Interleukin-23 concentrations of NREM-AHI greater than REM-AHI versus REM-AHI greater than NREM-AHI in obstructive sleep apnea syndrome

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Abstract

Objective: Although the presence of a systemic inflammatory response is known in obstructive sleep apnea syndrome (OSAS), there have not been adequate investigations on the association of such a response with the predominance of apneas either in rapid eye movement (REM) or in the non-REM (NREM) phase. In this study, we compared interleukin-23 (IL-23) concentrations, a marker of systemic inflammatory process, in individuals with sleep apnea syndrome, whose apneas where predominant in the REM or NREM phase.

Methods: Fifty-four patients aged over 18 years who were diagnosed as having OSAS based on polysomnography were included in the study. The apnea-hypopnea index (AHI) was used to divide patients into groups of AHI_{REM} > AHI_{NREM} and AHI_{NREM} > AHI_{REM}. In the blood samples drawn from the patients, IL-23 concentrations were measured using enzyme-linked immunosorbent assay.

Results: The study included patients with OSAS aged 28-65 years, of whom 29 were AHI_{REM} > AHI_{NREM} and 25 were AHI_{NREM} > AHI_{REM}. The AHI_{REM} > AHI_{NREM} OSAS group had no sex-based variance (14 males, 15 females), but the AHI_{NREM} > AHI_{REM} group was substantially composed of males (21 males, 4 females). The average AHI of the AHI_{REM} > AHI_{NREM} group (43.34±21.40) was significantly higher than in the AHI_{NREM} > AHI_{REM} group (26.79±17.32). IL-23 concentrations were higher in patients with AHI_{REM} > AHI_{NREM} sleep apnea (225.38±77.29) compared patients with AHI_{NREM} > AHI_{REM} sleep apnea (183.68±78.49).

Conclusion: In AHI_{REM} > AHI_{NREM} sleep apnea, the systemic inflammatory response may be more dramatic. Taking the AHI_{REM} value into consideration along with the average AHI value seems to be important for treatment modalities.

Keywords: Inflammation, interleukin-23, non-rapid eye movement sleep, obstructive sleep apnea syndrome, rapid eye movement sleep

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a sleep-related respiratory disorder that impairs quality of life, characterized by a partial or total collapse of the upper respiratory tract and recurrent episodes of apnea and hypopnea (1-3). Hypoxia and hypercapnia caused by such apneas and hypopneas result in fragmented sleep, excessive daytime sleepiness, increased oxidative stress, and a systemic inflammatory response (4, 5). The co-existence of OSAS with chronic diseases such as diabetes mellitus, hypertension, and obesity, and with auto-immune diseases is likely to be associated to the increased inflammatory response (2, 5).

In patients with OSAS, increased concentrations of C-reactive protein (CRP), tumor necrosis factor-α, interleukin (IL)-6 and IL-23 have been reported, as markers of systemic inflammation (2, 5, 6). IL-23 is a pro-inflammatory cytokine with an immune-modulatory effect (2, 5). An essential effector cell type in the immune response, Th17 cells, are proposed to be regulated by IL-23 (2, 5, 7). In the study by Can et al., the authors concluded that serum IL-23 concentrations reflected OSA-related systemic inflammation (2).

During sleep, obstruction of the upper respiratory tract may take place both in rapid eye movement (REM) and in the non-REM (NREM) phase (4). Airway collapse during REM sleep is suggested to arise from the suppression
of upper respiratory tract muscle tone (3, 4, 8). Furthermore, sympathetic activity and cardiovascular instability is known to increase along the REM phase of sleep (4, 9-11). Nevertheless, by virtue of a minor contribution by REM-sleep to the entire sleep duration, the apnea-hypopnea index (AHI) values of patients with REM-related sleep apnea tend to be low as recorded in overnight assessments (9). In this study, we wanted to compare patients with AHI\textsubscript{REM}>AHI\textsubscript{NREM} and AHI\textsubscript{NREM}>AHI\textsubscript{REM} sleep apnea for their IL-23 concentrations as a marker of systemic inflammation.

**METHODS**

The study included 54 patients aged over 18 years who were diagnosed as having sleep apnea syndrome based on polysomnography (PSG) conducted at the Neurophysiology Laboratory of Muğla Sıtkı Koçman University Research and Training Hospital, between May 2017 and August 2017. The selected patients’ sleep apnea was newly diagnosed, and none of patients had undergone continuous positive airway pressure therapy. Patients with chronic obstructive pulmonary disease, heart disease, morbid obesity (body mass index (BMI) >35 kg/m\(^2\)), and history of malignancy were excluded from the study. The PSG records of all patients were recorded using an PSG (Embla N7000, Natus, Kanata, Canada) system. Recording and scoring were performed in accordance to the American Academy of Sleep Medicine Manual for the Scoring of Sleep and Associated Events (12). AHI was taken as the total number of apneas and hypopneas during sleep in hours. Patients with an AHI value of >5 were diagnosed as having OSAS. Patients were divided into two groups as AHI\textsubscript{REM}>AHI\textsubscript{NREM} and AHI\textsubscript{NREM}>AHI\textsubscript{REM}. BMI was calculated by body weight (kg)/height (m\(^2\)).

In order to determine serum IL-23 concentrations, fasting blood samples were collected into gel-separated blood tubes. The tubes were left at room temperature for 10-20 min for separation of sera followed by centrifugation at 1000 x g for 10 min. Sera were then taken into Eppendorf tubes and stored at -80°C. Serum IL-23 concentrations were measured in the patients and healthy controls using a human IL-23 enzyme-linked immunosorbent assay (ELISA) kit (Thermo Scientific, Rockford, IL, USA; Cat No.: BMS2023-3TEN). Measurements were carried out using an ELISA plate reader (Bio-Tek Synergy HT, Biotek Instruments Inc., Winooski, VT, USA). The intra-assay coefficient of variation (CV) and inter-assay CV were <5.9% and <6.3%, respectively, and the assay range was between 15.6 pg/mL and 2000 pg/mL, with a sensitivity of 4 pg/mL.

Ethics Committee approval was obtained for the study ethics committee of Muğla Sıtkı Koçman University (11.05.2017/4). All participants were informed as per the Helsinki Declaration and their written consents were obtained.

**Statistical Analysis**

Study data were assessed using the SPSS computer software. The results are given as mean±standard deviation. A p-value of <0.05 was considered statistically significant. Inter-group differences were evaluated using statistical methods, the Mann-Whitney U test and the Chi-square test. Correlations between IL-23 concentrations and AHI-scores and O\(_2\) saturation were calculated using Pearson’s correlation coefficient.

**RESULTS**

The participating patients were aged 28-65 years, 29 of whom were diagnosed as having AHI\textsubscript{REM}>AHI\textsubscript{NREM} and 25 had AHI\textsubscript{NREM}>AHI\textsubscript{REM} sleep apnea syndrome. The AHI\textsubscript{REM}>AHI\textsubscript{NREM} OSAS group had no sex-based variance (14 males, 15 females), but the AHI\textsubscript{NREM}>AHI\textsubscript{REM} group was substantially composed of males (21 males, 4 females). The average age of the patients with AHI\textsubscript{REM}>AHI\textsubscript{NREM} OSAS was 49.24±10.42 years, and that of patients with AHI\textsubscript{NREM}>AHI\textsubscript{REM} OSAS was 51.44±6.10 years. The BMIs of the REM group and NREM group were 28.57±3.24 and 28.45±3.36 kg/m\(^2\), respectively. The two groups did not differ significantly in terms of age or BMI. On the other hand, the average AHI value of patients with AHI\textsubscript{NREM}>AHI\textsubscript{REM} OSAS (43.34±21.40) was significantly higher than that of patients with AHI\textsubscript{REM}>AHI\textsubscript{NREM} OSAS (26.79±17.32) (Table 1). There was no significant correlation between IL-23 and AHI, AHI\textsubscript{REM}, AHI\textsubscript{NREM}, mean O\(_2\) saturation or minimum O\(_2\) saturation (Table 2).

| Table 1. Comparison of patients with AHI\textsubscript{REM}>AHI\textsubscript{NREM} and AHI\textsubscript{NREM}>AHI\textsubscript{REM} sleep apnea syndrome |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| AHI\textsubscript{REM}>AHI\textsubscript{NREM} | AHI\textsubscript{NREM}>AHI\textsubscript{REM} | p               |
| Age             | 49.24±10.42     | 51.44±6.10      | 0.584           |
| BMI             | 28.57±3.24      | 28.45±3.36      | 0.972           |
| IL-23           | 225.38±77.29    | 183.68±78.49    | 0.03            |
| AHI             | 26.79±17.32     | 43.34±21.40     | 0.003           |
| AHI\textsubscript{REM} | 40.53±19.10     | 27.14±21.80     | 0.016           |
| AHI\textsubscript{NREM} | 23.17±19.12     | 46.57±21.73     | <0.001          |
| Mean O\(_2\) saturation | 91.91±1.82      | 90.80±3.51      | 0.317           |
| Minimum O\(_2\) saturation | 79.62±6.56      | 71.29±18.60     | 0.051           |

BMI: body mass index; IL-23: interleukin-23; AHI: apnea-hypopnea index; REM: rapid eye movement; NREM: Non-rapid eye movement

| Table 2. Correlations between IL-23 concentrations and AHI scores/O\(_2\) saturation |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| IL-23           | r               | p               |
| AHI             | -0.17           | 0.219           |
| AHI\textsubscript{REM} | -0.32           | 0.817           |
| AHI\textsubscript{NREM} | -0.187          | 0.175           |
| Mean O\(_2\) saturation | 0.201           | 0.149           |
| Minimum O\(_2\) saturation | 0.159           | 0.256           |

IL-23: interleukin-23; AHI: apnea-hypopnea index; REM: rapid eye movement; NREM: non-rapid eye movement
DISCUSSION

The obstruction of the upper respiratory tract the occurs in OSAS leads to chronic intermittent hypoxia, which is believed to be the cause of oxidative stress, increased systemic and vascular inflammation, and endothelial dysfunction (13-15). Studies on patients with OSAS have shown higher erythrocyte sedimentation rates, high-sensitivity CRP (hsCRP), and fibrinogen concentrations, which are inflammatory biomarkers (15, 16). A correlation has been argued between the level of inflammatory biomarkers and the severity of OSAS (15, 16).

The inflammatory process has an established role in atherosclerosis and the emergence of cardiovascular risk (14). For this reason, the resulting inflammation may be of importance in disease progression.

Although a chronic inflammatory response is known to exist in OSAS, there is a scarcity of evaluations on apneas by their dominant sleep phases. A previous study indicated no difference between REM-sleep and NREM-sleep when aspects of clinical nature and daytime sleepiness were compared in sleep-disordered breathing (17). Pharyngeal motor neuron inhibition and likewise inhibition of the genioglossus reflex in response to the negative pressure of the upper respiratory tract occurring during the REM phase of sleep may make the upper-airway vulnerable to collapse (4, 18). Moreover, the ventilatory response to hypoxia and hypercapnia diminishes in the REM phase (4, 19). In patients with OSAS, a more pronounced decrease in oxygen saturation has been reported during REM sleep relative to NREM sleep (20-22). On the contrary, we detected a lower average and minimum O₂ saturation for AHI_{REM}>AHI_{NREM} sleep apnea, albeit not to a statistically significant extent.

Although several studies have speculated that no clinical difference existed between AHI_{REM}>AHI_{NREM} OSAS and AHI_{NREM}>AHI_{REM} OSAS, some studies implicated that REM-related OSAS could be the cause of worsening in glycemic control of type 2 diabetes mellitus, and might also be associated with hypertension (3, 4, 20, 23, 24). Additionally, as the AHI_{REM} gets higher, the hypertension risk is also increased (4). In the presence of underlying coronary stenosis, along with the sympathetic nervous system activation at this phase, the heart rate increases and coronary blood flow is attenuated (4). Overall, these findings set forth the need for a separate evaluation for REM-related OSAS, which is reported more frequently for mild-to-moderate OSAS (4, 17). REM-related OSAS might be associated with a lower AHI values because REM sleep represents a smaller percentage of the total duration of sleep (9).

In our study, the increased IL-23 concentrations in patients with AHI_{REM}>AHI_{NREM} OSAS suggested that the inflammatory response was more common in this form of disease. REM sleep is characterized by attenuated vagal tone, whereas sympathetic activity and cardiovascular instability is augmented (4, 10, 11). In patients with OSAS, respiratory effort in response to upper airway occlusion is weaker and apneas are prolonged in REM as compared with NREM sleep (22). All of these mechanisms might explain the increased oxidative stress and inflammatory response inflicted in patients with OSAS. In our study, there were no statistical differences in REM versus NREM oxygen saturation, implying that blood oxygen saturation does not play a role on its own in inflammatory mechanisms.

In the English literature, we cannot find any studies that investigated the inflammatory response in AHI_{REM}>AHI_{NREM} and AHI_{NREM}>AHI_{REM} OSAS. However, there are some limitations in our study. First, our study includes a small number of patients. Further studies that contain larger numbers of patients are required. Likewise, future studies of the inflammatory response of REM and NREM-related apneas in mild, moderate, and severe OSAS may provide valuable results. Another limitation is that there is no generally accepted definition for REM-related apnea. Many studies have been examined apneas during REM sleep and during NREM sleep. Some studies define REM-related apnea as AHI_{REM}/AHI_{NREM} ≥ 2, some as AHI_{REM}/AHI_{NREM} ≥ 2 and AHI_{NREM} < 15, and others as AHI_{REM}/AHI_{NREM} ≥ 2 and AHI_{NREM}>8, and at least 10.5 min of REM sleep duration (25). Some of these studies compared features such as AHI_{REM}>AHI_{NREM} with AHI_{NREM}>AHI_{REM} (3). We compared patients with AHI_{REM}>AHI_{NREM} OSAS and patients with AHI_{NREM}>AHI_{REM} OSAS. The results may change according to the different criteria used. Our study is also restricted by the sole use of IL-23 as an inflammatory marker. Advanced studies to cover other ILs and inflammatory markers are needed.

In conclusion, we found a higher IL-23 concentration in AHI_{REM}>AHI_{NREM} OSAS compared with AHI_{NREM}>AHI_{REM} OSAS, which suggests that the inflammatory response is more pronounced in AHI_{REM}>AHI_{NREM} OSAS. Therefore, we would suggest that treatment modalities should take OSAS severity, total AHI values, and REM-specific AHI values into consideration.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Muğla Sıtkı Koçman University (11.05.2017/4).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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