a significant correlation between the PWV and CIMT values.

This study shows us that, even if there were no any coincidental risk factors for atherosclerosis, the OSAS patients had increased PWV and CIMT values which denote the atherosclerosis development. This increase in PWV and CIMT correlated with the disease severity which means that the atherosclerosis development increased along with the disease severity.

One limitation of our study was using the method of Epworth and Berlin scale rather than the PSG, in the selection of control individuals. However in daily clinical practice, we use those methods for the selection of appropriate patients for the polysomnography test.

In conclusion, early signs of atherosclerosis are present in OSAS patients, and these findings are correlated with the OSAS severity. This supports the hypothesis that, OSAS has an independent role in atherosclerosis progression. As a result of these reasons, in these patients, screening for cardiovascular diseases is important for the prevention of cardiovascular mortality and morbidity. The patients with increased CIMT and PWV have to be identified, and because of the high risk for atherosclerosis development in these patients, treatment should be initiated in the early stage. We think that, these significant findings of our analysis can guide for the further clinical practice. However these findings must be supported clinically with prospective cohort studies.

Conflict of interest: None declared

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