

Effect of continuous positive airway pressure on oxidative stress accompanied by obstructive sleep apnea

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Introduction In obstructive sleep apnea (OSA), there is increased oxidative stress.

Aim of this work This study aimed to examine the effect of continuous positive airway pressure (CPAP) on oxidative stress occurring in OSA.

Participants and methods The present study was carried out on 40 individuals classified into four groups: group I included 10 control participants, group II included 10 obese individuals without OSA, group III included 10 patients with mild to moderate OSA, and group IV included 10 patients with severe OSA. Sleep study was carried out, and Thiobarbituric acid-reactive substance and superoxide dismutase enzyme were measured.

Results Thiobarbituric acid-reactive substance was significantly increased, but superoxide dismutase was

significantly decreased in group IV, and CPAP led to an improvement in this condition.

Conclusion OSA leads to increased oxidative stress that improved with the use of CPAP.

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Keywords: continuous positive airway pressure, obstructive sleep apnea, superoxide dismutase, thiobarbituric acid-reactive substance

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Introduction

Obstructive sleep apnea (OSA) is characterized by repetitive interruption of ventilation during sleep caused by collapse of the pharyngeal airway associated with ongoing ventilatory effort [1,2]. Factors that increase for the risk of developing this disorder include age, male sex, obesity, family history, menopause, craniofacial abnormalities, and certain health behaviors such as cigarette smoking and alcohol use [2]. OSA is quantified on the basis of the apnea-hypopnea index (AHI). According to the American Academy of Sleep Medicine recommendations, OSA is defined as AHI more than 5, and it is classified as mild OSA with AHI of 5–15, moderate OSA with AHI of 16–30, and severe OSA with AHI more than 30 [3,4], leading to excessive daytime sleepiness, which can be assessed subjectively using the Epworth Sleepiness Scale [5–7] and treated with positive airway pressure [8]. Oxidative reactions are coupled with the continuous generation of highly reactive and potentially cytotoxic reactive oxygen species (ROS). Under normal conditions, the ROS produced during the course of metabolism are contained by the natural antioxidant system that protects the functional and structural molecules against ROS-mediated modifications, thereby preventing cytotoxicity and tissue damage [9]. Thus, oxidative stress results from an imbalance between radical-generating and radical-scavenging systems, that is OSA is characterized by recurrent nocturnal obstruction of the upper airway. Each episode of airway obstruction is usually followed

by a marked decrease in arterial oxygen saturation, which rapidly normalizes after ventilation resumes. Thus, OSA patients experience repeated episodes of hypoxia and normoxia. During the hypoxic phase, cells adapt to a low oxygen environment; however, the reoxygenation phase causes a sudden increase in oxygen in the cells. This reoxygenation phase is considered to result in the production of ROS and the promotion of oxidative stress [10,11] or reduced activity of antioxidant defenses, or both [12].

Aim of this work

The aim of this work was to assess the plasma levels of thiobarbituric acid-reactive substances (TBARS) and superoxide dismutase (SOD) enzyme in patients with OSA and correlate their levels with the severity of the disease, and to study the effect of continuous positive airway pressure (CPAP) therapy on their levels in severe cases after 1 and 7 days of treatment.

Participants and methods

The present study was carried out in the Sleep Laboratory Unit, Chest Department, Tanta University Hospital, on 40 individuals during the period from May 2011 till August 2012 classified into four groups. Group I included 10 control participants (seven men and three women) on the basis of both clinical and

polysomnographic criteria: AHI (1.6 ± 1.35), BMI (24.8 ± 1.47) (kg/m^2), and age (48.6 ± 19.02) years. Group II included 10 obese individuals (three men and seven women) without obstructive sleep apnea syndrome (OSAS) on the basis of both clinical and polysomnographic criteria: AHI (1.7 ± 1.33), BMI (33.9 ± 2.6) (kg/m^2), and age (48.5 ± 10.1) (years). Group III included 10 patients (four men and six women) with a diagnosis of mild to moderate OSAS on the basis of both clinical and polysomnographic criteria: AHI (14 ± 5.98), BMI (35.8 ± 4.39) (kg/m^2), and age (54.3 ± 5.88) (years). Finally, group IV included 10 patients (five men and five women) who were diagnosed with severe OSAS on the basis of both clinical and polysomnographic criteria: AHI (46.1 ± 13.64), BMI (42.2 ± 6.71) (kg/m^2), and age (47.2 ± 10.6) (years).

Exclusion criteria

- (1) Cardiovascular diseases: for example, hypertension, ischemia, and arrhythmia.
- (2) Pulmonary diseases: for example, COPD, bronchial asthma, and lung cancer.
- (3) Diabetes mellitus.
- (4) Renal failure.
- (5) Smoking.
- (6) Use of medications: for example, antioxidants, corticosteroids, vitamins, and iron.

The participants were subjected to the following:

- (1) Detailed assessment of personal history including age, sex, occupation, and special habits (smoking and alcohol).
- (2) Detailed assessment of medical history, with a special focus on symptoms of OSAS such as snoring, restless sleep, and excessive daytime sleepiness, and surgical history of any previous ENT operations.
- (3) Subjective evaluation of daytime sleepiness using The Epworth Sleepiness Scale.
- (4) Thorough clinical examination including measurement of blood pressure and heart rate, height and weight for calculation of BMI, measurement of neck circumference, and ENT examination to exclude major anatomical deformity.
- (5) Plain chest radiograph and ABG analysis.
- (6) Sleep study using the Res Med Apnea Link Plus system (ResMed Ltd., 1 Elizabeth Macarthur Drive Bella Vista, NSW 2153, Australia).
- (7) Venous blood samples were collected in the morning after sleep study and analyzed as follows:
 - (a) TBARS was measured spectrophotometrically through the color produced by the reaction of TBARS with malondialdehyde at 532 nm. For this purpose, TBARS levels were measured using a commercial assay such as the NWLSS

Malondialdehyde Assay (NWLSS, North of Portland, Oregon, USA) according to the manufacturer's instructions.

- (b) SOD enzyme activity was measured using a commercially supplied assay kit of the Superoxide Dismutase Assay Kit (Cayman Chemical, 1180 East Ellsworth Road, Ann Arbor, Michigan 48108, USA) according to the manufacturer's instructions.
- (8) Patients in group IV were subjected to an additional overnight CPAP autotitration study with the patient connected to the Apnea Link Plus system, and all the available parameters were measured. Arterial blood gas analysis and plasma levels of both TBARS and SOD were reassessed after one night and seven nights of CPAP therapy.

Statistical analysis

Mean, SD, ANOVA test, Tukey's test, and paired test were used in this study.

Informed consents were obtained from all participants.

Results

There was a significant increase in the OSA index in group IV compared with the other groups, but there was an insignificant difference between groups I, II, and III. Percent of total sleep less than 90% was significantly increased in group IV compared with the other groups. Group III showed a significant increase compared with groups I and II, but there was an insignificant difference between groups I and II, and the above two parameters improved significantly during CPAP autotitration. Serum TBARS were significantly increased in group IV compared with group II; also, they were significantly increased in groups III and IV compared with group I, but there was an insignificant difference between group III and groups II and IV, and also between groups I and II. It was significantly decreased after 1 and 7 days of CPAP treatment compared with before treatment. Serum SOD was significantly decreased in group IV compared with group II; also, it was significantly decreased in groups III and IV compared with group I, but there was an insignificant difference between group III and groups II and IV, and also between groups I and II. It was significantly increased after 1 and 7 days of CPAP treatment compared with before treatment (Tables 1–8).

Discussion

OSA syndrome is characterized by recurrent episodes of partial or complete pharyngeal collapse (hypopneas or apneas) occurring during sleep. It is a growing

Table 1 Range, mean value, SD, and statistical comparison of the obstructive apnea index in the four groups studied

Group	Obstructive apnea index		ANOVA		
	Range	Mean \pm SD	<i>F</i>	<i>P</i> -value	
Group I	0.000–1.000	0.500 \pm 0.527	33.826	<0.001	
Group II	0.000–3.000	0.700 \pm 1.059			
Group III	3.200–8.000	5.300 \pm 2.111			
Group IV	7.000–38.000	21.400 \pm 10.532			
Tukey's test					
I and II	I and III	I and IV	II and III	II and IV	III and IV
1.000	0.407	<0.001	0.454	<0.001	<0.001

ANOVA, analysis of variance.

Table 2 Range, mean value, SD, and statistical comparison of percent of total sleep time in which O₂ saturation less than 90% in the four groups studied

Group	Percent of total sleep time in which O ₂ saturation < 90%		ANOVA		
	Range	Mean \pm SD	<i>F</i>	<i>P</i> -value	
Group I	0.000–14.000	2.700 \pm 4.398	15.721	<0.001	
Group II	0.000–18.000	3.800 \pm 6.546			
Group III	4.000–99.000	28.000 \pm 30.540			
Group IV	29.000–91.000	50.900 \pm 18.424			
Tukey's test					
I and II	I and III	I and IV	II and III	II and IV	III and IV
0.999	0.019	<0.001	0.026	<0.001	0.039

ANOVA, analysis of variance.

Table 3 Range, mean value, SD, and statistical comparison of thiobarbituric acid-reactive substances (nmol/ml) in the four groups studied

Group	TBARS (nmol/ml)		ANOVA		
	Range	Mean \pm SD	<i>F</i>	<i>P</i> -value	
Group I	5.600–14.600	9.900 \pm 3.045	8.579	<0.001	
Group II	8.100–21.200	14.920 \pm 4.019			
Group III	14.200–30.100	20.500 \pm 5.220			
Group IV	12.400–46.800	23.080 \pm 10.432			
Tukey's test					
I and II	I and III	I and IV	II and III	II and IV	III and IV
0.306	0.004	<0.001	0.221	0.033	0.801

ANOVA, analysis of variance; TBARS, thiobarbituric acid-reactive substances.

Table 4 Range, mean value, SD, and statistical comparison of superoxide dismutase (U/ml) in the four groups studied

Group	SOD (U/ml)		ANOVA		
	Range	Mean \pm SD	<i>F</i>	<i>P</i> -value	
Group I	79.800–180.400	123.940 \pm 34.749	9.319	<0.001	
Group II	78.600–132.800	109.540 \pm 15.179			
Group III	61.200–121.400	95.550 \pm 18.911			
Group IV	45.700–97.400	71.220 \pm 19.187			
Tukey's test					
I and II	I and III	I and IV	II and III	II and IV	III and IV
0.517	0.046	<0.001	0.541	0.004	0.108

ANOVA, analysis of variance; SOD, superoxide dismutase.

Table 5 Range, mean value, SD, and statistical comparison of the obstructive apnea index before and during continuous positive airway pressure autotitration in group IV

Group	Obstructive apnea index		Paired differences		Paired samples test	
	Range	Mean \pm SD	Mean	SD	<i>t</i>	<i>P</i> -value
Before	7.000–38.000	21.400 \pm 10.532	8.577	2.712	6.157	<0.001
After one night	2.000–9.000	4.700 \pm 2.452				

Table 6 Range, mean value, SD, and statistical comparison of percent of total sleep time in which O₂ saturation less than 90% before and one night after continuous positive airway pressure therapy in group IV

CPAP autotitration	Percent of total sleep time in which O ₂ saturation < 90%		Paired differences		Paired samples test	
	Range	Mean ± SD	Mean	SD	<i>t</i>	<i>P</i> -value
Before	29.000–91.000	50.900 ± 18.424	18.615	5.886	7.220	<0.001
After one night	6.000–13.000	8.400 ± 2.914				

Table 7 Range, mean value, SD, and statistical comparison of thiobarbituric acid-reactive substances (nmol/ml) before, one night, and seven nights after continuous positive airway pressure therapy in group IV

CPAP therapy	TBARS nmol/ml			Paired differences		Paired samples test	
	Range	Mean ± SD		Mean	SD	<i>t</i>	<i>P</i> -value
Before	12.400–46.800	23.080 ± 10.432	<i>P</i> ₁	3.611	1.142	5.675	<0.001
After one night	9.900–32.800	16.600 ± 8.288	<i>P</i> ₂	6.316	1.997	6.819	<0.001
After seven nights	3.300–22.800	9.460 ± 6.400	<i>P</i> ₃	3.453	1.092	6.538	<0.001

TBARS, thiobarbituric acid-reactive substances.

Table 8 Range, mean value, SD, and statistical comparison of superoxide dismutase (U/ml) before, one night, and seven nights after continuous positive airway pressure therapy in group IV

CPAP therapy	SOD (U/ml)			Paired differences		Paired samples test	
	Range	Mean ± SD		Mean	SD	<i>t</i>	<i>P</i> -value
Before	45.700–97.400	71.220 ± 19.187	<i>P</i> ₁	6.608	2.090	-4.762	0.001
After one night	57.400–107.000	81.170 ± 17.737	<i>P</i> ₂	18.088	5.720	-7.588	<0.001
After seven nights	89.400–148.000	114.620 ± 18.326	<i>P</i> ₃	17.186	5.435	-6.155	<0.001

SOD, superoxide dismutase.

health concern affecting up to 5% of middle-aged men and women in the general population. This is a serious health hazard, being recognized as an independent risk factor for hypertension, arrhythmias, and coronary heart disease [13].

OSA patients experience repeated episodes of hypoxia (which can last for 10 s to as long as 2 min) and normoxia (2–3 min). During the hypoxic phase, cells adapt to a low oxygen environment. However, the reoxygenation phase causes a sudden increase in oxygen in the cells. This reoxygenation phase is considered to result in the production of ROS and the promotion of oxidative stress [11].

The present study showed that oxidative stress markers (TBARS and SOD) were significantly different between the four groups studied. Plasma levels of TBARS were significantly lower in the control group than in OSA patients, whereas plasma levels of SOD were significantly higher in the control group than in the OSA patients. These results were in agreement with those reported by Lavie *et al.* [14]; the morning plasma level of TBARS was found to be significantly higher in OSA patients with and without cardiovascular disease (CVD) than in nonapneic controls. Wysocka *et al.* [15] confirmed the above results. They found decreased SOD activity in the overweight OSAS patients compared with the overweight controls, but no significant difference in TBARS between

OSAS patients and controls. However, among obese participants, decreased SOD activity was found in obese OSAS patients compared with obese controls and increased TBARS concentration in obese OSAS patients compared with obese controls. Liu *et al.* [16] showed that plasma SOD activity was significantly lower in 107 patients with OSAHS than 69 control participants. Indices of oxidative stress increased in OSA patients for several reasons; first, sleep apneic patients are subjected repetitively each night to disturbed hypoxic sleep. These episodes of hypoxia/reoxygenation could facilitate free radical production, which would lead to lipid peroxidation and vascular damage. Second, increased inflammatory leukocytes in OSA patients have been shown to trigger free radical production. Third, catecholamine-induced changes, secondary to increased sympathetic nerve activity in OSA, can promote lipid peroxidation. Finally, long-term sleep deprivation has been shown to activate lipid oxidation, inhibit antioxidant defense systems, and inactivate mitochondrial enzymes [17,18]. In contrast to our findings Alzoughaibi *et al.* [19] reported no significant difference between severe OSA patients and controls in both SOD and TBARS. Savtikova *et al.* [20] measured plasma indices of oxidative stress and lipid peroxidation: TBARS, oxidized LDL, and isoprostanes in 41 men with moderate–severe OSA without other diseases and in 35 matched controls before sleep, and then after treatment with CPAP. Plasma levels of the three markers were similar in

patients with moderate–severe OSA and in the controls. The authors proposed that the absence of any evidence for oxidative stress in OSA patients is unlikely to be explained by a compensatory increase in the activity of antioxidative enzymes in these patients. Although their study was not designed to measure antioxidants, two preliminary reports suggested that antioxidant defense mechanisms are unaffected or even decreased in OSA. Wali *et al.* [21] reported no significant differences in glutathione peroxidase and catalase activities in red blood cells in hypoxic and non-hypoxic patients and Christou *et al.* [22] reported that in 14 patients with severe OSA (AHI>20), antioxidant capacity was reduced. Therefore, the lack of increased oxidative stress and lipid peroxidation in sleep apneic patients suggests that in the absence of significant comorbidities, sleep apnea does not initiate the generation of oxidative stress or lipid peroxidation. The main finding of this study is that short-term CPAP therapy in patients with severe OSA notably improved oxidative stress markers in these patients. We found a significant decrease in the plasma levels of TBARS after one night and seven nights of CPAP therapy and a significant increase in the plasma levels of SOD after one night and seven nights of CPAP therapy. In most studies, the CPAP treatment had been administered for periods ranging from 3 to 12 months, which may be bothersome to the patients. There is negligible information in the literature on the effects of short-term CPAP treatment on oxidative stress. Our results are consistent with those found by Singh *et al.* [23], who studied the effect of CPAP therapy for two nights on oxidative stress markers (lipid peroxidation by TBARS and antioxidant capacity by total reduced glutathione enzyme) in 20 male OSA patients. They repeated the measurement of both markers after 45 days of oral intake of antioxidant vitamins C and E. The baseline TBARS level was significantly higher in OSA patients compared with the control participants and CPAP therapy for two nights reduced the TBARS level significantly. The baseline glutathione levels were significantly lower in OSA patients compared with the control participants, and CPAP therapy for two nights led to a significant increase in the levels.

Not in complete agreement with our results, Alzoughaibi *et al.* [24] concluded that CPAP therapy decreases the levels of lipid peroxidation in OSA patients, but may not affect antioxidant defense after they studied the effects of one night of CPAP therapy on oxidative stress (lipid peroxidation) levels and the antioxidant activities of SOD in 34 hypertensive patients with severe OSA. However, Svatikova *et al.* [20] showed that the level of TBARS in sleep apneics was similar to that in controls after 4 h of effective treatment with CPAP.

Conclusion

OSA leads to increased oxidative stress, which was improved with the use of CPAP.

Acknowledgements

Conflicts of interest

None declared.

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