

Elevated C-Reactive Protein Levels and Increased Cardiovascular Risk in Patients With Obstructive Sleep Apnea Syndrome

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SUMMARY

Obstructive sleep apnea syndrome (OSAS) is associated with cardiovascular morbidity and mortality. Inflammatory processes associated with OSAS may contribute to cardiovascular morbidity in these patients. C-reactive protein (CRP) is an important serum marker of inflammation. We tested the hypothesis that patients with OSAS have increased plasma CRP.

After 173 male subjects underwent polysomnography, 94 were considered to have OSAS (apnea-hypopnea index (AHI) > 5), 38 cardiovascular disease (CVD), and 56 without CVD. Seventy-nine subjects were considered not to have OSAS (AHI < 5) and from among these 57 patients without CVD were enrolled as control subjects.

Serum CRP levels were significantly elevated in the OSAS + CVD group compared to the two other groups ($P < 0.05$). When we evaluated the association between the serum CRP level and severity of OSAS, CRP levels were positively correlated with AHI in OSAS patients ($r = 0.61$, $P < 0.001$).

OSAS, as a marker of inflammation and cardiovascular risk, is associated with elevated levels of CRP. According to these results, the link between cardiovascular morbidity and OSAS may be explained by the coexistence of other cardiovascular risk factors such as circulating CRP levels. (Int Heart J 2005; 46: 801-809)

Key words: CRP, Sleep apnea, Inflammation

OBSTRUCTIVE sleep apnea syndrome (OSAS) is a common disorder that affects both children and adults. It is characterized by periods of breathing cessation (apnea) and periods of reduced breathing (hypopnea). Both types of events have similar pathophysiology and are generally considered to be equal with respect to their impact on patients. There are several methods of quantifying the severity of the disorder such as measuring the number of apneas and hypopneas per hour of sleep (the apnea-hypopnea index: AHI), the severity of oxygen desat-

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uration during sleep, or the severity of the most commonly associated symptom, daytime sleepiness.¹⁾ In patients with moderate to severe disease who have excessive daytime sleepiness, the prevalence of OSAS is about 0.5% in middle-aged men with a mean body mass index (BMI) of 24.9 kg/m² and 1.5% in a similar population but with a mean BMI of 27.1 kg/m². If an all inclusive definition is used based entirely on polysomnographically defined AHI > 5, then the prevalence rises to 24% in the male population with a mean BMI of about 30 kg/m².²⁾

OSAS 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 constitutes a significant risk for cardiovascular diseases independently of other known risk factors.³⁻⁵⁾ Mechanisms such as increased sympathetic activity and endothelial dysfunction have been implicated. Inflammatory processes associated with OSAS may also act as potential mediators of cardiovascular morbidity in these patients.⁶⁾

C-reactive protein (CRP), an important serum marker of inflammation, is synthesized from the liver and regulated by cytokines. Unlike cytokines, CRP levels are quite stable in the same individual across 24 hours and may reflect the level of inflammatory response.⁵⁾

Ongoing inflammatory responses play important roles in atherosclerosis.^{7,8)} Although CRP is a nonspecific marker of inflammation, recent epidemiological studies suggest that CRP is an important risk factor in atherosclerosis and coronary artery disease.^{1,9)}

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

METHODS

Patients: Only obese male subjects with similar ages and body mass index (BMI) were included in order to eliminate other factors affecting CVD such as age, sex and obesity. Subjects were recruited from those who were referred for suspected sleep apnea to the Sleep Disorders Center of Gazi University. The study was approved by the Institutional Ethics Committee, and was performed in accordance with the guidelines of the Declaration of Helsinki. The control subjects and OSAS patients were examined using polysomnography (PSG) and clas-

sified according to their apnea-hypopnea index (AHI). BMI was calculated as weight in kilograms divided by the square of the height. Patients with hypertension (HT) and/or ischemic heart disease (IHD) were enrolled as patients with CVD. Before enrollment, all subjects gave written informed consent.

Sleep study: Overnight polysomnography (PSG) was performed in all patients by a computerized system (Somnostar alpha; Sensormedics, USA) and included the following variables: electrooculogram (2 channels), electroencephalogram (4 channels), electromyogram of submental muscles (2 channels), electromyogram of the anterior tibialis muscle of both legs (2 channels); and an electrocardiogram and airflow (with an oro-nasal thermistor). Chest and abdominal efforts (2 channels) were recorded using inductive plethysmography, and 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 stages were scored according to the standard criteria of Rechtschaffen and Kales.¹⁰⁾ Arousals were scored according to accepted definitions.¹¹⁾ Based on the American Sleep Disorders Association criteria for measurements, definitions, and severity ratings of the Sleep Related Breathing Disorders Task Force Report,¹²⁾ apnea is defined as the cessation of airflow ≥ 10 seconds and hypopnea is defined as a recognizable, transient reduction, but not a complete cessation of, breathing ≥ 10 seconds. A $> 50\%$ decrease in the amplitude of a validated measure of breathing must be evident or a $< 50\%$ amplitude reduction that is associated with either an oxygen desaturation of $\geq 3\%$ or an arousal. Obstructive apneas and hypopneas are typically distinguished from central events by the detection of respiratory efforts during the event. The apnea-hypopnea index (AHI) was defined as the number of obstructive apneas and hypopneas per hour of sleep. Patients with an AHI < 5 were assigned to the control group. Patients with an AHI ≥ 5 were considered as OSAS. Patients with sleep disorders, except OSAS, such as upper airway resistance syndrome (UARS), central sleep apnea syndrome (CSAS), periodic limbs movement (LPMs), or narcolepsy were excluded. Patients with cerebrovascular disease, a lung disease with hypoxemia such as chronic obstructive pulmonary disease (COPD), or an inflammatory disease were also excluded.

The patients were subdivided into 3 groups:

1. OSAS + CVD group: Patients with OSAS and CVD (HT or IHD or both) ($n = 38$)
2. OSAS - CVD group: Patients with OSAS without CVD ($n = 56$)
3. CVD group: Patients without OSAS but with CVD (HT or IHD or both) ($n = 57$)

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 for estimation of lipid levels. Cholesterol and triglyceride

concentrations in serum and lipoprotein fractions were determined enzymatically using standard laboratory procedures. Serum levels of CRP were measured with a latex particle-enhanced immunoturbidimetric assay. The normal range for CRP levels was considered to be 0 to 5 mg/L.

Statistical analysis: The mean and standard error of the mean (SEM) values were determined for continuous variables and percentage for categorical variables. The significance of differences within groups was analyzed using the Student *t* test. The Bonferroni correction was conducted for multiple comparisons. Differences in CRP levels among 3 groups were evaluated by ANOVA. Categorical data were analyzed using the χ^2 test with the Fisher Exact Probability test. Correlations were analyzed with the Pearson correlation coefficient.

To assess the relative strength of the association of OSAS as well as possible confounding factors with CRP, we employed multiple regression analysis for the patients with OSAS as a single group. In this analysis, serum levels of CRP were used as a dependent variable and the order of inclusion in the model of the following independent variables was evaluated: 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

After 173 male subjects underwent PSG, 94 were considered to have OSAS, including 38 patients with CVD and 56 patients without CVD. Seventy-nine subjects were considered not to have OSAS and from among these 57 patients with CVD were enrolled as control subjects.

The demographic and clinical data of the three groups are presented in the Table. The serum CRP levels were significantly elevated in the OSAS + CVD group compared to the two other groups ($P < 0.05$) (Table). There was no difference in CRP levels between the OSAS-CVD and CVD groups ($P > 0.05$).

When we classified the patients into two groups (OSAS with an AHI > 5 , non-OSAS with an AHI < 5), serum CRP levels were significantly elevated in the OSAS group compared to the non-OSAS group (3.36 ± 6.5 and 1.16 ± 5.8 , respectively) ($P < 0.05$).

CRP was positive (> 5 mg/L) in 23.4% of the patients (22/94) in the OSAS groups and in 5.3% of the patients (3/57) in the non-OSAS group ($P = 0.002$).

No significant differences were detected among the different groups with regard to serum total cholesterol, HDL, LDL, VLDL, and triglyceride levels (Table).

Table. Demographic and Clinical Data of the Three Study Groups

	OSAS + CVD (<i>n</i> = 38)	OSAS - CVD (<i>n</i> = 56)	CVD (<i>n</i> = 57)	<i>P</i>
Age, years	44.52 ± 9.18	42.96 ± 9.75	47.86 ± 11.55	> 0.05
BMI, kg/m ²	31.74 ± 4.89	32.26 ± 4.89	29.17 ± 4.09	> 0.05
AHI	32.93 ± 28.7	44.92 ± 41.53	2.11 ± 1.56 ¹	< 0.001
Total cholesterol	203.67 ± 62.31	216.05 ± 79.19	197.37 ± 39.45	> 0.05
HDL	46.15 ± 8.89	47.91 ± 16.62	47.26 ± 10.4	> 0.05
LDL	119.5 ± 29.33	127.68 ± 40.85	122.42 ± 35.59	> 0.05
VLDL	33.2 ± 18.66	31.63 ± 14.33	29.36 ± 16.1	> 0.05
Triglycerides	171.3 ± 118.7	174.01 ± 119.3	154.92 ± 97.64	> 0.05
CRP	4.72 ± 8.03 ¹	2.44 ± 5.11	1.16 ± 5.8	< 0.05

OSAS indicates obstructive sleep apnea syndrome; CVD, cardiovascular diseases; BMI, body mass index; AHI, apnea-hypopnea index; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; CRP, C-reactive protein.

¹*P* < 0.001, OSAS + CVD group versus CVD group and OSAS - CVD group versus CVD group.

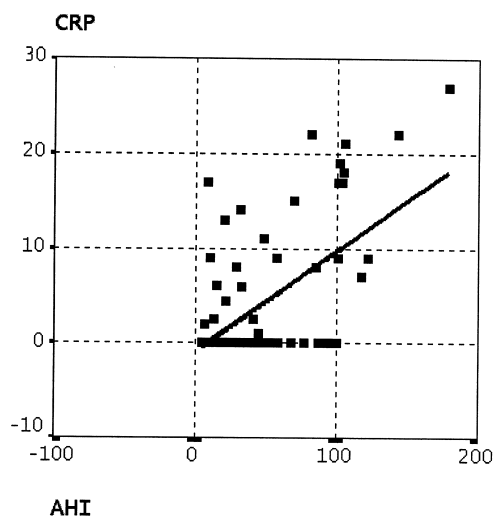


Figure 1. Correlation between AHI and serum CRP levels in OSAS patients (*n* = 94) (OSAS+CVD group (*n* = 38) and OSAS-CVD group (*n* = 56)). OSAS = obstructive sleep apnea syndrome; CVD = cardiovascular diseases; AHI = apnea-hypopnea index; CRP = C-reactive protein (mg/L)

Evaluation of the association between the serum CRP level and severity of OSAS revealed that CRP levels were positively correlated with AHI in OSAS patients (*n* = 94) (*r* = 0.61, *P* < 0.001) (Figure 1) as well as in CVD patients (*n* =

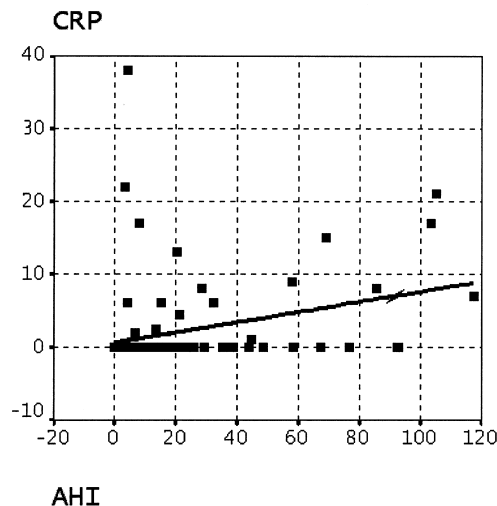


Figure 2. Correlation between AHI and serum CRP levels in CVD patients ($n = 95$) (OSAS+CVD group ($n = 38$) and CVD group ($n = 57$)). OSAS indicates obstructive sleep apnea syndrome; CVD, cardiovascular diseases; AHI, apnea-hypopnea index; and CRP, C-reactive protein (mg/L).

95) ($r = 0.32$, $P < 0.001$) (Figure 2). There was no correlation between serum lipid and CRP levels.

Evaluation of the relative strength of association using multiple regression analysis between CRP and OSAS severity (AHI) as well as the other possible confounding variables (total cholesterol, HDL, LDL, VLDL, triglycerides) showed that CRP levels were independently associated with AHI in both the OSAS + CVD ($F = 3.1$, $P < 0.05$) and OSAS-CVD groups ($F = 1.7$, $P < 0.005$).

DISCUSSION

The results of the present study confirm the previous finding that CRP levels are elevated in OSAS patients¹³⁻¹⁵ and show that serum CRP levels are independently correlated with the severity of OSAS. While there were no differences in conventional risk factors for cardiovascular diseases between the groups, a limitation of the study is that we had no data concerning the lipid lowering medications being taken by the patients. Therefore, the lack of a difference between the groups may be explained by decreases in the high lipid levels of some patients due to drug therapy.

A state of chronic systemic inflammation and elevated CRP levels in obese subjects has been demonstrated in previous studies.^{5,14,16)} Central obesity, measured by visceral fat distribution, is accompanied by higher degrees of inflammation. Visser, *et al* hypothesized that this was related to an increase in interleukin-6 (IL-6) production by an excess of adipose tissue.⁵⁾ IL-6 has proinflammatory properties. It is produced not only by immune cells and immune accessory cells including monocytes and macrophages, but also by cardiovascular components, such as endothelial cells, vascular smooth muscle cells, and ischemic myocytes. On the other hand, CRP functions to increase IL-6 and neutrophil IL-6 receptors and may serve to exaggerate the actions of IL-6 on the endothelium. Since IL-6 is a potent stimulus for CRP expression in liver, the increased vascular production of IL-6 may represent a positive feedback mechanism for the continued production of CRP from the liver.¹⁷⁾ Several recent studies have indicated that CRP and proinflammatory cytokines such as IL-6 were elevated in OSAS as well as obesity.^{13-15,18,19)} Thus, obesity and OSAS are inflammatory states, as evidenced by elevated levels of serum markers of systemic inflammation.⁵⁾ It is important to consider whether higher levels of CRP in subjects with visceral obesity may be a consequence of the obesity or OSAS. In our study, patients with OSAS had a BMI similar to controls in order to eliminate the effects of obesity.

Strong and consistent associations between CRP levels and cardiovascular disease have been established in several studies across different population groups.^{9,15,20-23)} Epidemiological studies have shown that CRP levels are a strong independent predictor of risk and an active pathogenic agent of future myocardial infarction, stroke, peripheral arterial disease, and vascular death among persons without known cardiovascular disease.¹⁵⁾ Possible mechanisms of cardiovascular dysfunction with increased levels of CRP have been described recently. CRP is found within atheromatous plaque, correlates with vascular dysfunction, and promotes the secretion of inflammatory mediators by vascular endothelium.^{14,20, 21,23)} CRP itself induces adhesion molecules and the production of monocyte chemoattractant protein-1 in human endothelial cells and sensitizes endothelial cells to the cytotoxic process by CD4 + T cells.^{24,25)}

We showed that the number of OSAS patients whose CRP levels were above the limits was significantly higher than for non-OSAS patients (5.3% of patients in non-OSAS group and 23.4% in OSAS groups had positive CRP (> 5 mg/L), $P = 0.002$). In addition, OSAS patients with CVD had significantly higher levels of CRP compared with patients with CVD without OSAS. This suggests that, among CVD patients, patients with OSAS may have higher CRP levels due to OSAS. The significant correlation between levels of CRP and AHI also suggest a possible causal interaction between OSAS and increased CRP.

Mechanisms linking OSAS to increased CRP may include repetitive hypoxemic stress and sleep deprivation. Increased plasma CRP levels have been noted during hypoxic conditions at high altitude.²⁶⁾ Increased daytime plasma levels of IL-6, which is one of the key regulators of CRP synthesis by the liver, were also noted after sleep deprivation and in patients with excessive daytime sleepiness.²⁷⁾ Thus, both hypoxia and sleep disturbances in OSAS may contribute to elevated CRP in patients with OSAS.

In view of the increasing levels of CRP, its high levels in the OSAS + CVD group may confer an added risk in addition to the risk conferred by the repeated apneic and hypoxemic events. This may at least partially explain the high cardiovascular mortality risk reported in OSAS.

The close relationships between increased CRP and OSAS and between CRP and CVD have been reported previously, however, in the present study that included only BMI-matched and age-matched male patients, we eliminated the factors affecting CVD. On the other hand, we demonstrated a correlation between AHI and CRP in CVD patients with OSAS as well as in OSAS patients.

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