Plasma cytokine levels in patients with obstructive sleep apnea syndrome: a preliminary study

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SUMMARY The levels of some pro- and anti-inflammatory cytokines [interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, IL-10, and transforming growth factor (TGF)-β], were measured by enzyme-linked immunosorbent assay (ELISA) method in the plasma of patients affected by obstructive sleep apnea syndrome (OSAS) at 22:00 hours before polysomnographic recording and immediately after the first obstructive apnea causing an SaO2 below 85%. Significantly higher levels of TNF-α were found in OSAS patients assessed before polysomnography compared with the control group (P < 0.01). A slight but significant increase in the plasma levels of IL-6 was also present (P < 0.05). Conversely, a significant decrease in the plasma levels of IL-10 was evident at baseline in OSAS patients (P < 0.04). No significant difference emerged between the mean values of IL-1α and TGF-β between OSAS patients and controls. The present data support a prevailing activation of the Th1-type cytokine pattern in OSAS patients, which is not associated with the severity and duration of OSAS. This can have important consequences for the outcome of OSAS patients, especially with regard to the increased risk for developing atherosclerosis and cardiovascular and cerebrovascular diseases. Immediately after the first obstructive apnea causing an SaO2 <85%, a significant variation was observed in the plasma levels of TNF-α in OSAS patients compared with those measured before the beginning of polysomnographic recording (P < 0.001). The role played by this further increase in TNF-α levels after the obstructive apnea in OSAS patients remains to be established in the light of the pathogenic mechanisms of this sleep disorder.

KEYWORDS interleukin-1β, interleukin-10, interleukin-6, obstructive sleep apnea, plasma cytokines, transforming growth factor-β, tumor necrosis factor-α

INTRODUCTION

Variations in certain immune parameters in obstructive sleep apnea syndrome (OSAS) have been established in the last few years, but whether these changes play a causative role or are merely a consequence of pathogenic mechanisms underlying OSAS remains to be elucidated.

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The most relevant findings in OSAS patients are increased tumor necrosis factor (TNF)-α and interleukin (IL)-6 secretion from peripheral mononuclear cells stimulated with lipopolysaccharide, as well as the increase in plasma levels of these two proinflammatory cytokines compared with control subjects (Liu et al., 2000). Their levels appeared significantly correlated with the percentage of time of apnea and hypopnea, as well as the percentage of time spent at SaO2 below 90% during the total sleep period. Increased plasma levels and mononuclear cell production of IL-6 were also found in obese patients without OSAS, but these values were lower than those detected.
hypersomnia. by excessive daytime sleepiness, and therefore can be detected
rhythms of IL-1, IL-6, and IFN-γ and TNF-α were also investigated ex vivo by the short-term culture of blood samples from OSAS patients. Whereas circadian rhythms of IL-1, IL-6, and IFN-γ, and of the immunoregulatory hormones cortisol and melatonin did not differ from those in controls, the circadian rhythm of TNF-α was profoundly disturbed. The nocturnal physiologic peaks in this cytokine almost disappeared, and an additional daytime peak developed. Moreover, nasal continuous positive airway pressure (nCPAP) therapy did not normalize TNF-α levels. On the basis of these findings, the pathophysiologic link between TNF-α and OSAS has been hypothesized considering the pivotal role played by this cytokine as a sleep modulator (Entzian et al., 1996).

Short-term variations in cytokine levels as a result of sleep apnea in OSAS patients have never been investigated until now. In this preliminary study, we determined the plasma levels of the proinflammatory cytokines IL-1, TNF-α, and IL-6, and of the anti-inflammatory cytokines IL-10 and transforming growth factor (TGF)-β before beginning nocturnal polysomnography (22:00 hours) and immediately after the first obstructive apnea causing an SaO2 <85%.

**Patients and Controls**

Eighteen patients with OSAS (three females and 15 males, mean age 52.4 ± 12.7 years) attending the Neuroscience Department of the University of Perugia who underwent sleep polysomnography from December 2001 to June 2002 participated in the study.

All patients gave their written informed consent and the protocol was approved by the Ethics Committee of Umbria. They underwent a complete physical examination, including neurologic, cardiovascular, and ear, nose, and throat evaluations. Exclusion criteria were: narcolepsy or idiopathic hypersomnia, airway obstruction, overt cardiopulmonary disease, neuromuscular diseases, endocrinologic diseases, psychiatric disorders, alcohol and drug abuse, recent or concomitant upper or lower airway or systemic infections.

The one-night polysomnographic study was performed according to the Guidelines of the American Electromyelographic Society (1994) and included the following parameters according to standard methods: electroencephalography (EEG), electrooculographic activity, submental electromyographic activity, intercostal electromyographic activity, chest and abdominal movements, snoring, airflow (oronasal flussimetry), oxygen saturation and plethysmography, lower limb movement and electrocardiographic activity. The 8-h polysomnogram was attended by a trained technologist, manually scored and interpreted by a trained clinical polysomnographer according to standardized Criteria (Rechtschaffen and Kales, 1968).

OSAS diagnosis was based on the Criteria of the International Classification of Sleep Disorders (American Sleep Disorders Association, 1990). Obstructive apnea was defined as a cessation of airflow for at least 10 s in the presence of a respiratory effort despite cessation of airflow. Hypopnea occurred when there was a reduction of airflow by 50% or more for at least 10 s. The number of apnea or hypopnea events per hour was obtained by dividing the total number of such events per total sleep time as defined by the apnea–hypopnea index (AHI) (American Sleep Disorders Association, 1990).

For each patient, sleep efficiency, Epworth score (Johns, 1991) as a measure of excessive diurnal somnolence, and body mass index (BMI) were also calculated.

The control values for plasma cytokine levels were obtained from a group of 20 age- and sex-matched apparently healthy individuals. They did not suffer from sleep disturbances, hypertension, cardiovascular or cerebrovascular diseases, diabetes, renal or hepatic diseases, nor were they affected by other diseases of the central or peripheral nervous systems. They had a negative history for infections in the last 2 months. The BMI in all control subjects was below 25. They were tested for blood cytokine levels but did not undergo polysomnography.

Details of OSAS patients and controls are reported in Table 1.

### Determination of Plasma Cytokine Levels

Peripheral blood was drawn from OSAS patients at 22:00 hours, before beginning polysomnographic recording and after the first sleep apnea causing an SaO2 <85%. The first significant apnea occurred for all patients between midnight and 02:00 hours (mean time 01:05 hours). Blood

<table>
<thead>
<tr>
<th>Table 1 Characteristics of patients with OSAS and control subjects</th>
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<tbody>
<tr>
<td><strong>OSAS (n = 18)</strong></td>
</tr>
<tr>
<td>Age (years) (mean ± SD)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
</tr>
<tr>
<td>BMI (kg m⁻²) (mean ± SD)</td>
</tr>
<tr>
<td>Arterial hypertension (%)</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
</tr>
<tr>
<td>Smoking (actual or past) (%)</td>
</tr>
<tr>
<td>Epworth Sleepiness Scale (mean ± SD)</td>
</tr>
<tr>
<td>AHI (h⁻¹) (mean ± SD)</td>
</tr>
<tr>
<td>Mean oxygen desaturation (%)</td>
</tr>
<tr>
<td>Mean apnea duration (s) (mean ± SD)</td>
</tr>
</tbody>
</table>

OSAS, obstructive sleep apnea syndrome; SD, standard deviation; BMI, body mass index; AHI, apnea–hypopnea index.
SaO2 < 85%, as well as those measured in controls at measured immediately after the first obstructive apnea with an plasma levels measured before beginning recording and those The same test was also used to compare the mean cytokine plasma levels of OSAS patients assessed before beginning Analysis of variance was used to compare the mean cytokine STATISTICAL ANALYSIS cytokines measured at 22:00 hours and those measured to other cytokines.

The optical density at 490 nm of each well was measured by an Immuno Reader J 2000. The detection limits were the following: IL-1β < 1 pg mL⁻¹, TNF-α < 2 pg mL⁻¹, IL-6 < 0.8 pg mL⁻¹, IL-10 < 1 pg mL⁻¹, and TGF-β < 2 pg mL⁻¹.

The intra- and inter-assay variabilities were, respectively: IL-1β, 3 and 6%; TNF-α, 4 and 7%; IL-6, 5 and 9%; IL-10, 4 and 6%; and TGF-β, 3 and 5%.

Table 2 Cytokine plasma levels (mean ± 2 SD) of OSAS patients at the beginning of polysomnographic recording and after the first obstructive apnea causing an SaO2 below 85% and of control subjects

<table>
<thead>
<tr>
<th>Cytokine levels (pg mL⁻¹)</th>
<th>OSAS patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before obstructive apnea (22:00 hours)</td>
<td>After obstructive apnea</td>
</tr>
<tr>
<td>IL-1β</td>
<td>2.5 ± 0.6</td>
<td>2.7 ± 0.8†</td>
</tr>
<tr>
<td>TNF-α</td>
<td>9.7 ± 8.5***</td>
<td>26.9 ± 6.9††</td>
</tr>
<tr>
<td>IL-6</td>
<td>6.4 ± 3.7***</td>
<td>6.9 ± 3.3†††</td>
</tr>
<tr>
<td>IL-10</td>
<td>3.9 ± 2.6***</td>
<td>3.5 ± 2.7††††</td>
</tr>
<tr>
<td>TGF-β</td>
<td>3.9 ± 1.8</td>
<td>3.5 ± 1.9</td>
</tr>
</tbody>
</table>

OSAS, obstructive sleep apnea syndrome; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin.

OSAS patients at baseline versus control subjects:
*TNF-α = P < 0.005 ; ***IL-6 = P < 0.05 ; ****IL-10 = P < 0.04.
OSAS patients at baseline versus OSAS patients after obstructive apnea:
**TNF-α = P < 0.001.
OSAS patients after obstructive apnea versus controls assessed between midnight and 02:00 hours:
†IL-1β = P < 0.05; ††TNF-α = P < 0.002; †††IL-6 = P < 0.04; ††††IL-10 = P < 0.02.

Spearman correlation coefficients were calculated between plasma cytokine levels and age, duration of OSAS (years), percentage of time of apnea and hypopnea, AHI, sleep efficiency, Epworth score, and BMI.

RESULTS

The levels of IL-1β, IL-6, TNF-α, and IL-10 in patients (both before and after sleep apnea) and controls (both at 22:00 hours and between midnight and 02:00 hours) are shown in Table 2.

Controls

No significant variations were detected between the levels of all cytokines measured at 22:00 hours and those measured between midnight and 02:00 hours.

Before the beginning of polysomnographic recording (baseline)

Significantly higher levels of TNF-α emerged in OSAS patients compared with the control group (P < 0.005). A slight but significant increase in the plasma levels of IL-6 was also found (P < 0.05). IL-1β plasma values were also slightly higher than those of controls, but the difference did not reach statistical significance between the two groups. A significant decrease in the plasma levels of IL-10 was also present at baseline in OSAS patients (P < 0.04). No significant difference emerged in the mean values of TGF-β between OSAS patients and controls.

After obstructive apnea

Immediately after the first obstructive apnea causing an SaO2 desaturation <85%, a significant variation was observed in the plasma levels of TNF-α in OSAS patients compared with those measured at the beginning of
polysomnographic recording \( (P < 0.001) \). Values of TNF-\( \alpha \) measured in OSAS patients after the first significant apnea were significantly higher than those measured in control subjects at 02:00 hours \( (P < 0.002) \). The variations in the plasma levels of TNF-\( \alpha \) for each patient with OSAS are shown in Fig. 1. A slight but not significant increase in IL-6 levels was also observed compared with baseline. The values measured after obstructive apnea remained significantly greater than those measured in controls, both at 22:00 hours and between midnight and 02:00 hours \( (P < 0.03 \text{ and } P < 0.04, \text{ respectively}) \).

No significant changes were observed in the levels of IL-1\( \beta \), IL-10, and TGF-\( \beta \) compared with baseline. Nevertheless, the levels of IL-1\( \beta \) measured after obstructive apnea were slightly but significantly higher \( (P < 0.05) \), and those of IL-10 significantly lower \( (P < 0.02) \) than those measured in the control group between midnight and 02:00 hours.

Correlation with clinical parameters

The plasma levels of cytokines measured before the beginning of polysomnographic recording and immediately after the first obstructive apnea showed no significant correlation with age, duration of OSAS (years), sleep efficiency, Epworth score, AHI, percentage of time of apnea and hypopnoea, and BMI. No correlation was found between the plasma levels of all cytokines measured after obstructive apnea and its duration and SaO\(_2\) measurement.

DISCUSSION

In this preliminary study, we observed an increase in the plasma levels of the proinflammatory cytokines TNF-\( \alpha \), and to a lesser extent IL-6, in a small group of OSAS patients assessed at bedtime (22:00 hours) before polysomnographic recording. A significant decrease in the peripheral plasma levels of IL-10 was instead found in the same patients compared with the control group. After the first obstructive apnea causing a desaturation below 85\%, a significant further increase in the levels of TNF-\( \alpha \) was found, with no variations in the plasma levels of the other cytokines.

These findings support a systemic activation of the inflammatory response in OSAS patients, which does not appear to be associated, however, with its severity and duration, as shown by the lack of correlation between cytokine plasma levels measured both before and after obstructive apnea and the number of years with OSAS, sleep efficiency, Epworth score, AHI, and percentage of time of apnea and hypopnea.

The prevailing Th1-type cytokine pattern of the immune cells in the peripheral blood of OSAS patients can partially be explained by hypoxia. Experimental evidence supports, in fact, the role of hypoxia in inducing the expression of the proinflammatory cytokines, in particular TNF-\( \alpha \) but also IL-1\( \beta \) and IL-6 (Naldini et al., 1997). This has clearly been delineated in in vitro studies involving peripheral blood mononuclear cells, human macrophage lines and alveolar macrophages stimulated with LPS, and may be mediated by
the induction of haem oxygenase and NF-kappa B activation, at least for IL-6 (Muraoka et al., 1997; Naldini et al., 1997; Tamion et al., 1999). Nevertheless, few studies have been carried out on the influence of systemic hypoxemia on the production of proinflammatory cytokines (Ghezzi et al., 1991; Hempel et al., 1996; Scannell et al., 1993). The activation of the TNF-\alpha system has been clearly shown in chronic obstructive pulmonary disease, a condition characterized by chronic hypoxemia; however, the effect of brief but repeated hypoxic conditions, such as that characterizing OSAS, on cytokine production is poorly known (Takabatake et al., 2000).

It can be hypothesized that short-lasting but recurrent hypoxic episodes and reactive oxygen species in OSAS patients can increase the production of proinflammatory cytokines by mononuclear cells, as suggested by experimental evidence. This may be responsible for the rise in their peripheral blood levels, as a result of their spill-over into the circulation from these cells (Ghezzi et al., 1991).

Our finding of increased plasma levels of IL-6 in OSAS patients confirms both previous observations of an enhanced production of this cytokine by stimulated mononuclear cells (Liu et al., 2000), and the most recent evidence of a significant increase in levels of IL-6 in breath condensate associated with an increase in the oxidative stress marker 8-isoprostane in the same patients (Carpagnano et al., 2002). The increased production of IL-6 in the breath condensate of OSAS patients has been attributed to local adipose tissue based on the observation of a significant correlation between neck circumference and IL-6 levels (Carpagnano et al., 2002). In another study, the concomitant presence of OSAS in obese patients appeared to be associated with the highest levels of IL-6 (Roytblat et al., 2000). Although the above results suggest obesity to be a pivotal determinant for the increase of IL-6, in our study we did not find any correlation between the level of this cytokine and BMI.

In a further study, variations in IL-6 and TNF-\alpha in OSAS patients appeared to be associated with daytime sleepiness and fatigue and their increase appeared to be strongly and independently correlated with insulin resistance and visceral obesity (Vgontzas et al., 2000). As in previous research, visceral fat was not measured in our study, and thus no correlation with cytokine levels could be obtained.

The central role played by TNF-\alpha and IL-6 in modulating sleep has been clearly elucidated (Gudewill et al., 1992; Krueger et al., 2001; Shearer et al., 2001; Vgontzas et al., 1999). There is evidence in experimental animals that systemic TNF-\alpha enhances sleep in animals, and conversely, that inhibition of this cytokine suppresses sleep (Krueger et al., 1999; Takahashi and Krueger, 1997; Takahashi et al., 1995).

Based on the above findings, it can be hypothesized that increased levels of TNF-\alpha in our OSAS patients contributed to sleep apnea. By inducing sleepiness, which is associated with more apnea, increased levels of TNF-\alpha would establish a vicious cycle in the pathogenic events of OSAS.

Contrary to previous literature, we found no correlation between the severity of OSAS, as measured by AHI, and the high levels of cytokines. However, differences in cytokine levels between severe-moderate OSAS and mild OSAS patients cannot be excluded, although the latter were not included in the present research. It can be hypothesized that beyond certain values of AHI and lower mean values of O_2 saturation a linear correlation cannot be found, suggesting that additional unknown mechanisms other than hypoxia may be involved in inducing higher levels of proinflammatory cytokines in OSAS patients.

The circadian rhythm of TNF-\alpha has been demonstrated to be disturbed in OSAS patients, but this aspect was not investigated in our research. Our attention was rather focused on the immediate effect of acute hypoxemia occurring during obstructive apnea on cytokine levels in the peripheral blood. The cytokine that seems more sensitive to rapid changes in O_2 saturation is TNF-\alpha, although the role of its fluctuations in the pathogenic events of OSAS remains to be elucidated.

In our preliminary study, the controls were matched to OSAS patients only for blood sampling before bed time and at night when the first significant apnea was detected. No additional blood samples were taken during the night, and therefore, we can at the moment only partially address the question whether the higher TNF-\alpha levels seen after the first apnea are potentially a time of day effect and not apnea-induced.

In a study carried out in healthy controls and HIV patients, Darko et al. (1995) observed a cyclic nocturnal variation in TNF-\alpha levels in six of 10 subjects studied, and a coupling between the increase in TNF-\alpha levels and sleep EEG delta spectral amplitude.

This finding suggests that cyclic nocturnal variations of TNF-\alpha should be assessed in OSAS not only in relation to the time of night but also to power spectral analyses of the EEG. This aspect needs to be investigated in future research, along with serial analysis of the soluble TNF-\alpha receptor. However, because the majority of our OSAS patients were examined for the first apnea during the REM phase (\(n = 12\)) or phase 2 of sleep, we could hypothesize a smaller contribution of delta power to the short-term variation in TNF-\alpha determined within 20–45 s after the first significant apnea.

The increase in proinflammatory cytokine production in OSAS patients can have important consequences for patient outcomes, especially with regard to the increased risk for developing atherosclerosis and cardiovascular and cerebrovascular diseases (Aboyans et al., 1999; Bassetti et al., 1996; Mohsenin, 2001; Schafer et al., 1999). Due to the pivotal role played by IL-6 and TNF-\alpha in the development and severity of the above disorders (Anguera et al., 2002; Bermudez et al., 2002; Emsley and Tyrrell, 2002; van Exel et al., 2002; Grau et al., 2001; Jenny et al., 2002; Kaplan and Frishman, 2001; Kell et al., 2002; Lowe, 2001; Pradhan et al., 2002; Skoog et al., 2002), their determination should be considered in future studies (Strohl, 1997), together with the well-known risk factors such as hyper-homocysteinemia, obesity, hypertension,
atherosclerosis, and platelet hyperaggregability associated with OSAS (Aboyans et al., 1999; Lavie et al., 2000, 2001; Macko et al., 1996; Peker et al., 1999; Rangemark et al., 1995; Schafer et al., 1999; Yudkin et al., 2000).

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