Serum Cardiovascular Risk Factors in Obstructive Sleep Apnea*

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Background: Obstructive sleep apnea (OSA) patients have increased cardiovascular morbidity and mortality. The cardiovascular markers associated with OSA are currently not defined.

Objectives: The aims of this study were to determine whether OSA is associated with serum cardiac risk markers and to investigate the relationship between them.

Methods: Sixty-two male patients were classified into two groups with respect to apnea-hypopnea index (AHI): group 1, sleep apnea (n = 30), with AHI > 5; and group 2 (n = 32), with AHI < 5. We compared cardiovascular risk factors in both groups with control subjects (n = 30) without OSA (AHI < 1). Serum cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A-I, apolipoprotein B, lipoprotein (a), C-reactive protein (CRP), and homocysteine were measured. Statistical significance was assessed with analysis of variance at p < 0.05. In correlation analysis, Pearson correlation was used.

Results: There was no significant difference between group 1 and group 2 in total cholesterol, LDL-C, HDL-C, triglyceride, apolipoprotein A-I, apolipoprotein B, and lipoprotein (a). All of the M-mode echocardiographic parameters were in the normal reference range. Serum homocysteine and CRP levels were significantly increased in group 1 compared to group 2 (p < 0.05). Serum CRP values were increased in both group 1 and group 2 when compared with control subjects (p < 0.05). Serum homocysteine values were higher in group 1 than in control subjects (p < 0.05).

Conclusions: Our results show that OSA syndrome is associated not only with slight hyperhomocysteinemia but also with increased CRP concentrations. Increased plasma concentrations of homocysteine and CRP can be useful in clinical practice to be predictor of long-term prognosis for cardiovascular disease and the treatment of OSA.

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Key words: C-reactive protein; homocysteine; obstructive sleep apnea

Abbreviations: AHI = apnea-hypopnea index; CPAP = continuous positive airway pressure; CRP = C-reactive protein; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; OSA = obstructive sleep apnea

Obstructive sleep apnea (OSA) is a common chronic respiratory disorder. OSA occurs in approximately 4% of men and 2% of women > 30 years old. Increase in the ratio with age may depend on the role of OSA on the complications of the disease.

OSA is well-defined syndrome that includes one or two of the following symptoms: severe snoring, nocturnal respiratory arrest, repeated nocturnal awakening, nonrecuperative sleep, diurnal fatigue, and altered concentration. These clinical findings are related to the extent of hypoxemia and hypercapnia that develop as a result of disordered breathing.
Although OSA patients have increased cardiovascular morbidity and mortality, how much of their cardiovascular diseases are due to OSA rather than to other risk factors such as upper-body obesity; insulin resistance; increased age; alcohol and caffeine consumption; and cigarette smoking is yet unknown. Therefore, identifying the possible risk factors involved in OSA cardiovascular morbidity and mortality is of great clinical importance. Several studies report a strong association between homocysteine, C-reactive protein (CRP), total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride, apolipoprotein A-I and B, lipoprotein (a) levels, and coronary heart disease.

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The pathophysiology of the underlying mechanisms and complications of OSA is complex and multifactorial. The aims of this study were to determine whether OSA syndrome is associated with serum cardiac risk markers, and to investigate the relationship between serum cardiac risk markers.

Materials and Methods

Study Design

Patients were recruited for the study based on medical history and written consent. The study protocol was approved by the university ethics committee and was performed in accordance with the current revision of the guidelines in accordance with the Declaration of Helsinki.

Patients

OSA was diagnosed on the basis of the International Classification of Sleep Disorders. Sixty-two male patients who were referred for suspected sleep apnea underwent an overnight sleep study. Patients were classified into two groups with respect to apnea-hypopnea index (AHI); group 1 (n = 30), AHI > 5; group 2 (n = 32), AHI < 5. We compared cardiovascular risk factors in both groups with control subjects (n = 30) without OSA (AHI < 1). All subjects with OSA snored and reported excessive daytime sleepiness or two or more other features such as impaired concentration, unrefreshing sleep, witnessed apneas, restless sleep, and irritability/personality change. Before enrollment, the subjects were asked about their regular medications and medical history regarding diabetes mellitus, renal diseases, and ischemic heart disease. The patients were assessed for coronary artery disease with resting and stress 12-lead precordial ECG. Previous myocardial infarction, unstable angina, prior coronary intervention, arrhythmia, conduction abnormalities, heart failure, digoxin therapy, inability to perform tests, hypertension (BP ≥ 140/90 mm Hg or receiving medication), chronic renal disease, and diabetes mellitus were the exclusion criteria. Subjects who smoked or had systemic infections at the time of the study were also excluded. Before polysomnography, baseline demographic data, BF, ECG, and echocardiography were assessed throughout the day, and blood samples were collected between 8 PM and 9 PM.

Polysomnography

Polysomnography was started at 9 PM and ended at 6:30 AM. Surface electrodes were applied using standard techniques to obtain an EEG, an electromyogram of the chin, an ECG, and an electrooculogram. Sleep was defined according to the criteria of Rechtschaffen and Kales. Ventilation was monitored using inductive plethysmography. Airflow was monitored by thermistors placed at the nose and mouth, while arterial oxygen saturation was monitored continuously with a pulse oximeter. A polygraph was run continuously at 10 mm/s to record all of the above physiologic data simultaneously throughout the course of the experiment. All parameters were stored in a data recorder for subsequent analysis. Apnea was defined as the cessation of airflow at the nose and mouth lasting for > 10 s. Hypopnea was defined as a decrease of ≥ 50% in thoracoabdominal motion associated with a fall in the baseline oxygen saturation of ≥ 4%. All AHI values were calculated to express the number of episodes of apnea and hypopnea per hour of total sleep time.

Echocardiographic Analyses

We performed echocardiography with M mode (GE-VingMed System 5 Ultrasound System; GE-VingMed Sound AB; Horten, Norway). All studies were performed and interpreted by the same operator and recorded on videotape. Left ventricular end-diastolic dimension, left ventricular end-systolic dimension, and thickness of the interventricular septum and posterior wall were measured. The ejection fraction was calculated from area measurements using the area-length method applied to the average apical area. Echocardiographic data were recorded according to the guidelines of the American Society of Echocardiography.

Blood Collection

All blood samples were obtained in the nonfasting state. The subjects did not perform any specific exercise or apply any specific diet during the study period. For homocysteine, serum samples were centrifuged immediately and placed on ice prior to separation. After centrifugation, the serum aliquots were frozen and stored at −80°C.

Biochemical Analysis

Serum cholesterol, triglyceride, and HDL-C were measured by enzymatic colorimetric methods with commercially available kits (Cobas Integra 800; Roche Diagnostics GmbH; Mannheim, Germany), and LDL-C was calculated according to the Friedewald formula: total cholesterol − HDL-C − (0.45 × triglyceride). Apolipoprotein A-I and apolipoprotein B were measured by immunoturbidimetric method, and lipoprotein (a) and CRP were determined by particle-enhanced immunoturbidimetric method on the Roche Integra 800 analyzer. Serum homocysteine was measured by enzyme-linked immunosorbent assay (Axis Homocysteine ELIA; Asiquid Diagnostics; Dundee, Scotland) on a diagnostic instrument (LP 400: Diagnostics Pasteur; Chaska, MN). Vitamin B12 and folate levels were measured by electrochemiluminescent immunoassay on a Roche Elecsys 2010 analyzer (Vitamin B12 and folate kit; Roche Diagnostics). Erythrocyte count, hemoglobin concentration, mean cell volume, and mean cell hemoglobin concentration were measured (MAX; Beckman Coulter; Fullerton, CA), and stained RBC examinations of the patients were studied.

Statistical Analysis

Results are expressed as mean ± SE. We used analysis of variance to analyze any differences in demographic and hemo-
When we compared the patients with the healthy control subjects, there were no significant differences between group 1, group 2, and the control group with respect to age, body mass index, and BP (Table 1). All of the M-mode echocardiographic parameters (left ventricular end-diastolic dimension [LVEDD], 4.46 ± 0.45 cm; left ventricular end-systolic dimension [LVESD], 2.93 ± 0.41 cm; EF, 64.6 ± 2.66%; IVS, 1.28 ± 0.09 cm; and PW, 1.17 ± 0.10 cm) were in normal reference range. With regard to the conventional parameters (total cholesterol, LDL-C, HDL-C, triglycerides, apolipoprotein A-I, apolipoprotein B, and lipoprotein (a)) there were no significant differences between group 1 and group 2. Total cholesterol, LDL-C, triglyceride, apolipoprotein B, and lipoprotein (a) values were increased in both group 1 and group 2 when compared with control group (p < 0.05). There were no significant differences between group 1, group 2, and the control group in apolipoprotein A-I and HDL-C results (Table 2). Serum CRP values were increased in both group 1 and group 2 when compared with control group (p < 0.05). Serum homocysteine values were higher in group 1 than in control subjects (p < 0.05). Comparison of serum homocysteine and CRP levels revealed a significant difference (p < 0.05) between group 1 and group 2. (Table 2). Distribution of plasma homocysteine and CRP is shown in Figure 1. We did not find a significant correlation between CRP and homocysteine levels (r = 0.06, p > 0.05). There was no relationship between AHI and both CRP and homocysteine (r = 0.12, p > 0.05; r = 0.31, p > 0.05, respectively). The values of vitamin B12, folate, erythrocyte count, hemoglobin, mean cell volume, and mean cell hemoglobin concentration were in reference range. Stained RBC examination results were in the normal range.

**RESULTS**

Homocysteine is a thiol-containing amino acid that is an intermediate substance produced during intracellular demethylation of methionine. Elevated levels of homocysteine are found in patients with cardiovascular diseases. A clear correlation was shown between mildly elevated total blood homocysteine concentrations and premature coronary artery diseases, stroke, peripheral artery diseases, or venous thrombosis.

Elevated homocysteine levels in OSA patients were reported only in patients with associated ischemic heart disease and/or hypertension. Jordan et al reported 30% decrease in homocysteine level after long-term continuous positive airway pressure (CPAP) treatment in a small group of patients mostly with hypertension or diabetes. However, Svatikova et

**DISCUSSION**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Control</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>47.14 ± 1.62</td>
<td>45.31 ± 1.21</td>
<td>42.6 ± 3.2</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>29.63 ± 0.67</td>
<td>31.12 ± 0.76</td>
<td>20.2 ± 0.82</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>124.4 ± 2.29</td>
<td>125 ± 2.28</td>
<td>122 ± 4.5</td>
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<tr>
<td>Diastolic BP, mm Hg</td>
<td>76.43 ± 1.17</td>
<td>76.88 ± 1.03</td>
<td>75.4 ± 2.1</td>
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<td>AHI</td>
<td>25.04 ± 3.85</td>
<td>2.72 ± 0.28</td>
<td>&lt;1</td>
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*Data are presented as mean ± SE.

<table>
<thead>
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<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Control</th>
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<tbody>
<tr>
<td>Homocysteine, μmol/L</td>
<td>5.08 ± 3.25</td>
<td>44.0 ± 10.0</td>
<td>4.40 ± 10.0</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>121.8 ± 46.0</td>
<td>133.3 ± 33.3</td>
<td>126.4 ± 20.6</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>133.8 ± 69.0</td>
<td>132.7 ± 65.0</td>
<td>110.8 ± 33.7</td>
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<tr>
<td>Triglycerides, mg/dL</td>
<td>44.0 ± 10.0</td>
<td>43.2 ± 10.9</td>
<td>101.8 ± 23.3</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>133.3 ± 33.3</td>
<td>124.6 ± 27.1</td>
<td>132.1 ± 27.9</td>
</tr>
<tr>
<td>Lipoprotein (a), mg/dL</td>
<td>16.6 ± 11.0</td>
<td>13.0 ± 9.3</td>
<td>8.7 ± 3.2</td>
</tr>
<tr>
<td>Apolipoprotein A-I, mg/dL</td>
<td>126.4 ± 20.6</td>
<td>121.7 ± 39.1</td>
<td>101.8 ± 23.3</td>
</tr>
<tr>
<td>Apolipoprotein B, mg/dL</td>
<td>110.8 ± 33.7</td>
<td>101.8 ± 23.3</td>
<td>80.4 ± 16.7</td>
</tr>
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</table>

*Significant difference between group 1 and group 2 (p < 0.05).
†Significant difference between group 1 and control group (p < 0.05).
‡Significant difference between group 2 and control group (p < 0.05).
have shown that plasma levels of homocysteine are not elevated in OSA patients, and neither acute untreated OSA nor treatment with CPAP and disturbed sleep affect plasma homocysteine levels or obscure its diurnal variation. This result is in contrast to the findings of our study, in which we found slightly enhanced homocysteine levels in OSA patients. The OSA patients in our study did not have any heart disease and/or hypertension. Thus, we can say that OSA might be independently associated with mildly increased blood homocysteine levels.

A recent epidemiologic study\textsuperscript{23} has shown that enhanced levels of CRP are a strong independent predictor of risk of future myocardial infarction, stroke, peripheral arterial disease, and vascular death among persons without known cardiovascular disease. In OSA patients, hypoxia and reoxygenation episodes can also cause activation of inflammatory cells, as observed for neutrophils and monocytes,\textsuperscript{24} and ongoing inflammatory responses play important roles in atherosclerosis.\textsuperscript{25}

Although CRP is a nonspecific marker of inflammation, epidemiologic studies\textsuperscript{23,26} suggest that CRP is an important risk factor in atherosclerosis and coronary artery disease. CRP that was found at high concentrations in the atherosclerotic lesion\textsuperscript{27} has a direct role on secretion of inflammatory mediators by vascular endothelium,\textsuperscript{28} up-regulates the expression of adhesion molecules in endothelial cells, and increases low-density lipoprotein uptake into macrophages.\textsuperscript{29} The development of systemic inflammation, characterized by elevated levels of certain potent proinflammatory mediator such as CRP, may have an important and direct role in the development of atherosclerotic lesions and in promoting cardiovascular morbidity.\textsuperscript{30}

Population-based cross-sectional studies\textsuperscript{31} have shown that plasma CRP concentrations are elevated in obesity.\textsuperscript{34} In this study, CRP levels of both groups were found to be significantly higher than those of the control group. Chronic subclinical inflammation effects may be one pathophysiologic mechanism explaining the enhancement of CRP levels in OSA patients. Shamsuzzaman et al.\textsuperscript{32} reported higher CRP values in patients with moderate-to-severe OSA than in control subjects. Yokoe et al.\textsuperscript{33} observed elevated CRP values in patients with moderate-to-severe OSA as well, and they noted a decrease in CRP levels by treatment with nasal CPAP. In agreement with these studies, we found high levels of CRP in OSA patients, but this enhancement did not have any correlation with the severity of OSA. Similarly, we did not find any correlation between CRP and homocysteine. Both of the cited authors\textsuperscript{32,33} reported significant positive relationship between CRP and AHI; however, our results disagree with these authors. The lack of correlation between AHI and CRP levels is explained by the fact that apnea-related hypoxia was not sufficient in patients with mild-to-moderate OSA. This finding shows that CRP may be an independent risk factor in patients with mild-to-moderate OSA for future cardiovascular events.

Our results show that OSA syndrome is associated not only with slight hyperhomocysteinemia but also with increased CRP concentrations. The lack of...
correlation between homocysteine and CRP supports the possibility that homocysteine and CRP are independent risk factors for cardiovascular disease in OSA patients. Increased plasma concentrations of homocysteine and CRP can be useful in clinical practice to be predictor of long-term prognosis for cardiovascular disease and the treatment of OSA, providing many benefits to the patients and society.

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