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Serum paraoxonase, arylesterase activity and oxidative status in patients with obstructive sleep apnea syndrome (OSAS)

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Abstract. – *Introduction:* **The aim of this study was to evaluate the serum paraoxonase (PON), arylesterase activities, lipid hydroperoxide (LOOH), sulfhydryl (-SH), and ceruloplasmin (Cp) levels, total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) in adults with obstructive sleep apnea syndrome (OSAS) and to determine whether these oxidant and antioxidant levels can be used as OSAS markers. The results were compared with measurements from healthy control subjects.**

Methods and Methods: **This study was a prospective, controlled trial including 26 OSAS patients and 35 healthy controls (61 subjects total). Peripheral venous blood samples were taken from the OSAS patients and from the healthy volunteers. The serum PON, arylesterase activities LOOH, -SH, Cp, TAS, TOS, and OSI levels were measured.**

Results: **LOOH and Cp levels were higher in the OSAS group than in the control group ($p < 0.01$). The -SH levels were lower in the OSAS group than in the control group ($p < 0.01$). The PON and arylesterase enzyme activity levels were lower in the OSAS group than in the control group ($p < 0.05$). The TOS and OSI levels were significantly higher in the OSAS group ($p < 0.01$), while their TAS levels were significantly lower ($p < 0.01$) compared with the control group.**

Conclusions: **Patients with OSAS have increased systemic oxidative stress and reduced levels of circulating antioxidant enzymes. Oxidative stress appears to be an underlying condition associated with OSAS.**

Key Words:

Paraoxonase, Obstructive sleep apnea syndrome, Total antioxidant status, Total oxidant status, Free radical.

Introduction

Obstructive sleep apnea (OSAS) is characterized by recurrent apnea and hypopnea episodes caused by upper airway obstruction[1,2]. The repeated upper airway obstruction episodes lead to a significant hypoxemia[2]. The Working Group of the American Academy of Sleep Medicine proposed the definition and classification of OSAS and noted that the most important indicator of the disease's severity is the apnea/hypopnea index (AHI)3. The prevalence of OSAS is approximately 2-4% among adults, and it is the second most common respiratory system disorder[2,3]. OSAS is also associated with cardiovascular complications, including hypertension, myocardial infarction and cerebrovascular disease[2,4]. Some Authors have reported an increase in the levels of systemic biomarkers of inflammation and oxidative stress in patients with OSAS[2]. Multiple different proteins control oxidative stress, including paraoxonase, arylesterase[5]. Paraoxonase-1 (PON) is mainly synthesized in the liver and secreted into the blood. PON is a high-density lipoprotein (HDL)-associated antioxidant enzyme that protects low-density lipoprotein (LDL) from oxidation by hydrolyzing the lipid peroxides in the oxidized lipoprotein[6,7].

Lipid hydroperoxide (LOOH) is a well-known marker of oxidative stress formed from unsaturated phospholipids, glycolipids, and cholesterol[8]. Enzymes and proteins with free SH groups have antioxidant characteristics[9]. Ceruloplasmin (Cp) is considered a preventive plasma antioxidant because it sequesters transition metals, thereby, preventing them from participating in free radical reactions[10].

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Antioxidant enzymes, including their activities and overall levels, and the total oxidant or antioxidant status have been previously studied[2,11,12]. However, serum paraoxonase (PON) and arylesterase activities, and other markers of the antioxidant system have not been previously investigated in the same patients at the same time. The aim of this study was to investigate serum PON and arylesterase activities the total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), and LOOH, Cp and sulfhydryl (-SH) levels in serum of patient and control groups to confirm the link between oxidative stress and OSAS.

Materials and Methods

The local Ethics Committee approved the study protocol (02/2011-03). Patient consent was obtained before the procedures began. A total of 61 subjects (26 patients with OSAS and 35 healthy controls) were evaluated in this study. All of the patients underwent a baseline evaluation, including a detailed medical history, a standard otorhinolaryngological and chest examination, and an evaluation with a Viasys Sleep Screen polysomnography device (Viasys Healthcare, Hoechberg, Germany), at the Gaziantep University Department of Pulmonary Sciences. The patients underwent blood testing after 12 hours of fasting. The main inclusion criteria were age between 18 and 65 years and the presence of OSAS. The main exclusion criteria were a history of any systemic chronic disease and the use of any systemic medication.

Measurement of Paraoxonase and Arylesterase Activities

The basal activity of paraoxonase was measured. Paraoxon is a toxic organophosphate that is also known as diethyl-p-nitrophenylphosphate. The rate of paraoxon hydrolysis was measured by monitoring the absorbance increase at 412 nm at 37 °C. The amount of p-nitrophenol generated was calculated from the molar absorptivity coefficient at pH 8, which was 17,000[13] M^{-1} cm^{-1}. Paraoxonase activity was expressed as U/L serum. Phenylacetate was used as a substrate to measure the arylesterase activity by monitoring its absorbance increase at 270 nm at 37°C. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol, which was 1,310 M^{-1} cm^{-1}. One unit of arylesterase activity was defined as 1 mol phenol generated/min under the above conditions and expressed as kU/L serum[13].

Measurement of Total Antioxidant Status

The serum TAS levels were measured using an automated method[14]. The Rel Assay (Rel Assay®, Diagnostics kits, Mega Tıp, Gaziantep, Turkey) was used. In this method, the antioxidant molecules in the sample decolorize the 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)* cationic radical. The decolorization rate is proportional to the amount of the antioxidant molecule present. Trolox, a vitamin E analog, was used as a calibrator. The data were expressed as mmol Trolox equivalents/L.

Measurement of the Total Oxidant Status

TOS measurement was performed using the Erel method[15]. The reagent was manufactured by Rel Assay (Rel Assay®, Diagnostics kits, Mega Tıp, Gaziantep, Turkey). In this method, oxidants that are present in the sample oxidize the ferrous-ion-o-dianisidine complex to ferric ion, and glycerol molecules that are abundantly present in the reaction medium enhance the oxidation reaction. The ferric ion produces a complex colored with xylenol orange in an acidic medium. The color intensity is related to the number of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide, and the results were expressed in terms of the micromolar hydrogen peroxide equivalent per liter (mmol H_2O_2 equivalent/L)[15].

OSI Calculation

The OSI is the ratio of the TOS to the TAS. To calculate the OSI, the TAS is multiplied by 10 and equalized relative to the TOS[16]. The results are reported as arbitrary units (AU).

$$OS = \frac{\text{TOS, } \mu\text{mol } H_2O_2 \text{ equiv./L.}}{\text{TAS, mmol Trolox equiv./L.} \times 10}$$

Measurement of Sulfhydryl Groups in the Serum Samples

The free -SH groups in the serum samples were assayed according to the method described by Ellman[17] and modified by Hu et al[18]. Briefly, 1 mL of buffer containing 0.1 M Tris, 10 mM EDTA, pH 8.2, and 50 µL serum was added to cuvettes, followed by the addition of 50 µL of 10 mM DTNB in methanol. Samples prepared containing no DTNB in the methanol were used as a blank for each sample as a test. Following incubation for 15 min at room temperature, the sam-

ple absorbance was read at 412 nm on a Cecil 3000 spectrophotometer. The samples with reagent blanks were subtracted. The concentration of the -SH groups was calculated using reduced glutathione as a standard for free -SH groups, and the result was expressed as mmol/L.

Measurement of LOOH Level

LOOH levels were measured with the ferrous ion oxidation-xylenol orange (FOX-2) method as previously described[19].

Measurement of Ceruloplasmin

Cp enzymatic activity was measured according to Erel's method[20]. Using this assay, ferrous ion is oxidized to ferric ion via ceruloplasmin ferroxidase activity. The results are expressed as U/L.

Statistical Analysis

The results are reported as the mean ± SD. Independent sample *t*-tests were used for statistical analysis of the OSAS and control groups. The Statistical Program for the Social Sciences (SPSS Inc.,Version 16.0, Chicago, IL, USA) was used for the analysis. In our evaluation of the significance of the differences, $p < 0.05$ was considered statistically significant.

Results

The subjects included 26 OSAS patients whose apnea and hypopnea index (AHI) values were ≥5 and 35 normal controls. The control group consisted of 15 males and 20 females aged 18 to 65 years (mean age: 45 ± 14 years). There were no significant differences in either age or gender between the patient and the control groups. The demographics of the patients and the control group are presented in Table I.

Table I. Demographics of the control group and OSAS cases.

Parameter	OSAS cases n = 26	Control n = 35
Age	49.29 ± 11.88	47.15 ± 10.56
Height	164.81 ± 8.36	163.76 ± 7.40
Weight	74.5 ± 15.84	67.5 ± 12.43
BMI	26.61 ± 5.61	23.34 ± 4.27
AHI	31.21 ± 28.11	2.41 ± 0.34

BMI: Body mass index; AHI: Apnea hypopnea index.

The TOS and OSI levels were significantly higher in the OSAS patients than in the controls ($p < 0.01$). The TAS level was significantly lower in the OSAS group than in the control group ($p < 0.01$). The PON and arylesterase enzyme activity levels were lower in the OSAS group than in the control group ($p < 0.05$). The other markers of oxidative stress, such as SH, were lower, and the ceruloplasmin and lipid peroxide levels were higher in the OSAS group compared with the control group. The results are shown in Table II.

Additionally, there was a negative correlation between AHI and TAS ($p < 0.05$; correlation coefficient: r = –0.42).

Discussion

Reactive oxygen species (ROS) can cause damage to all classes of biochemical compounds and can impair cells' activities[21]. An imbalance between oxygen free radical production and antioxidative defense mechanisms may increase ROS levels. High concentrations of ROS can be cytotoxic and neurotoxic[22]. The enzymatic activity of paraoxonase has been previously reported to play a protective role in such diseases as active

Table II. Mean ± SD of PON and arylesterase activities TAS, TOS and OSI, levels.

Oxidative stress markers	OSAS	Control	*p* value
TAS (mmol Trolox equivalents/L)	0.96 ± 0.14	1.13 ± 0.14	< 0.05
TOS (mmol H_2O_2 equivalent/L)	22.32 ± 5.08	11.19 ± 2.11	< 0.01
OSI (arbitrary units)	2.35 ± 0.68	1.00 ± 0.22	< 0.01
PON (U/L)	144.75 ± 43.28	165.90 ± 37.44	< 0.05
Arylesterase (kU/L)	144.80 ± 41.50	166.25 ± 29.68	< 0.05
Lipid hydroperoxide (µmol/L)	10.80 ± 2.48	8.27 ± 1.43	< 0.01
Ceruloplasmin (U/L)	665.18 ± 102.81	605.50 ± 59.11	< 0.01
Sulfhydryl (mmol/L)	0.37 ± 0.08	0.48 ± 0.056	< 0.01

pulmonary tuberculosis, asthma, cardiovascular diseases, rheumatoid arthritis, and chronic adenotonsillitis[23-27]. The current knowledge regarding the presence of increased oxidative stress in OSAS is controversial. Dyugovskaya et al[28] reported increased ROS production in leukocytes, and Barcello et al[29] reported increased systemic oxidative stress in severe cases of OSAS. Lavie et al[30] reported that lipid peroxidation was increased in patients with OSAS compared with controls. In contrast, a number of studies have failed to confirm increased oxidative stress in plasma by finding no differences in the lipid peroxidation products of OSAS patients and control groups[32-34].The role of free radical scavengers in OSAS remains unclear. Christou et al[11] showed that patients with severe OSAS have reduced antioxidant capacity values, and a negative correlation was found between antioxidant capacity and disease severity. Recently, additional information about the antioxidant status of OSAS patients has been provided. Barcelo et al[29] reported that patients with OSAS had lower TAS levels. In accordance with Barcelo et al[29], our study's results indirectly support the presence of oxidative stress in OSAS through decreased TAS.

In this investigation, the serum PON, arylesterase activities, TAS, TOS and OSI levels were studied in OSAS and control groups. This report's findings are as follows:

1. Patients with OSAS presented reduced antioxidant capacity and increased levels of oxidative stress.
2. OSAS patients exhibited decreased levels of antioxidant enzymes, such as paraoxonase and arylesterase.
3. LOOH levels were higher in OSAS patients, and -SH levels were lower in OSAS patients compared with healthy controls.

Several papers have evaluated the role of ROS in the pathology of OSAS. However, there is little information about the relationships among TAS, TOS, OSI, and PON and arylesterase activity. The levels of plasma PON, arylesterase, activities, the LOOH, -SH levels, TAS, the TOS, and OSI seem to be appropriate markers of antioxidant capacity. Plasma PON and arylesterase activities were low in the OSAS patients in this study. The results of several researches on oxidative stress and OSAS suggest an increased level of oxidative stress in OSAS patients[2,23]. However, to our knowledge, few studies have evaluated both antioxidant capacity and oxidative stress together with PON and arylesterase activities. Kotani et al[35] reported that PON and arylesterase activities were lower in OSAS patients, and they concluded that the enzymes' activity increased after CPAP (Continuous Positive Airway Pressure) treatment. Their findings were similar to those of our report. The number of their study was limited but they performed the measurements of enzyme activity increased after the CPAP treatment. One of our study's limitations was the absence of the measurement of oxidative stress marker changes after the treatment. A further limitation was our limited number of cases. The reduction in antioxidant enzyme activities shows that there is systemic oxidative stress in patients with OSAS[11,29]. The negative correlation between AHI and TAS indicates that patients with severe OSAS have excessive oxidative stress along with decreased antioxidant capacity and antioxidant enzyme activities. In chronic inflammatory diseases, PON and arylesterase activities are decreased[23-28]. Reduced serum antioxidant capacity is an index of excessive oxidative stress. Antioxidant status is an indicator of redox homeostasis[28].

Conclusions

Patients with OSAS have increased oxidative stress levels and reduced antioxidant enzyme activities. Increased oxidative stress in OSAS patients may explain some of the associations among OSAS, hypoxia, and the risk of cardiovascular disease in OSAS patients. Further studies are needed to more clearly define the role of oxidative stress in these associations and the use of these oxidant and antioxidant parameters as markers of oxidative stress in OSAS.

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