Serum homocysteine levels and cardiovascular morbidity in obstructive sleep apnea syndrome

Oguz Kokturk, Tansu Ulukavak Ciftci*, Elif Mollarecep, Bulent Ciftci

Faculty of Medicine, Department of Pulmonary Disease, Gazi University, Besevler, Ankara, Turkey

Received 7 March 2005; accepted 30 May 2005

Summary

Background: Obstructive sleep apnea syndrome (OSAS) is associated with cardiovascular morbidity and mortality. Elevated levels of serum homocysteine are also associated with cardiovascular morbidity and mortality. We aimed to investigate serum homocysteine levels and conventional cardiovascular risk factors (cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides) in OSAS patients with and without cardiovascular diseases (CVD).

Methods and Results: Levels of homocysteine, cholesterol, LDL, HDL and triglycerides were measured in 114 obese, male participants after overnight fasting. The presence of OSAS was determined by standard overnight polysomnography. The cases included OSAS patients (apnea–hypopnea index: AHI ≥ 5) with CVD (OSAS+CVD group) (n:25) and without CVD (OSAS–CVD group) (n:47). Control group was patients without OSAS (AHI < 5) with CVD (CVD group) (n:42). The serum homocysteine levels were significant.

© 2005 Elsevier Ltd. All rights reserved.

Introduction

Obstructive sleep apnea syndrome (OSAS), characterized by repeated episodes of upper airways obstruction during sleep leading to significant hypoxemia, is a very prevalent disorder particularly among middle-aged, obese men, although its existence in women is increasingly recognized. Epidemiological studies estimate that 2–5% of the population meets the minimal diagnostic criteria (snoring, witnessed apnea and excessive daytime sleepiness), and two community-based studies have found that about 2% of women and 4% of men are affected by OSAS. The syndrome is associated with cardiovascular morbidity, such as systemic hypertension, coronary heart disease, atherosclerosis, and stroke. Studies of random samples obtained from the general population suggest that the existence of OSAS constitute a significant risk for cardiovascular diseases (CVD) independently of other known risk factors.
Neurohumoral and hemodynamic responses to untreated sleep apnea are likely mechanisms that produce functional and structural changes within the cardiovascular system. Obesity, higher blood pressure, and advancing age, which are common characteristics of patients with OSAS, contribute to the risk for CVD. Recent studies indicate that OSAS is associated with or aggravates other risk factors for CVD and one of these risk factors is hyperhomocysteinemia.

Homocysteine is an intermediate amino acid in methionine–cysteine metabolism, as it represents a branching point at which it can be remethylated to methionine or converted to cysteine. It was described by McCully, in infants with inborn errors of metabolism, as an atherogenic compound that accelerates atherosclerosis. Many clinical and epidemiological studies confirmed this observation that mild elevation in total plasma homocysteine confers an increased risk for peripheral arterial occlusive disease, coronary artery disease, and cerebrovascular disease similar to other conventional risk factors such as hyperlipidemia or smoking.

Pertaining to the associations between OSAS and cardiovascular morbidity and between homocysteinemia and cardiovascular morbidity, we investigated the serum levels of homocysteine and conventional cardiovascular risk factors such as total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density of lipoprotein (VLDL) and triglycerides in patients diagnosed to have OSAS with and without CVD. We compared them to control subjects with CVD without OSAS.

Materials and methods

Patients

Age and Body Mass Index (BMI) matched male subjects were included in order to eliminate the factors affecting CVD such as age, sex and obesity in the present study. All patients were obese with BMI > 27 kg/m². Subjects were recruited from those who were referred for suspected sleep apnea to our Sleep Disorders Center, Gazi University, Faculty of Medicine. The study was approved by the institutional Ethics Committee, and study was performed in accordance with the guidelines of declaration of Helsinki. The control subjects and OSAS patients were examined with polysomnography (PSG) and classified according to data of the apnea–hypopnea index (AHI). BMI was calculated as weight in kilograms divided by the square of the height in meters measured by a scale. Patients with hypertension (HT) and/or ischemic heart disease (IHD) were enrolled as patients with CVD.

None of the patients has used folat or other food supplements like it, but data about lipid lowering medications of patients were not available.

Before enrollment, all subjects gave written informed consent.

Sleep study

Overnight PSG was performed in all patients by a computerized system (Somnostar alpha; Sensormedics, USA) and included the following variables: electrooculogram (two channels), electroencephalogram (four channels), electromyogram of submental muscles (two channels), electromyogram of the anterior tibialis muscle of both legs (two channels), and electrocardiogram and airflow (with an oro-nasal thermistor). Chest and abdominal efforts (two channels) were recorded using inductive plethysmography, arterial oxyhemoglobin saturation (SaO₂: 1 channel) by pulse oximetry with a finger probe. The recordings were conducted at a paper speed of 10 mm/s, and sleep stage were scored according to the standard criteria of Rechtschaffen and Kales. Arousals were scored according to accepted definitions. Apneas were defined as complete cessation of airflow ≥ 10 s. Hypopneas were defined as reduction of > 50% in one of three respiratory signals, airflow signal or either respiratory or abdominal signals of respiratory inductance plethysmography, or with a fall of ≥ 3% in oxygen saturation or an arousal. The AHI was defined as the number of apneas and hypopneas per hour of sleep. Patients with AHI < 5 were included in control group. Patients with AHI ≥ 5 were considered as OSAS.

Patients were divided into three groups:

1. OSAS+CVD group: patients with OSAS and CVD (HT and/or IHD)
2. OSAS–CVD group: patients with OSAS without CVD
3. CVD group: patients without OSAS with CVD (HT and/or IHD).

Circulating parameters assay

All subjects had fasting blood samples taken between 07:00 AM and 08:00 AM. Blood samples were immediately sent to the hospital laboratory. Cholesterol and triglyceride concentrations in serum and lipoprotein fractions were determined enzymatically using standard laboratory procedures. Serum homocysteine levels were measured using Chromsystems HPLC with Agilent 1100 series...
fluorescence detector. Normal range is considered as $5–14 \mu\text{mol/L}$ for homocysteine levels.

**Statistical analysis**

Means and standard errors of measurement (SEM) were determined for continuous variables and percentage for categorical variables. The significance of differences between three groups was analyzed with the Kruskal–Wallis variance analysis. Differences between two groups were analyzed with the Mann–Whitney U test. We applied a Bonferroni correction for multiple comparisons. Categorical data were analyzed by $x^2$ with Fisher Exact Probability test. The correlation was analyzed with Spearman correlation coefficient.

To assess the relative strength of association of OSAS as well as possible confounding factors with homocysteine, we employed a multiple regression analysis to the patients with OSAS as a single group. In this analysis, we used serum levels of homocysteine as dependent variable and evaluated the order of inclusion in the model of the following independent variables: AHI, BMI, total cholesterol, HDL, LDL, and VLDL.

All statistical analyses were carried out using statistical software (SPSS, version 11.0 for Windows; SPSS Inc; Chicago, IL). Differences were considered significant at $P < 0.05$.

**Results**

Newly diagnosed obese (BMI $> 27 \text{kg/m}^2$) men with OSAS and age- and BMI matched male control subjects with HT and/or IHD were enrolled in present study.

After 157 obese, male subjects underwent PSG, 72 were considered to have OSAS, which included 25 patients with CVD and 47 patients without CVD. Eighty-five subjects were considered not to have OSAS and of these, 42 patients, who were with CVD, were enrolled as control subjects. The 114 patients were subdivided into three groups:

1. OSAS+CVD group: patients with OSAS and CVD (HT and/or IHD) ($n = 25$)
2. OSAS–CVD group: patients with OSAS without CVD ($n = 47$)
3. CVD group: patients without OSAS with CVD (HT and/or IHD) ($n = 42$).

The demographic and clinical data of the three groups are presented in Table 1. The serum homocysteine levels were significantly elevated in OSAS+CVD group more than both the other two groups ($P < 0.001$). When differences between two groups were analyzed, we showed that serum homocysteine levels significantly increased more in the OSAS–CVD group compared to the CVD group (Table 1, Fig. 1). Hyperhomocysteinemia ($>14 \mu\text{mol/L}$) was found in 17 patients (68%) of OSAS+CVD group, in 22 patients (46.8%) of OSAS–CVD group and in seven (16.7%) of CVD group. Therefore, hyperhomocysteinemia was found highest in OSAS+CVD groups ($P < 0.05$). No significant difference was detected among the different groups with regard to serum total cholesterol, HDL, LDL, VLDL and triglyceride levels (Table 1).

When we evaluated the association between the serum homocysteine level and AHI as a severity measure of OSAS, homocysteine levels were positively correlated with the severity of OSAS both in OSAS+CVD ($r = 0.73$, $P < 0.001$) and OSAS–CVD

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The demographic and clinical data of study groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>OSAS–CVD ($n = 47$)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.56 ± 10.02</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.53 ± 5.1</td>
</tr>
<tr>
<td>AHI</td>
<td>32.93 ± 28.7</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>218.47 ± 84.99</td>
</tr>
<tr>
<td>HDL</td>
<td>48.5 ± 17.87</td>
</tr>
<tr>
<td>LDL</td>
<td>129.5 ± 41</td>
</tr>
<tr>
<td>VLDL</td>
<td>29.48 ± 11.87</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>168.22 ± 123.44</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>14.7 ± 4.52</td>
</tr>
</tbody>
</table>

OSAS: obstructive sleep apnea syndrome; CVD: cardiovascular disease; BMI: body mass index; AHI: apnea–hypopnea index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; SD: standard deviation. $*P < 0.001$, OSAS–CVD group vs. CVD group and OSAS+CVD group vs. CVD group. $\dagger P < 0.001$, OSAS–CVD group vs. CVD group and OSAS+CVD group vs. CVD group.
There was no correlation between serum lipid and homocysteine levels in all groups.

Evaluation of the relative strength of association using multiple regression analysis between homocysteine and OSAS severity as well as the other possible confounding variables showed that homocysteine levels were independently associated with OSAS severity both in OSAS+CVD ($F = 3.1, P < 0.05$) and OSAS–CVD group ($F = 1.7, P < 0.005$).

**Discussion**

The acute hemodynamic alterations of obstructive sleep apnea include systemic and pulmonary hypertension, increased right and left ventricular afterload, and increased cardiac output. Earlier reports attributed the coexistence of OSAS with CVD to the shared risk factors such as age, sex, and obesity. However, recent epidemiologic data confirm an independent association between OSAS and the different manifestations of CVD. Possible mechanisms may include a combination of intermittent hypoxia and hypercapnia, repeated arousals, sustained increase in sympathetic tone, reduced baroreflex sensitivity, increased platelet aggregation, elevated plasma fibrinogen levels and also hyperhomocysteinemia.

Pertaining to the associations between OSAS and cardiovascular morbidity and between homocysteine and cardiovascular morbidity, our study confirms the previous finding by Lavie et al.\cite{15,16} that levels of homocysteine are elevated in OSAS patients. The most important difference in our study is that it consists of obese males with completely similar age and weights. Therefore, some parameters affecting cardiovascular morbidity such as sex, age and BMI has been eliminated in this study. In Lavie’s study, however, age and BMI have been differed between groups.

We showed that OSAS patients with and without CVD had significantly higher levels of homocysteine when compared with patients with CVD without OSAS, and serum homocysteine levels

---

**Figure 1** Serum homocysteine levels in the study groups.

**Figure 2** Simple correlation between serum homocysteine levels and AHI in OSAS+CVD and OSAS–CVD groups.
were independently associated with severity of OSAS. There was no difference in conventional risk factors for CVD among the three groups, but there was also a limitation in our study because we had no data about lipid lowering medications of patients. Therefore, the difference between groups may be explained by decreasing high lipid levels of some patients with medication.

We found that only 16.6% of CVD group had hyperhomocysteinemia (>14 µmol/L) and the mean homocysteine levels in CVD group was 10.35 ± 3.63 µmol/L. This mean value is similar to the level of homocysteine observed in seven studies investigating large groups of cardiovascular patients (12.2 ± 1.3 µmol/L) and in Lavie’s study investigating patients with IHD (11.92 ± 5.77 µmol/L). Our CVD group contained both HT and IHD patients. Although it is known to increase in CVD morbidity by hyperhomocysteinemia, considering the other factors also affecting CVD, it would not be surprising to find low homocysteine levels in this CVD group.

In patients with OSAS and CVD, serum homocysteine levels were higher than patients with CVD without OSAS. Besides, in OSAS patients with CVD, the number of patients whose homocysteine levels were above the limits was found to be significantly higher. This suggests that, among CVD patients, patients with OSAS may have higher homocysteineemia due to OSAS. The essential issue deserving to be researched is the mechanism that is responsible for the increased serum homocysteine levels in OSAS.

In patients with OSAS, cyclical alterations of arterial oxygen saturation are observed with oxygen desaturation developing in response to apnea followed by the resumption of oxygen saturation during hyperventilation. This phenomenon has been referred to as hypoxia/reoxygenation and might alter the oxidative balance through the induction of excess oxygen free radicals quite like in the sequelae of ischemia/reperfusion injury. Some authors have reported that systemic biomarkers of oxidative stress has increased in patients with sleep apnea suggesting a possible role in the pathologic consequences of OSAS. Excess superoxide production due to hypoxia/reoxygenation may also inactivate nitric oxide resulting in the formation of the toxic peroxynitrite. This may further prevent the inactivation of homocysteine leading to an increase in its levels, putting OSAS patients with cardiovascular morbidity at a greater risk.

Endothelial dysfunction, which is the first step in the development of atherosclerosis according to the response to injury hypothesis, was initially implicated with hypercholesterolemia. However, endothelial dysfunction could result from many seemingly unrelated pathological conditions such as increased plasma homocysteine levels. Cumulative data in vivo and in vitro clearly demonstrate that homocysteine affects multiple vascular functions such as promoting a prothrombotic phenotype of the endothelium by increasing platelet aggregation and activation, and stimulating vascular smooth muscle cell proliferation. Currently, the leading mechanisms proposed for the adverse effects of homocysteine on endothelial function implicate oxidative stress and depletion in nitric oxide bioavailability.

In conclusion, the impact of alleviating OSAS on cardiovascular risk factors such as hyperhomocysteinemia has not been fully elucidated. Given the compelling epidemiological and mechanistic evidence implicating increased homocysteine in CVD, homocysteine may be an important factor linking OSAS to cardiovascular morbidity.

Since CPAP is a very efficient treatment method which eliminates the oxygen desaturation and other consequences resulting from obstructive respiratory events, we speculate that CPAP-related improvement in OSAS also translates into lower serum homocysteine level and, by inference, a decrease in cardiovascular risk. Further studies are needed to evaluate the effect of CPAP on the serum homocysteine levels.

References


