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TITLE: Relationship of oxidative stress and endothelial dysfunction in sleep apnoea.

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ABSTRACT

OBJECTIVES: To evaluate ischemic reactive hyperemia (IRH) in obstructive sleep apnoea (OSA) and its relationship with oxidative stress.

PATIENTS AND METHODS: We studied 69 consecutive patients referred to our Sleep Unit. Patients with chronic diseases or taking medication were excluded. IRH was assessed before and after the polysomnogram. Morning IRH and oxidative stress markers were compared between patients with (apnoea-hypopnoea index (AHI) ≥ 5) and without (AHI < 5) OSA. Measurements were repeated in 25 severe OSA patients after CPAP therapy.

RESULTS: We included 46 OSA patients (AHI 49 ± 32.1) and 23 non-OSA subjects (AHI 3 ± 0.9). The OSA patients showed a significant worsening of morning IRH and a significant increase in malondialdehyde and 8-hydroxydeoxyguanosine. Only the oxygen desaturation index independently explained morning IRH while malondialdehyde showed a weak effect on IRH. In severe OSA patients, IRH improved significantly after CPAP treatment, as did the malondialdehyde, 8-hydroxydeoxyguanosine and protein carbonyls.

CONCLUSIONS: In OSA patients, endothelial dysfunction and oxidative stress were observed, and IRH worsened after sleep. The increase in oxidative stress was not associated with IRH while intermittent hypoxia was strongly associated with IRH. In severe OSA patients, CPAP treatment improved oxidative stress and endothelial function.

INTRODUCTION

Obstructive sleep apnoea syndrome (OSA) is characterized by snoring, witnessed apnoeas, unrefreshing sleep and excessive daytime sleepiness [1]. These symptoms are due to frequent episodes of upper airway collapse, resulting in arousals and sleep disruption. The respiratory events are accompanied by dips in peripheral oxygen saturation ($SapO_2$). Hypoxia-reoxygenation episodes can occur repeatedly during the night, and have been associated with an increase in cardiovascular diseases, including systemic hypertension, coronary artery disease, cerebrovascular disease and cardiac arrhythmias [2-5]. Nevertheless, evaluating the involvement of OSA in cardiovascular disease is complex due to the high prevalence of smoking, high blood pressure and diabetes mellitus in OSA patients. Moreover, OSA may cause hypertension and insulin resistance.

Various mechanisms link OSA to an increase in vascular diseases [6-9].These mechanisms can produce endothelial dysfunction, an early indicator of vascular disease. This is of great epidemiological importance as it can favour or accelerate the process of atherogenesis and the development of cardiovascular disease [6,8,10,11].

Oxidative stress affects important macromolecules, especially lipid peroxidation [12], as well as producing DNA and protein damage [13]. To date, the impact of oxidative stress on these macromolecules in patients with OSA has only been partially studied and its role in endothelial dysfunction is not conclusive [14-16].

It has been proposed that nocturnal hypoxaemia may be the source of oxidative stress in OSA and that it is this mechanism that produces endothelial dysfunction. These changes can improve after CPAP treatment.

A prospective study was designed with the following objectives: 1) to evaluate whether endothelium-dependent, ischemic reactive hyperemia (IRH) is altered in OSA patients as compared with a control group; 2) to study the relationship between oxidative stress and IRH in these patients; 3) to determine whether CPAP treatment (continuous positive airway pressure) in OSA patients improves endothelial function and oxidative stress.

PATIENTS AND METHODS

Setting: The study was carried out in the Sleep Unit of the Reina Sofía University Hospital, Córdoba, Spain.

Subjects. The sample was recruited from consecutive patients who were referred to the Sleep Unit between October 2007 to June 2008, and underwent polysomnographic

studies due to the following symptoms: snoring, unrefreshing sleep and excessive daytime sleepiness (Epworth scale score >11) [17]. Patients were eligible for the study if they were between 35 to 65 years old and agreed to participate. Patients were excluded from the study if they had $SapO_2$ < 94% (breathing room air), congestive cardiac failure, hepatic cirrhosis, chronic renal insufficiency, Stages III and IV (GOLD) chronic obstructive pulmonary disease (COPD), thyroid dysfunction, rheumatoid arthritis or any other chronic or severe inflammatory diseases. Smokers, patients with drug addictions and hypertensive patients treated with calcium antagonists, nitrates, α- and ß-blockers or ACE inhibitors were also excluded. After polysomnography, subjects were classified as either OSA patients (n = 46; apnoea-hypopnea index, AHI ≥ 5) or non-OSA subjects (n = 23; control group, AHI < 5).

Study design. This was a prospective study, with consecutive sampling of those subjects evaluated in our Sleep Unit who met the inclusion criteria. All patients underwent a complete physical examination, and their medical histories were taken, paying special attention to the symptoms and signs suggesting respiratory sleep disorders. They were asked about tobacco usage and medication consumption. Their body mass index (BMI) was calculated using the formula: weight in kg/(height in meters)^2.

After ≥15 minutes rest, $SapO_2$ was measured with a Pulsox 300i pulse oximeter (Konica Minolta Sensing, Shanghai, China) while the subject was awake and breathing room air. Sleep was studied by overnight polysomnography, and endothelial function was determined before polysomnography (night-time measurement) and afterwards (morning measurement). After evaluating nocturnal endothelial function, blood pressure was taken with the patient lying face up and resting for ≥ 5 minutes (HG Erkameter 300, Erka, Bad Tolz, Germany).

Patients with moderate or severe OSA received CPAP treatment [18]. Pressure was titrated by an auto-CPAP device (GoodKnight 420 E auto-CPAP, Nellcor Puritan Bennett, Boulder, CO, USA) following ASDA guidelines [19]. After three months of treatment (CPAP > 5 hours/night), all tests were repeated under identical conditions. In this phase, we excluded those patients who presented significant weight changes (> 3 % gain or loss), were being treated with new medications or who presented a new pathology.

Informed consent was obtained from all subjects. The study was approved by the ethics committee of the Reina Sofía University Hospital.

Methods

Polysomnography. A polysomnograph was used (SomnoScreen™, SomnoMedics, Randersacker. Germany). The test began at 12 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 $SapO_2$ by digital pulse oximetry were also monitored. Recordings were staged according to the system of Rechtschaffen and Kales. Apnoea was defined as a significant decrease (> 90%) in oronasal flow of at least 10 seconds, and hypopnoea as an evident decrease in airflow, > 30% and < 90%, and associated with either oxygen desaturation of ≥ 3% or and arousal. The following respiratory variables were monitored: the apnoea-hypopnoea index (AHI), determined by the sum of apnoeas and hypopnoeas per hour of sleep, minimum $SapO_2$ reached during sleep, the oxygen desaturation index, defined as the number of falls in $SapO_2 \geq 3\%$ per hour of sleep. Finally, the sleep time spent with $SapO_2 < 90\%$ was estimated. All studies were reviewed and interpreted by a study-blinded, board-certified, sleep medicine physician. Polysomnograms were considered valid for diagnosis when at least 180 minutes of sleep were obtained.

Endothelial function. A Laser-Doppler linear Periflux 5000 (Perimed S.A., Stockholm, Sweden) was used to measure IRH. The methodology has been described previously [20]. 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 palmar surface of the second finger of the same 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). The data obtained were recorded and stored using PeriSoft Software for Windows. The values of the area under the curve (AUC) of the t0 and td times were analyzed. These data were used to calculate the increase in post-ischemic flow by means of the formula: IRH = (AUCtd - AUCt0) x 100 AUCt0. The first IRH registration took place at 11pm and the second at 7:30 am after the polysomnography and blood extraction. This method has an interstudy variability of 8.85 % and intrastudy variability of 8.7% [20].

Oxidative stress biomarker determination. Blood samples were obtained at 7 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 then centrifuged at 1500 x *g* and 4º C for 10 minutes. The resulting plasma was aliquoted and frozen at -80º C for subsequent analysis. The entire process was carried out in ≤ 60 minutes post-extraction. Total plasma proteins were measured with Coomassie Brilliant Blue G-250 (Bio-Rad, Richmond, CA, USA), using the Bradford method (1976).

Protein carbonyls were determined in plasma with an enzyme immunoassay kit (ELISA) (BioCell PC, Papatoetoe, NZ), measuring absorbency at 620 nm according to the manufacturer's instructions. The concentration of protein carbonyls (nmol/mg protein) was established using a calibration curve with known concentration patterns. The standard curve was linear in the range of 0-3.36 nmol /mg protein. The intra-assay variation of samples was around 5%, based on the material provided with the kit.

DNA oxidative damage was measured with an ELISA test, Bioxytech 8-OHdG-EIA Kit (Oxis International, California, USA) on 8x12 microtiter plates, following the manufacturer's specifications. The concentration of 8-hydroxydeoxyguanosine (ng/ml) was calculated with a calibration line obtained from known 8-hydroxydeoxyguanosine concentrations. The standards range were from 0.5 ng/ml to 200.0 ng/ml. The intra-assay variation of samples were 2.7%.

Plasma malondialdehyde values, that 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. Malondialdehyde concentration (μM) was calculated from a calibration line with known malondialdehyde 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 homogenous 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 586nm. The total variation coefficient was 2%.

Variables and statistical analysis

Data were expressed as means, standard deviations, minima and maxima for continuous variables, and frequencies and percentages for categorical variables. Continuous

variables before and after polysomnography were compared using the Mann-Whitney U-test. Confidence intervals for the differences between two means were determined at a confidence level of 95%. 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 dependent variable (IRH) and predictive variables, a multivariate analysis was carried out using a multiple lineal regression model. In the analysis, endothelial function was taken as the dependent variable, determined by analysis of the AUC values at t0 and td. Independent variables were nocturnal $SapO_2$ values (oxygen desaturation index, sleep time spent with $SapO_2$ <90%, mean $SapO_2$) and oxidative stress biomarkers (malondialdehyde, 8-hydroxydeoxyguanosine, protein carbonyls). The differences between values before and after CPAP treatment were compared using the Wilcoxon test.

Data were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows 14.0 (SPSS, Chicago, IL, USA).

RESULTS

Basal Parameters. Seventy-six subjects were pre-selected, of whom seven patients were excluded. The reasons for exclusion were the following: two patients were smokers, two were taking betablockers and calcium antagonists, and three had $SapO_2$ values < 94% while awake on the night of the polysomnographic study. The remaining 69 subjects were included in the study. After polysomnography, the subjects were classified as either OSA patients (n = 46, AHI ≥ 5) or non-OSA subjects (n = 23; control group, AHI < 5). Figure 1 shows the study sequence and the composition of the different groups. Table 1 summarizes the baseline characteristics of the patients included in this study. There were no significant differences between the two groups regarding age, gender or BMI. No differences were observed either in awake $SapO_2$ or in biochemical parameters, except for triglyceride values that were significantly elevated in the OSA patients.

Endothelial function and oxidative stress. Table 2 shows that there were no significant differences in nocturnal IRH between the two groups studied before sleep onset ($p = 0.904$). However, compared with the control group, OSA patients showed a significant decrease in morning IRH ($p < 0.001$). In these patients, differences were observed in all variables related to the disease (AHI) and nocturnal $SapO_2$ (oxygen desaturation index, sleep time spent with $SapO_2$ < 90%, minimum $SapO_2$ and mean $SapO_2$). As for the

oxidative stress study, plasma levels of malondialdehyde and 8-hydroxydeoxyguanosine were significantly higher in OSA patients than in the control group.

The correlations of IRH with various sociodemographic, respiratory and oxidative stress variables in OSA patients are shown in Table 3, where a correlation between nocturnal $SapO_2$, malondialdehyde, 8-hydroxydeoxyguanosine and IRH values can be observed.

To examine independent predictors of IRH in patients with OSA, a stepwise multiple linear regression was performed. The oxygen desaturation index was the only significant independent predictor of IRH (adjusted $R^2 = 0.181$, $F = 2.986$, ß = -0.557, $p = 0.011$).

As explained previously, significant correlations were observed between IRH and both malondialdehyde and 8-hydroxydeoxyguanosine, although the multiple lineal regression showed only a tendency to explain the IRH results (adjusted $R^2 = 0.195$, $F = 2.419$, ß = -0.234, $p = 0.082$).

Of the 46 patients with OSA, CPAP treatment was prescribed for 30 patients according to ASDA criteria [19]. Of these, one refused treatment and four were excluded for non-compliance with at least four hours of daily CPAP therapy. After three months of CPAP treatment, these patients once again underwent the same tests under the same conditions, including polysomnography, IRH measurement and blood analysis for the oxidative stress study. In the 25 patients who completed CPAP treatment (Table 4), no significant changes were observed in BMI. After at least three months of treatment, CPAP corrected respiratory events (AHI), alterations in nocturnal $SapO_2$ (minimum and mean $SapO_2$, oxygen desaturation index and sleep time spent with $SapO_2 < 90\%$) and decreased blood pressure significantly (Table 4). Moreover, an improvement in oxidative stress was observed, with significantly lower malondialdehyde ($p = 0.001$), 8-hydroxydeoxyguanosine ($p = 0.001$) and protein carbonyl levels ($p = 0.021$). When comparing values before and after CPAP treatment, it was seen that the differences in IRH values were correlated with AHI, oxygen desaturation index, malondialdehyde and 8-hydroxydeoxyguanosine (Figure 2), while there were no significant correlations with sleep time spent with $SapO_2 < 90\%$ (rho = -0.205; $p = 0.325$) or protein carbonyls (rho = -0.194 ; $p = 0.353$).

DISCUSSION

To our knowledge, this study is the first in which patients with OSA were assessed for the impact of oxidative stress on lipids, DNA and proteins, as well as its association with endothelial function. This study shows that, in comparison with a control group, OSA patients suffer from greater endothelial dysfunction. AHI and the variables evaluating nocturnal $SapO_2$ were correlated with IRH, although the number of decreases in $SapO_2$ (oxygen desaturation index) was the only parameter that was independently associated with endothelial dysfunction. Malondialdehyde and 8-hydroxydeoxyguanosine were significantly elevated in patients with OSA, although these variables were not independent predictors of IRH. Furthermore, after three months of CPAP treatment, the patients showed significant improvements in oxidative stress markers and endothelial function.

Endothelial dysfunction favours atherosclerosis and is considered to be a cardiovascular risk factor [8,10]. Our study provides interesting data, and to the best of our knowledge it is the first to compare nocturnal and morning IRH in the same patients, in order to accurately evaluate the effects of respiratory events on endothelial function. Both groups (patients with OSA and patients without OSA) had similar values for nocturnal IRH (Table 2). However, significant worsening of morning IRH was observed in OSA patients.

In our study, malondialdehyde and 8-hydroxydeoxyguanosine levels differed in the OSA and control groups, confirming that oxidative stress is greater in OSA patients. However, the association of oxidative stress with endothelial function was not observed. There have been several studies with a limited sample sizes that have studied oxidative stress and endothelial dysfunction. They compare the impact of CPAP [14], allopurinol [15] or vitamin C [16]. They reported, as did our study, correlations between some oxidative stress markers and endothelial dysfunction, Nevertheless, these markers were not predictors of IRH. The works mentioned above did not study causality and carried out therapeutic interventions to evaluate oxidative stress [14-16]. Furthermore, some of the markers used were different and may not have the same biological role as those used in our study [14]. The markers used in our study have been shown to be valid in human pathology [13].

The relationship between endothelial function and respiratory parameters is controversial. Nieto et al [21], observed a significant association between sleep time spent with $SapO_2 < 90\%$ and both baseline arterial diameter and the percentage of flow-

mediated dilation. In contrast, Kato et al [22], did not observe significant endothelial function differences between patients with OSA and a control group. In our study, IRH was not associated with the severity of hypoxaemia as determined by sleep time spent with $SapO_2 < 90\%$, but with intermittent hypoxia as evaluated by the oxygen desaturation index. In a recent article, OSA patients with greater nocturnal desaturation had poorer endothelial function [23].

It is known that oxygen partial pressure regulates the expression of nitric oxide synthase. The systemic production of nitric oxide also worsens in OSA and the administration of oxygen significantly increases it [24].

Thus, the close relationship of both oxidative stress and endothelial dysfunction with nocturnal hypoxaemia is possible. Intermittent hypoxia was independently associated with a worsening of IRH in our study. These results are consistent with those reported by other authors [14-16]. Overall, our study suggests that there are probably other biological mechanisms related to intermittent hypoxemia, besides oxidative stress, that can affect endothelial function. The release of inflammatory molecules and endothelial apoptosis, among others, have been described [5-8,11,25-27].

It can therefore be predicted that correcting intermittent hypoxia with CPAP can have an important influence on these parameters. In previous studies, improvement in endothelial dysfunction is observed in response to CPAP treatment [11,23,24,28,29]. It is also postulated that CPAP can improve oxidative stress [11,30]. Carpagnano et al [31] showed that an increase in 8-isoprostane in exhaled breath condensate, decreased significantly after CPAP treatment. Our study shows that, compared to the situation prior to treatment in severe OSA patients, IRH and oxidative stress improved significantly after CPAP treatment. This finding is not surprising, as CPAP treatment effectively corrects AHI and the effect of these events in nocturnal $SapO_2$. It was interesting to see that protein oxidation also improved with CPAP. To our knowledge, only one study has assessed the effect of oxidative stress on serum proteins, although it was carried out in patients on hemodialysis, a treatment which produces oxidative stress *per se* [32]. It is important to emphasize that protein carbonylation indicates a more severe oxidative stress. This may explain the trend to higher levels observed in OSA patients and that the most severe cases, when treated with CPAP, showed a global decrease in oxidative stress, including protein oxidation.

Potential limitations of the study. Our study was designed to compare the results obtained in OSA patients with those of a control group. However, the effect of the

treatment was not controlled with a placebo, given the cardiovascular risk inherent in leaving severe OSA patients untreated [2-5,33]. In our patients with severe OSA (median AHI = 71 events per hour of sleep) it does not seem ethical to use suboptimal pressure (sham CPAP) for a period of at least three months. In the study design, we controlled for factors not related to OSA that could also be associated with endothelial dysfunction. Therefore, patients with severe diseases, including hypertension under treatment with drugs that affect endothelial function were excluded. Obesity has been associated with endothelial dysfunction. Nevertheless, there was no change in BMI in those patients treated with CPAP, although significant improvements were observed in oxidative stress and IRH in this group. In our study, small, statistically significant differences were observed in oxidative stress markers. These were elevated in patients with OSA and the values decreased after CPAP treatment. The clinical relevance of these changes is unclear, although our study has shown that they are significantly relevant in themselves to produce changes in IRH.

Therefore, in conditions of clinical practice and using an ample number of subjects, our study demonstrated that oxidative stress is not a factor that explains the IRH variability independently, reinforcing the idea that other mechanisms are involved, factors associated with intermittent hypoxemia which can cause endothelial dysfunction. In fact, our study demonstrates a close relationship between intermittent hypoxia and deterioration of endothelial function, showing a significant improvement in the severe patients who were treated with CPAP. This finding is of great clinical importance, as treating OSA could have an impact on a preclinical vascular risk factor, possibly preventing the vascular complications subsequently associated with it.

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Competing interests: None.

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LEGENDS FOR ILLUSTRATIONS

Figure 1. Flow chart showing study cohort and different subgroups.

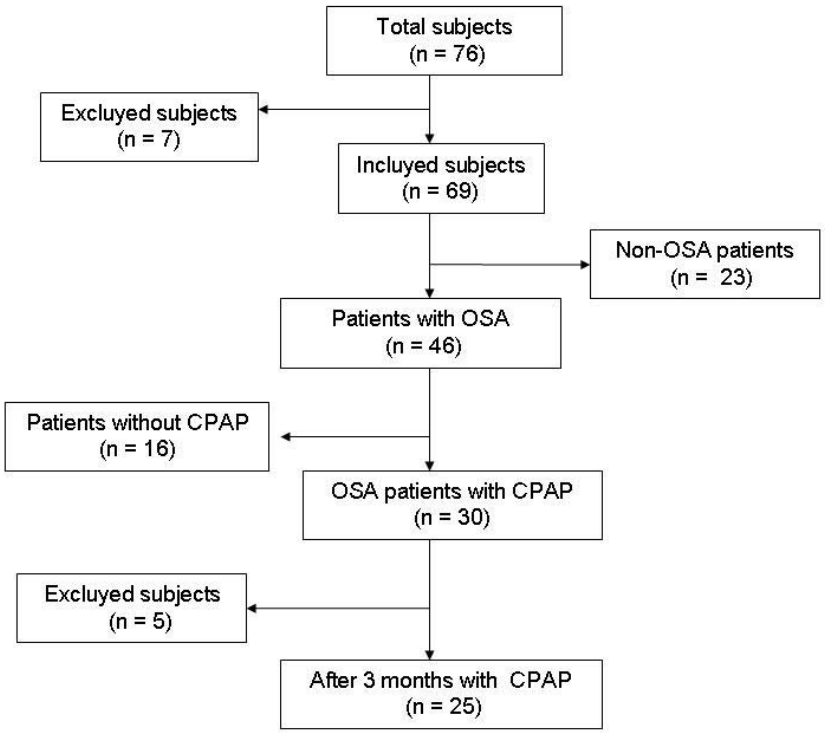
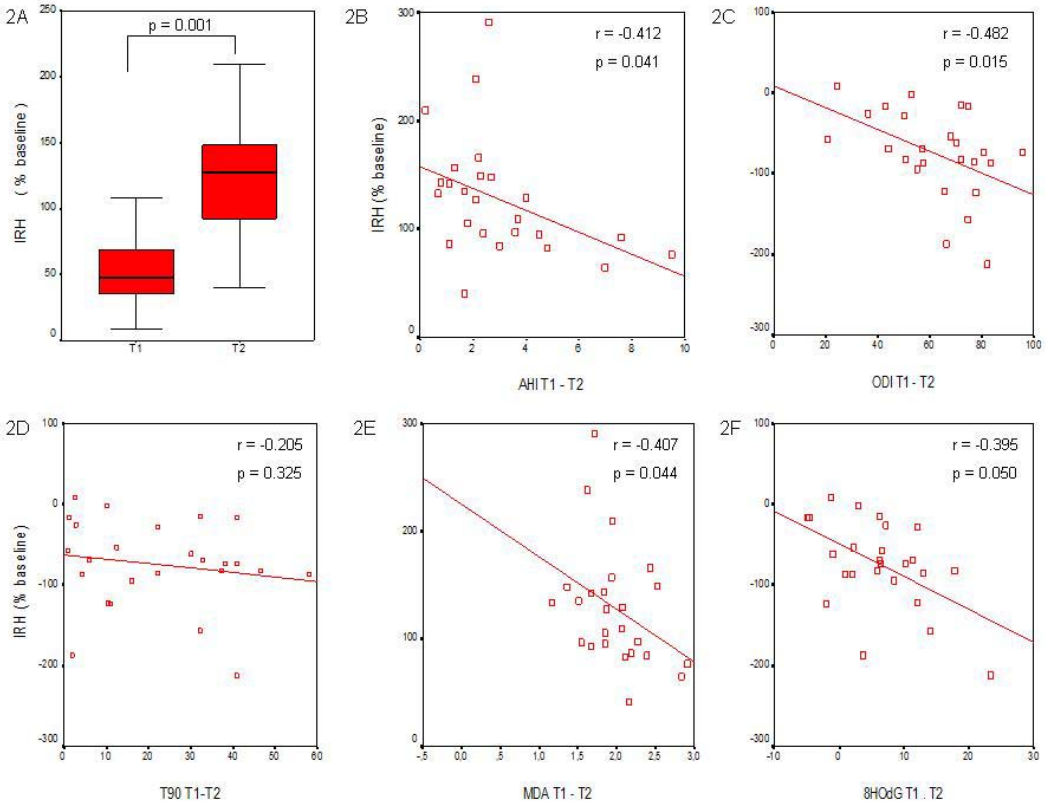


Figure 2. Differences in values before (T1) and after (T2) CPAP treatment. Correlations of ischemic reactive hyperemia with both respiratory variables and oxidative stress markers.



IRH: ischemic reactive hyperemia. AHI: sum of apnoeas and hypopnoeas per hour of sleep, ODI: number of dips in $SapO_2$ ≥3% per hour of sleep, T90: sleep time spent with $SapO_2$ <90%, MDA: malondialdehyde, 8OHdG: 8-hydroxydeoxyguanosine

Table 1. Characteristics of patients with obstructive sleep apnoea (OSA) and controls. Data are presented as median and interquartile range for continuous variables and n (%) for categorical variables.

Variable	**Patients with OSA** ***(n = 46)***	**Pacients without OSA** ***(n = 23)***	**p-value**
Age, yr	47 (40-47)	48 (44-51)	0.738
Male gender, n (%)	34 (73%)	15(61%)	0.575*
Body Mass Index, Kg/m^2	31 (27-34)	30 (28-31)	0.061
$SapO_2$ awake, **%**	95 (95-96)	95 (94-96)	0.438
Ex-smokers, n (%)	14 (30%)	5 (22%)	0.572*
Diabetes Mellitus, n (%)	2 (4%)	2 (8%)	0.596*
Fasting glucose, mg/dL	91 (88-98)	91(85-108)	0.728
Creatinine clearance ml/minuto	105 (102-111)	109 (102-115)	0.642
Total cholesterol, mg/dL	182 (164-205)	181 (165-200)	0.478
HDL-cholesterol, mg/dL	48 (38-52)	46 (38-63)	0.631
Triglycerides, mg/dL	109 (77-147)	79 (67-99)	0.044

Values shown are estimated difference in medians and the 95% CI on Mann-Whitney testing and * Chi-2 test.

Table 2. Comparison of the ischemic reactive hyperemia, respiratory variables and oxidative stress markers (median and interquartile range) between the groups of the study.

Variable	Patients with OSA *(n = 46)*	Patients without OSA *(n = 23)*	p-value
Nocturnal * IRH, % baseline	108 (70-136)	80 (60-149)	0.604
Morning * IRH, % baseline	56 (38-82)	168 (69-212)	0.001
† SBP, mm Hg	130 (120-140)	120 (110-130)	0.077
‡ DBP, mm Hg	80 (71-82)	70 (67-75)	0.023
§AHI, events per hour sleep	46 (15-74)	3 (2-4)	0.001
¶ODI, events per hour sleep	49 (19-75)	7 (3-11)	0.001
****T90**, %	6 (1-29)	0.2 (0-0.6)	0.001
$SapO_2$ minimum, %	81 (69-85)	89 (85-91)	0.001
$SapO_2$ mean, %	93 (91-94)	94 (94-95)	0.003
Arousals, per hour sleep	18 (13-30)	6 (3-9)	0.001
Malondialdehyde, μM	2.6 (1.9-3.7)	1.6 (1.5-1.8)	0.001
8-hydroxydeoxyguanosine, ng/ml	107 (104-111)	103 (88-105)	0.001
Protein carbonyls, nmol/mg protein	0.09 (0.04-0.12)	0.07 (0.05-0.15)	0.498

* IRH: ischemic reactive hyperemia. † SBP: systolic blood pressure. ‡ DBP: diastolic blood pressure. § AHI: sum of apnoeas and hypopnoeas per hour of sleep. ¶ ODI: number of dips in $SapO_2 \geq 3\%$ per hour of sleep. ** T90: sleep time spent with $SapO_2 < 90\%$.

Table 3. Correlations (Spearman's Rho) between clinical variables, respiratory events oxidative stress markers and ischemic reactive hyperemia (IRH).

Variable	**IRH** **Rho-value**	**p-value**
Age, yr	-0.068	0.579
* **BMI**, kg/m^2	- 0.076	0.537
Gender	0.053	0.913
AHI, events per hour sleep	0.542	0.001
‡ **ODI**, events per hour sleep	-0.565	0.001
§**T90**, **%**	-0.480	0.001
SapO minimun, %	0.311	0.051
$SapO_2$ mean, %	0.426	0.002
$SapO_2$ awake, %	0.193	0.126
Arousals, per hour sleep	-0.189	0.209
Total cholesterol, mg/dL	0.088	0.689
HDL-cholesterol, mg/dL	-0.203	0.352
Triglycerides, mg/dL	0.209	0.315
Malondialdehyde, μM	-0.371	0.002
8-hydroxydeoxyguanosine, ng/ml	-0.271	0.025
Protein carbonyls, nmol/mg protein	0.163	0.180
¶**SBP**, mm Hg	-0.117	0.355
****DBP**, mm Hg	-0.243	0.051

* BMI: body mass index; ‡ ODI: number of dips in $SapO_2 \geq 3\%$ per hour of sleep. §T90: sleep time spent with $SapO_2 < 90\%$. ¶ SBP: systolic blood pressure. **DBP: diastolic blood pressure.

Table 4. Respiratory variables, oxidative stress markers and ischemic reactive hyperemia in 25 patients with severe SAHS treated with CPAP. Parameters before and after three months with CPAP treatment. Values expressed as median and interquartile range.

Variable	Before CPAP	After CPAP	p-value
* **BMI**, kg/m^2	33 (30-36)	32 (30-35)	0.091
†**AHI**, events per hour of sleep	71 (52-85)	2 (1-3)	0.001
‡ **ODI**, events per hour sleep	68 (54-83)	5 (2-9)	0.001
$SapO_2$ minimum, %	72 (62-82)	91 (86-92)	0.001
$SapO_2$ mean,%	92 (87-93)	95 (93-96)	0.035
§**T90**, %	22 (5-40)	0.4 (0-1.4)	0.001
Malondialdehyde, μM	3.2 (2.5-4.2)	1.9 (1.6-2.2)	0.001
8-hydroxydeoxyguanosine, ng/ml	107 (105-114)	102 (101-106)	0.001
Protein carbonyls, nmol/mg protein	0.10 (0.05-0.14)	0.10 (0.04-0.12)	0.021
¶**SBP**, mm Hg	140 (120-142)	120 (118-130	0.001
****DBP**, mm Hg	80 (77-90)	70 (68-80)	0.001
†† **Morning IRH,** % baseline	47 (33-74)	127 (89-148)	0.001

* BMI: body mass index; †AHI: sum of apnoeas and hypopnoeas per hour of sleep. ‡ ODI: number of dips in $SapO_2 \geq 3\%$ per hour of sleep. §T90: sleep time spent with $SapO_2 < 90\%$. ¶SBP: systolic blood pressure. **DBP: diastolic blood pressure, †† IRH: ischemic reactive hyperemia.