Association between the severity of newly diagnosed obstructive sleep apnea and subclinical carotid atherosclerosis in patients without overt cardiovascular disease


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Abstract. – OBJECTIVE: Obstructive Sleep Apnea (OSA) has been associated with both subclinical and accelerated atherosclerosis; however, it still remains unknown whether this association is unique or is mediated by the higher burden of co-existing cardio-metabolic disorders frequently seen in patients with OSA.

PATIENTS AND METHODS: A total of 40 subjects without clinically diagnosed cardiovascular disease (CVD) referred for polysomnography test were included in the study. Subjects with apnea/hypopnea index (AHI > 15/h) were classified as moderate/severe OSA. Subclinical changes in carotid atherosclerosis were assessed using mean carotid intima-media thickness (cIMT) and presence of atheromatic plaques on both carotid arteries. The measurement was performed using B-mode ultrasonogram. Framingham risk score was used in the approximation of cardiovascular risk.

RESULTS: The mean age of our cohort was 56.8 years, 70% (n = 28) of whom were males. Moderate/severe OSA was diagnosed in 21 subjects. Both groups were well matched in terms of clinical and demographic characteristics, and cardiovascular risk profile, as shown in their respective Framingham risk scores (10.4 ± 6.6 vs. 11.8 ± 8.8, p = NS). Patients with moderate/severe OSA had a higher mean AHI, 3% oxygen desaturation index, and lower minimum nocturnal oxygen saturation than controls. No significant differences were detected in terms of C-reactive protein levels. The two groups had similar cIMT (0.66 ± 0.17 vs. 0.75 ± 0.20, p = 0.33) and presence of atheromatic plaque (50% vs. 45%, p = 1.00).

CONCLUSIONS: Our study suggests that among patients with similar cardiovascular risk profile and free of overt CVD, the severity of newly diagnosed OSA was not correlated with increased inflammation or subclinical carotid atherosclerosis.

Key Words: Obstructive sleep apnea (OSA), Subclinical atherosclerosis (SCA), Carotid intima-media thickness (cIMT), Inflammation.

Introduction

Obstructive sleep apnea (OSA) is a sleep-related breathing disorder characterized by recurrent episodes of complete or partial obstruction of the upper airway, specifically the apnea and hypopnea. An apnea/hypopnea index ≥ 5 events/hour is defined as OSA. The prevalence of OSA in the US is approximately 20-30% in males and 10-15% in females, and it is estimated that over 15 million Americans suffer from OSA. Its’ prevalence is slated to increase in light of increasing rates of obesity worldwide. While OSA has gained popularity among medical society and patients, the vast majority of patient with moderate to severe OSA have remained undiagnosed.

Despite recent advances in pharmacotherapy and medical technology, cardiovascular dis-
ease (CVD) remains the leading cause of death worldwide resulting in a shift of modern practice towards early detection of atherosclerosis, proper risk stratification and more aggressive preventing strategies. Markers of sub-clinical atherosclerosis including carotid intima-media thickness (cIMT), presence of carotid artery plaque, coronary artery calcification (CAC), and abdominal aortic calcification have been used as predictors of future cardiovascular events and mortality.

Cumulating evidence suggests link between OSA and accelerated atherosclerosis and endothelial dysfunction. Recently, a series of studies have associated OSA with subclinical atherosclerosis, however exact mechanisms remain unknown. Moreover, OSA patients are of high cardiovascular risk profile, given the high prevalence of conventional cardiovascular risk factors including obesity, diabetes mellitus, and hypertension. Consequently, whether the observed increased cardiovascular disease burden in OSA patients is due to OSA itself or mediated mostly by co-existing cardio-metabolic disorder remains unknown.

Patients and Methods

Patients

We enrolled 40 subjects who were referred for full overnight polysomnography and were free of cardiovascular disease. Moderate to severe OSA was defined as Apnea-Hypopnea Index [AHI] > 15, and the control group consisted of those with none/mild OSA (AHI < 15). All study subjects underwent overnight polysomnography for the detection and assessment of OSA presence and severity. Patients with definite history of CVD including those with previous myocardial infarction, peripheral arterial disease, stroke, unstable angina, prior coronary intervention, arrhythmias, aortic aneurysm, chronic kidney disease, use of statins or aspirin, or any chronic inflammatory disease were excluded.

A full medical history and sleep history was obtained from each patient. Blood samples were obtained in the morning post-polysomnography, and carotid ultrasound was performed within a week post-polysomnography. The study was approved by the Institutional Ethics Committee of our University Hospital and informed written consent was obtained from all the participants.

Polysomnography

A full overnight polysomnography was performed in all participants to determine the presence and severity of OSA. Apnea and hypopnea episodes were defined based on the recommendations of the American Academy of Sleep Medicine (AASM). Apnea was defined as continuous complete cessation of breathing for more than 10 seconds, and hypopnea was defined as a transient reduction in airflow of 50% or more lasting for at least 10 seconds accompanied by arousal or a decrease in SpO2 of at least 3%. Polysomnography recordings and data were interpreted by two experienced sleep medicine physicians. The degree of severity was defined by the number of apneas and hypopneas per hour over the total sleep time. OSA severity was defined as none-mild (AHI < 15), moderate (AHI 15-30), and severe OSA (AHI > 30).

Carotid Ultrasound and Measurements

All patients underwent two-dimensional echocolor Doppler of the carotid arteries adopting a Philips Sonos 5500 (Bothwell, WA, USA) high definition vascular echograph and a 10-3 MHz lineal electronic probe. All the examinations were performed by an experienced radiologist ultrasound specialist. The cIMT was defined as the distance between the lumen intima and media adventitia borders of the vessel. For intima-media thickness measurements images were obtained in three zones: (1) proximal zone about 2 cm before the flow divider, (2) distal zone- about ½ cm before the flow divider, (3) the middle zone. These data were used to obtain the arithmetical mean cIMT value. The assessment of atheromatic plaque presence was made by scanning the common, internal, and external carotid arteries and at the bifurcation area on both sides. A focal area of hyperechogenicity or a focal protrusion into the lumen of the vessel (> 25% of the vessel diameter) were defined as atheromatic plaques.

Statistical Analysis

Continuous variables are presented as mean ± standard deviation or median (interquartile range) and compared using an unpaired Student’s t-test or the Wilcoxon rank-sum test, respectively. Correlations were described with the Pearson correlation coefficient (r). Multivariate Cox regression analysis was used to determine covariate-adjusted odds ratio (ORs) of the prevalence of elevated cIMT and plaque after adjustment for sleep apnea and other covariates including smoking status,
BMI, diabetes mellitus, Framingham risk score. \( p \)-values < 0.05 were considered statistically significant. All statistical analyses were performed using STATA version 12.1 (StataCorp LP, College Station, TX, USA).

**Results**

Our study population comprised of 40 subjects who were screened for OSA and had no overt cardiovascular disease. Moderate/severe OSA was diagnosed in twenty-one subjects. Baseline characteristic of study participants are summarized in Table I. The mean age of our cohort was 56.8 years, and 70% (n = 28) of them were males. Both groups comprised of obese patients. While OSA group patients had higher BMI it was not statistically significant \((33.7 \pm 7.6 \text{ vs. } 30.7 \pm 6.3, p = 0.34)\). Similarly, the two groups were well matched for all major conventional cardiovascular risk factors including hypertension (50% vs. 55%, \( p = 1.00 \)), diabetes mellitus (25% vs. 10%, \( p = 0.41 \)), LDL-cholesterol levels \((133 \pm 21.5 \text{ vs. } 129 \pm 16.6, p = 0.87)\) and smoking (36% vs. 33%, \( p = 1.00 \)). No differences were detected with regards to the cardiovascular risk profile as expressed by Framingham risk score \((10.4 \pm 6.6 \text{ vs. } 11.8 \pm 8.8, p = \text{NS})\).

No/Mild OSA was diagnosed in 19 subjects, moderate in 6 subjects (15%) and severe (AHI > 30/hr) in 15 subjects (38%) (Table II). The mean AHI in the two groups was 5.8 and 42.3 respectively. Patients with moderate to severe OSA had significantly lower levels of oxygen saturation \( (sO_2) \) during awake time \((88.5 \pm 16.9 \text{ vs. } 93.9 \pm 4.8, p = 0.03)\) as well as minimum nocturnal oxygen \( sO_2 \) levels \((73.4 \pm 9.1 \text{ vs. } 88.9 \pm 6.6, p < 0.001)\). Likewise, 3% oxygen desaturation index (ODI) was considerably more prominent among moderate/severe OSA patients than control subjects \((60\% \text{ vs. } 5\%, p = 0.001)\). The occurrence of COPD and asthma were similar in the two groups as well as lung volumes and capacities as measured by spirometry.

No differences were found in the C-reactive protein levels in the two groups \((3.47 \pm 2.3 \text{ vs. } 3.51 \pm 1.7, p = 0.82)\). Moderate to severe OSA patients had comparable to control group cIMT

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<th>Table I. Baseline demographic and clinical characteristics.</th>
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<td><strong>AHI ≤ 15 (n = 19)</strong></td>
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<td>Age</td>
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<td>Gender (female)</td>
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<tr>
<td>Hypertension</td>
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<td>Diabetes mellitus</td>
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<td>Body Mass Index (kg/m²)</td>
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<th>Table II. Imaging and sleep study parameters.</th>
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<td><strong>AHI ≤ 15 (n = 20)</strong></td>
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<tr>
<td>Apnea/Hypopnea Index (AHI)</td>
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<td>Sleep total time (min)</td>
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<td>Sleep ( sO_2 ) awake</td>
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<td>Time &lt; 90% ( sO_2 ) (minutes)</td>
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<td>Time &lt; 90% ( sO_2 )/Total sleep time</td>
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<td>ODI &gt; 5</td>
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<td>EPWORT sum</td>
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<td>CRP (mg/dl)</td>
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<td>Mean cIMT (mm)</td>
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<td>Atheromatous plaque (present)</td>
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<td>Sleep total time (min)</td>
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(0.66 ± 0.17 vs. 0.75 ± 0.20 p = 0.33) and similar prevalence of carotid atheromatic plaque (50% vs. 45%, p = 1.00). While multivariable analysis failed to show any correlation between severity of OSA and carotid atherosclerosis or cIMT, age (1.74, 95% CI: 1.48-2.16, p < 0.001) and Framingham score (1.86, 95% CI: 1.23-2.94, p < 0.001) were identified as predictors of subclinical carotid atherosclerosis.

Discussion

In this prospective case-control study, we sought to investigate whether patients with newly diagnosed moderate/severe OSA carry a higher burden of carotid atherosclerosis as compared to healthy, well-matched control patients for all standard Framingham risk factors. In our study, we found no association between OSA and SCA and inflammation.

Our study has some strengths including its' prospective nature, the fact that all our study population underwent polysomnography and thus both groups were accurately defined, the use of comprehensive validation examination of carotid disease, exclusion of patients with overt CVD, and the examination of inflammatory markers.

The two main physiologic disturbances that occur in OSA patients are obstructive episodes and hypoxemia. The repetitive desaturation-re-oxygenation leads to the reflective activation of sympathetic nerve system, vasoconstriction, and increased oxidative stress. Over-production of reactive oxidative species results in endothelial vasodilator dysfunction through suppression of nitric oxide synthase pathway. The subsequently enhanced lipid peroxidation induces alterations in endothelial function and triggers the inflammatory signaling pathways promoting atherosclerosis.

Suzuki et al demonstrated that hypoxemia correlated with cIMT even after adjustment for AHI, suggesting that perhaps hypoxemia may be associated with atherosclerosis regardless of obstruction.

While theoretically OSA has been well correlated with accelerated inflammatory process and atherosclerosis, clinical data regarding OSA and carotid atherosclerosis is somewhat controversial and it yet remains unclear whether this association is unique or it is driven mainly by confounders.

Data from a series of studies and systematic reviews suggest a direct link between OSA and cIMT. Ali et al in a comprehensive review of 52 studies examined the correlation between OSA and SCA as assessed by coronary artery calcification (CAC), cIMT, brachial artery flow-mediated dilation and pulse wave velocity. It was demonstrated that OSA patients suffer from accelerated atherosclerosis and endothelial dysfunction independent of other confounders. Similar results were demonstrated by other studies which all showed direct link between OSA and SCA. For instance, Fox et al recruited OSA patients free of CVD and conventional risk factors and compared them to controls matched for BMI, age, and gender. OSA was found to be independently correlated with greater cIMT with a stronger correlation for those with severe sleep apnea. However, among OSA patients, severity of OSA was not associated with either increased cIMT or presence of carotid plaques. Of note, the control group patients did not undergo polysomnography to rule out OSA limited considerably study results and strength.

However, these studies and systematic reviews should be interpreted cautiously given some limitations in their design and methodology including cross-sectional, retrospective or matched cohort nature. Furthermore, data regarding inflammatory markers.
ry markers (i.e. CRP, IL-6) which would elaborate on elucidating the relationship between OSA and inflammation were not available in the majority of studies.

In agreement with our findings, a large cross-sectional study and a subset of the Sleep Heart Study\textsuperscript{33} reported no association between OSA and cIMT. In this study, 985 subjects with no history of coronary heart disease and stroke and with mild to severe OSA were recruited. No association between increasing RDI and CIMT and carotid plaques was detected [adjusted OR 1.0, 1.04 (0.71-1.54), 1.07 (0.71-1.61), and 1.25 (0.81-1.95)]. A major difference with other researches is that subjects of this work were recruited from the general community rather than just referred patients to the sleep clinics. Subsequently, most of the study participants were middle-aged Caucasians who had only mild-to-moderate OSA as the median RDI was 8.7 events per hour. Similarly, Gorzewska et al\textsuperscript{34} showed that in non-obese group of patients free of cardiovascular risk factors OSA had no correlation with increased cIMT. In this report, median cIMT was 0.41 mm in OSA patients (n = 28 subjects) and 0.46 mm in the control group (p = 0.087) suggesting that isolated OSA may not contribute to increased risk of cardiovascular events.

Our multivariate regression analysis, in contrast to previous investigations, showed no correlation between cIMT and any of the sleep parameters including AHI, ODI or nadir sO\textsubscript{2} levels. It did, however, demonstrate a strong correlation between carotid atherosclerosis and Framingham risk score which has been well documented previously and further validates our results\textsuperscript{35}. Our study also showed that almost 50% of study population suffered from carotid atherosclerosis as evidenced by the presence of atheromatic plaque throughout the carotid arteries. Our data are in line with the Progression of Early Subclinical Atherosclerosis (PESA) study\textsuperscript{36}. The PESA study prospectively enrolled 4184 asymptomatic subjects 40 to 54 years of age and evaluated the extent of subclinical atherosclerosis in different anatomic territories (carotid, iliofemoral, coronary arteries and aorta). It was reported that carotid SCA was present in 40% in patients with moderate CV risk (10-year FHS score of 10-20).

Lack of association between OSA and carotid SCA might be attributable to a number of reasons. First, clinical comorbidities including obesity, male gender, smoking, diabetes, and hypertension may actually be the driving factors of atherosclerosis observed in OSA patients rather than hypoxia. The atherogenic effects of those medical conditions have been well evidenced. Second, it might be a late rather than acute effect. The Wisconsin Cohort study\textsuperscript{15}, a large longitudinal prospective study of the natural history of OSA in adults, demonstrated that OSA even after multivariable adjustment is associated with long-term increased cIMT and plaque, implying that the cardiovascular effects of sleep apnea may be more prominent long- rather than short- term.

Third, conventional 2-D carotid U/S as compared to novel 3-D ultrasounds is less sensitive in the detection of atheromatic plaques. Also, CIMT and the presence of atheromatic plaques might not be the ideal marker of atherosclerosis as suggested by Sillesen et al\textsuperscript{37} which showed that carotid plaque burden a quantitative metric of the total plaque area across carotid artery confers greater prognostic value as compared to cIMT. Data also support that iliofemoral atherosclerosis may precede carotid or coronary atherosclerosis due to more potent dynamic flow changes that trigger inflammation and atherosclerosis. Otherwise, the fact that our paper

\begin{figure}
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\includegraphics[width=0.8\textwidth]{fig1.png}
\caption{Carotid media thickness (CMT) and number of atheromatic plaque in the two groups.}
\end{figure}
showed no correlation between OSA and cIMT does not necessarily exclude any association between OSA and subclinical atherosclerosis since CAC, iliofemoral, cIMT, and PWV were not assessed in our work. The PESA study sought to examine the presence, distribution, and extent of SCA in the carotid, aortic, coronary and iliofemoral territories. In this study, almost half of the participants (41%) were found to have intermediate to generalized atherosclerosis (presence of plaques in ≥ 2 territories). Atheromas were most common in iliofemoral (44%) as compared to carotids (31%) and aorta (25%) whereas CAC was present in 18% suggesting added value of imaging for diagnosis and prevention of SCA.

Carotid plaques and cIMT are surrogate markers of atherosclerosis and predict incidence of stroke and coronary heart disease. The use of more than one imaging modality for the identification of subclinical atherosclerosis seems to confer extra prognostic value. The Bioimage study\textsuperscript{7} which enrolled 5805 participants with no known atherosclerotic CVD (ASCVD) sought to evaluate the role of vascular imaging (CAC and carotid U/S) on cardiovascular risk prediction. It was demonstrated that detection of subclinical carotid or coronary atherosclerosis via carotid ultrasound and CAC score respectively improves both risk predictions and reclassification compared with conventional risk factors. It was shown that almost 1/3 of the patients who would not qualify for statins based on the ACC/AHA risk assessment tool (10-year ASCVD risk score of ≤ 7.5%) had positive CAC or carotid U/S test, whereas a substantial percentage of high risk patients (20%) (10 year ASCVD risk score of ≥ 15%) had no imaging evidence of coronary and carotid vasculopathy. Notably, in our report, 50% of asymptomatic participants were found to have evidence of carotid atherosclerosis and they would, therefore, qualify for statin treatment underscoring the clinical importance of aggressive preventive screening strategies. Multi-territorial noninvasive assessment of subclinical atherosclerosis can act complementary to traditional risk scales assisting to achieve the goal of individualized risk assessment.

Our study limitations include the relatively modest small sample size, the single-center nature, and that we may have been underpowered to detect potential associations. However, majority of published papers are single-center and, additionally, our study population is comparable to those of others. To identify and enroll OSA patients without overt CVD is challenging and limits sample size given the extensive overlap between OSA and CV comorbidities. Lastly, other imaging modalities for the detection of early signs of atherosclerosis such as calcium score, iliofemoral or aortic U/S would have enhanced our study results.

Conclusions

We suggest that among patients with similar cardiovascular risk profile and free of overt CVD, the severity of newly diagnosed OSA was not correlated with increased inflammation or subclinical carotid atherosclerosis. Larger and multicenter studies with the use of multiple imaging modalities for the presence of subclinical atherosclerosis are warranted to determine the exact correlation between OSA and SCA.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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