Homocysteine Levels and Echocardiographic Findings in Obstructive Sleep Apnea Syndrome

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Abstract
Background: The obstructive sleep apnea syndrome (OSAS) is characterized by repeated upper airway obstruction during sleep together with decreases in oxygen saturation leading to a series of pathological events, primarily in the cardiovascular system. Elevated plasma homocysteine levels have recently been considered as an independent risk factor for vascular disease, and increased levels are attributed to cardiovascular diseases. Objectives: We aimed to investigate the possible relationship between homocysteine levels and echocardiographic findings in OSAS patients at different stages of disease. Methods: Thirty-eight patients (23 males and 15 females) with polysomnographically verified OSAS (mean age, 49 ± 12 years, range 27–74) and a mean body mass index of 31.27 ± 5.24 kg/m² (range 22.60–47.90) were prospectively studied. Plasma levels of homocysteine, cholesterol, triglycerides, vitamin B12 and high-sensitive C-reactive protein (hsCRP), as well as echocardiographic and lung function parameters were assessed. Results: Homocysteine levels were elevated in all OSAS groups and were statistically significantly different between the mild and moderate/severe groups. Significant differences were present between the variables nocturnal oxygen desaturation (NOD), respiratory arousal and light sleep among the mild and moderate/severe groups. We found a significant positive correlation between homocysteine levels and NOD duration, and hsCRP levels were positively correlated with the apnea-hypopnea index and NOD duration. Conclusions: In all OSAS groups, homocysteine levels were elevated regardless of the presence of cardiac dysfunction. Echocardiographic abnormalities were primarily left-ventricular (LV) hypertrophy and LV diastolic dysfunction and could be observed in all OSAS severity groups.
The OSA syndrome (OSAS) is characterized by repetitive episodes of absent or reduced airflow during sleep despite inspiratory efforts leading to increased pleural pressure swings, decreases in oxyhemoglobin saturation and arousal from sleep. Thus, airway patency is maintained and airflow resumes. These episodes last at least 10 s, end in loud snoring and can occur even hundreds of times a night.

As a result, a series of secondary physiological events occur, which in turn give rise to clinical complications of the syndrome. Potentially fatal systemic illnesses frequently associated with this disorder include systemic arterial hypertension, pulmonary hypertension, heart failure, nocturnal cardiac dysrhythmias, myocardial infarction, coronary heart disease, ischemic stroke and sudden death [1–4]. The aim of this study was to investigate the possible relation between homocysteine levels, and echocardiographic and lung function findings in OSAS patients at different stages of disease.

Patients and Methods

Thirty-eight patients (23 males and 15 females) referred to our sleep laboratory for snoring, suspected OSAS or excessive daytime sleepiness with a mean age of 49 ± 12 years (range 27–74) and a mean body mass index (BMI) of 31.27 ± 5.24 kg/m² (range 22.60–47.90) were prospectively studied. The sleep test was the first test to be applied because patients were included in the study on the basis of polysomnographically verified OSAS. An apnea-hypopnea index (AHI) ≥ 5, >15 or ≥30 events/h associated with typical clinical features was accepted as mild, moderate or severe OSAS, respectively. Overnight PSG (Compumedics E Series Sleep System; Compumedics, Melbourne, Australia) was performed according to internationally approved methods. It consisted of polygraphic recordings from surface electrodes for electroencephalography, electro-oculography, electromyography (including chin and lower extremity muscles) and electrocardiography, and from noninvasive sensors for nasal and oral airflow, tracheal sounds (microphone), and thoracic and abdominal respiratory efforts. Transcutaneous oxygen saturation was measured continuously with a finger-pulse oximeter. Positional changes during sleep were recorded. During the test period, full-night video recordings were also made. The test was terminated after final waking in the morning.

Sleep was defined according to the criteria of Rechtschaffen and Kales [5]. Obstructive, mixed and central apneas were defined according to the usual criteria. Hypopneas were defined as a 50% fall in tidal volume compared with its basal value during quiet wakefulness, lasting at least 10 s, accompanied by a decrease ≥ 3% in oxygen saturation. AHI was defined as the number of apnea and hypopnea events per hour of sleep. The sleep test was staged manually.

A fasting venous blood sample was taken between 07:00 and 08:30 a.m. following PSG and stored at −20°C until homocysteine, high-sensitive C-reactive protein (hsCRP), cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL), triglyceride (TG) and vitamin B12 levels were analyzed. Lung function tests and echocardiographic evaluation were performed the day after the PSG evaluation when the patients returned to get the test results. Pulmonary volumes and flows were measured by trained technicians using a spirometer (Vitalograph II), and the results were expressed as percentages of the reference values. Echocardiography was performed at the cardiology department of our hospital by the same cardiologist involved in the study. Echocardiographic measurements were made using a Siemens Acuson CV 70 Doppler echocardiography system. The cardiologist was blinded to the OSAS severity of the patients.

Homocysteine, hsCRP, total cholesterol, HDL, LDL, triglyceride and vitamin B12 levels were measured in serum samples. Homocysteine levels were detected by fluorescence polarization immunoassay (IMX; Abbott Pharmaceuticals, Abbott Park, Ill., USA). For homocysteine levels, 5–15 μmol/l were considered as the normal laboratory reference range. Vitamin B12 levels were measured by immunnoassay-chemiluminescence (Access-Beckman-Coulter, Fullerton, Calif., USA). Biochemical parameters and hsCRP were measured using a Dimension RXL automated analyzer (Dade Behring, Deerfield, Ill., USA). Study patients confirmed that they did not use lipid-lowering and folate-containing drugs. Our study was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from each subject. The study protocol was approved by the Ethics Committee of the Medical Faculty of the Maltepe University.

Statistical Analysis

The results are presented as means ± SD and percentages. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) for Windows (version 12; SPSS, Chicago, Ill., USA). Differences were considered significant at p < 0.05. Differences between two groups were analyzed by Mann-Whitney U test. Categorical variables were compared using the χ² test and Fisher’s exact test. Correlations were analyzed with Pearson’s correlation coefficients. Multiple linear regression analysis was used to determine variables that affect homocysteine levels.

Results

The characteristics and PSG data of the study population are shown in tables 1 and 2. The patients were classified into two groups according to their AHI: mild OSAS (5 ≤ AHI ≤ 15; group 1) and moderate/severe OSAS (AHI > 15; group 2). Newly diagnosed OSA patients (n = 38), 15 (39.5%) females and 23 (60.5%) males (mean age 49 ± 12 years, range 27–74), were enrolled in this study. Of the patients, 9 (23.7%) were mild and 29 (76.3%) were moderate/severe OSAS cases. OSAS groups were similar with regard to age, sex and BMI (p > 0.05). We excluded patients with chronic obstructive pulmonary disease, hypertension and diabetes mellitus. None of the patients had a family history of heart disease.
All patients complained of at least one of the cardinal symptoms of OSAS as snoring, witnessed apnea periods during sleep or excessive daytime sleepiness. Regarding PSG variables, there were significant differences between median values of REM sleep, sleep stages I/II (light sleep; %), nocturnal oxygen desaturation (NOD) duration (min) and the number of respiratory arousals (\( p < 0.05, p < 0.01, p < 0.0001 \) and \( p < 0.0001 \), respectively).

Lung function parameters and biochemical data of the patients were presented in tables 3 and 4. There were no significant differences between median values of the lung function parameters (FEV\(_1\), FVC, PEF and FEF\(_{25–75}\); all in %) in both study groups (Mann-Whitney U test).

When biochemical data were compared, there was a statistically significant difference between both groups with regard to homocysteine levels (\( p < 0.01 \); table 4; fig 1). No statistically significant correlation between hsCRP and BMI was found (\( s: 0.286, p = 0.086 \)).

However, AHI and NOD duration were positively correlated with hsCRP (\( s: 0.339, p = 0.040 \), and \( s: 0.393, p = 0.016 \), respectively). Patients in both (mild and moderate/severe OSAS) groups were obese (\( n = 34; 89.5\% \)) and there was no significant difference between the groups, but a positive significant correlation between AHI and BMI was noted (\( r: 0.328, p = 0.044 \); fig 2).

There were significant differences between both study groups when the \( \chi^2 \) test was applied to determine the relationship between the echocardiographic findings and the severity of OSAS. There was a significant difference regarding cardiac dysfunction between mild and moderate/severe OSAS patients. In moderate/severe OSAS patients, cardiac dysfunction was significantly increased...
compared with the mild OSAS group (p = 0.001; table 5).

Mean homocysteine values did not significantly differ between the patients with and without cardiac dysfunction (23.75 ± 8.73 and 21.87 ± 4.02, respectively; Mann-Whitney U test, p = 0.697).

Pearson’s correlation analysis identified that the serum homocysteine level and AHI were not correlated (r: 0.184, p = 0.275). There was also no correlation between homocysteine and BMI (r: 0.264, p = 0.115) or hsCRP (r: −0.009, p = 0.956) in the patients, but homocysteine levels were positively correlated with NOD duration (r: 0.341, p = 0.039; fig. 3).

Using multiple regression analysis, we found significant relations between homocysteine levels and the following four variables: presence of moderate/severe OSAS, presence of cardiac dysfunction, AHI and duration of NOD (table 6). The four variables combined explained 35% of the total variance that determined homocysteine levels. According to the standardized regression coefficient (β), the ranking of the variables according to their importance was as follows: OSAS type, AHI, NOD duration and cardiac dysfunction. Results of t tests were examined to evaluate the significance of the regression coefficient (β); we found that OSAS type, AHI and NOD duration were associated with homocysteine levels – but not cardiac dysfunction.

**Discussion**

The mechanisms linking OSA with cardiovascular disease remain to be elucidated despite a vast amount of studies. There is evidence that OSAS is associated with...
a group of proinflammatory and prothrombotic factors that are also involved in the development of cardiac diseases, e.g. atherosclerosis. OSAS is associated with endothelial dysfunction, increased CRP and cytokine expression, elevated fibrinogen levels and decreased fibrinolytic activity [2, 3, 6–8].

Nocturnal and daytime sympathetic activity is elevated in the sleep apnea syndrome. Surges in sympathetic activity, blood pressure, ventricular wall tension and afterload adversely affect ventricular function [9]. Many studies have shown that OSAS patients have an increased risk of cardiovascular mortality [1–4, 6, 7]. Clinical and epidemiological studies have shown that there is a clear association between elevated blood homocysteine levels and coronary heart, peripheral artery and cerebrovascular diseases, stroke and venous thrombosis [3, 4, 6, 7].

The results of the present study revealed that in both OSAS groups homocysteine levels were elevated above the normal laboratory ranges and were significantly different between mild and moderate/severe OSAS patients. However, high homocysteine levels were not correlated with disease severity (AHI). On the other hand, homocysteine levels were positively correlated with NOD duration (s: 0.341, p < 0.039).

Homocysteine is a sulfur-containing amino acid formed by the demethylation of methionine in dietary products. It can be remethylated to methionine or converted to cysteine. In healthy subjects, the fasting plasma homocysteine level is between 5 and 15 µmol/l. Moderate hyperhomocysteinemia (≤30 µmol/l) is a major independent risk factor for a number of diseases, e.g.
occlusive vascular, coronary, cerebral and peripheral diseases [10]. Our patients had moderate hyperhomocysteinemia, and homocysteine levels were positively correlated with NOD duration (s: 0.341, p < 0.039), although no correlation with AHI was found, suggesting that oxygen desaturation and not AHI may be the determinant factor of pathological processes observed in OSA; thus using AHI alone may not be the best way of determining disease severity.

In a previous study, plasma homocysteine levels increased with advancing age, and they were higher in men than in women. High homocysteine levels are associated with impaired renal function, high plasma creatinine, smoking, coffee consumption, alcoholism and certain drugs, including folate antagonists, nitrous oxide and L-DOPA [11]. A limitation of our study is the lack of a control group to be able to compare biochemical data (mainly homocysteine levels) between patients and healthy controls.

Moderate/high homocysteine levels are known to cause oxidative damage, possibly leading to inflammation especially in the endothelium [11]. One of the mechanisms suggested for the adverse vascular effects of homocysteine is its ability to alter endothelial function via oxidative stress and depletion of bioactive nitric oxide. It is oxidized in the plasma during hydrogen peroxide production to form free radicals which are toxic to endothelial cells. Due to the damaged endothelium, high homocysteine levels also limit NO production, and recent studies have shown that circulating NO levels are decreased in OSAS patients [3, 4, 6].

CRP is a well-known inflammatory biomarker. It is considered to be an important independent predictor of future cardiovascular events, and a predictor of myocardial infarction, hypertension, stroke and diabetes. CRP promotes endothelial cell activation and dysfunction, has effects on vascular smooth muscle cells, and directly affects monocyte and macrophage activity. Its role in atherothrombotic processes is being evaluated in clinical research studies [7, 8, 12, 13]. It is also proposed that CRP may block the effect of leptin on satiety and weight gain resulting in leptin resistance [14]. We did not measure serum leptin levels in this study. Previous studies have reported that adipose tissue is claimed to be a potent source of IL-6 production which leads to increased CRP levels [7]. In our study, we could not find a statistically significant correlation between hscRP and BMI but there was a significant positive correlation between hscRP and NOD duration (s: 0.393, p = 0.016). On the other hand, AHI was correlated with hscRP (s: 0.339, p = 0.040).

Ryan et al. [7] found that homocysteine and CRP levels were not associated with OSAS severity in men. In the same study, they reported that CRP is independently associated with obesity. Can et al. [12] showed that OSAS is associated with slight hyperhomocysteinemia and increased CRP concentrations, the latter being a possible marker of long-term prognosis for cardiovascular disease and OSAS.

Boudjeltia et al. [15] observed an independent association between the AHI and hscRP in 49 patients referred to the sleep laboratory for suspected sleep apnea. In multivariate analysis, including age, BMI, gender, hypertension, diabetes, tobacco, microarousal index, AHI, total cholesterol, triglycerides and LDL cholesterol, AHI was the only variable independently associated with hscRP. They proposed that not only the severity of the disease, but also the duration of sleep-disordered breathing may result in CRP increases [15].

Observational studies have shown that there is a causal relation between excess body weight and OSAS. Excess body weight has been hypothesized to affect breathing in many ways, including alterations in upper airway structure or function, and by exacerbating OSAS events via obesity-related reductions in functional residual capacity and increased whole body oxygen demand [1]. In the present study, patients in both OSAS groups (mild and moderate/severe OSAS groups) were overweight and we found a significant positive correlation between AHI and BMI (r: 0.328, p = 0.044; fig. 2).

When biochemical data were compared, both study groups had statistically significantly different mean homocysteine levels. Cardiac dysfunction was noted in all study groups, e.g. left ventricular (LV) diastolic dysfunction, LV hypertrophy and right ventricular (RV) tissue Doppler dysfunction, but there was a significant difference among the groups with regard to cardiac dysfunction with increasing severity of the disease. Comparisons between the patients with and without cardiac dysfunction revealed no significant difference regarding mean homocysteine values (p = 0.697).

Romero-Corral et al. [16] found that OSAS, particularly moderate/severe OSAS, is associated with impaired RV/LV function and increased left atrial volume. Similarly, Otto et al. [17] reported that otherwise healthy obese patients with OSAS have impaired diastolic function measured by tissue Doppler echocardiography compared to similarly obese control subjects without OSAS. In a study by Cloward et al. [18], LV hypertrophy is a common echocardiographic abnormality in severe OSA and reverses with nasal continuous positive airway pressure therapy.
Dursunoğlu et al. [19] evaluated uncomplicated OSAS patients by echocardiography using the myocardial performance index (MPI) reflecting global function. They excluded subjects with hypertension, diabetes mellitus and any known cardiac and lung disease. LV MPI (global function) differed significantly between OSAS patients and controls, while it did not significantly differ between OSA groups. On the other hand, RV diastolic dysfunction was only observed in moderate/severe OSAS patients and RV MPI was statistically higher in patients with moderate/severe OSAS than in those with mild OSAS. RV MPI showed a positive correlation with AHI reflecting the severity of OSAS. They concluded that patients with moderate/severe OSAS had RV global dysfunction in addition to the presence of a diastolic dysfunction [19].

Lavie et al. [3] reported the results of a large study and only detected increased homocysteine levels in OSAS patients with coexisting cardiovascular disease. In the study by Kokturk et al. [4], OSAS patients with and without cardiovascular disease had significantly higher homocysteine levels compared with patients with cardiovascular disease without OSAS, and serum homocysteine levels were independently associated with the severity of OSAS.

Yavuz et al. [20] compared homocysteine levels between OSAS patients and healthy subjects (control group) and found increased levels in the OSAS group. They concluded that high levels of serum homocysteine were correlated with age, smoking status, diabetes mellitus and coronary heart disease in OSAS patients.

Although the pathophysiological mechanisms underlying OSAS are multifactorial, repeated apneic episodes in OSAS patients result in hypoxic attacks that initiate oxidative stress by increasing the expression of adhesion molecules (ICAM-I and VCAM-I) and the production of reactive oxygen species in leukocytes, resulting in an increase in inflammatory markers, e.g. TNF-α, IL-6 and CRP, lipid peroxidation and neutrophil superoxide release. As a result, there is a decrease in NO synthesis and bioavailability, and an increase in its degradation products which lead to exaggerated endothelial cell damage and dysfunction.

Recent studies suggest that in patients with OSAS antioxidant function is impaired and antioxidant capacity decreased [21]. Homocysteine, which is generated by the catabolism of methionine, an essential amino acid, is a naturally occurring thiol amino acid. It has been proposed that the thiol group of homocysteine readily undergoes autoxidation in plasma to generate reactive oxy-

References:

1. Lavie et al. [3]
2. Kokturk et al. [4]
3. Yavuz et al. [20]
4. Dursunoğlu et al. [19]

OSAS is a multisystem disease that affects metabolism in different aspects, leading to hypertension, insulin resistance (diabetes), obesity, neurological diseases and altered hormonal metabolism. In the literature, controversy exists regarding homocysteine and CRP levels in OSAS patients. The reasons for the different outcomes may be ascribed to methodological factors, small numbers in the study groups, lack of control groups including matched healthy individuals and difficulties in grouping patients with coexisting diseases (heterogeneous groups).

In conclusion, in the present study, OSAS patients were grouped according to disease severity. Echocardiographic abnormalities were primarily LV hypertrophy and LV diastolic dysfunction in our OSAS patients. Homocysteine levels were elevated in all OSAS study groups independent of the presence of cardiac dysfunction, but NOD duration was positively correlated with homocysteine levels. In our opinion, hypoxia during apneic-hypopneic periods seemed responsible for the pathological condition leading to elevated homocysteine levels. Also, disease severity should not only be determined by AHI (an index counting the numbers of apneas and hypopneas per hour) but primarily by NOD duration, possibly a determinant of pathological processes associated with OSAS.
References