

## Investigation of Lipid Peroxidation and Antioxidant Enzyme Activity in Sleep Apnea Patients

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### Abstract

**Objective:** The goal of this study was to investigate the activities of malondialdehyde (MDA), a product of lipid peroxidation, and glutathione peroxidase (GPx), an antioxidant enzyme, in sleep apnea patients.

**Material and Method:** The study involved 40 healthy control patients and 40 sleep apnea patients. Participants in the experimental group were between the ages of 18 and 65, were diagnosed and treated in the Respiratory Diseases Clinic of Yüzüncü Yıl University Medical Faculty. Malondialdehyde (MDA) and glutathione peroxidase (GPx) levels in blood samples were measured using spectrophotometry.

**Results:** According to our results, the difference in the enzyme activity of glutathione peroxidase (GPx) between the experimental group ( $0.03 \pm 0.04$  U/ml) and the control group ( $0.06 \pm 0.03$  U/ml) was significant ( $p < 0.001$ ). MDA levels were determined to be  $3.292$  U/L ( $\pm 0.724$  U/L) in the experimental group and  $0.882$  U/L ( $\pm 0.226$ ) in the control group ( $p < 0.001$ ).

**Conclusion:** Based on our results, we can conclude that sleep apnea causes a decrease in the GPx enzyme, by means of the body's antioxidant defence system and an increase in malondialdehyde, a symptom of oxidative stress.

**Key Words:** Sleep Apnea, MDA, GPx

### Introduction

Sleep apnea syndrome (SAS) is the involuntary cessation of respiration during sleep for a minimum of 10 seconds, with a reduction in the amount of oxygen in the blood due to obstruction of the upper respiratory tract. It is characterized by the occurrence of hypopnea, in which respiration decreases by more than 50% at least 5 times per hour. These metabolic changes trigger oxidative stress and systemic inflammation that subsequently cause the release of reactive oxygen species, anti-oxidant enzymes and inflammatory indicators (1).

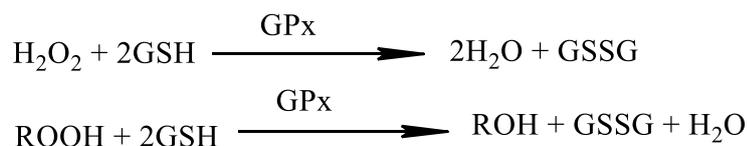
When free radicals exceed the antioxidant capacity of cells, lipid peroxidation occurs. Lipid peroxidation results in the transformation of lipid hydroperoxides into aldehydes and other carbonyl compounds. One of the end products of lipid peroxidation is malondialdehyde (MDA), which is frequently used to determine lipid peroxide activity (2).

Sleep apnea indicates the presence of high oxidative stress levels (1-3) and decreased antioxidant enzyme activity (4). Increased oxidative stress has also been observed in sleep apnea patients (5).

Antioxidants are grouped into two categories: enzymatic and non-enzymatic. Enzymatic antioxidants include superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), while vitamins E, C, and A, selenium, transferrin, and lactoferrin are non-enzymatic antioxidants. Antioxidants are either endogenous or exogenous (7-8-9).



Glutathione peroxidase (GSH-Px; E.C. 1.11.1.9), a cytosolic enzyme, is responsible for the reduction of hydroperoxides. Erythrocytes are the most effective antioxidant enzymes against GPx oxidative stress, and phagocytic cells have some important immune functions (7).



Glutathione peroxidase catalyzes the detoxification of  $\text{H}_2\text{O}_2$  and lipid peroxides together with reduced glutathione, thus protecting membrane lipids and hemoglobin against oxidation by peroxides. GSH-Px also plays a role in the excretion of drugs and other substances which are foreign to a living system. In mammalian cells, GSH-Px is the antioxidant enzyme system which provides the most important defense against peroxidative damage of biological membranes (8).

The goal of this study was to observe changes in levels of glutathione peroxidase (GPx) and malondialdehyde (MDA) in the blood serum of sleep apnea patients.

## Material and Methods

The study participants included 40 healthy control patients and 40 sleep apnea patients between the ages of 18 and 65 who had been diagnosed and treated at the Respiratory Diseases Clinic at Yüzüncü Yıl University Medical Faculty. Before blood samples were taken, approval was received from both the Education and Research Hospital and the Laboratory Research Ethical Board of Yüzüncü Yıl University Medical Faculty. Venous blood in the amount of 3 ml was drawn from the patients and healthy subjects and then subjected to centrifugation for approximately 5 minutes at 5,000 rpm/min. The serum and plasma were subsequently separated and stored at  $-45^\circ\text{C}$  until analysis.

Glutathione peroxidase activity and malondialdehyde levels were determined from blood serum samples taken from the study participants.

### Determination of glutathione peroxidase activity

Beutler's method was used to determine glutathione peroxidase activity. This method is based on monitoring the activity of glutathione peroxidase (glutathione  $\text{H}_2\text{O}_2$  oxidoreductase, EC 1.11.1.9), which catalyzes the oxidation of reduced glutathione to oxidized glutathione, a product of the reaction with hydrogen peroxide. The glutathione reductase (GSH-R) enzyme then reduces oxidized glutathione to GSH by the presence of NADPH, and the decrease in NADPH absorbance is measured at 340 nm (9).

### Determination of malondialdehyde (MDA) activity

Malondialdehyde, one of the products of peroxidation formed by the reaction of fatty acids with free radicals, is measured using thiobarbituric acid; the fluorescence of the resulting derivative is then determined via spectrophotometry. 200  $\mu\text{l}$  of whole blood was taken from a tube. 800  $\mu\text{l}$  of phosphate buffer, 25  $\mu\text{l}$  of BHT solution and 500  $\mu\text{l}$  of 30% TCA were then added to the whole blood. The tubes were mixed in a vortex and stored on ice for 2 hours. Next, they were placed in a centrifuge for 15 minutes at 2,000 rpm. 1 ml of the supernatant was taken and transferred to other tubes. 75  $\mu\text{l}$  of EDTA and 250  $\mu\text{l}$  of TBA were added to these tubes. The tubes were mixed in a vortex and placed in a hot water bath for 15 minutes. Finally, the tubes were brought to room temperature and absorbance was read on a UV/Vis spectrophotometer at 532 nm (10).

### Statistical Analysis

The key statistics are expressed as the mean and standard deviation. In comparing the two groups, the T-test was used where normal distribution obtained, while the Mann Whitney U test was used where normal distribution did not obtain. Statistical significance was set at a level of 5% and the SPSS statistical package program was used for the calculations.

## Results

According to our findings, GPx enzyme activities (Table 1) of the experimental group ( $0.03 \pm 0.04$  U/ml) were lower than that of the control group ( $0.06 \pm 0.03$  U/ml). This difference was determined to be statistically significant ( $p < 0.001$ ).

MDA (malondialdehyde) levels were found to be significantly elevated in the experimental group ( $3.292$  U/L  $\pm$   $0.724$ ) than in the control group ( $0.882$  U/L  $\pm$   $0.226$ ) ( $p < 0.001$ ).

**Table 1:** GPx serum activities and MDA levels of the study population.

Parameters	Patients $\pm$ SEM(n=40)	Control $\pm$ SEM(n=40)
MDA ( $\mu$ mol/L)	$0.882 \pm 0.226^*$	$3.292 \pm 0.724^*$
GPx (U/L)	$0.06 \pm 0.03^*$	$0.03 \pm 0.04^*$

\*:  $p < 0.001$

## Discussion

Obstructive sleep apnea syndrome is a respiratory disorder characterized by recurring attacks of hypoxia and reoxygenation during sleep. The brain attempts to activate the sympathetic nervous system in order to awaken sufferers of sleep apnea to prevent recurring attacks of hypoxia. Hypoxia and an overactive sympathetic nervous system lead to oxidative stress and thus disrupt the oxidant-antioxidant balance (23-24-25).

Antioxidants inhibit, reduce and/or delay the effects of oxidation in proteins found in living cells, lipids, carbohydrates and DNA. The mechanism by which antioxidants function is known as antioxidant defense. There are several defense mechanisms aimed at prevention of the formation of reactive oxygen species (ROS) and the damage that they cause. These mechanisms, as stated above, are referred to as "antioxidant defense systems" or simply "antioxidants". Antioxidants act in four distinct ways:

- 1) By interacting with free oxygen radicals, either by holding onto them or transforming them into new weaker molecules, they have a cumulative effect.
- 2) Antioxidants have a suppressive effect by interacting with free oxygen radicals by transferring hydrogen to them, reducing their activities, or rendering them inactive. Vitamins and flavonoids have a suppressive effect.
- 3) By linking free oxygen radicals, antioxidants break their chains and have an inhibitory effect. Hemoglobin, seruloplasm, and minerals possess this chain-breaking effect.
- 4) Finally, antioxidants, by repairing the damage caused by free radicals, have a reparative effect (6).

The general definition of lipid peroxidation is the breakdown of membrane lipids due to oxidative damage. The unsaturated bonds of cholesterol and fatty acids in cell membranes generate products of peroxidation by reacting with free radicals. According to some epidemiological and experimental studies, the excessive consumption of fats (both liquid and solid) increase the risk of diseases such as cancer of the rectum, large intestine, ovaries, breasts, testes and prostate, while high cholesterol intake increases the risk of lung and pancreatic cancers (11-12).

In this study, we examined the levels of malondialdehyde (MDA), which is an indicator of oxidative stress in sleep apnea patients, and glutathione peroxidase (GPx), an antioxidant enzyme, in sleep apnea patients. The products of lipid peroxidation can be measured not only in tissues but also in serum due to transference of these products. The data in our study showed that malondialdehyde (MDA), a secondary product of lipid peroxidation, was greater in the experimental group than in the control group. Our results also showed that OSAS patients had lower levels of GPx than the control group.

Recurring incidents of hypoxia during sleep characterize the pathology of OSAS. Production of ROS may be increased by repeated fluctuations in oxygen saturation levels in the arteries (15). Nonetheless, it is still not entirely clear to what extent oxidative stress is involved in the pathogenesis of OSAS. The release of free radicals, which may be augmented by cycles of hypoxia-reoxygenation, can cause damage to the vascular

endothelium (15). Thus, the relationship between cardiovascular morbidities and OSAS may be strengthened by oxidative stress (10, 15).

The results of studies investigating the role of oxidative stress in OSAS have been inconclusive. While some studies have found that OSAS patients had higher levels of oxidative stress when compared to healthy control subjects (16,17), the results of other studies seemed to indicate that OSAS patients and control groups had no appreciative difference in their levels of oxidative stress (18,19). Factors such as coexisting morbidities and heterogeneity of study participants may explain these discrepancies (14).

Reactive oxidative stress (ROS) has been determined to be linked to OSAS in a number of studies (14, 15). Elevated levels of reactive oxygen metabolites, which may lead to cellular damage, have been detected in the blood of OSAS patients (20). Endothelial dysfunction, which may increase the incidence of cardiovascular and cerebrovascular diseases in OSAS patients, is also associated with OSAS and oxidative stress (21). Nevertheless, whether or not OSAS patients have elevated levels of oxidative stress markers has yet to be ascertained (15).

Our data indicated that OSAS patients have increased levels of oxidative stress indicators. Elevated levels of oxidative stress in OSAS patients may have important clinical implications for diagnosis, treatment and prognosis. Discrepancies in findings regarding the role played by oxidative stress in OSAS may be a result of different pathways and the various factors involved, whether metabolic, systemic, genetic, or inflammatory (14, 15).

In this study, we examined malondialdehyde (MDA) levels, which indicate oxidative stress in sleep apnea patients, and the activity of glutathione peroxidase (GPx), an antioxidant enzyme. Measurement of the products of lipid peroxidation can be made not only from tissues but also from serum due to transference of these products. The data in our study showed that levels of malondialdehyde (MDA), a secondary product of lipid peroxidation, were significantly greater in the experimental group than in the control group.

In patients with sleep apnea, the oxidative stress generated by recurring hypoxia during sleep initiates a series of inflammatory reactions. Whether or not the mechanism of equilibration between antioxidant enzymes and the markers which constitute oxidative stress damage the patient is an important topic. Over the years, numerous studies on MDA, an indicator of oxidative stress, and GPx, an antioxidant enzyme, have been conducted. Due to the low level of GPx and high level of MDA found in the experimental group, the difference shown by our study between oxidative stress and the antioxidant enzymes which counter this stress appears to indicate that oxidative stress has excessively harmful effects on these patients.

The limitations of the current study should be acknowledged. The sample size of this study is relatively small and the design is cross-sectional. Secondly, differences in such metabolic, inflammatory or dietary confounding factors may result in data discrepancies, as may a different methodology or the instability of reactive oxygen species. For this reason, any attempt to extrapolate from our results should only be undertaken with great caution. Additional research is still needed on this topic.

## Conclusion

In conclusion, our findings indicated that that sleep apnea results in a decrease in the GPx enzyme, by means of the body's antioxidant defense system, and an increase in malondialdehyde, a symptom of oxidative stress. Thus, the present study represents an important contribution to the literature on this topic

**Conflict of interest:** The authors declare they have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article, and declare study has ethical permissions if required..

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