Adhesion Molecules in Patients With Coronary Artery Disease and Moderate- to-Severe Obstructive Sleep Apnea*

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Study objectives: It has been suggested that obstructive sleep apnea (OSA)-induced hypoxic stress might contribute to cardiovascular disorders by promoting expression of soluble adhesion molecules. The reported increase of circulating adhesion molecules in patients with OSA remains controversial because confounders such as cardiovascular risk factors and left ventricular function have not been adequately controlled for. We hypothesized that soluble intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, L-selectin, and E-selectin levels are correlated with OSA independent of coexisting coronary artery disease (CAD).

Settings: University-affiliated teaching hospitals.

Design and participants: A prospective study of 61 consecutive subjects with angiographically proven CAD deemed to have stable angina. Fifteen patients (mean ± SD) 61.2 ± 1.9 years old with moderate-to-severe OSA (apnea-hypopnea index [AHI] ≥ 20/h) were matched to a control group (AHI ≤ 5/h) for age, gender, body mass index, and severity of CAD. Venous blood samples were collected the morning of the sleep study and assayed for human ICAM-1, VCAM-1, L-selectin, and E-selectin with commercially available enzyme-linked immunosorbent assay kits.

Results: All but L-selectin were significantly increased in the OSA group compared to the control subjects (ICAM-1, 367.4 ± 85.2 ng/mL vs 252.8 ± 68.4 ng/mL, p = 0.008; VCAM-1, 961.5 ± 281.7 ng/mL vs 639.1 ± 294.4 ng/mL, p = 0.004; E-selectin, 81.0 ± 30.4 ng/mL vs 58.1 ± 23.2 ng/mL, p = 0.03, respectively). The increased levels of adhesion molecules correlated with the AHI and the oxygen desaturation index but not with the severity of hypoxemia or the frequency of arousals.

Conclusions: These findings suggest that OSA modulates the expression of proinflammatory mediators. Further studies should evaluate the influence of adhesion molecules on cardiovascular outcome in CAD patients with OSA.

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Key words: cell adhesion molecules; coronary artery disease; hypoxia; sleep apnea

Abbreviations: AHI = apnea-hypopnea index; CAD = coronary artery disease; ICAM = intercellular adhesion molecule; Ln = logarithmic transformation; ODI = oxygen desaturation index; OSA = obstructive sleep apnea; VCAM = vascular cell adhesion molecule

Sleep apnea may turn out to be one of the important independent risk factors for cardiovascular diseases, including hypertension, ischemic heart disease, and cerebrovascular accidents.1,2 Cross-sectional and longitudinal studies have documented a higher prevalence of obstructive sleep apnea (OSA) among selected patients with coronary artery disease (CAD),3 and an increased risk of cardiovascular mortality among untreated OSA in patients with ischemic heart disease.4 The risk of mortality was pronounced particularly in those with moderate-to-severe sleep apnea syndrome.5,6 One of the potential mechanisms advanced in linking the association between OSA and cardiovascular events postulates that OSA-induced hypoxic stress modulates circulating inflammatory mediators causing accelerated atherogenesis.7
Adhesion of circulating leukocytes to the endothelial cells is considered one of the initial steps in the pathogenesis of atherosclerosis. This process is thought to be mediated by cellular adhesion molecules in response to several inflammatory cytokines, including interleukin-1 and tumor necrosis factor. Clinical and pathologic data have implicated an array of adhesion molecules in acute atherothrombotic syndromes, and pointed to an increased expression of adhesion molecules in several components of the atherosclerotic plaque.

Although the pathologic role of these cellular adhesion molecules in OSA is uncertain, studies have shown elevated circulating levels of adhesion molecules in subjects with OSA compared to those without sleep-disordered breathing. These studies, however, did not take into account ventricular function, which is a confounding variable, and they included a heterogeneous population with various risk factors for cardiovascular diseases. The purpose of this study was to extend these studies by testing the hypothesis that there is a direct correlation between plasma intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, L-selectin, and E-selectin levels in patients with angiographically proven CAD and moderate-to-severe OSA, as determined by full polysomnography independent of coexistent heart disease.

**Materials and Methods**

**Subjects**

Seventy-two consecutive patients referred for evaluation of CAD at the Veterans Affairs Medical Center of Western New York were recruited for participation in the study. All study participants were free from previous stroke, transient ischemic attack, and cancer at study entry. Patients with valvular heart disease, cardiomyopathy (left ventricular ejection fraction ≤ 45%), malignant arrhythmias, severe pulmonary disease (FEV1 < 1.0 L), or those who were receiving oxygen or continuous positive airway pressure treatment were excluded. All patients underwent outpatient ventriculography and coronary angiography using the standard Judkins approach. Those deemed to require hospitalization or urgent surgical intervention were excluded from further participation. The stratification of diseased coronary artery vessels was made according to the criteria used in the Coronary Artery Surgery Study Registry.

Risk factors at baseline were assessed according to the following criteria: hypertension was defined as ongoing pharmacologic antihypertensive treatment and or recorded systolic BP > 160 mm Hg or diastolic BP > 95 mm Hg measured on at least 3 different days. Diabetes mellitus was recorded if insulin or oral hypoglycemic agents were used or if the fasting blood sugar concentration was > 140 mg/dL at three separate occasions. Hypercholesterolemia was defined as total serum cholesterol concentration > 200 mg/dL. Subjects were classified as current smokers, former smokers (for those who had quit smoking for ≥ 6 months), or never smokers. Data were collected on surgical intervention for CAD such as coronary artery bypass grafting or percutaneous transluminal angioplasty and pharmacologic treatment for CAD. The information was retrieved from reviews of clinical charts and direct patient interviews.

**Sleep Studies**

Overnight polysomnography was conducted within 3 months of coronary angiography. All participants were asked to refrain from alcohol consumption or use of a sedative in the 48 h prior to undergoing the sleep study. A questionnaire was administered to each patient in the presence of the bed partner when available. The questionnaire inquired about the presence of any history of snoring, witnessed opnea, excessive daytime sleepiness, choking or gasping during sleep, restless leg syndrome, falling asleep while driving, or decreased libido. Demographic information (age and gender) and anthropometric measurements (neck circumference, height, and weight) were obtained on presentation to the sleep center.

Continuous EEG, electro-oculogram, ECG, and submental and anterior tibial electromyogram were recorded on a 16-channel polygraph using standard technique, and digitized on a computerized system (Acquitytron; Mallinckrodt; St. Louis, MO). Airflow was measured qualitatively by an oral-nasal thermistor (Graphich Control; Buffalo, NY). Measurement of arterial oxyhemoglobin saturation was performed with a pulse oximeter (Ohmeda 3740; Ohmeda; Boulder, CO) with the probe placed on the patient’s finger. Thoracoabdominal movements were recorded using piezoelectric belts.

Sleep stages were scored in 30-s epochs using the Rechtschaffen and Kales sleep scoring criteria. Each epoch was analyzed for the number of apneas, hypopneas, arousals, oxyhemoglobin desaturation, and disturbances in cardiac rate and rhythm. Apnea was defined as the absence of airflow for > 10 s. An obstructive apnea was defined as the absence of airflow in the presence of rib cage and/or abdominal excursions. Hypopnea was defined as a visible 20% reduction in the airflow or discernible reduction in the thoracoabdominal excursions lasting > 10 s associated with either a 4% decrease in arterial oxyhemoglobin saturation or an EEG arousal, or both. The apnea-hypopnea index (AHI) was defined as the number of apneas and hypopneas per hour of sleep. A sleep study finding for OSA was considered positive when the AHI was ≥ 10/h. Moderate and severe OSA were defined as AHI ≥ 20/h and < 40/h, and ≥ 40/h, respectively. Desaturation during sleep was defined by the computer software system (Acquitytron; Mallinckrodt) as a fall in baseline oxygen saturation of ≥ 4%. The oxygen desaturation index (ODI) was derived from the number of episodes of desaturation per hour of total sleep time.

**Study Design**

Sixty-four of 72 eligible patients met the inclusion criteria for enrollment. Three patients did not appear for the sleep study. Twenty-seven of 61 patients (44%) met the definition of OSA. Eleven patients (18%) had mild OSA (AHI, 11 to 20/h), 12 patients (19%) had moderate OSA (AHI, 20 to 40/h), and 4 patients (7%) had severe OSA (AHI > 40/h). Of the 16 patients with moderate-to-severe OSA, 1 patient refused to have his blood drawn after two attempts, leaving 15 patients for analysis. These were matched consecutively to a control group without OSA for the number of diseased vessels.

**Serum Assays**

Whole blood was obtained by venipuncture without tourniquet application in all subjects between 8 AM and 10 AM of the
morning of the interview. The blood samples were centrifuged at 3,000 g at 4°C for 10 min, and plasma was frozen at −80°C until measurement of adhesion molecules. Stored plasma was thawed and assayed for human ICAM-1, VCAM-1, L-selectin, and E-selectin with a commercially available enzyme-linked immunosorbent assay kit (R&D Systems; Minneapolis, MN). The minimal detectable dose was determined by adding two standard deviations to the mean optical density value of 20 zero standard replicates and calculating the corresponding concentrations. The minimal detectable dose for ICAM-1, VCAM-1, L-selectin, and E-selectin were < 0.35 ng/mL, 0.2 ng/mL, 0.3 ng/mL, and 0.1 ng/mL, respectively. The intra-assay coefficient of variation for the soluble adhesion molecules was 3.3 to 4.8% (n = 10) for ICAM-1, 4.3 to 5.9% (n = 10) for VCAM-1, 2.5 to 5.0% (n = 20) for L-selectin, and 4.7 to 5.0% (n = 10) for E-selectin. The interassay coefficient of variation was 6.0 to 10.1% (n = 18) for ICAM-1, 8.5 to 10.2% (n = 14) for VCAM-1, 7.1 to 11.9% (n = 21) for L-selectin, and 5.7 to 8.8% (n = 18) for E-selectin. Laboratory personnel were unaware of case or control status.

Statistical Analyses

Descriptive statistics were performed. Data are expressed as mean ± SD. Means were compared using Student’s t test and the Mann-Whitney test. Proportions were analyzed using the χ2 analysis with Yates continuity correction or Fisher’s Exact Test where appropriate. A logarithmic transformation (Ln) of the soluble adhesion molecules was performed. All reported p values are two tailed. In all cases, p values < 0.05 were considered to be significant. Statistical analysis was performed with software (Statistical Package for the Social Sciences for Windows; SPSS; Chicago, IL).

RESULTS

Baseline clinical characteristics of patients and control subjects were similar for age, gender, anthropometric measurements, and major risk factors (Table 1). Patients with OSA and those without OSA did not differ in the use of β-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors, or lipid-lowering agents. The angiographic data were comparable in the two groups after controlling for the number of coronary arteries diseased (Table 2). In particular, the difference in left ventricular ejection fraction between the two groups was not significant (p = 0.823). Subjective symptoms regarding history of snoring, witnessed apneas, or falling asleep while driving did not differ significantly between the two groups, whereas excessive daytime tiredness, and choking and gasping during sleep were more likely to be reported in the OSA group (Table 3). The results of the overnight polysomnography are presented in Table 4. There was no significant difference in the total sleeping time or sleep efficiency between the two groups. The oxygen saturation nadir was, however, more pronounced in the OSA group, and the percentage of time spent with an oxygen saturation of < 90% was significantly longer for the OSA group compared to the control group.

Patients with OSA syndrome had significantly greater soluble adhesion molecule levels than the control subjects. Specifically, circulating ICAM-1 levels were higher (367.4 ± 85.2 ng/mL vs 252.8 ± 68.4 ng/mL, p = 0.008), as were VCAM-1 levels (961.5 ± 281.7 ng/mL vs 639.1 ± 294.4 ng/mL, p = 0.004), and E-selectin levels (81.0 ± 23.2 ng/mL vs 58.1 ± 23.2 ng/mL, p = 0.03), respectively. We did not find, however, a significant difference in the levels of L-selectin between the two groups.

Table 1—Clinical Characteristics of OSA and Non-OSA Groups*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>OSA (n = 15)</th>
<th>Non-OSA (n = 15)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>61.2 ± 1.9</td>
<td>59.3 ± 2.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Male/female gender</td>
<td>15/0 (100)</td>
<td>15/0 (100)</td>
<td>1.0</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>31.47 ± 1.12</td>
<td>29.02 ± 1.40</td>
<td>0.153</td>
</tr>
<tr>
<td>Neck circumference, cm</td>
<td>45.13 ± 1.07</td>
<td>43.83 ± 0.81</td>
<td>0.344</td>
</tr>
<tr>
<td>Former smoker</td>
<td>5 (33)</td>
<td>4 (27)</td>
<td>0.638</td>
</tr>
<tr>
<td>Current smoking history</td>
<td>4 (26)</td>
<td>7 (46)</td>
<td>0.321</td>
</tr>
<tr>
<td>Never smoked</td>
<td>3 (20)</td>
<td>2 (13)</td>
<td>1.0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>13 (86)</td>
<td>9 (60)</td>
<td>0.106</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>8 (53)</td>
<td>4 (26)</td>
<td>0.702</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>7 (46)</td>
<td>7 (46)</td>
<td>1.0</td>
</tr>
<tr>
<td>History of myocardial infarction</td>
<td>12 (80)</td>
<td>10 (66)</td>
<td>0.426</td>
</tr>
<tr>
<td>History of CABG</td>
<td>6 (40)</td>
<td>4 (26)</td>
<td>0.456</td>
</tr>
<tr>
<td>Medications used</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>14 (93)</td>
<td>15 (100)</td>
<td>1.0</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>8 (53)</td>
<td>9 (60)</td>
<td>1.0</td>
</tr>
<tr>
<td>Diuretics</td>
<td>2 (13)</td>
<td>0</td>
<td>0.45</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>11 (73)</td>
<td>9 (60)</td>
<td>0.7</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>5 (33)</td>
<td>7 (47)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD or No. (%). CABG = coronary artery bypass grafting; ACE = angiotensin-converting enzyme.
VCAM-1 and E-selectin (ICAM-1 and VCAM-1) molecules revealed a significant relation between OD1. Pairwise correlations among the adhesion molecules revealed a significant relation between ICAM-1, VCAM-1, and the Ln of AHI (r = 0.41, p = 0.02 and r = 0.5, p = 0.005, respectively) (Fig 1). A positive correlation was also noted between E-selectin and Ln AHI; however, a statistical significance was not attained between these two variables (r = 0.32, p = 0.08). When Ln ODI was correlated with ICAM-1, VCAM-1, and E-selectin, all three also showed a positive trend, but the correlation was significant for VCAM-1 (r = 0.39, p = 0.03) and E-selectin (r = 0.41, p = 0.02; Fig 2). L-selectin was not related to either Ln AHI or Ln ODI. Pairwise correlations among the adhesion molecules revealed a significant relation between ICAM-1 and VCAM-1 (r = 0.60, p < 0.001), ICAM-1 and E-selectin (r = 0.41, p = 0.03), and VCAM-1 and E-selectin (r = 0.44, p = 0.01).

The severity of hypoxemia assessed by the lowest nocturnal oxygen saturation and the percentage of time with oxygen saturation < 90% did not correlate with the circulating levels of adhesion molecules. Similarly, the frequency of cortical arousals had no correlation with ICAM-1, VCAM-1, L-selectin, and E-selectin levels.

**Discussion**

The results of this study demonstrate the following: (1) circulating adhesion molecules are significantly elevated in CAD patients with moderate-to-severe OSA compared to those without OSA, and (2) the concentration of circulating adhesion molecules correlated with the severity of sleep apnea and the desaturation index but not with the severity of hypoxemia or the frequency of arousals.

Epidemiologic studies have suggested an asso-
Association between OSA and systemic hypertension independent of age, gender, and body mass index. It has also been suggested that OSA significantly raises the risk of cardiovascular events and may in fact be an independent risk factor of cardiovascular mortality.\textsuperscript{1–6} We have found that the circulating levels of ICAM-1, VCAM-1, and E-selectin are significantly increased in CAD patients with moderate-to-severe OSA compared with those of a matched control group. Since cellular adhesion molecules mediate cellular interactions and transmigration of leukocytes across the vascular endothelial wall,\textsuperscript{19} our findings suggest that OSA could contribute potentially to the inflammatory process implicated in atherogenesis.

The present data confirm previous results that OSA syndrome is associated with a rise in circulating levels of adhesion molecules. Ohga and coworkers\textsuperscript{7} reported increased levels of ICAM-1, VCAM-1, and L-selectin levels in seven patients with OSA compared to a control group of “normal subjects.” The authors suggested that the repetitive hypoxic stress during sleep might induce activation and provoke sustained release of these inflammatory mediators. The study had, however, significant limitations. The OSA group was older than the control group, although not significantly different (48.6 ± 1.8 years in the OSA group vs 42.2 ± 7.2 years in the control group), but the power of the study was too small to exclude a potential significance. The subjects of both groups were not evaluated for coexisting cardiac disease, and no detail was provided regarding their cardiac function. Since elevated adhesion molecules have been reported in the failing heart,\textsuperscript{20} the correlation between OSA and adhesion molecules could be interpreted as an epiphenomenon rather than a true correlation. Moreover, Ohga and colleagues\textsuperscript{7} suggested that the elevation in circulating adhesion molecules are related to hypoxia, yet the study did not establish a correlation between the degree of hypoxia and the levels of adhesion molecules.

Patients with OSA present with repetitive apneas and hypopneas that result in oxygen desaturations with arousals as well as increased sympathetic nerve activity. While hypoxia has been implicated in the
induction of interleukin-1 and cellular adhesion molecules gene expression, other studies have found no significant change. In our study, we found no relation between cellular adhesion molecules and the severity of hypoxemia as indicated by the percentage of time arterial oxygen saturation remained < 90%, or the lowest nocturnal oxygen saturation. We did observe, however, a significant correlation between VCAM-1 and E-selectin, and the oxygen desaturation index suggesting that the risk of cardiovascular events is perhaps related to the intermittent hypoxia observed during sleep rather than the time spent in hypoxemia. It is plausible that intermittent hypoxia promotes oxygen radical formation that leads to activation of transcriptional factors that upregulate the genetic expression of adhesion molecules. In vitro studies have shown that in perfused cell cultures, hypoxia/reoxygenation causes an increase in the levels of adhesion molecules. Furthermore, Mooe and colleagues, in a study of the relation between nocturnal myocardial ischemia and sleep-disordered breathing in patients with CAD, noted that ST-segment depressions occurring within 2 min after a breathing event were often preceded by repetitive episodes of apneas/reoxygenation. Alternatively, sleep fragmentation and chronic intermittent hypoxia could lead to increased sympathetic discharge with subsequent modulation of the adhesion molecule expression. There is, however, little evidence to support this idea at the present time.

Potential limitations of these data merit consideration. First, we have made measurements of circulating levels of adhesion molecules to assess the expression of cell-associated adhesion molecules. Although this approach has been widely used in previous studies, there is always the concern that circulating levels may not totally reflect what is happening at the tissue level, and vascular biopsy would be needed to confirm these findings. Second, because baseline plasma samples were obtained on only one occasion, we could not take into account variation in concentrations that may have occurred over time. The impact of this potential limitation would be to increase random variability, an effect that, if present, could lead to an underestimation of the magnitude of any relation. Third, smokers have significantly higher concentrations of ICAM-1 than nonsmokers, raising the possibility of a confounder. However, the association between adhesion molecules and the severity of sleep-disordered breathing was present independent of the smoking status. It is noteworthy to indicate that there was no significant relationship between the severity of CAD and the levels of adhesion molecules, but the investigation was not designed to specifically explore this question.

In summary, the present findings indicate that OSA increases the circulating levels of adhesion molecules independent of the severity of CAD. It remains unclear at present how universal our findings might be with regard to other mediators of the inflammatory process. Further studies are needed to elucidate the interaction between intermittent hypoxia and activation of cellular adhesion molecules.

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