Original Article

Associations between mean oxygen saturation and thiobarbituric acid reacting substances after an intervention program in obstructive sleep apnoea syndrome patients

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Conflict of interest
None of the authors had any potential conflict of interest.

Running title : MSaO2 and TBARS in OSAS patients.

Abstract

Background and objectives : Several studies have suggested the presence of increased lipid peroxidation in patients with obstructive sleep apnoea syndrome (OSAS) using various indices. The aim of this study was to investigate the most important factors that determine lipid peroxidation in obese patients with obstructive sleep apnoea syndrome (OSAS) after a weight reduction program.

Methods : Nine hundred patients were evaluated during a 1-year period (November 2008–October 2009), and 21 obese patients with moderate OSAS based on overnight attended polysomnography were included. These subjects had undergone a lifestyle- intervention program (11 followed the Mediterranean diet and 10 a prudent diet plus counselling to increase in physical activity) in parallel with continuous positive airway pressure for a 6-month period. Thiobarbituric acid reactive substances (TBARS) were measured in serum.

Results : At the end of the intervention, multiple linear regression analysis showed a negative association between TBARS and mean oxygen saturation.

Conclusion : This study supports the important role of mean oxygen saturation in the association between OSAS and lipid peroxidation.

Keywords : TBARS, OSAS, obesity, diet, weight loss.

Introduction

Oxidative stress forms an integral part of the development of cardiovascular diseases. Currently, there is an interest with regards to lipid peroxidation and its relationships to cardiovascular risk factors like obstructive sleep apnoea syndrome (OSAS). Several studies have suggested the presence of increased lipid peroxidation in patients with (OSAS) using various indices[1-4]. One important mechanism explaining this association is intermittent hypoxia, in which patients are subjected to repeated episodes of brief oxygen desaturation in the blood, followed by reoxygenation[5]. Such cycles of hypoxia/reoxygenation may result in the generation of reactive oxygen species. In contrast, other studies[6] have not demonstrated increased oxidative stress in OSAS patients when compared with controls. One possible explanation could be that oxidative stress has been related to many factors that could be acting as confounding factors in the relationship with OSAS. These include obesity, smoking, age, antioxidant nutrient intake, hypertension, hyperlipidemia and diabetes[7,8]. Thiobarbituric acid reactive substances (TBARS) are one of the earliest markers of lipid peroxidation in human studies and are used as predictors for atherosclerosis. The TBARS assay should be considered as a global test, allowing a global approach of lipid peroxidation[9]. A previous study that investigated the most important factors that determine lipid peroxidation in predominantly male patients with moderate to severe OSAS revealed an independent association between lowest oxygen saturation and TBARS levels after controlling for age, gender, diet and obesity[10]. Then, these patients underwent repeated measures of TBARS, anthropometry and polysomnography after the implementation of a healthy lifestyle program in parallel with continuous positive airway pressure (CPAP) treatment. Whether the association between lowest oxygen saturation and TBARS levels persisted after the program would be of interest and is examined in this study.

Material & Methods

Participants

A series of consecutive patients, who were diagnosed with OSAS by overnight attended polysomnography (PSG) (Alice 5; Philips Respironics, Best, the Netherlands) in the Sleep Disorders Unit, Department of Thoracic Medicine, Medical School, University of Crete, during a one-year period (November 2008-October 2009) were evaluated and the study population was selected based on the following criteria. Inclusion criteria were as follows: a) age 18-65 years; b) body mass index ≥ 30 kg/m^2; c) apnoea-hypopnoea index >15 events/h. Exclusion criteria were: a) continuous positive airway pressure treatment, b) smoking, c) diseases such as cardiac ischemic disease, diabetes mellitus, thyroid disorders, and malignancies, d) upper airway surgery, e) gestation, f) alcoholism, g) therapy with sleeping pills, h) use of anti-depressive medication and i) ages < 18 and >65 years. Twenty-one obese patients (17 males and 4 females) composed the sample (Table 1). None of them was taking medications that could have an effect on oxidative stress such as statins[11]. After the visit to the study physicians and the confirmation that the patients fulfilled the inclusion/exclusion criteria, the subjects were allocated randomly to two study groups using a computer-generated random number sequence. This study was approved by the ethical committee of the University of Crete and all the participants provided signed consent.

Table -1
Demographic/ pornographically characteristics and thiobarbituric acid reactive substances levels of the subjects (n=21) at baseline.

Demographic characteristics	**Mean ± S.D.**
Age (years)	48.1 ± 12.4
Body mass index (kg/m2)	36.6 ± 3.7
Waist circumference (cm)	115.5 ± 10.3
Mediterranean diet score	27.8 ± 5.4
Polysomnographic characteristics	
Apnoea-hypopnoea index (events/h)	45.5 ± 31.4
Desaturations per hour (events/h)	43.6 ± 28.5
Mean oxygen saturation	92.1 ± 2.4
Lowest oxygen saturation	77.3 ± 7.9
Thiobarbituric acid reactive substances (μmol/L)	0.9 ± 0.5

Intervention

Two groups of patients with moderate to severe OSAHS were formed. In both groups, the patients received CPAP therapy and lifestyle interventions. The lifestyle interventions included an initial weight reduction programme that involved increasing physical activity, mainly by walking for at least 30 min daily, and following a low-calorie diet (a prudent diet or the Mediterranean diet). Patients of both groups were offered a specific individualized programme and received education in reducing calorie intake. The energy intake restriction was limited to 1,500 kcal/day for women and 1,800 kcal/day for men. The general guidelines to the participants asked to follow the Mediterranean diet were consumption of six servings per day of non-refined cereals, five or more servings per week of potatoes, five servings per day of various vegetables (two of them as salad), four servings per day of various fresh fruits, three or more servings per week of legumes, three servings per week of fish (at least one serving of fatty fish), one serving per day of nuts, three servings per week of poultry without skin, three servings per week of red meat and seven glasses each week of red wine. The recommended intake of fruits, vegetables, legumes, non-refined cereals and fish was three times higher in Mediterranean diet group than in the other group, whereas the red meat intake in Mediterranean diet group was one third of that in the other. The moderate daily consumption of nuts and alcohol (red wine) was only recommended in Mediterranean diet group. In both groups, the moderate consumption of olive oil was recommended because the people living on the island of Crete produce and consume this type of oil. Patients in both groups were also advised to eliminate or limit the consumption of cream, butter, margarine, carbonated and/or sugared beverages, commercial bakery products (e.g. sweet desserts, cakes, biscuits/cookies, puddings and custard), chips (French fries) or potato crisps (chips) and processed meats (i.e. burgers and sausages) and to consume two servings per day of low fat dairy products. The intervention lasted for 6 months and consisted of seven visits with the study dietician. The study dietician was responsible for providing dietary and exercise counseling at each visit. Additionally compliance with CPAP therapy was monitored. All patients exhibited good compliance, using CPAP >4 h per day and >5 days per week, which are the criteria of regular use[12]. The end of the PSG study was performed without CPAP. One day prior to PSG, CPAP was ceased.

Procedures and measurements

Patients underwent a full diagnostic PSG study, according to standard techniques, with monitoring of the electroencephalogram (EEG) using frontal, central and occipital leads, electro-oculogram (EOG), electromyogram (EMG), flow (by oronasal thermistor and nasal air pressure transducer), thoracic and abdominal respiratory effort by uncalibrated impedance plethysmography belts, oximetry, and body position. Snoring was recorded by a microphone placed on the anterior neck. A single modified type II EKG lead was used for cardiac monitoring. Two sleep recordings were performed during the study. The first was conducted at baseline and the second at 6 months. Anthropometric measurements (weight, height, waist circumference) were carried out by an expert. Weight was assayed using a digital scale instrument with accuracy of ±0.1 kg. The subject was standing without shoes and wearing light clothes. Height was measured on bare-foot to the nearest 0.5 cm using a stadiometer with the shoulders in relaxed position and arms hanging freely. A subject was defined as obese when body mass index was ≥ 30.0 kg/m^2 [13]. Adherence to the diets was evaluated by a semi-quantitative food frequency questionnaire[14] at baseline, at the 3-month visit and at the 6-month visit. In addition, overall dietary habits were evaluated through a special diet score (MedDietScore, range 0–55) that assessed adherence to the Mediterranean dietary pattern. Greater adherence to the Mediterranean dietary pattern has been associated with a reduction in TBARS levels[15]. Physical activity was assessed using the long version of the self-reported International Physical Activity Questionnaire16 at baseline, at the 3-month visit and at the 6-month visit.

Four millilitres of venous blood was also obtained from each patient in the morning (7 a.m.) after an overnight fast at baseline and at the 6-month visit. The concentration of TBARS, reflecting lipid peroxidation products, was determined following a previously published method with a slight modification[17]. Briefly, serum, spiked solutions or standard (400 μL) was mixed with 50 μL butylated hydroxytoluene in ethanol (concentrations ranging from 0 to 1 μmol/L) and 400 μL orthophosphoric acid (0.2 mol/L) in test tubes and stirred well. Fifty microlitres of thiobarbituric acid reagent (0.11 mol/L prepared by adding 800 mg of TBA in 50 mL NaOH 0.1 M) was added and again stirred well. The reaction mixture was incubated at 90°C for 45 min in a water bath. Then the test tubes were put on ice to stop further reaction. TBARS were extracted once with 1,000 μL n-butanol. To facilitate phase separation, 50-μL saturated NaCl solution was added, and the test tubes were centrifuged at 4,000 rpm for 5 min. Absorption of the upper butanol phase was read at 535 and 572 nm to correct baseline in a UV spectrometer (Shimadzu UV-1800). The range of the standard curve was 0–1.0 μmol/L. The within-day relative standard deviation (in percent) was determined by preparing and extracting five spiked blood samples at three concentration levels (0.1, 0.25 and 1 μmol/L) and measuring them during one working day. Within-day precision was estimated to be 19.5%, 6.6% and 2.2% at concentration levels of 0.1, 0.25 and 1 μmol/L, respectively.

Statistical analysis

Mean values and standard deviations are used to describe post intervention characteristics of the treatment subjects. Multiple linear regressions with backward selection was performed after 6 months of intervention, with TBARS levels as the dependent variable and age, gender, MedDietScore, waist circumference, body mass index, apnoea-hypopnoea index, desaturations/hour, mean oxygen saturation (haemoglobin), lowest oxygen saturation (haemoglobin) as the initial independent variables set. Due to the small sample size we did not examine these associations in each intervention group that would be interesting, but in both groups together as one. All data were analyzed with the Statistical Package for Social Sciences (SPSS) version 18.0. A p-value less than 0.05 was considered significant.

Results

Demographic and polysomnographic characteristics of the studied patients and their TBARS levels after 6 months of intervention are presented in Table 2. In general, the studied patients were middle-aged and the majority was men (80.9 %) with moderate OSAS and class 1 obesity.

Table - 2

Demographic/polysomnographic characteristics and thiobarbituric acid reactive substances levels of the subjects (n=21) after 6 months of intervention.

	After 6 months
Demographic characteristics	**Mean ± S.D.**
Age (years)	48.1 ± 12.4
Body mass index (kg/m2)	33.3 ± 3.6
Waist circumference (cm)	107.8 ± 9.4
Mediterranean diet score	34.6 ± 8.5
MET (min/week)	1692.5± 1756.6
Polysomnographic characteristics	
Apnoea-hypopnoea index (events/h)	35.9 ± 30.1
Desaturations per hour (events/h)	34.8 ± 28.8
Mean oxygen saturation	93.2 ± 2.2
Lowest oxygen saturation	81.1 ± 8.3
Thiobarbituric acid reactive substances (μmol/L)	0.3 ± 0.2

Standard deviation (S.D.), Metabolic equivalent of task (MET).

Multiple linear regression analysis after 6 months showed that mean oxygen saturation during sleep was independently associated with TBARS levels (p: <0.05, 95%CI: -0.09 to 0.00, beta: -0.41). Regarding other parameters in the regression model only age was found to be inversely associated with TBARS (p: <0.05, 95%CI: -0.23 to -0.005, beta: -0.66).

Table - 3

Multiple linear regression predictors of thiobarbituric acid reactive substances levels measured after 6 months of intervention.

Predictor	Beta	95% Lower CI	95% Upper CI	p-value
MSaO2 after 6 months	-0.41	-0.09	0.00	<0.05
Age	-0.66	-0.23	-0.005	<0.05
Gender	0.36	-0.04	0.49	0.89

Mean oxygen saturation (haemoglobin) (MSaO2), Confidence interval (CI).

Discussion

Among the indicators of OSAS severity, mean oxygen saturations were found to be most strongly related with TBARS at the end of the weight loss program in patients with OSAS. This is an interesting finding that contributes to the current discussion regarding whether the cyclic alterations of arterial oxygen saturation observed during sleep in OSAS patients represent a significant pathway related to enhanced production of free radicals. Cyclic intermittent hypoxia leads to mitochondrial dysfunction, activation of enzymes that utilise oxygen such as xanthine oxidase or NADPH, leukocyte activation and endothelial cell dysfunction thus producing generation of oxidizing agents[18]. Tissue hypoxia also has been implicated in transient depletion of cellular reductants resulting in disruption of the oxidation-reduction state and thus contributing to the generation of oxidative stress[18]. A recent study suggested that the reduction of antioxidant capacity of blood, due to hypoxia related to respiratory events, could be a possible sleep apnoea-mediated oxidative-mechanism[19]. Oxidative stress alters signaling pathways and activates inflammatory/immune responses via increased interactions of blood cells with endothelial cells, facilitating endothelial cell injury and dysfunction. Such events can play a role in the onset of cardiovascular complications seen in OSAS patients[20]. The findings of this study argue that oxygen saturation should be considered in studies examining the association between OSAS and oxidative stress. A previous study indicated that oxidative stress, as evaluated by urinary 8-hydroxy-29-deoxyguanosine excretion, was related with the oxygen desaturation index (ODI) after adjustment for confounding factors (age, obesity, smoking, hyperlipidemia, hypertension, and diabetes mellitus)[2]. This is interesting because ODI reflects the frequency of transient episodes of hypoxemia with subsequent rapid reoxygenation during which reactive oxygen species are assumed to be produced. A more recent study that examined the role of systemic inflammation in cardiovascular disease seen in OSAS patients showed that intermittent hypoxia, and not the apnoea-hypopnoea index, was related to systemic inflammation[21]. The loss of the statistical significance of the associations between other OSAS severity indices and TBARS in the adjusted model could be attributed to the small sample size.

It is of interest that after 6 months of intervention accompanied with a reduction in TBARS levels and an improvement in mean oxygen saturation (p=0.008) their association was highlighted while that with the lowest oxygen saturation (this OSAS parameter was statistically increased (P<0.001) was weakened compared to baseline analysis[10]. This observation is difficult to be interpreted. Mean oxygen saturation has the advantage of being less vulnerable to artifacts and does not capture a short snapshot of time compared to lowest oxygen saturation and probably more reliable parameter of OSAS. On the other hand, a previous study that examined the role of arterial oxygen saturation measurement in identifying apnoeic snorers from non-apnoeic snorers and in the assessment of the severity of OSAS, only lowest oxygen saturation was found to be useful in the assessment of the severity of OSAS[22].

The present study has several potential limitations. TBARS have been claimed to be a nonspecific index of lipid peroxidation[23]. However, it has been shown that under conditions of induced oxidative stress, which is presumably a characteristic of patients with OSAS, there is a good correlation between TBARS and the levels of isoprostane, which is a more specific marker of lipid peroxidation[24]. The study is underpowered because of the small sample. The recruitment of a larger number of participants during the planned selection period was difficult in this study because of certain factors such as the characteristics of the population, the rigorous exclusion criteria and the lack of willingness of 23 patients to participate[25]. Finally, the selection of all individuals with certain clinical characteristics and the exclusion of comorbidities decrease the external validity of our results. Then, the clinical application of the data should be considered with great caution as they may be valid only for patients with similar characteristics with the studied population.

In conclusion, TBARS were found to be independently associated with mean oxygen saturation after an intervention program in predominantly male patients with moderate to severe OSAS. These findings may help to explain the presence of lipid peroxidation in patients with OSAS, which is characterized by cyclical hypoxia – reoxygenation, as well as to understand the pathogenesis of OSAS cardiovascular complications.

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