Association of Cellular Adhesion Molecules and Oxidative Stress with Endothelial Function in Obstructive Sleep Apnea

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Abstract

Objective To evaluate the impact of oxidative stress and cellular adhesion molecules on ischemic reactive hyperemia (IRH) in patients with OSA.

Materials and Methods Consecutive patients treated at a sleep laboratory and whose polysomnography showed an apnea hypopnea index (AHI) ≥5 were included in the study. Patients with acute illness receiving vasoactive medications were excluded. Based on their oxygen desaturation index (ODI), subjects were assigned to the mild-moderate (ODI ≤30) or the severe desaturation group (ODI >30). Then IRH and oxidative stress markers [malondialdehyde (MDA)] and proinflammatory markers (ICAM-1 and P-selectin) were measured.

Results Sixty-eight subjects with OSA were included, 31 in the mild-moderate desaturation group and 37 in the severe group. No differences by age, gender and body mass index were observed. The severe desaturation group showed significantly higher values in the AHI, MDA, ICAM-1 and P-selectin (p<0.005), as well as a worsening of IRH (p=0.001). Only ICAM-1 (p=0.019) and P-selectin (p=0.033) were independently associated with IRH in a multiple-linear regression model.

Conclusion Patients with OSA and greater intermittent hypoxia showed worse endothelial function, and higher levels of MDA, ICAM-1 and P-selectin. Nevertheless, ICAM-1 and P-selectin rather than MDA were independently associated with IRH.

Key words: atherosclerosis, cellular adhesion molecules, endothelial function, obstructive sleep apnea, oxidative stress


Introduction

OSA is characterized by snoring, witnessed apnoeas, un-refreshing sleep and excessive daytime sleepiness. Respiratory events are accompanied by hypoxia-reoxygenation episodes which may recur many times during the night. These respiratory disturbances are associated with an increased risk for cardiovascular disease, including hypertension, coronary artery disease and cerebrovascular events (1-4).

Endothelial dysfunction is an early indicator of vascular disease. Previous research indicates that this may promote or accelerate the process of atherogenesis and the subsequent development of cardiovascular disease (4-7). There are several mechanisms that link OSA with increased vascular pathology, probably due to oxygen deficit during sleep. However, the precise mechanisms involved are unknown, although the release of proinflammatory substances and the
increase in oxidative stress are commonly suggested (8, 9). Nevertheless, the current studies are not conclusive in OSA patients and the two known biological pathways have not been studied yet.

Malondialdehyde (MDA) is a lipid peroxidation marker, commonly used in human pathology (10-12). P-selectin is largely responsible for the rolling phase of the leukocyte adhesion cascade (13). ICAM-1 (Inter-Cellular Adhesion Molecule 1) is a type of intercellular adhesion molecule, ubiquitous in low concentrations in the membranes of leukocytes and endothelial cells. Therefore, cellular adhesion molecules are good indicators of endothelial dysfunction and vascular inflammation (14, 15).

The OSA facilitates the development of endothelial dysfunction, although the underlying mechanism is unknown. Therefore, we have designed a prospective study in OSA patients with the following objectives: 1) Assess ischemic reactive hyperemia (IRH) as a function of the level of intermittent hypoxemia; 2) Study the relationship of IRH with oxidative stress and cellular adhesion molecules.

Materials and Methods

Study design

Prospective study, with consecutive subjects, carried out in the Sleep Unit of a University Hospital.

All patients underwent a complete physical examination, review of clinical records and were asked about medications taken. Body mass index (BMI) was calculated using the formula weight/(height in meters)$^2$ and awake peripheral oxygen saturation $\text{SaO}_2$ was measured with a pulse oximeter (Pulsox 300i Konica-Minolta™, Japan).

Subjects

Subjects were selected from among consecutive patients that were seen at the sleep unit and who fulfilled the study inclusion criteria.

Subjects with symptoms of OSA an AHI ≥5 in the overnight polysomnography, between 30-70 years old, and who agreed to participate in the study were selected. Patients were excluded if they had a $\text{SaO}_2$ ≤93%, acute inflammatory disease, symptomatic vascular disease, and hypertension treated with calcium antagonists, α nitrates and beta-blockers, or angiotensin-converting enzyme inhibitors. Based on the number of falls in $\text{SaO}_2$ ≥3% (ODI), subjects were assigned to the mild-moderate (ODI ≤30) or to the severe desaturation group (ODI >30). After polysomnography, oxidative stress markers (MDA) and those related to inflammation (ICAM-1, P-selectin) were measured. Ischemic vascular reactivity was assessed after overnight polysomnography. Observed values were compared between the two groups.

This study was carried out with the approval of the Research Ethics Committee of the Reina Sofia de Córdoba University Hospital.

Methods

Polysomnography. A polysomnograph was used (Somnoseen™, Somnomedics, Randersacker, Germany). The test began at 12 : 00 PM and concluded at 7 : 30 AM. We registered two electroencephalogram channels (C4/A1 and C3/A2), electrooculogram, submental and tibial electromyogram, and airflow by pressure signal. Snoring, thoracic and abdominal effort, electrocardiographic derivation (V2) and $\text{SaO}_2$ by digital pulse oximetry were also monitored. Recordings were staged using the Rechtschaffen and Kales system. Apnea was defined as a significant decrease (>90%) in oronasal flow of at least 10 seconds, and hypopnea as an evident decrease in airflow ≥30% and <90%, and associated with either oxygen desaturation of ≥3% and/or arousal. The following respiratory variables were monitored: apnea-hypopnea index (AHI), determined by the sum of apneas and hypopneas per hour of sleep, minimum $\text{SaO}_2$ reached during sleep, and the ODI, defined as the number of falls in $\text{SaO}_2$ ≥3% per hour of sleep. Finally, sleep time spent with $\text{SaO}_2$ <90% was estimated. All studies were reviewed and interpreted by a study-blinded, board-certified, sleep medicine physician. Polysomnography was considered valid for diagnosis when a total of at least 180 min of sleep was recorded.

Endothelial function

A Laser-Doppler linear Periflux 5,000 (Perimed S.A., Stockholm, Sweden) was used to measure IRH after polysomnography using a previously described methodology (16). Briefly, with the patient lying in the supine position in a room with a stable temperature (20°- 22°C), the blood pressure cuff (HG Erkameter 300, Erka, Bad Tolz, Germany) was placed five centimeters above the elbow, while the laser probe was attached to the palm surface of the second finger of the dominant hand. After a five minute resting period, basal capillary flow was measured for one minute (t0). Thereafter, four minute distal ischemia was induced by inflating the cuff to suprasystolic pressure (200 to 220 mm Hg). The cuff was then deflated and, after 30 seconds, the flow was recorded for one minute (td). Data obtained were recorded and stored using PeriSoft Software for Windows. The values for area under the curve (AUC) of the t0 and td times were analyzed. These data were used to calculate the increase in posts ischemic flow by means of the formula: $\text{IRH}=\left(\text{AUCtd} - \text{AUCt0}\right) \times 100 / \text{AUCt0}$. The IRH recording took place at 8:15 AM after polysomnography and blood extraction. This method has an interstudy variability of 8.85% and intrastudy variability of 8.7% (17).

Serum markers

Blood samples were obtained at 8 : 00 AM, after one night of fasting. Whole blood was collected in vacutainer tubes (BD Diagnostic Systems, Franklin Lakes, NJ, USA) following our standard hospital extraction protocol. Blood was allowed to cool and coagulate for 30 minutes, and was
Table 1. Characteristics of the Patients Mild-moderate Desaturation Group and Severe Desaturation Group. Data are Presented as Median and Interquartile Range for Continuous Variables and n (%) for Categorical Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mild-moderate (n = 31)</th>
<th>Severe desaturation group (n = 37)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>48 (43-53)</td>
<td>48 (41-59)</td>
<td>0.278</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>22 (71%)</td>
<td>27 (73%)</td>
<td>0.854*</td>
</tr>
<tr>
<td>Body Mass Index, kg/m²</td>
<td>30 (28-33)</td>
<td>31 (28-34)</td>
<td>0.142</td>
</tr>
<tr>
<td>Epworth</td>
<td>13 (12-15)</td>
<td>14 (13-16)</td>
<td>0.489</td>
</tr>
<tr>
<td>SaO₂ awake, %</td>
<td>96 (95-97)</td>
<td>95 (94-96)</td>
<td>0.392</td>
</tr>
<tr>
<td>Ex-smokers, n (%)</td>
<td>4 (13%)</td>
<td>6 (16%)</td>
<td>0.742*</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>5 (16%)</td>
<td>7 (19 %)</td>
<td>0.822*</td>
</tr>
<tr>
<td>Diabetes Mellitus, n (%)</td>
<td>2 (6 %)</td>
<td>3 (8 %)</td>
<td>1*</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>3 (9%)</td>
<td>2 (5 %)</td>
<td>0.653*</td>
</tr>
<tr>
<td>COPD</td>
<td>6 (19%)</td>
<td>3 (8 %)</td>
<td>0.282*</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>94 (87-101)</td>
<td>95 (88-98)</td>
<td>0.848</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>106 (102-114)</td>
<td>105 (101-112)</td>
<td>0.739</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>189 (171-220)</td>
<td>187 (173-219)</td>
<td>0.739</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>42 (39-50)</td>
<td>43 (35-49)</td>
<td>0.917</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>168 (145-178)</td>
<td>165 (146-169)</td>
<td>0.956</td>
</tr>
</tbody>
</table>

Values shown are estimated difference in medians and the 95% CI on Mann-Whitney testing and * Chi² or Fisher test.

Table 1: Characteristics of the Patients Mild-moderate Desaturation Group and Severe Desaturation Group. Data are Presented as Median and Interquartile Range for Continuous Variables and n (%) for Categorical Variables.

Serum levels of ICAM-1 and P-selectin were measured using a microsphere fluorescent immunoassay and multiplex cytometry detection, in compliance with the manufacturer’s recommendation (Flow Cytomix®, Bender MedSystems®, Vienna, Austria). The intra-assay coefficient of variation for the measurements of soluble adhesion molecules was 1.4% to 4.3% (n=10), and the inter-assay coefficient of variation was 4.4% to 6.9% (n=5). The calculation of the results was done with the FlowCytomix Pro 2.2 software.

Serum MDA values, which measure lipid peroxidation, were determined at 586nm in triplicate for each subject with the Bioxytech LPO 586 test (Oxis International), in accordance with the manufacturer’s specifications. MDA concentration (μM) was calculated from a calibration line with known MDA levels. The measurements were done on microtiter plates using a DTX 880 Multimode Detector (Beckman-Coulter, Fullerton, CA, USA). The unused wells on the sides were filled with water to maintain a homogeneous temperature throughout the plate. The curve standards were linear from 0.5 to 4 μM and the lower limit of detection was defined as 5,185 SD from blank absorbance at 586 nm. The total variation coefficient was 2%.

**Variables and statistical analysis**

Data were expressed as median and interquartile range for continuous variables and frequencies and percentages for categorical variables. Continuous variables were compared using the Mann-Whitney U test. Spearman’s rank test was used for correlation analysis. A p-value <0.05 was considered to be statistically significant.

To analyze the relationship between the IRH and predictive variables, a multivariate analysis was carried. Data were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows 14.0 (SPSS, Chicago, IL, USA).

### Results

A total of 68 subjects were included in this study. After polysomnography, the subjects were classified as mild-moderate desaturation group (n=31) or severe desaturation group (n=37). Table 1 summarizes the baseline characteristics of the patients included in the study. There were no significant differences between the two groups regarding age, gender and BMI.

The percentage of smokers or ex-smokers and rates of diagnosis with COPD, diabetes mellitus or hypertension were similar between the two groups. No differences were observed in awake SaO₂, sleepiness or biochemical parameters. Except for a higher arousal index in severe desaturation observed group, there was no significant difference in sleep architecture between patients with severe desaturation and mild-moderate group (Table 2).

Compared with the mild-moderate desaturation group (Fig. 1), the severe desaturation group showed significantly worse IRH. In these patients, differences were observed in all variables related to the disease (AHI) and nocturnal SaO₂ (oxygen desaturation index, sleep time spent with SaO₂<90% and mean SaO₂). As for the levels of MDA, ICAM-1 and P-selectin were significantly higher in severe desaturation group than in the mild-moderate group (Table 2).
Table 2. General Parameters of Sleep in the Mild-moderate Desaturation Group and Severe Desaturation Group. Comparison of the Ischemic Reactive Hyperemia, Respiratory Variables, Malondialdehyde, ICAM-1 and P-selectin (Median and Interquartile Range) between the Groups of the Study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mild-moderate group (n = 31)</th>
<th>Severe desaturation group (n = 37)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>* IRH, % baseline</td>
<td>91 (58-183)</td>
<td>48 (30-71)</td>
<td>0.001</td>
</tr>
<tr>
<td>§AHI, events per hour sleep</td>
<td>19 (15-26)</td>
<td>46 (35-60)</td>
<td>0.001</td>
</tr>
<tr>
<td>¶ODI, events per hour sleep</td>
<td>16 (13-21)</td>
<td>44 (35-51)</td>
<td>0.001</td>
</tr>
<tr>
<td>¶T90, %</td>
<td>1.3 (0.7-2.1)</td>
<td>11 (7-16)</td>
<td>0.001</td>
</tr>
<tr>
<td>SaO2 mean, %</td>
<td>95 (94-95)</td>
<td>92 (89-93)</td>
<td>0.001</td>
</tr>
<tr>
<td>Malondialdehyde, μM</td>
<td>1.7 (1.3-2.6)</td>
<td>2.9 (1.7-3.9)</td>
<td>0.002</td>
</tr>
<tr>
<td>ICAM-1, ng/mL</td>
<td>265 (201-306)</td>
<td>410 (347-548)</td>
<td>0.001</td>
</tr>
<tr>
<td>P-selectin, ng/mL</td>
<td>118 (109-147)</td>
<td>163 (145-217)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total Sleep Time, (%)</td>
<td>82 (78-85)</td>
<td>83 (78-86)</td>
<td>0.64</td>
</tr>
<tr>
<td>Sleep-onset latency (min)</td>
<td>7 (5-16)</td>
<td>8 (5-15)</td>
<td>0.77</td>
</tr>
<tr>
<td>Stage 1 + 2, %TST</td>
<td>88 (86-90)</td>
<td>88 (85-91)</td>
<td>0.380</td>
</tr>
<tr>
<td>Stage 3 + 4, %TST</td>
<td>7 (4-12)</td>
<td>6 (5-9)</td>
<td>0.374</td>
</tr>
<tr>
<td>REM, % TST</td>
<td>5 (3-7)</td>
<td>4 (2-6)</td>
<td>0.479</td>
</tr>
<tr>
<td>† WASO</td>
<td>7 (3-10)</td>
<td>6 (3-9)</td>
<td>0.118</td>
</tr>
<tr>
<td>Arousal index, events per hour sleep</td>
<td>19 (12-26)</td>
<td>31 (23-43)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* IRH: ischemic reactive hyperemia. § AHI: sum of apneas and hypopneas per hour of sleep. ¶ ODI: number of dips in SaO2 ≥3% per hour of sleep. ¶ T90: sleep time spent with SaO2 < 90%. † WASO: wakefulness after sleep onset.

The correlations of IRH with respiratory, cellular adhesion molecules and MDA variables in OSA patients were performed. No correlations between IRH and age (rho=0.828) or with BMI (rho=0.943) were seen. Nevertheless, a correlation between AHI (rho=-0.447, p=0.001), ODI (rho=-0.477, p=0.001), T90 (rho=-0.469, p=0.001), mean SaO2 (rho=0.329, p=0.006) and IRH values was observed. A significant correlation between IRH and MDA, ICAM-1 and P-selectin was also observed (Fig. 2).

To examine the independent predictors of IRH in patients with OSA, a stepwise multiple linear regression was performed. Age, gender, BMI and arousal index were included in addition to MDA, ICAM-1 and P-selectin (Table 3). The model showed an R²=0.377 (p=0.001). Only ICAM-1 (β=-0.284; p=0.019) and P-selectin (β=-0.281; p=0.033) were significant independent predictors of IRH, while MDA was not associated with IRH variability.

**Discussion**

The present study shows that, in OSA patients, the subjects with the greatest nighttime oxygen desaturation showed greater oxidative stress, an increase in proinflammatory markers and worse endothelial function. ICAM-1 and P-selectin were the variables that were independently associated with IRH. These findings are relevant and point to the inflammatory pathway being directly related to endothelial dysfunction.

OSA appears in middle age and can present with few symptoms in up to 26% of the population (18). In addition to its high prevalence, it is considered to be a cardiovascular risk factor (4, 5, 19). OSA has been reported to produce endothelial dysfunction and to facilitate atherosclerosis. Therefore it is important to study the pathological mechanisms involved in endothelial dysfunction. In this respect, the hallmark of OSA is intermittent hypoxia (20-23). Our group has described the impact of intermittent hypoxia on the development of endothelial dysfunction (16). In addition, a recent study in OSA patients showed that subjects with greater nocturnal desaturation had worse endothelial function (24).
endothelial function. However, most clinical research has studied the correlation of this impact via therapeutic interventions without analyzing causality (10, 25, 26). Other studies have shown improvements in oxidative stress with CPAP treatment (7, 27-30). Recently, our group has observed an increase in MDA in OSA patients with greater desaturation, although this increase was not related to worsening of endothelial function (16). Therefore, it is probable that there are other biological pathways involved in the relationship between OSA and endothelial dysfunction, including reduced antioxidant capacity, inflammation, oxidative stress and cellular apoptosis, which are activated during sleep apnoea.

In this vein, research into subclinical cardiovascular disease has identified associations between cellular adhesion molecules and the severity of atherosclerosis (14, 15). Furthermore, it has been observed that ICAM-1 levels are associated with an increase in cardiovascular mortality in patients with coronary artery disease (31, 32). El-Solh et al, found significantly elevated levels of ICAM-1, VCAM-1 and E-selectin in patients with coronary disease and OSA when compared to similar patients without OSA (33). In the present study, the group with the greatest desaturation had significantly elevated levels of ICAM-1 and P-selectin. These results are similar to those found by other authors (34-36). Thus, it has been observed that OSA patients show an elevated level of cellular adhesion molecules with respect to controls (34, 35), and a decrease after CPAP treatment (36). These results, and those obtained in the present study, indicate a close relationship between sleep apneas and endothelial dysfunction.

However, it should not be forgotten that the release of cellular adhesion molecules can also be stimulated by oxidative stress. It is possible that increased oxidative stress worsens endothelial function and increases the release of cellular adhesion molecules. This suggests that both oxidative stress and inflammatory pathways can interact and that endothelial dysfunction is the sum of various causes (5, 9, 22, 23, 37, 38).

**Potential limitations of this study**

An issue in any research regarding OSA and its relationship to vascular disease involves controlling for confounding factors. The design of our study controlled for OSA-related factors which could be associated with endothelial dysfunction. Patients with symptomatic vascular disease, including hypertension treated with medications that could modify IRH were excluded.

The endothelial function can be impaired due to obesity. The present patients were obese (BMI >30). Nevertheless, no correlation between IRH and BMI was observed. In addition, in the multiple regression analysis BMI was not associated with IRH variability. Therefore, in a sample of OSA patients with obesity, BMI does not play an important role.

In summary, our study shows that intermittent hypoxemia favors increased oxidative stress and an increase in cellular adhesion molecules in OSA patients. Furthermore, proinflammatory molecules are independently associated with the poorer endothelial function observed in these patients. These results confirm the evidence that sleep apnea is associated with inflammation, which plays a key role in atherogenesis.
The authors state that they have no Conflict of Interest (COI).

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References


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