

## Evaluation of Dynamic Thiol/Disulfide Homeostasis in Patients with Obstructive Sleep Apnea

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### Abstract

**Background and Objective:** Hypoxia-reoxygenation cycles may cause oxidative stress in Obstructive sleep apnea (OSA). We aimed in the study to evaluate the OSA association with oxidative stress, using thiol-disulfide homeostasis method. We also evaluated the correlations between oxygen desaturation index (ODI) and oksidative stress.

**Patients and Methods:** Patients with OSA group described as obstructive apnea/hypopnea index (AHI)  $\geq 5$ . Healthy control group were chosen from our outpatient clinic who never snored with AHI  $< 5/h$ . Patients with smoking history, infectious disease, comorbidities that increase oxidative stress and using any antioxidant agent were excluded. After excluded patients with high BMI ( $\geq 31$  kg/m<sup>2</sup>) which may affect the thiol-disulfide homeostasis and matched the groups in terms of mean age, sex and BMI in the final study population there were 20 patients with OSA and 17 patients with controls.

**Results:** The disulfide level and ratios were significantly higher and mean native/total thiol ratio was significantly lower in the OSA group than in the control group ( $p < 0.001$ ). When we compare AHI  $< 30$  and AHI  $\geq 30$  while native thiol was significantly higher ( $p = 0.034$ ), disulfide ratios were lower in severe group but not statistically significant ( $p = 0.052$ ). Although we did not find any correlation between AHI and thiol/disulfide homeostasis we explored negative correlations between the ODI and the disulfide level, ratios and positive correlations between the ODI and the native/total thiol ratio.

**Conclusion:** Thiol/disulfide homeostasis can be a indicator of oxidative stress in OSA. By increasing of OSA severity reduction of oxidative stress, was shown in our study, suggests that some factors like duration of disease and compensation in early time that influence the thiol levels might be.

**Keywords:** Thiol; Disulfide; Oxidative Stres; Obstructive Sleep Apnea

### Introduction

Obstructive sleep apnea (OSA) is characterized by episodes of complete or partial upper-airway obstruction occurring during sleep resulting with apnea or hypopnea. Each episode of apnea or hypopnea is usually followed by a decrease in arterial oxygen saturation which rapidly normalizes after ventilation. This hypoxia-reoxygenation cycles may cause oxidative stress and than development of vascular injury and endothelial dysfunction can form the basis of cardiovascular disease [1,2]. A number of studies have shown that markers of oxidative stress are associated with OSA [3,4]. Because of unstability of reactive oxygen metabolites (ROS) it is very difficult to measure them directly. Indirect markers include lipid peroxidation products, oxidized protein, amino acids, peptides and DNA. Thiol groups (-SH),

situated in amino acids such as sulfur containing cysteine, methionine, homocysteine, glutathione and contribute to oxidation reactions, by forming disulfide (-S-S) bonds. Dynamic thiol/disulfide homeostasis is play a role in detoxification, apoptosis, regulation of signaling pathways and enzymatic reactions [5,6]. But increasing release of oxygen-free radicals beyond physiological antioxidant capacity, abnormal thiol/disulfide concentrations can be seen associated with oxidative stress in OSA. For measuring thiol disulfide levels one by one and cumulatively method can be used. Thiol-disulfide homeostasis is a easy and new method to measure oxidative stress [7].

On the basis of these considerations, we performed the prospective case control study to confirm that the presence and the severity of OSA is associated with oxidative stress, using thiol-disulfide homeostasis method. We also evaluated the correlations between oxygen desaturation index (ODI) and oxidative stress.

### Methods

#### Patients and controls

This prospective case control study was conducted in the Sleep Laboratory of Sureyyapaşa Chest Disease and Thoracic Surgery Training and Research Hospital, Istanbul, Turkey; the study was approved by the local Ethics Committee of Yıldırım Beyazıt University School of Medicine, Department of Clinical Biochemistry, Ankara, Turkey and adhered to the tenets of the Declaration of Helsinki and informed consents were obtained from the all participants. 44 patients who had signs and symptoms of a sleep disturbance including snoring, mouth breathing, and witnessed breath holding were included in the study and an overnight polysomnography (PSG) was done. The objective criteria for the diagnosis of OSA based on PSG were an obstructive apnea/hypopnea index (AHI) equal to or greater than 5. In addition, 38 healthy persons that attended to our outpatient clinic who never snored as reported by their parents were also included in this study and AHI < 5/h were considered as the control group. Patients with smoking history, infectious disease, comorbidities that increase oxidative stress (diabetes mellitus, cardiovascular disease, hypertension, thyroid disorders, chronic obstructive pulmonary disease, asthma, nasal polyposis), malignancy, autoimmune disease, renal insufficiency, chronic liver diseases, chronic inflammatory disease; and using any antioxidant agent were excluded. We have noticed that 24 of the OSA patients (54.5%) and 9 of the control groups (23.7%) BMI were  $\geq 31$  kg/m<sup>2</sup> and also we found that there was a significant correlation between BMI and oxidative parameters (native thiol and total thiol;  $p < 0.01$ ). We have also excluded patients with smaller than 27 years in control group for matching the groups. So there is not any significant difference between the groups in terms of mean age, sex and BMI.

#### Polysomnography

All subjects were monitored with a nocturnal polysomnography which was performed with multichannel monitoring that includes neurophysiological electrodes (electroencephalography electrodes), chest wall motion, abdominal motion, arterial oxygen saturation, and electrocardiography electrodes (Grass- Telefactor Cephalo, An Astro-med Inc. Product Group, 2005, USA). Oronasal airflow was measured by a thermistor. The oxyhemoglobin saturation was monitored with a finger pulse oximeter with a sampling rate of 1 Hz. The body position was measured by a position sensor attached to the anterior chest wall. Signals recorded in the sleep period were manually analyzed. Apneas were scored when the airflow decreased by at least 90% from the baseline for at least 10 s and classified as central, mixed, or obstructive depending on the occurrence of thoracoabdominal movements [8]. Hypopneas were scored when airflow decreased by at least 30% for  $\geq 10$ s and were associated with a SpO<sub>2</sub> (oxygen saturation) fall  $\geq 3\%$ . Apnea/ hypopnea index (AHI) was calculated as the average number of apneas and hypopneas per hour of recording in the sleep period. Oxygen desaturation index (ODI) is the hourly average number of desaturation episodes, which are defined as at least 4% decrease in saturation from the average saturation in the preceding 120 seconds, and lasting 10 seconds [9]. SpO<sub>2</sub> in the sleep period was automatically analyzed, and after manual elimination of possible artifacts, mean SpO<sub>2</sub> and minimum nocturnal SpO<sub>2</sub> values were detected. Subjects with AHI  $\geq 5$ /h were considered to have OSA; AHI = 5-15/h: mild, 15-30/h: moderate, > 30/h: severe OSA was defined.

#### Biochemical analysis

Venous blood samples were collected from the patients with OSA before polysomnography and from the controls at baseline. Samples were centrifuged in the cold at 2300 x g for 10 minutes and stored at -80°C. Serum thiol-disulfide homeostasis measurement method was

used by an automated clinical chemistry analyser (Roche, cobas 501, Mannheim, Germany) [7]. Native thiol (-SH) and total thiol (-SH + -SS-) were measured directly, and disulfide (-S-S-) level, disulfide/ total thiol ratio (-S-S-/-SH + -S-S-), disulfide/native thiol ratio (-SS-/-SH) and native/total thiol ratio (-SH /-SH + -S-S-) were calculated [7].

**Statistical Analysis**

The statistical analyses of our study were done using SPSS program (SPSS inc.,IL, USA). If normally distribution was observed the descriptive data were given as mean ± standard deviation if not median and (25 - 75 percentile) were expressed. Categorical variables are shown as numbers and percentages. Thiol/disulfide homeostasis in blood were statistically compared with control group using Student’s t-test. The test was also used for comparisons of OSA severity in spite of Thiol/ disulfide homeostasis. The significance was taken as p < 0.05. The relationship between ODI and oxidative parameters were investigated with pearson correlation test.

**Results**

The initial study was comprised of 44 patients with OSA and healthy control group including 38 patients. After excluded patients with high BMI (31 ≥ kg/m<sup>2</sup>) and matched the groups in terms of mean age, sex and BMI in the final study population there were 20 patients with OSA and 17 patients with controls. The demographic characteristics of the OSA patients and control group are summarized in table 1.

	<b>OSA group (n = 20)</b>	<b>Control group (n = 17)</b>	<b>p value*</b>
Age	45.65 ± 10.69	42.12 ± 8.17	0.27
Sex: Male/ Female	17/3	13/4	0.68
BMI; kg/m <sup>2</sup>	27.25 ± 2.22	27.31± 2.62	0.93
AHI Mean	37.19 ± 24.51		
Median (%25-%75)	37.09 (15.14-50.25)		
ODI	36.27 ± 21		

**Table 1:** Demographic and clinical characteristics of the OSA and control groups.

Abbreviations: OSA: Obstructive Sleep Apnea; BMI: 5 Body Mass Index; AHI: Apnea Hypopnea Index; ODI: Oxygen Desaturation Index.

Values are reported as mean ± standard deviation , because of the not normally distribution AHI were also given median and %25 - 75 percentile.

\*P value < 0.05 considered significant.

When we compare the groups in terms of oxidative parameters as seen in table 2; while native thiol and total thiol were not different between the groups, the disulfide level, the disulfide/native thiol, disulfide/total thiol ratios were significantly higher and mean native/total thiol ratio was significantly lower in the OSA group than in the control group (p < 0.001).

	<b>OSA group (n = 20)</b>	<b>Control group (n = 17)</b>	<b>p value*</b>
-SH mmol l <sup>-1</sup>	314.34 ± 45.65	303.17 ± 50.72	0.48
-SH + -SS- mmol l <sup>-1</sup>	354.75 ± 42.16	328.25 ± 54.46	0.10
-SS- mmol l <sup>-1</sup>	20.20 ± 8.07	12.53 ± 4.89	0.002
-SS- / -SH,%	6.72 ± 3.20	4.17 ± 1.66	0.006
-SS-/-SH + -SS-,%	5.79 ± 2.43	3.81 ± 1.40	0.006
-SH /-SH + -SS-,%	88.41 ± 4.87	92.37 ± 2.81	0.006

**Table 2:** Thiol/disulphide levels and ratios between the OSA and control groups.

Abbreviations: -SH: Native Thiol; -SH + -S-S-: Total Thiol; -S-S-: Disulfide.

Results were given as mean ± sd.

\*P value < 0.05 considered significant. Bolds are statistically significant

When we grouped OSA patients according to severity; 4 patients mild, 4 moderate and 12 were severe. As seen in table 3 to be comparable we divide patients into two groups (moderate + mild group: AHI < 30 and severe group: AHI ≥ 30). While native thiol was significantly higher (p = 0.034), disulfide/native thiol and disulfide/total thiol ratios were lower in severe group but not statistically significant (p = 0.052).

	AHI < 30 (n = 20)	AHI 30 ≥ (n = 17)	p value*
-SH mmol l <sup>-1</sup>	288.42 ± 52.51	331.62 ± 32.11	0.034
-SH + -SS- mmol l <sup>-1</sup>	335.23 ± 54.83	367.77 ± 26.36	0.091
-SS- mmol l <sup>-1</sup>	23.40 ± 6.18	18.07 ± 8.70	0.153
-SS- / -SH, %	8.34 ± 2.66	5.63 ± 3.17	0.062
-SS- / -SH + -SS-, %	7.07 ± 1.94	4.93 ± 2.42	0.052
-SH / -SH + -SS-, %	85.84 ± 3.88	90.12 ± 4.85	0.052

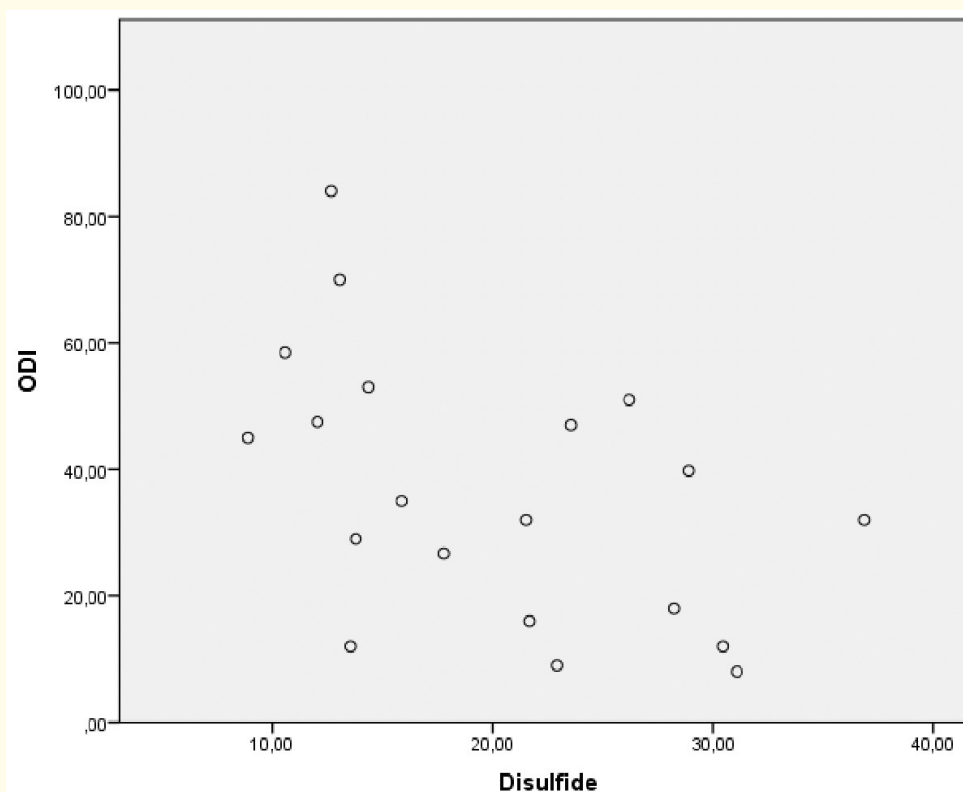
**Table 3:** The mean blood levels of thiol/disulfide homeostasis of the study group according to OSA severity.

Abbreviations: -SH: Native Thiol; -SH + -S-S-: Total Thiol; -S-S-: Disulfide.

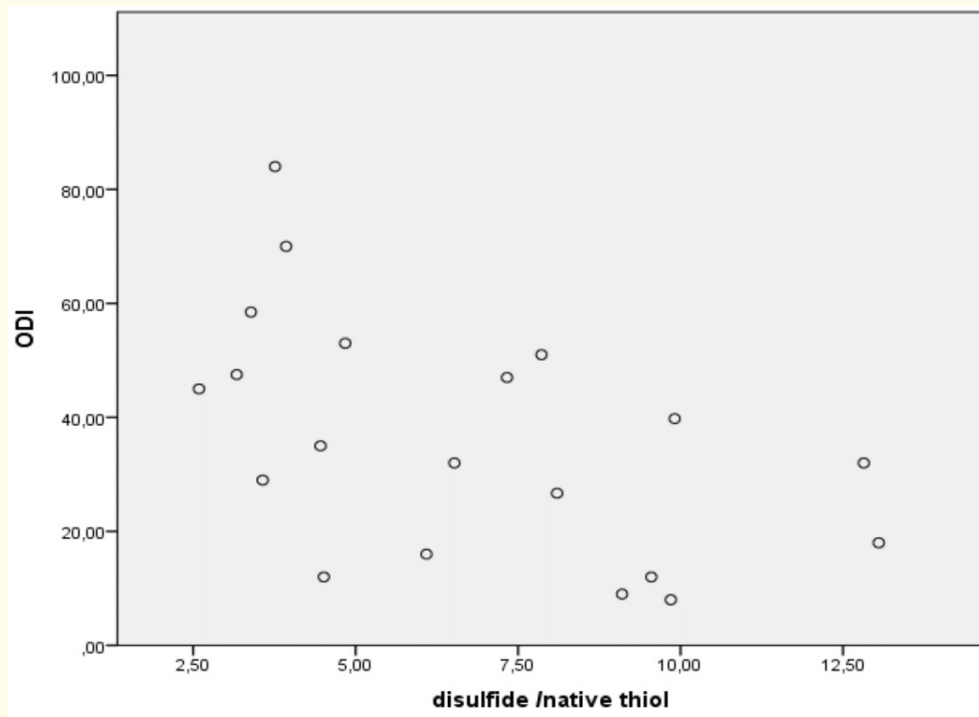
Results were given as mean ± sd.

\*P value < 0.05 considered significant. Bolds are statistically significant

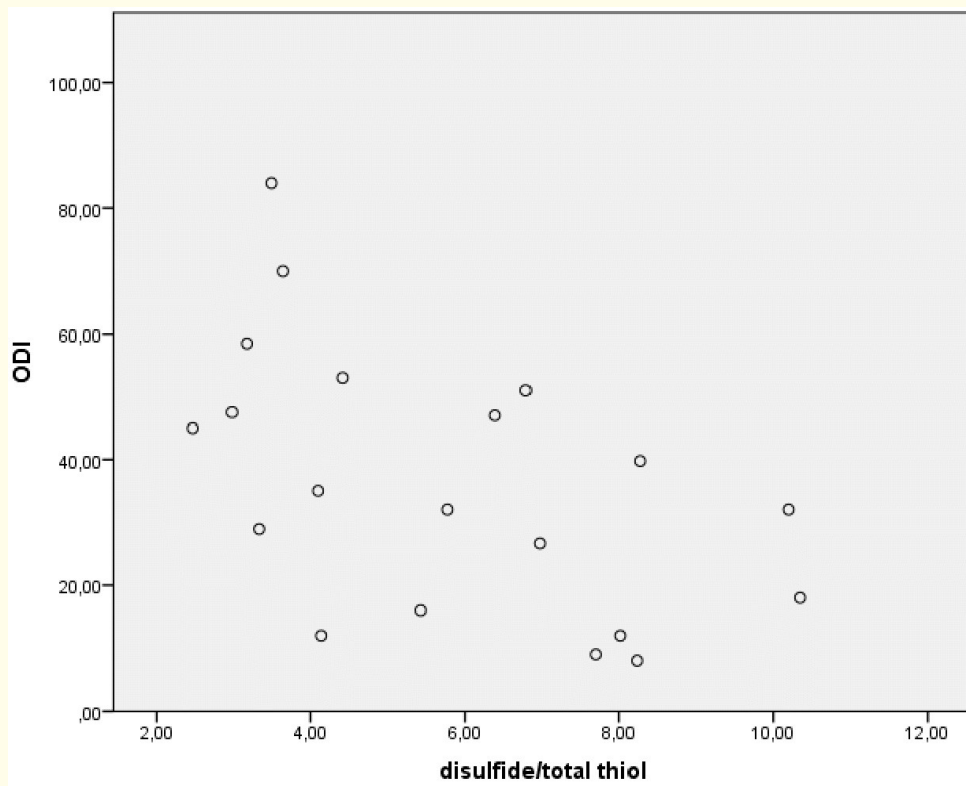
Although we did not find any correlation between AHI and thiol/disulfide homeostasis we explored correlations between the ODI and thiol/disulfide homeostasis. We found significant negative correlations between the ODI and the disulfide level, disulfide/native thiol, disulfide/total thiol ratios (r = -0.495, p = 0.027 vs r = -0.520, p = 0.019 vs r = -0.529 p = 0.016) (Figures 1-3). We found no significant correlation between the ODI and native thiol and total thiol level. We found significant positive correlations between the ODI and the native/total thiol ratio (r = 0.529, p = 0.017) (Figure 4).



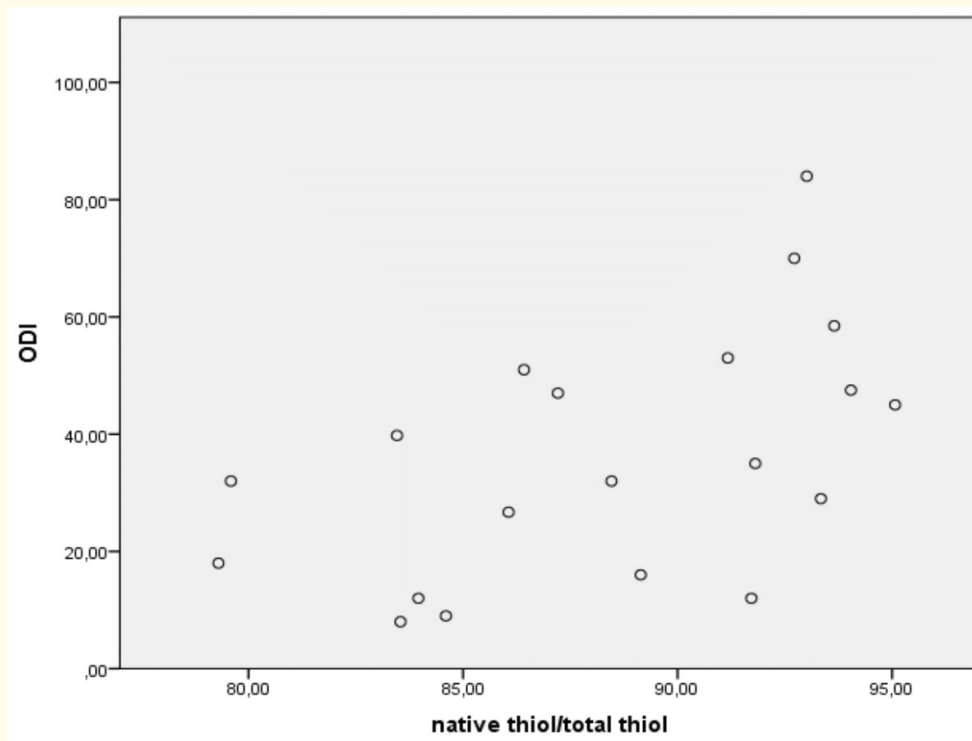
**Figure 1:** There was a significant negative correlation between ODI and disulfide levels in obstructive sleep apnea patients. ODI: Oxygen desaturation index, disulfide; -S-S-.



**Figure 2:** There was a significant negative correlation between ODI and disulfide /native thiol ratio in obstructive sleep apnea patients . ODI: Oxygen desaturation index , disulfide /native thiol ratio: -S-S-/ -SH.



**Figure 3:** There was a significant negative correlation between ODI and disulfide /total thiol ratios in obstructive sleep apnea patients. ODI: Oxygen desaturation index, disulfide/total thiol: -S-S-/ -SH + -S-S-.



**Figure 4:** There was a significant positive correlation between ODI and native/total thiol ratio in obstructive sleep apnea patients. ODI: Oxygen desaturation index, native/total thiol: -SH/ -SH + -S-S-.

**Discussion**

In this study, higher disulfide levels and ratios were demonstrated in patients with OSA compared to control group. When patients with AHI < 30 and AHI 30 ≥ were compared interestingly we have found that native thiol level and ratio which are important antioxidants; were significantly higher in severe OSA (AHI 30≥). There was not any confounder like BMI differences between the OSA groups that explain this situation. Moreover we have found that ODI was significantly negative correlated with disulfide levels and ratios and positive correlated with native/total thiol ratio. So we have demonstrated that when the the severity of oxygen desaturation increased oxidant antioxidant balance is shifted to the side of the native thiol which protects against oxidative stress.

Many studies have shown that oxidative stress levels are elevated in OSA patients compared to healthy subjects [10-12]. But there is a few study investigate thiol-disulfide homeostasis in patients with OSA. Compliance with our work which is the first study show the thiol / disulfide homeostasis as a novel marker of oxidative stress, conducted by Dinc ME., *et al.* found the disulfide level and disulfide/total thiol ratios were significantly higher in patients with OSA than in controls [13]. But in their study the OSA groups BMI was significantly higher than in simple snoring controls (38.39 to 30.03 kg/m<sup>2</sup>) and they did not find any differences between the second control OSA group which have similar BMI with snoring controls. In anouther study conducted in children with adenotonsillar hypertrophy and diagnosed OSA excluded BMI at the 95<sup>th</sup> percentile or higher was demonstrated that disulfide level and ratios were higher than in controls [14]. But contrast to our study they found that disulfide levels and ratios increased as the severity of OSA increased. Passali and colleagues investigated

oxidative stress in severe OSA (AHI > 30) with not only thiol/disulfide levels but also non-protein bound iron (NPBI), plasma isoprostane levels, advanced oxidation protein products (AOPP), urine isoprostane [15]. They found that only thiol/disulfide levels were not different between OSA and controls; 0% sensitivity and 100% specificity were analyzed for thiols. They thought that thiols may be a late rather than an early marker of oxidative stress. Another reason that is why that they evaluated only patients with severe OSA. In our study severe patients have significantly higher native thiol level and ratio which is a significant antioxidant. Moreover, reduction of disulfide level and ratios which are a sign of oxidative stress by increasing ODI may be depend on the compensatory mechanism.

The limitation of our study is at the beginning many of our patients with OSA who do not have any comorbide disease might have high BMI and control group have younger patients and so we had to exclude most of the patients to ensure the homogeneity of the groups. Another limitation is we did not evaluate change of oxidative parameters after CPAP therapy.

### Conclusion

Thiol/disulfide homeostasis can be a indicator of oxidative stress in OSA. But most of the OSA patients may have high BMI so it should not be forgotten that the measurements may be affected by this situation. In the reason of sensitivity of thiol measurements in severe OSA is 0% detected before and reduction of oxidative stress, by increasing of OSA severity, was shown in our study, suggests that some factors like duration of disease and compensation in early time that influence the thiol levels might be.

### Conflict of Interest

None declared.

### Bibliography

1. Bradley TD and Floras JS. "Sleep apnea and heart failure. Part I: Obstructive sleep apnea". *Circulation* 107.12 (2003): 1671-1678.
2. Lanfranchi P and Somers VK. "Obstructive sleep apnea and vascular disease". *Respiratory Research* 2.6 (2001): 315-319.
3. Lavie L. "Obstructive sleep apnoea syndrome: an oxidative stress disorder". *Sleep Medicine Reviews* 7.1 (2003): 35-51.
4. Suzuki YJ, *et al.* "Oxidative stress and oxidant signaling in obstructive sleep apnea and associated cardiovascular diseases". *Free Radical Biology and Medicine* 40.10 (2006): 1683-1692.
5. Witko-Sarsat, *et al.* "Advanced oxidation protein products as a novel marker of oxidative stress in uremia". *Kidney International* 49.5 (1996): 1304-1313.
6. IA Cotgreave and RG Gerdes. "Recent trends in glutathione biochemistry glutathione-protein interactions: a molecular link between oxidative stress and cell proliferation?" *Biochemical and Biophysical Research Communications* 242.1 (1998): 1-9.
7. Erel and S Neselioglu. "A novel and automated assay for thiol/disulfide homeostasis". *Clinical Biochemistry* 47.18 (2014): 326-332.
8. Silber MH, *et al.* "The visual scoring of sleep in adults". *Journal of Clinical Sleep Medicine* 3.2 (2007): 121-131.
9. Berry RB, *et al.* "Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine". *Journal of Clinical Sleep Medicine* 8.5 (2012): 597-619.
10. Lavie L, *et al.* "Evidence for lipid peroxidation in obstructive sleep apnea". *Sleep* 27.1 (2004): 123-128.
11. Barcelo A, *et al.* "Abnormal lipid peroxidation in patients with sleep apnoea". *European Respiratory Journal* 16.4 (2000): 644-647.

12. Carpagnano GE., *et al.* "8-Isoprostane, a marker of oxidative stress, is increased in exhaled breath condensate of patients with obstructive sleep apnea after night and is reduced by continuous positive airway pressure therapy". *Chest* 124.4 (2003): 1386-1392.
13. Dinc ME., *et al.* "Thiol/Disulfide Homeostasis As a Novel Indicator of Oxidative Stress in Obstructive Sleep Apnea Patients". *Laryngoscope* 127.7 (2016): E244-E250.
14. Gul F., *et al.* "A novel method for evaluation of oxidative stress in children with OSA". *International Journal of Pediatric Otorhinolaryngology* 89 (2016): 76-80.
15. Passali D., *et al.* "Oxidative stress in patients with obstructive sleep apnoea syndrome". *Acta Otorhinolaryngologica Italica* 35.6 (2015): 420-425.

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