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Lipid peroxidation and nitric oxide metabolites in a group of subjects with obstructive sleep apnea syndrome

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Abstract. It is known that in OSAS the plasma lipid peroxidation has an opposite behavior in comparison with nitric oxide metabolites. In the re-examination of our survey of OSAS subjects we calculated the ratio between thiobarbituric acid reactive substances (TBARS) and nitric oxide metabolites (NOx) in relation to OSAS severity. The study has regarded 48 OSAS subjects subdivided in two subgroups according to the apnea/hypopnea index – AHI- (Low = 21 subjects with AHI <30 and High = 27 subjects with AHI >30). From the obtained data it is evident that the TBARS/NOx ratio is significantly higher in the H subgroup compared to L subgroup as well as this ratio is reduced in L subgroup in comparison with the whole group of OSAS subjects. In the entire group of OSAS subjects the TBARS/NOx ratio results positively correlated with AHI and ODI and inversely correlated with mSO_2.

Keywords: Lipid peroxidation, nitric oxide metabolites, obstructive sleep apnea syndrome

1. Introduction

In observational studies, subjects with obstructive sleep apnea syndrome (OSAS) have an elevated risk for cardiovascular diseases, including stroke and myocardial infarction [24]. The incidence of cardiovascular events seems to be related to OSAS severity, considering that the apnea/hypopnea index and the time spent with oxygen saturation <90% are strong predictors of cardiovascular outcome [17].

This higher risk of fatal events is due to the effects of OSAS on cardiovascular system. First of all, OSAS is commonly associated with the development of arterial hypertension, often occurring as resistant hypertension [19]. During the apneic event, the hypoxia may cause an imbalance between the myocardial oxygen demand and supply, which may induce myocardial ischemia or arrhythmias [12]. Nocturnal angina or ST-segment depression can be triggered by OSAS [10] and the Sleep Hearth Health Study has effectively demonstrated in OSAS subjects a 4-fold increased risk for atrial fibrillation [25]. The continued hypoxia-reoxygenation episodes have a pivotal role in the pathogenesis of the endothelial dysfunction in OSAS: the intermittent hypoxia may induce the production of reactive oxygen species (ROS) that contribute to the generation of adhesion molecules, leukocyte activation, and an enhanced systemic inflammation leading to endothelial damage [21]. In OSAS an increase in lipid and protein oxidation [3, 7, 9, 13, 18, 26] and a decrease in antioxidant defenses [7, 9, 16, 23, 27, 30] have been demonstrated. In OSAS subjects the hypoxia-reoxygenation phenomena influence nitric oxide (NO)

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synthesis by NO synthase (NOS) inducing a down-regulation of the eNOs expression [32, 33] and activation [15] and a simultaneous increase in inducible NOS (iNOS) expression [15]. Some studies have demonstrated that plasma nitric oxide (NO) metabolites, such as nitrites and nitrates (usually expressed as NOx), are reduced in subjects with OSAS [6, 26, 28, 29], but other authors found no difference in NO levels between hypertensives with or without OSAS. Considering that in OSAS the lipid peroxidation has an opposite behavior compared to nitric oxide, in this re-examination of our group of OSAS subjects we evaluated the TBARS (thiobarbituric acid-reactive substances)/NOx ratio in relation to OSAS severity.

In OSAS the TBARS/NOx may be considered as an integrated index of plasma lipid peroxidation and endothelial dysfunction. Up to now this ratio has been evaluated in preeclampsia [20], in juvenile essential hypertension [31], in hypertensive adolescents with obesity or uraemia [1, 2], in subjects with mild essential hypertension [5], in sportsmen before and after a cardiopulmonary test [22] and in a group of subjects with metabolic syndrome [4].

2. Subjects and methods

We consecutively recruited 48 subjects (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. OSAS was diagnosed after a 1-night cardiorespiratory sleep study. The apnea/hypopnea index (AHI) was defined as the number of obstructive apneas and hypopneas per hour of sleep. Patients with an AHI $\geq$5 were considered as affected by OSAS and then they were subdivided according to the AHI value in two subgroups: Low (L = 21 subjects with AHI <30) and High (H = 27 subjects with AHI >30). Means and S.D. of age, BMI, waist and neck circumference, AHI, oxygen desaturation index (ODI), and mean nocturnal SO_2 (mSO_2) are reported in Table 1. Twenty-three of the OSAS subjects had arterial hypertension, 10 had diabetes mellitus and 6 had cardiovascular disease (history of myocardial infarction or stroke). Each subject gave the informed consent and the Ethical Committee approved the study.

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:

Table 1
Means ± S.D. of anthropometric and polysomnographic parameters in the whole group of OSAS patients and in the two subgroups

	All OSAS patients	L-OSAS	H-OSAS	F
Age (years)	49.7 ± 14.6	45.3 ± 14.4	52.8 ± 14.2	1.549
BMI (kg/m^2)	35.4 ± 7.3	35.7 ± 8.5	35.1 ± 6.5	0.037
Waist circumference (cm)	118.8 ± 16.1	114.2 ± 14.5	122.5 ± 16.6	1.341
Neck circumference (cm)	44.4 ± 4.5	41.5 ± 3.2	46.6 ± 4.1**	6.80[2]
ESS	11.1 ± 5.1	9.2 ± 3.7	12.4 ± 5.6	2.07
AHI	38.5 ± 25.7	15.1 ± 8.1¶	56.6 ± 18.9§***	22.7[1]
mSO_2 (%)	91.1 ± 3.7	93.4 ± 2.7	89.5 ± 3.4**	6.82[2]
ODI	39.3 ± 29.0	14.3 ± 9.4	55.4 ± 25.7#***	12.8[1]

[1]$p < 0.001$ [2]$p < 0.01$ (ANOVA). #$p < 0.05$ §$p < 0.01$ ¶$p < 0.001$ vs all OSAS (Bonferroni's test). **$p < 0.01$ ***$p < 0.001$ vs L-OSAS (Bonferroni's test). OSAS = Obstructive Sleep Apnea Syndrome. BMI = Body Mass Index. ESS = Epworth Sleepiness Scale. mSO_2 = mean oxygen saturation. AHI = apnea/hypopnea index. ODI = oxygen desaturation index.

– **Lipid peroxidation** was evaluated in plasma by detection of thiobarbituric acid-reactive substances (TBARS), generated by peroxidative processes, which include lipid peroxides and malonildialdehyde. The evaluation of TBARS was made by fluorimetry, using 1,1,3,3-tetramethoxypropane as standard.
– **Nitric oxide metabolites** (NOx): considering that *in vivo* NO has a very short life (less than 0.1 sec) and it is converted into nitrite (NO_{2^-}), which has a half-life of few minutes, and into the more stable nitrate (NO_{3^-}), NOx represents almost only the nitrate concentration. In the laboratory method adopted by us at first nitrate was converted into nitrite by a nitrate reductase, and then nitrite was assessed by spectrophotometry after addition of Griess reagent.

3. Statistical analysis

The values were expressed as means ± SD. The comparison among the entire group, the L subgroup and the H subgroup of OSAS subjects was performed using the one-way analysis of variance (ANOVA), integrated with Bonferroni's multiple post test. The values of TBARS/NOx were correlated with the anthropometric parameters and with polysomnographic parameters using the linear regression test. The null hypothesis was rejected for p values less than 0.5.

4. Results

We found that the TBARS is significantly increased as well as the NOx is decreased in the H subgroup in comparison with the entire group and especially with the L subgroup of OSAS subjects. The TBARS/NOx ratio is significantly higher in the H subgroup in comparison with the L subgroup of OSAS subjects as well as this ratio is significantly reduced in the L subgroup compared to the entire OSAS group (Table 2). Only in the whole group we found a positive correlation between TBARS/NOx ratio and waist circumference and between TBARS/NOx and neck circumference (Table 3). Examining indeed the correlation between TBARS/NOx ratio and the polysomnographic parameters, we observed in the entire group a positive correlation between TBARS/NOx and AHI ($r = 0.763$, $p < 0.001$) and between TBARS/NOx and ODI ($r = 0.705$, $p < 0.001$) and a negative correlation between TBARS/NOx and mSO_2 ($r = –0.390$, $p < 0.01$); in the L subgroup only a positive correlation between TBARS/NOx and AHI was present, while in the H subgroup TBARS/NOx was positively correlated with AHI and ODI (Table 3).

5. Discussion

The behavior of the TBARS/NOx ratio in OSAS subjects shows in a peculiar way that the trend of TBARS and NOx is clearly different in the two subgroups; the values of each parameter in the

Table 2
Means ± S.D. of TBARS, NOx and TBARS/NOx ratio in the whole group of OSAS patients and in the two subgroups

	All OSAS patients	L-OSAS	H-OSAS	F
TBARS (nmol/ml)	6.431 ± 1.635	5.247 ± 0.469[§]	7.351 ± 1.629[#***]	12.2[1]
NOx (nmol/ml)	27.49 ± 10.25	33.47 ± 10.05	22.84 ± 7.79[***]	7.27[2]
TBARS/NOx ratio	0.284 ± 0.176	0.175 ± 0.068[#]	0.370 ± 0.188[***]	8.43[1]

[1]$p < 0.001$ [2]$p < 0.01$ (ANOVA). [#]$p < 0.05$ [§]$p < 0.01$ vs all OSAS (Bonferroni's test). [***]$p < 0.001$ vs L-OSAS (Bonferroni's test). OSAS = Obstructive Sleep Apnea Syndrome. TBARS = Thiobarbituric Acid Reactive Substances. NOx = nitric oxide metabolites (nitrite+nitrate).

Table 3
Values of r for linear correlations between TBARS/NOx ratio and anthropometric parameters (A) and between TBARS/NOx ratio and polysomnographic parameters (B) in the whole group of OSAS patients and in the two subgroups

	All OSAS patients	L-OSAS	H-OSAS
(A) TBARS/NOx vs			
Age	0.029	0.234	–0.257
BMI	0.203	0.235	0.360
Waist circumference	0.314*	0.152	0.238
Neck circumference	0.504**	0.313	0.276
(B) TBARS/NOx vs			
ESS	0.022	–0.157	–0.223
AHI	0.763***	0.434*	0.670***
mSO_2	–0.390**	–0.267	–0.125
ODI	0.705***	0.210	0.588**

*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$ (linear regression). OSAS = Obstructive Sleep Apnea Syndrome. TBARS = Thiobarbituric Acid Reactive Substances. NOx = nitric oxide metabolites (nitrite+nitrate). BMI = Body Mass Index. ESS = Epworth Sleepiness Scale. mSO_2 = mean oxygen saturation. AHI = apnea/hypopnea index. ODI = oxygen desaturation index.

H subgroup of OSAS subjects reflect how the severity degree of this clinical condition increases the plasma lipid peroxidation and reduces the endothelial NO synthesis.

The role carried out by oxidative stress, endothelial dysfunction and sympathetic activation in the pathogenesis of the cardiovascular risk may be worsened by the simultaneous deregulation of the gelatinases (MMP-2 and MMP-9) and their tissue inhibitors (TIMPs), as found by us [14] in OSAS subjects. Considering the cardiovascular risk that follows the OSAS, it is useful to underscore that the gelatinases activity produces elevated levels of angiostatin through the proteolytic cleavage of plasminogen. As it is known, angiostatin inhibits endothelial cell proliferation and migration, induces apoptosis, reduces vascular endothelial growth factor expression and decreases eNOS activity [8], influencing significantly the angiogenesis.

Assembling therefore the presence of oxidative stress, the reduction of the NO metabolites and the increase in gelatinase activity, it results more easy to explain the involvement of the cardiovascular system in OSA syndrome. Another observation regards the significant correlation between the TBARS/NOx ratio and the principal polysomnographic parameters (AHI, ODI, mSO_2) in the whole group of OSAS subjects and partially in the H subgroup; this latter datum is interesting also considering that this ratio correlates positively with the waist circumference and with the neck circumference in the entire group of OSAS.

All these information seem to warrant our interest for the examination of this ratio in OSAS also bearing in mind that the TBARS/NOx ratio is able to discriminate clearly OSAS subjects subdivided according to the AHI values.

This research complies with the requirement for ethical publication in Clinical Hemorheology and Microcirculation as published in Clin Hemorheol Microcirc. 2010;44(1):1-2.

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