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Lipid peroxidation and protein oxidation are related to the severity of OSAS

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Abstract. – OBJECTIVE: Obstructive sleep apnea syndrome (OSAS) is associated with elevated cardiovascular morbidity and mortality. Considering that oxidative stress is involved in endothelial dysfunction and atherosclerosis development, our aim was to examine lipid peroxidation and protein oxidation, two parameters of oxidative status, in a group of subjects with OSAS.

PATIENTS AND METHODS: We consecutively enrolled 48 patients (36 men and 12 women; mean age 49.7±14.6 yrs) with OSAS, subsequently subdivided according to the apnea/hypopnea index (AHI) value in two subgroups: Low (L= 21 subjects with AHI<30) and High (H= 27 subjects with AHI>30). We examined lipid peroxidation, expressed as TBARS, and protein oxidation, measured as carbonyl groups in plasma samples from fasting venous blood.

RESULTS: We observed that TBARS and carbonyl groups were significantly higher in subjects with AHI > 30 in comparison with the L subgroup and the whole group of OSAS subjects. In addition, we found that these parameters were positively correlated with neck and waist circumference, with the AHI value and with the oxygen desaturation index, and negatively correlated with the mean oxygen saturation.

CONCLUSIONS: Lipid peroxidation and protein oxidation in OSAS patients are significantly correlated with the severity of the disease.

Key Words:
Cardiovascular risk, Lipid peroxidation, OSAS, Protein oxidation.

Introduction

Obstructive sleep apnea syndrome (OSAS) is characterized by repeated partial or complete obstructions of upper airways, usually occurring at the level of the oropharynx, during sleep with resulting apnea or hypopnea, with consequent intermittent arterial oxygen desaturation and sleep disruption[1]. The gold standard for the treatment of OSAS is continuous positive airway pressure (CPAP) therapy, with or without associated O_2, for correction of the upper airway obstructive episodes and of the hypoxemia during sleep[2].

Intermittent hypoxemia can increase sympatetic tone, oxidative stress, pro-inflammatory cytokine production, platelet aggregation, and metabolic dysregulation[3773]. A recent meta-analysis shows that OSAS is significantly and independently associated with an increased risk of cardiovascular diseases, cerebrovascular events and all-cause mortality and that the prevalence of cardiovascular events is related to OSAS severity[4]. Patients with severe untreated OSAS have an higher occurrence of fatal and non fatal cardiovascular events in comparison with simple snorers or OSAS patients treated with cPAP[5]. Some authors have demonstrated early atherosclerotic lesions in OSAS subjects, which can be reduced by CPAP treatment; therefore, OSAS is considered an independent risk factor for coronary artery disease (CAD) and it also increases CAD mortality[6]. In a large observational cohort study[78] OSAS patients had a 2-fold increased risk of stroke and this association was independent from other cardiovascular and cerebrovascular risk factors, such as hypertension.

Several papers have demonstrated an altered inflammatory[3] and oxidative status[8] in OSAS. As it is known, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in several clinical conditions. The main ROS is the superoxide anion (O_2^-), which physiologically is rapidly neutralized by anti-oxidant enzymes, while, if generated in excess, oxidizes nitric oxide (NO) producing peroxinitrite and starting a cascade of ROS generation that leads to oxidation of carbohydrates, lipids and proteins. Lipid peroxidation generates some relatively stable end products, such as malonyldialdehyde (MDA), hydroxynonenal (HNE), oxononenal (ONE) and isoprostanes, while the oxidation of proteins produces advanced oxidation protein products (AOPPs)[9]. Carbonylation is another irreversible ROS-induced protein modification: ROS may directly oxidize lysine, arginine, proline or threo-

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nine residues or may react with carbohydrates and lipids generating reactive carbonyl species (RCS), such as ketoamine, ketoaldehydes, MDA, HNE and ONE, that subsequently interact with proteins[9].

Examining the oxidative/antioxidant status of patients with OSAS, an increased lipid[10-12] and protein oxidation[10,13] can be found, with a parallel decreased nitric oxide metabolites[8], and antioxidant defenses[10,11,14].

Celec et al[10] have observed elevated levels of thiobarbituric acid reacting substances (TBARS), marker of lipid peroxidation, in OSAS subjects, that decreased after only 1 month of cPAP therapy. TBARS are positively correlated with OSAS severity[12,16-18], expressed as apnea/hypopnea index (AHI) or oxygen desaturation index (ODI), and negatively correlated with the mean oxygen saturation[16]. However, recently Ntalapascha et al[19] found no difference in TBARS levels between OSAS and normal subjects. Similarly, some authors[17] have found higher plasma MDA levels in subjects with OSAS, while others[20,21] demonstrated no significant differences in MDA levels between normal and OSAS subjects. In the paper of Vatansever et al[17], MDA concentrations were higher in subjects with severe OSAS, but not in those with mild OSAS, in comparison with normal controls, and significantly correlated with AHI values. MDA levels may be improved by cPAP therapy, as demonstrated by Yagihara et al[22]. Also the levels of plasmatic 8-isoprostane are higher in subjects with OSAS without pulmonary or cardiac diseases, than in normal controls and they are reduced by cPAP therapy[23].

Regarding protein oxidation, in OSAS patients an increase in AOPPs levels has been found[10,24], but no correlation between AOPPs and AHI has been demonstrated[25]. However, protein carbonyl content is elevated in subjects with severe OSAS and significantly correlated with AHI value[17]. In sample of mucosal and muscle tissue from upper airways of severe OSAS patients an increased protein carbonylation was observed[13]. Advanced glycation end products (AGEs) and fructosamine, indicators of carbonyl stress, are increased in OSAS subjects and their production can be influenced by cPAP treatment[10].

Considering all these data, our aim was to examine lipid peroxidation and protein oxidation in a group of subjects with OSAS, subsequently subdivided according to the apnea/hypopnea index.

Patients and methods

We consecutively recruited 48 patients (36 men and 12 women; mean age 50.3±14.68 yrs) with obstructive sleep apnea syndrome from those with suspected OSAS referred to our center. Clinical history and physical examination were performed in all subjects and Epworth Sleepiness Scale (ESS) was also given. OSAS was diagnosed after a 1-night cardiorespiratory sleep study: apneas were defined as the cessation of airflow for ≥10 seconds and hypopneas were defined as a transient reduction of breathing ≥ 50% with an oxygen desaturation of ≥3% or as a reduction of breathing ≥ 30% with an oxygen desaturation of ≥ 4% for ≥10 seconds. Obstructive apneas and hypopneas were distinguished from central events by the detection of respiratory efforts during the event. AHI was defined as the number of obstructive apneas and hypopneas per hour of sleep. Patients with an AHI ≥ 5 were considered as OSAS and then they were subdivided

Table I. Mean ± S.D. of age, anthropometric characteristics and OSAS parameters in the whole group of OSAS patients and in the two subgroups with respectively mild and severe disease

	All OSAS patients	Mild OSAS	Severe OSAS	F
Age (years)	49.7 ± 14.6	45.3 ± 14.4	52.8 ± 14.2	1.549
BMI (kg/m^2)	35.37 ± 7.31	35.72 ± 8.49	35.10 ± 6.47	0.037
Waist circumference (cm)	118.8 ± 16.1	114.2 ± 14.5	122.5 ± 16.6	1.341
Neck circumference (cm)	44.41 ± 4.53	41.50 ± 3.25	46.62 ± 4.15*	6.80[a]
ESS	11.07 ± 5.12	9.18 ± 3.69	12.42 ± 5.62	2.07
AHI	38.47 ± 25.66	15.13 ± 8.15¶	56.63 ± 18.90§**	22.7[b]
mSO2 (%)	91.1 ± 3.68	93.4 ± 2.68	89.5 ± 3.45*	6.82[a]
ODI	39.34 ± 29.03	14.28 ± 9.39§	55.38 ± 25.75#**	12.8[b]

[a] $p < 0.01$ [b] $p < 0.001$ (ANOVA)
# $p < 0.05$ § $p < 0.01$ ¶ $p < 0.001$ vs all OSAS patients (Bonferroni)
* $p < 0.01$ ** $p < 0.001$ vs mild OSAS (Bonferroni)

according to the AHI value in two subgroups: Low (L= 21 subjects with AHI<30) and High (H= 27 subjects with AHI>30). Therefore, the Low subgroup included patients with mild to moderate OSAS, while the H subgroup included the patients with severe OSAS. Means and S.D. of age, BMI, waist circumference, neck circumference, AHI, oxygen desaturation index (ODI), mean nocturnal SO_2 are reported in Table I. 23 of the OSAS patients had arterial hypertension, 10 patients had diabetes mellitus and 6 had cardiovascular disease (history of myocardial infarction or stroke). Each subject gave the informed consent and the study was approved by the Ethical Committee.

On fasting venous blood, collected by puncture from the antecubital vein of each subject and immediately transferred to glass tube anticoagulated with EDTA-K3, we evaluated lipid peroxidation and protein carbonyl (PC) groups.

Lipid peroxidation was evaluated in plasma by detection of thiobarbituric acid-reactive substances (TBARS), generated by peroxidative processes, which include lipid peroxides and malonyldialdehyde. The evaluation of TBARS was made by fluorimetry, using 1,1,3,3-tetramethoxypropane as standard[26].

The PC groups were measured by an enzyme-linked immunosorbent assay (ELISA) kit (Bio-Cell PC test kit, Enzo Life Sciences AG, Lausen, Switzerland). It uses the classic PC reagent 2,4dinitrophenyl-hydrazyne (DNP), which reacts with the PC forming a stable hydrazone product. In brief, plasma samples were incubated with DNP, and then plasma proteins were nonspecifically adsorbed to the wells of an ELISA plate. Unconjugated DNP and non-protein constituents were washed away. The absorbed proteins were probed with a biotinylated anti-DNP antibody, followed by streptavidin-linked horseradish peroxidase. A chromatin reagent was added, and the reaction was stopped by adding an acid solution. Absorbance for each well was measured at 450 nm and related to a standard curve prepared for serum albumin, containing increasing proportions of hypoclorous acid-oxidized protein, calibrated colorimetrically. Total protein concentration in plasma samples was evaluated by the method of Lowry et al[27].

Statistical Analysis

Data were expressed as means ± S.D. The statistical difference between the entire group of OSAS patients, the L subgroup and the H subgroup was estimated using the 1-way analysis of variance (ANOVA) integrated with the Bonferroni test. The correlations were performed employing the linear regression test. The null hypothesis was rejected for p values < 0.05.

Results

Subdividing the entire group of OSAS according to the AHI value, we observed that the lipid peroxidation, expressed as TBARS, was significantly increased in the H subgroup in comparison with the L subgroup and the entire group (Table II). The same behavior was observed evaluating the protein oxidation, and in fact the carbonyl groups were significantly increased in the H subgroup in comparison with the L subgroup and the entire group of OSAS patients (Table II). Regarding the demographic characteristics, in the entire group of OSAS patients we found a positive correlation between TBARS and neck circumference ($r = 0.88$, $p < 0.0002$). About the polysomnographic parameters we found a positive correlation between TBARS and AHI value ($r = 0.88$, $p < 0.0001$), and between TBARS and ODI ($r = 0.88$, $p < 0.0001$) and a negative correlation between TBARS and mean oxygen saturation ($r = –0.52$, $p < 0.0003$) (Figure 1). Regarding the protein oxidation, we observed a positive cor-

Table II. Mean ± S.D. of oxygen status parameters in the whole group of OSAS patients and in the two subgroups with respectively mild and severe disease.

	All OSAS patients	Mild OSAS	Severe OSAS	F
TBARS (nmol/ml)	6.431 ± 1.635	5.247 ± 0.469§	7.351 ± 1.629#*	12.2[a]
PCO (nmol/mg prot.)	0.316 ± 0.120	0.230 ± 0.088§	0.382 ± 0.099#*	11.6[a]

[a] $p < 0.001$ (ANOVA)
# $p < 0.05$ § $p < 0.01$ vs all OSAS patients (Bonferroni's test)
* $p < 0.001$ vs mild OSAS (Bonferroni's test)

relation not only between carbonyl groups and neck circumference (r = 0.61, $p < 0.0001$), but also between carbonyl groups and waist circumference (r = 0.35, $p < 0.02$). We also observed a positive correlation between carbonyl groups and AHI values (r = 0.68, $p < 0.0001$), and between carbonyl groups and ODI (r = 0.63, $p < 0.0001$) and a negative correlation between carbonyl groups and mean oxygen saturation (r = –0.46, $p < 0.001$) (Figure 1).

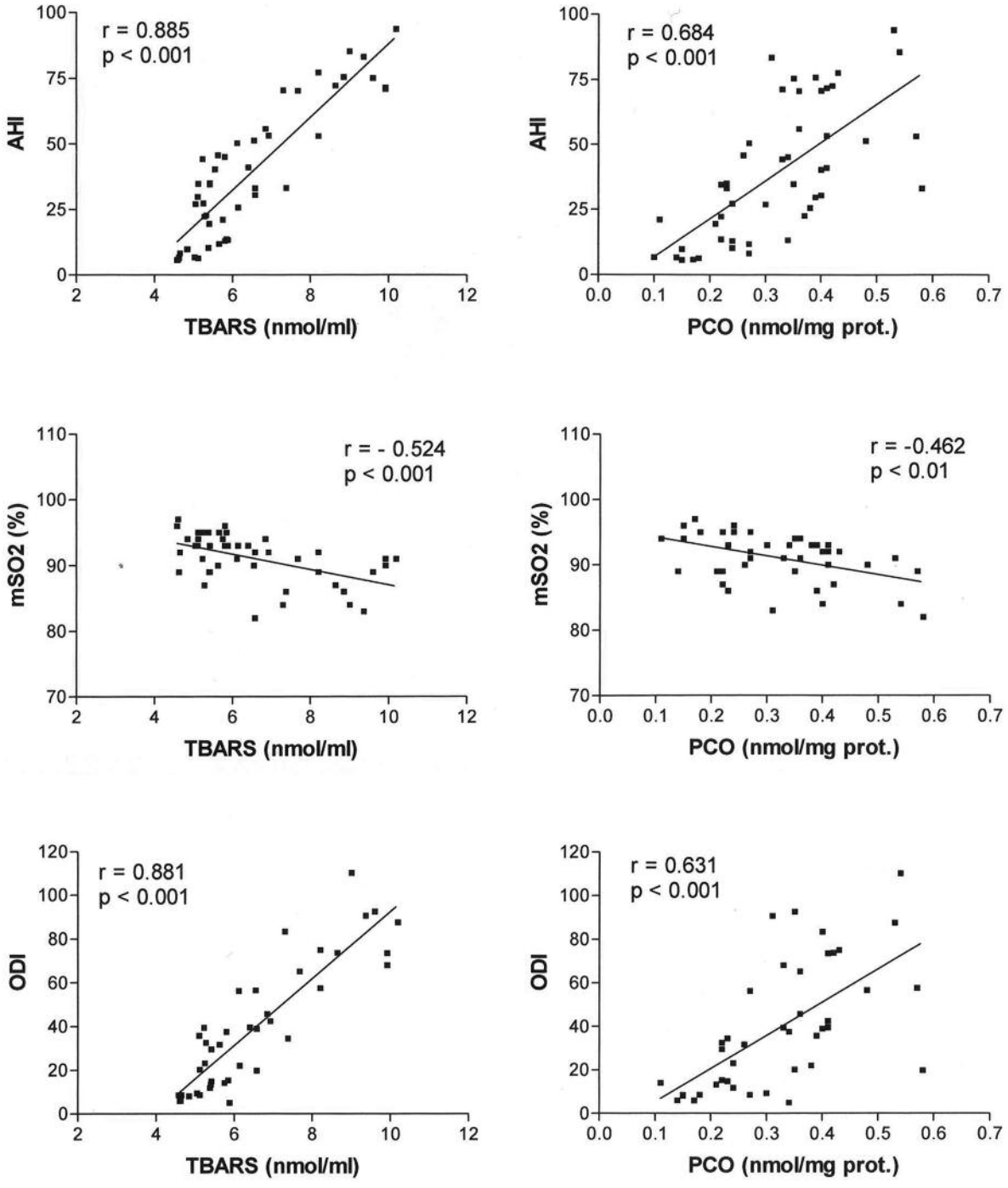

Figure 1. Correlations between oxidative stress parameters and polysomnographic parameters.

Discussion

The principal information that emerges from this research is the behavior of lipid peroxidation and protein oxidation, between them significantly interrelated ($r = 0.61$, $p < 0.0001$), that results influenced by the degree of severity of this syndrome. In these last decades, the particular attention addressed towards the oxidative status in OSAS patients has been related especially to the fact that its impairment, associated with the increase in sympatethic tone, might be considered one of the links between OSAS and the increase in cardiovascular events.

Our data show clearly that lipid peroxidation and protein oxidation discriminate subjects with mild to moderate OSAS from those with severe OSAS. Considering that the literature data underline how the cardiovascular morbidity and mortality[4,5,7,28], especially in OSAS patients with previous cardiovascular diseases, are significantly related to the degree of the OSAS severity, these data deserve to be considered.

In this group of OSAS patients no correlation was found among lipid peroxidation and protein oxidation with age and body mass index and this datum agrees with those of other authors[16,17]. However, we observed a positive correlation of these two parameters with neck and waist circumference. These findings partially agree with those of Vatansever et al[17] and contrast with those of Papandreu[16].

Another aspect of this research is the interrelationship between these two parameters of the oxidative status and the polysomnographic characteristics. In agreement with others[12,16-18], we found a positive correlation between TBARS and AHI values and between TBARS and ODI, and a negative correlation between TBARS and mean oxygen saturation. In contrast with Mancuso et al[25], but in agreement with Vatansever et al[17], we observed a significant correlation between protein oxidation and AHI values. We also found a positive correlation between carbonyl groups and ODI, and a negative correlation between carbonyl groups and mean oxygen saturation.

The impairment of the oxidative status, and in particular of the lipid peroxidation and protein oxidation, is dependent on the hypoxia-reoxygenation episodes that characterize OSAS[3,29]. An increased mitochondrial ROS production in cultured endothelial cells exposed to hypoxia has been demonstrated[29]. *In vitro* hypoxia induces also the activation of leukocytes and the production of ROS and Lavie et al[29] have demonstrated an increased ROS generation by monocytes and neutrophils from OSAS patients activated by phorbol myristate acetate. In addition, ROS are involved in signaling pathways activation, as they influence the activity of mitogen-activated protein kinase (MAPK), able to induce several nuclear transcription factors[29]. In particular, NF-kB starts a cascade of inflammatory pathways leading to the production of cytokines and adhesion molecules implicated in atherosclerosis development, while the hypoxya-inducible factor 1α (HIF-1α) increases the sympathetic nerve activity [3,29]. Jelic et al[30] observed an increased NF-κB activation in freshly venous endothelial cells from obese OSAS in comparison with obeses without OSAS, which was associated with endothelial dysfunction demonstrated by reduced levels of activated eNOS.

Conclusions

Lipid peroxidation and protein oxidation are dependent on the degree of severity of OSAS patients. In the next future we may observe if the treatment of OSAS using cPAP will came out able to modify at the same time and proportionally these two oxidative status parameters.

Conflict of interest

The Authors declare that they have no conflict of interests.

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