

## SERUM COPPER AND ZINC STATUS IN OBSTRUCTIVE SLEEP APNEA PATIENTS

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### Abstract

*Obstructive Sleep Apnea Syndrome (OSAS) is a chronic disease characterized by oxygen desaturation and awakens follow from repetitive apnea and hipopnea during the night. It is thought that diversity of oxygen levels during night in OSAS cause oxidative stress with the increment of the free radical production. In this study, serum levels of copper (Cu) and zinc (Zn), two of the most important elements of antioxidant defense mechanism, were investigated. Patients to be participated into the study were divided into 4 groups according to the result of polysomnography test. Levels of serum Cu and Zn are measured by using flame atomic absorption spectrophotometry equipment. Glucose, blood lipid profile (HDL, LDL, trigliserid, total cholestrol), urea and creatinine were taken into account in all patients. Serum Cu levels were similar with OSAS patients (mild OSAS: 109±37 µg/dL; moderate OSAS: 93±20 µg/dL; severe OSAS: 97±14 µg/dL) and healthy controls (99±37 µg/dL, p>0.05). Serum Zn levels were significantly low in OSAS patients, especially in severe OSAS (mild OSAS: 79±9 µg/dL; moderate OSAS: 79±12 µg/dL; severe OSAS: 74±18 µg/dL) when compared to controls (88±12 µg/dL, p<0.05). These results suggest that patients with severe OSAS are under considerable oxidative stress. Supporting OSAS patients with Zn would be beneficial to decrease oxidative stress and its effects.*

**Key words:** Obstructive sleep apnea syndrome, Cu, Zn, Oxidative stress, Flame atomic absorption spectrophotometry.

### Obstrüktif Uyku Apneli Hastalarda Serum Bakır ve Çinko Düzeyleri

*Obstrüktif Uyku Apne Sendromu (OUAS) gece içinde sık tekrarlayan nefes durmaları ve azalmaları, sonrasında oluşan oksijen desaturasyonu ve uyanıklık reaksiyonu ile karakterize bir hastalıktır. OUAS'da gece içindeki oksijen değişimlerinin serbest radikal üretimini arttırarak oksidatif strese neden olduğu düşünülmektedir. Bu çalışmada, OUAS'lı hastalarda oksidatif stresin göstergesi olabilecek, antioksidan savunma mekanizmasının önemli yapı taşlarından Bakır (Cu) ve Çinko (Zn) elementleri serum düzeyleri araştırıldı. Çalışmaya alınan hastalar polisomnografi testinin sonucuna göre 4 gruba ayrıldı. Serum Cu ve Zn düzeyleri alevli atomik absorpsiyon cihazında ölçüldü. Tüm hastalarda açlık kan şekeri düzeyi, kan lipid profili (HDL, LDL, trigliserid, total kolestrol), üre ve kreatinin değerlendirmeye alındı. Serum Cu seviyelerinin, OUAS hastalarında (hafif derecede OUAS: 109±37 µg/dL; orta derecede OUAS: 93±20 µg/dL; ileri derecede OUAS: 97±14 µg/dL) ve sağlıklı kontrollerde (99±37 µg/dL, p>0.05) benzer olduğu görüldü. Serum Zn düzeyleri, OUAS'lı hastalarda, özellikle ileri derecede OUAS'lı hastalarda (hafif derecede OUAS: 79±9 µg/dL; orta derecede OUAS: 79±12 µg/dL; ileri derecede OUAS: 74±18 µg/dL) kontrole (88±12 µg/dL, p<0.05) kıyasla istatistiksel olarak anlamlı düzeyde düşüktür. Bu çalışma ile, ileri OUAS hastalarının daha fazla oksidatif stres altında olduğu ileri sürülmektedir. OUAS'lı hastalarda Zn alımının, oksidatif stres ve etkilerini azaltmada faydalı olabileceği düşünülmektedir.*

**Anahtar kelimeler:** Obstrüktif uyku apne sendromu, Cu, Zn, Oksidatif stres, Alevli atomik absorpsiyon spektrofotometri.

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## INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a chronic disease caused by repetitive episodes of partial or complete upper airway collapse, due to complete or partial pharyngeal obstruction (1). These obstructions result with interruption of sleep with frequent arousals (sleep fragmentation); loss of rapid eye movement (REM) sleep and slow wave sleep (stage 3-4); repetitive decreases in oxygen saturation with rapid reoxygenation causing cyclical deoxygenation/reoxygenation; and repeated changes in intrathoracic pressure and episodic hypercapnia (2-4). The apnea/hypopnea index (AHI) shows the severity of OSAS, interpreted as the average number of apneas and hypopneas per hour (5).

Male gender, overweight/obesity, and being over the age of forty are considered as the typical risk factors for sleep apnea, listed in the public health literature (6). Though most data suggest that OSAS is more common among middle-aged adults between 40 and 60 years old, it affects individuals of all ages (1). The prevalence in the general population is about 3-7% in adult men and 2 to 5 % in adult women (7, 8), increasing significantly in the elderly from 5% to 9 % (9). The prevalence is much higher in specific patient groups, such as those with congestive heart failure (40 %) (10), end-stage kidney disease (50%) (11) and stroke (60 %) (12), where less than 5 % of all OSAS patients receive treatment (8, 10-13).

OSAS has been strongly associated with increased cardiovascular morbidity and mortality and is highly common in patients with established cardiovascular disease (CVD) (14–16). The relative contribution of oxidative stress has been examined to explain the associations between OSAS and CVD (17, 18). Studies demonstrate that hypoxia, a pathological condition commonly observed in sleep apnea, promotes the formation of reactive oxygen species (ROS), particularly during the reoxygenation period (1).

Zinc (Zn), copper (Cu) are involved in many homeostatic mechanisms of the body, such as specific immunity, inflammation, and oxidative stress (19-22). Increased oxidative stress (23) and decreased content of antioxidant elements such as Zn has been shown to have a relationship with CVD. Moreover, the oxidative stress leading to cardiac functional disorders may be elevated due to increased content of some elements like Cu (23, 24).

The objective of this study was to examine serum Zn and Cu levels in OSAS patients in order to see whether these markers reflect the severity of OSAS.

## EXPERIMENTAL

### *Patients*

Our study group comprised recruited volunteers with suspected OSAS admitted to our sleep clinic through unselected referral. In total, 27 males and 3 females with the mean average age  $43.2 \pm 9.4$  years were recruited into the study after their informed consent were obtained. The Local Ethics Committee of Marmara University and Turkish Ministry of Health, Central Ethics Committee, approved the study protocol. The detailed medical histories of all volunteers were evaluated together with general examination.

### *Procedures and measure*

Physiological and respiratory variables such as central and occipital electroencephalography (EEG), oblique electrooculography (EOG), submental and tibialis electromyography (EMG) activity, electrocardiography (EKG), nasal and oral airflow via nasal pressure transducer and thermister, thoracic and abdominal excursions with peizo belts and continuous oxygen saturation were evaluated by clinical polysomnography (PSG) to diagnose OSAS with a full night sleep in laboratory. Standard criteria's were used by trained technicians to score the sleep stage. Recommended guidelines were used to score apneas and hypopneas (25). Analysis of sleep stages was done manually at 30-second intervals by the criteria of Rechtschaffen and

Kales (26). Apnea was described as complete cessation of airflow lasting  $\geq 10$  s, and hypopnea as a  $>50$  % reduction in respiratory airflow lasting  $\geq 10$  s linked with an arousal or oxygen desaturation of  $>3\%$ . The total sleep time is divided by the number of respiratory events to obtain the apnea/ hypopnea index (AHI). The diagnostic of OSAS is described with an AHI of more than five events per hour of sleep (mild OSAS: AHI  $\geq 5$  to  $<15/h$ ; moderate OSAS: AHI 15 to  $<40/h$ ; severe OSAS: AHI  $\geq 40/h$ ). 24 subjects were diagnosed with OSAS and 6 subjects without OSAS according to PSG evaluation. Peripheral venous blood samples were collected in the morning (between 8.00-10.00 am) and immediately transferred to the laboratory for analyses. Biochemical profiles such as cholesterol, LDL, HDL, triglyceride, glucose, urea, and creatinine were analyzed (Beckman Coulter Synchron LX 20, UK).

#### *Determination of serum Cu and Zn levels by flame atomic absorption spectrophotometry*

Levels of serum Cu and Zn were measured by using flame atomic absorption spectrophotometry equipment (Shimadzu AA-6800). A 10 mL blood sample was collected from subjects; the samples were centrifuged at 2500 rpm for 10 min. The serum obtained was separated and frozen at  $-20$  °C until the time of analysis. All samples were diluted 1:5 by volume with 0.5 %  $\text{HNO}_3$  and analyzed two times. Zn and Cu hollow cathode lamps were operated at a spectral width of 0.5 nm, which were selected to isolate the 213.9 nm and 324.8 nm lines, respectively. Analytical reference solutions were prepared by successive dilution of 1000 mg/l Cu and Zn Titrisol stock solutions (Merck) and by high purity de-ionized water (18 M $\Omega$ . cm) obtained from a Milli Q RG water purification system (Millipore Corp; Bedford, MA, USA). The values of Cu and Zn were measured against aqueous standards by direct calibration (calibration standards at concentration of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mg/L). The linear regression line for Cu and Zn were  $y=0.5172x+0.0089$   $r=0.9990$ ;  $y=5172x+0.0089$   $r=0.9998$  respectively.

#### *Statistical analysis*

The statistical analysis was performed with Matlab 7.0 Statistic Toolbox. All results were expressed as means  $\pm$ SD and  $p$  value less than 0.05 was defined to be statistically significant. One way ANOVA test was used to compare the mean values between two different groups. The correlation between continuous variables was evaluated by the Pearson's correlation. To calculate the serum Cu and Zn levels according to markers of AHI and oxygenation levels, a logistic regression analysis was executed.

## **RESULTS**

All subjects had a mean age of  $43.2 \pm 9.4$  years. Based on the results of polysomnography, twenty-four out of 30 subjects had an AHI greater than 5 (OSAS group), and 6 subjects had an AHI equal to or less than 5 (healthy group). The healthy control group and OSAS groups of patients with different stages of the syndrome (mild, moderate and severe OSAS) did not differ with respect to age, fasting glucose concentration, lipid parameters, serum uric acid and creatinine ( $p>0.05$ ). Significant positive correlation was found between body mass index (BMI) and AHI ( $r=0.46$ ,  $p=0.02$ ).

It was observed that, serum Cu levels were similar with OSAS patients (mild OSAS:  $109 \pm 37$   $\mu\text{g/dL}$ ; moderate OSAS:  $93 \pm 20$   $\mu\text{g/dL}$ ; severe OSAS:  $97 \pm 14$   $\mu\text{g/dL}$ ) and healthy controls ( $99 \pm 14$   $\mu\text{g/dL}$ ,  $p>0.05$ ). Serum Zn levels were significantly low in OSAS patients, especially in severe OSAS (mild OSAS:  $79 \pm 90$   $\mu\text{g/dL}$ ; moderate OSAS:  $79 \pm 12$   $\mu\text{g/dL}$ ; severe OSAS:  $74 \pm 18$   $\mu\text{g/dL}$ ) when compared to controls ( $88 \pm 12$   $\mu\text{g/dL}$ ,  $p<0.05$ ). Cu/Zn ratio was not different in OSAS group (mild OSAS:  $1.4 \pm 0.6$ ; moderate OSAS:  $1.2 \pm 0.3$ ; severe OSAS:  $1.4 \pm 0.6$ ) compared to controls ( $1.2 \pm 0.2$ ,  $p<0.05$ ). Demographic data and sleep characteristics of the participants and corresponding concentrations of serum Zn and Cu are shown in Table 1.

The serum Zn and Cu levels, Cu/Zn ratio according to markers of AHI and oxygenation levels have been calculated with regression analysis. Serum Zn levels were significantly correlated with oxygenation levels ( $r=-0.42$  and  $p=0.0019$ ). The correlations between Cu and Zn levels and polysomnography data are presented in Table 2.

**Table 1.** Demographic data, sleep characteristics and biochemical profiles of the OSAS patients, healthy controls and corresponding concentrations of serum copper (Cu) and zinc (Zn)

	Controls <5	Mild OSAS ≥5 to <15	Moderate OSAS 15 to <40	Severe OSAS ≥40	P values
<b>Total number</b>	6	6	8	10	
<b>Demographics</b>					
Age (years)	35.8±8	42±8	48±8	46±9	>0.05
BMI (kg/m <sup>2</sup> )	26±3	29±5	30±4	32±4	<0.05*
Female/male ratio	2/4	1/5	0/8	0/10	
<b>Polysomnographics values</b>					
AHI (events/h)	2±1	8±1	24±7	67±17	<0.05*
Lowest SaO <sub>2</sub> I (%)	91±2	86±2	82±7	72±10	=0.0002**
Mean SaO <sub>2</sub> I (%)	96±1	95±2	94±1	91±4	=0.0002**
t SaO <sub>2</sub> I <90(%)	0±0	2±2	4±4	28±23	<0.05*
Desaturation (%)	4±1	5±1	6±2	9±3	=0.0002**
Desaturation index	2±1	6±2	19±8	58±19	=0.0002**
TTA (min)	402±29	386±25	386±59	393±17	>0.05
TSP (min)	389±28	357±39	369±67	382±13	>0.05
WASO (min)	22±13	10±11	14±13	28±37	>0.05
TST (min)	367±39	348±35	356±73	354±37	>0.05
SEI	91±3	90±7	92±7	90±12	>0.05
SCI	94±4	97±3	96±4	93±10	>0.05
Non-REM 1-2 (%)	64±4	51±14	62±10	91±5	<0.05*
Non-REM 3-4 (%)	24±6	33±10	22±10	4±5	<0.05*
REM (%)	45±38	53±24	55±36	16±17	<0.05*
<b>Biochemical profiles</b>					
Cholesterol (mg/dL)	193±65	209±63	220±56	212±45	>0.05
HDL (mg/dL)	41±7	40±4	41±13	35±7	>0.05
LDL (mg/dL)	130±54	136±58	125±56	134±32	>0.05
LDL/HDL	3±1	3±1	3±1	4±1	>0.05
Triglyceride (mg/dL)	110±35	166±58	191±177	217±67	>0.05
Glucose (mg/dL)	100±3	97±6	107±19	111±10	>0.05
Urea (mg/dL)	31±4	36±9	34±6	34±9	>0.05
Creatinine (mg/dL)	1±0.1	1±0.1	1±0.3	1±0.2	>0.05
<b>Serum Cu and Zn Values</b>					
Serum Cu (µg/dL)	99±14	109±37	93±20	97±14	>0.05
Serum Zn (µg/dL)	88±12	79±90	79±12	74±18	<0.05*
Cu/Zn	1.2±0.2	1.4±0.6	1.2±0.3	1.4±0.6	>0.05

OSAS: Obstructive sleep apnea; BMI: body mass index; AHI: apnea-hypopnea index; SaO<sub>2</sub>I: oxygen saturation; TTA: total time analyzed; TSP: total sleep period; WASO: wake time during sleep period; TST: total sleep time; SEI: sleep efficiency index; SCI: sleep continuous index; REM: rapid eye movement.

\*  $p$ -Value < 0.05

**Table 2.** The correlations between copper (Cu) and zinc (Zn) levels and polysomnography data.

<i>Polysomnographics values</i>	<b>Cu</b>	<b>Zn</b>	<b>Cu/Zn</b>
AHI (events/h)	r=-0.15, p=0.42	r=-0.37, p=0.042*	r=0.21, p=0.25
Lowest SaO <sub>2</sub> I (%)	r=0.18, p=0.33	r=0.34, p=0.067*	r=-0.18, p=0.32
Desaturation (%)	r=-0.20, p=0.28	r=-0.26, p=0.15	r=0.10, p=0.57
Desaturation index	r=-0.21, p=0.25	r=-0.42, p=0.019*	r=0.20, p=0.27
t SaO <sub>2</sub> I <90(%)	r=-0.13, p=0.49	r=-0.20, p=0.28	r=0.12, p=0.53

AHI: apnea-hypopnea index; SaO<sub>2</sub>I: oxygen saturation\* *p*-Value < 0.05

## DISCUSSION

Obstructive sleep apnea syndrome might be seen similar to ischemic/reperfusion injury, especially with its hypoxia/reoxygenation episodes, which causes the generation of ROS (27). Patients which have sleep apnea are considered to be at risk for CVD (14, 15). The associations between OSAS and CVD have been examined by means of the relative contribution of oxidative stress (28).

A previous study focusing into OSAS as an oxidative stress disorder showed contradictory results. Barcelo et al. (29) and Lavie et al. (30) have found increased lipid peroxidation in patients with OSAS compared to controls. Vatansever (31) reported that patients with moderate-severe OSAS (characterized by elevated AHI) have shown proof for greater oxidative stress (both serum lipid peroxidation and serum protein oxidation) than healthy controls. Carpagnano et al. (32) have found a positive correlation between 8-isoprostane levels, which is a marker of oxidative stress in OSAS patients' breaths, and AHI. On the other hand, Ozturk et al. (33), Wali et al. (34) and Alzoghaibi and BaHammam (35) have demonstrated that there was no significant difference between OSAS patients and healthy controls in terms of lipid peroxidation concentrations and glutathione activities. Besides, Alzoghaibi et al. showed that there is not an adaptive increase in serum superoxide dismutase (SOD) activities of OSAS patients. Christou et al. (36), found that the antioxidant capacity in OSAS patients was similar to the healthy subjects. Yet, patients with severe OSAS usually had low antioxidant capacity which indicates an excessive oxidative stress. Wysocka et al. (37) evaluated OSAS effect on blood antioxidant status of overweight and obese subjects. They presented that SOD was decreased in the overweight OSAS and obese OSAS patients; while the total antioxidant status of obese OSAS patients was significantly lower than controls. Ntalapascha et al. (38) investigated the systemic stress level in OSAS patients. They have shown that the difference between overnight changes in plasma markers was not significant between OSAS and controls but GSH levels of controls were increased, while OSAS did not. They suggested that increased oxidative stress in OSAS would be associated with protein oxidation-GSH/GSSG pathway.

Male gender, overweight/obesity, and being over the age of forty are listed as typical risk factors for sleep apnea in the public health literature (6). In our study, OSAS groups of patients with different stages of the syndrome and the healthy control group did not differ with respect to age, fasting glucose concentration, lipid parameters, serum uric acid and creatinine. However, significant positive correlation was found between BMI and AHI.

The relationship between the obesity and severity of OSAS has been reported by several studies done at obese patients. Namyslowski et al. (39) demonstrated that there was no significant relationship between the BMI and sleep study parameters in overweight patients (BMI 25-30 kg/m<sup>2</sup>) yet there were significant correlation between increasing BMI and respiratory disturbance index in obese patients. A significant correlation between BMI and AHI was observed by Pillar et al. (40) in a large group of patients. Akita et al. (41) and Vgontas et al.

(42) found no difference in mean BMI in the group with and without apnea. However, Akita et al. have showed that there was a tendency between the increase of AHI and the increase in BMI.

Like glutathione peroxidase (GPx), SOD catalyzes oxidation–reduction reactions (43) Zn and Cu are essential micronutrients for enzymes. In epidemiological studies, serum copper has been shown to be increased in patients with a history of acute myocardial infarction, where serum zinc was normal or decreased (44, 45). The reference ranges of serum Cu and Zn levels in healthy individuals are 70-150 µg/dL; 60-110 µg/dL, respectively. In this study we found that, serum Cu levels were similar with OSAS patients and healthy controls. Serum Zn levels were significantly low in OSAS patients, especially in severe OSAS when compared to controls. Cu/Zn ratio was not different in OSAS group compared to controls.

Kosar et al. (46) reported that when compared to healthy controls, heart failure patients had lower serum zinc and higher copper concentrations. Leone et al. (47) showed that serum zinc and copper were linked to all-cause mortality in men. Their findings indicated that combined low serum zinc with either high serum Cu values lead to an increased mortality risk. Volna et al. (48) measured serum Zn and Cu levels by inductively-coupled plasma mass spectrometry as biochemical oxidative stress-related markers in OSAS patients. They found that Cu levels were higher in severe OSAS patients, a correlation further explained by association of ventilation parameters to BMI. On the other hand, no significant correlation was found between Zn concentration and AHI.

It is observed that Cu and Zn elements play an important role in carcinogenesis. Zowczak et al. (49) measured the serum Cu and Zn concentrations of patients with breast, lung, gastrointestinal and gynecological cancer and found that serum Zn level in lung cancer was significantly lower than control group. Yücel et al. (50) reported that the mean serum copper level and the mean Cu/Zn ratio in patients with breast cancer were significantly higher when compared with the control group. Maes et al. (51) investigated the serum Zn levels in chronic fatigue syndrome and they showed that patients with chronic fatigue syndrome were affected by a low serum Zn status and that was related to markers of inflammation and immune activation.

However, it is important to mention that the present study was performed using a limited study sample and further studies are needed to confirm the interactions between serum zinc and serum copper and their potential contribution to the prediction of oxidative stress and OSAS in clinical practice.

In our study we have observed that serum Cu levels did not change significantly through all groups, but Zn level had a tendency to decrease while AHI increase. This result leads us to an argument that high level OSAS patients are subject to higher oxidative stress. As it is mentioned in a study with Angina pectoris patients, having high dosage of Zn (50-300 mg/day) would be supportive for treatment of disease symptoms (52). Supporting OSAS patients with Zn may help to decrease oxidative stress and the effects depending on it.

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