RESEARCH LETTER

Evaluation of oxidative stress in obstructive sleep apnea and its comorbidities using serum concentrations of oxidized guanine species

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Introduction Obstructive sleep apnea (OSA) is a common respiratory disease characterized by periodic pauses in breathing during sleep. The severity of OSA is measured by the apnea-hypopnea index (AHI), one of the variables derived from polysomnography (PSG), which is calculated as the sum of apnea and hypopnea episodes per 1 hour of sleep. The standard treatment of OSA is continuous positive airway pressure (CPAP) therapy. The prevalence of OSA reaches up to 38% and increases with age. Moreover, OSA is closely related to many comorbidities, such as type 2 diabetes mellitus (T2DM) or hypertension.[1]

Pauses in breathing in OSA lead to numerous desaturations and intermittent hypoxia (IH). In turn, IH and consequent reoxygenation result in the generation of an extensive amount of reactive oxygen species (ROS), which cause oxidative stress. ROS damage lipids, proteins, and nucleic acids, thus aggravating systemic inflammation through the nuclear factor κβ (NF-κβ).[1] As a transcription factor, NF-κβ increases the expression of many inflammatory markers, such as interleukins and tumor necrosis factor.[1] Elevated levels of these markers were shown in OSA patients.

Oxidative stress measurement may be performed indirectly. Since ROS are very unstable and react immediately with different cell compounds, oxidative stress can be evaluated through oxidative damage and the performance of the antioxidant system. Assessment of superoxide dismutase (SOD) activity is the easiest way to evaluate the antioxidant function performance of the antioxidant system. Wysocka et al[2] previously reported decreased SOD activity in OSA patients, which is probably the reason for the inflammatory state in these individuals, particularly those with severe OSA. Other studies also reported increased levels of oxidative damage markers in OSA, such as of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG),[3] salivary advanced oxidation protein products (AOPPs),[4] or lipid peroxidation products.[5] Moreover, hyperoxidized peroxiredoxin 2 in red blood cells was also shown to be a possible marker of both oxidative stress and OSA diagnosis.[6]

The present study aimed to evaluate oxidative stress in patients with OSA based on serum concentrations of oxidized guanine species, and to compare the results with those obtained in a healthy control group. A secondary aim was to assess the relationship between the oxidized guanidine species concentrations and selected PSG parameters.

Patients and methods **Patients** The study group consisted of 66 individuals who were referred to the Sleep and Respiratory Disorders Center in Łódź, Poland with a presumptive diagnosis of OSA. All patients underwent standard nocturnal PSG examination. The following exclusion criteria were applied: presence of chronic respiratory disorders, chronic inflammatory diseases, psychiatric disorders, cancer, and pregnancy. Moreover, the patients who developed an infection within 1 month of blood collection, those who had taken international flights at least 2 weeks prior to the PSG examination, and those who had used medications known to affect sleep at least 2 weeks before or during PSG were also excluded. The study protocol was approved by the Ethics Committee of the Medical University of Lodz (RNN/432/18/KE). All patients provided their written informed consent to participate in the study.

Polysomnography The participants were admitted to the sleep laboratory at approximately 9 PM (or up to 30 minutes before/after that time) and underwent physical examination. The following channels were recorded during nocturnal PSG: electroencephalography (C4\A1, C3\A2), chin

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Received: April 27, 2023.
Revision accepted: July 31, 2023.
Published online: August 7, 2023.
Pol Arch Intern Med. 2023;
133 (9): 16542
doi:10.20452/pamw.16542

TABLE 1 Baseline characteristics of the study population

Parameter		Control group (n = 30)	OSA group (n = 36)	*P* value
Sex	Men	10 (33.3)	10 (27.8)	0.15
	Women	20 (66.7)	26 (72.2)	
Age, y, mean (SD)		42.5 (9.6)	49.4 (9)	0.004
BMI, kg/m^2		26.8 (24.6–30.3)	34 (30.7–40.7)	0.001
Oxidized guanine species, ng/ml, mean (SD)		16.4 (4.7)	21.0 (5.9)	0.001
Total sleep time, h, mean (SD)		6.2 (1.3)	6.3 (1)	0.64
Arousal index, events/h		10.7 (6.8–13.7)	22.9 (14.8–29.5)	<0.001
AHI, events/h		1.8 (1–3.1)	50.4 (40–65.4)	<0.001
Total number of desaturations		12 (5–16)	323 (212.5–433.8)	<0.001
Desaturation index		2 (1–3)	52.1 (37.3–71.8)	<0.001
Basal SpO_2, %		94.4 (93.6–95.1)	90.9 (87–93.6)	<0.001
Mean SpO_2 at desaturations during sleep, %		91.9 (90.7–92.7)	85.5 (78.6–89)	<0.001
Minimal SpO_2, %		89.9 (90.7–91.2)	71.5 (54.4–79)	<0.001
T2DM	Yes	28 (93.3)	29 (80.6)	0.17
	No	2 (6.7)	7 (19.4)	
Hypertension	Yes	18 (60)	17 (47.2)	0.33
	No	12 (40)	19 (52.8)	
T2DM or hypertension	Yes	18 (60)	15 (41.7)	0.22
	No	12 (40)	21 (58.3)	

Data are presented as number (percentage) of patients or median (interquartile range) unless indicated otherwise.

Abbreviations: AHI, apnea-hypopnea index; BMI, body mass index; OSA, obstructive sleep apnea; T2DM, type 2 diabetes mellitus

muscle and anterior tibialis electromyography, electrooculography, measurements of the oronasal airflow, snoring, body position, respiratory movements of the chest and abdomen, unipolar electrocardiogram, and oxygen saturation (SpO_2). The examination was performed using an Alice 6 device (Phillips-Respironics, Murrysville, Pennsylvania, United States). The criteria based on the 30-second epoch standard were used to score sleep stages in the recorded PSG. Apnea was defined as a reduction of airflow to less than 10% of the baseline for at least 10 seconds. Hypopnea was described as a reduction of airflow by 30% or more for at least 10 seconds, accompanied by an over 3% decrease in SpO_2 or arousal. The American Academy of Sleep Medicine guidelines[7] were used to score the arousals. The PSG parameters included the total number of desaturations, total sleep time (h), arousal index (events/h), AHI (events/h), desaturation index, basal SpO_2 (%), mean SpO_2 (%), level of desaturation during sleep (%), and minimal SpO_2 (%).

OSA severity was assessed based on AHI and classified as mild (AHI ≥5 and <15), moderate (AHI ≥15 and <30), or severe (AHI ≥30).[8] Only the patients with severe OSA were included in the study group.

Oxidative stress assessment In the morning (6:00–6:30 AM) following the PSG examination, peripheral blood samples were collected and centrifuged. Serum was isolated and stored at –80 °C. Enzyme-linked immunosorbent assay kits (DNA/RNA Oxidative Damage [High-Sensitivity] ELISA Kit; Cayman Chemical, Ann Arbor, Michigan, United States) were used to assess the aggregate serum concentration of major DNA/RNA oxidative damage markers, such as 8-hydroxyguanosine, 8-hydroxy-2'-deoxyguanosine (8-OHdG), and 8-hydroxyguanine. The absorbance was measured at a wavelength of λ = 405 nm by an absorbance reader (BioTek 800 TS, Agilent, Santa Clara, California, United States).

As oxidative damage is considered a marker of oxidative stress, the term "oxidative stress level" is used in this work as a synonym of oxidized guanine species concentration for ease of understanding.

Statistical analysis The level of significance was set at *P* below 0.05. Statistical analysis was performed with SPSS 28.0 software (IBM, Chicago, Illinois, United States). The distribution of variables was evaluated by the Shapiro–Wilk test. The groups with normally distributed data were compared by the independent samples *t* test, with results presented as mean (SD). Comparisons of variables that did not follow a normal distribution were performed using the Mann–Whitney test, and the results were presented as median and interquartile range. The χ^2 test was used to compare categorical variables. The Spearman rank correlation coefficient was used to assess correlations. Linear regression was performed to analyze the effect of variables on the oxidative stress level in the study group.

Results Based on AHI, the patients were divided into 2 groups. The individuals with an AHI below 5 were included in the control group (n = 30), while the OSA group (n = 36) comprised individuals with an AHI greater than or equal to 30. Baseline characteristics of both groups are shown in TABLE 1.

The patients in the OSA group had a higher oxidized guanine species concentration than the controls (mean [SD], 21 [5.9] vs 16.4 [4.7] ng/ml; *P* = 0.001). Furthermore, the oxidative stress level correlated positively with age (R = 0.463; *P* <0.001), body mass index (BMI; R = 0.442; *P* <0.001), AHI (R = 0.435; *P* <0.001), number of desaturations (R = 0.541; *P* <0.001), desaturation index (R = 0.463; *P* <0.001), and negatively with basal SpO_2 (R = –0.397; *P* <0.001) and minimal SpO_2 (R = –0.342; *P* = 0.005) (Supplementary material, *Figure S1*). No significant correlations were found between the oxidative stress level and total sleep time (R = 0.016; *P* = 0.9) or the arousal index (R = 0.212; *P* = 0.09).

The linear regression model evaluating the factors predictive of the oxidative stress level included age (B = 0.375; *P* = 0.01), total number of desaturation (B = 1.489; *P* = 0.02), and AHI (B = –1.144;

$P = 0.07$). The model explained 42.8% of the oxidative stress level variability. Detailed results of the regression analysis are shown in Supplementary material, *Table S1*.

Additionally, in the OSA group, the individuals with comorbidities, such as T2DM or hypertension, had a higher oxidized guanine species concentration than those with OSA alone (mean [SD], 22.8 [5.2] vs 18.56 [6.1] ng/ml, respectively; $P = 0.03$).

Discussion Oxidative stress in patients with OSA is the consequence of IH. In this preliminary study, we demonstrated that the oxidative stress level, reflected by the aggregate serum concentration of oxidized guanine species, was greater in the OSA patients than in the healthy controls. The total number of desaturations may be a predictive factor of oxidative stress. Moreover, the patients with comorbidities, such as T2DM or hypertension, had a higher level of oxidative stress.

Recent studies showed increased levels of oxidative damage markers from the peripheral blood in OSA.[9] However, none of them assessed the levels of oxidized guanine species. A meta-analysis by Hu et al[9] demonstrated that malondialdehyde (MDA), asymmetric dimethylarginine, total oxidant capacity (TOC), AOPPs, and thiobarbituric acid reactive substances (TBARS) are elevated in patients with OSA, as compared with healthy controls. MDA and TBARS are markers of lipid peroxidation. They may also be associated with dyslipidemia and further lead to the development of arteriosclerosis through increased compounding lipid deposition into the arterial wall.[10] AOPPs represent the protein damage caused by oxidative stress, and TOC is an unspecific indicator of ROS.[9]

However, DNA/RNA oxidative damage markers are also significantly increased in OSA.[11] Peres et al[11] found elevated 8-isoprostane levels in OSA patients, and this increase was independently related to OSA severity. Moreover, the same study showed that co-occurrence of heart diseases with OSA was associated with decreased SOD activity.[11] Wan et al[12] also found elevated plasma levels of 8-OHdG and interleukin-1β in OSA, and both of these markers positively correlated with AHI.[12] In other studies,[13,14] 8-OHdG and MDA were found to be increased in OSA patients. Jurado-Gámez et al[13] showed that the levels of both of these oxidative markers negatively correlated with ischemic reactive hyperemia (IRH; a marker of endothelial functioning), indicating the role of oxidative stress in endothelial dysfunction. Furthermore, after 3 months of CPAP treatment, improvements in oxidative stress markers and IRH were observed.[13]

Our findings of increased oxidative stress levels in patients with OSA are in line with these results. To our best knowledge, this is the first study in which the assessment of oxidative damage in OSA patients was performed using a kit that measures the total serum concentration of several oxidized guanine species simultaneously.

Furthermore, we observed that oxidative stress correlated positively with age, AHI, BMI, and desaturation index, and negatively with basal and minimal SpO_2. The correlation with age and BMI is not surprising. Oxidative damage accumulates with age, which is related to longer exposure to ROS and decreased antioxidant activity. Recent studies have suggested that oxidative stress increases along with BMI, and it is expressed through an elevated total peroxide level and decreased total antioxidant status.[15] Obesity, which is present in more than 50% of patients with OSA, is itself a chronic inflammatory state related to systemic oxidative stress and increased cardiovascular morbidity.[16] The presence of correlations with the PSG variables associated with breathing disruption highlights the importance of IH in oxidative stress development.

Quite interestingly, this study was the first to show that the total number of desaturations was a predictive factor of oxidative stress in OSA. Probably, the greater total number of desaturations increases the frequency of IH periods, thereby increasing the production of ROS and aggravating oxidative stress.

It is also worth mentioning the importance of oxidative stress in the pathophysiology of OSA and its comorbidities, including hypertension and T2DM. While the role of oxidative stress in the development of hypertension is relatively clear—it occurs through increased lipid peroxidation and atherosclerotic plaque formation—it is not so straightforward for T2DM. T2DM is one of many conditions that frequently co-occur with OSA. Multiple studies suggested that OSA may increase insulin resistance through various mechanisms. Oxidative stress seems not to be the direct cause of T2DM, but rather the effect of the disease.[17] In persistent hyperglycemia, around 30% of glucose in the body is metabolized via the polyol pathway, leading to a NAD^+/NADH redox imbalance and increased ROS production. In this process, NADH is used in the conversion of glucose to sorbitol, further leading to deficiency of NADH, which is a cofactor needed for regenerating the critical antioxidant, that is, reduced glutathione. Consequently, this process further aggravates oxidative stress.[18]

Moreover, the systemic oxidative stress found in OSA may represent a key mechanism of endothelial dysfunction, and be a primary reason for an increased cardiovascular risk observed in this patient population.[19,20] Individuals with OSA are characterized by increased levels of many endothelial dysfunction markers, such as von Willebrand factor, intercellular adhesion molecule-1, vascular cell adhesion molecule, or E-selectin. All of them are overexpressed due to the oxidative damage caused by oxidative stress and IH. Their levels may be decreased with OSA treatment, such as surgical intervention, and their improvement correlates

with ameliorated oxyhemoglobin saturation and glucolipid metabolism.[19]

To sum up, the study showed that patients with severe OSA were characterized by increased oxidative stress, measured for the first time through the serum level of DNA/RNA oxidative damage markers, which is a very sensitive test. Moreover, in the patients with comorbid T2DM or hypertension, oxidative damage was more intense than in the group with OSA alone. The number of desaturations was found to be a predictive factor of oxidative stress.

There are some limitations that should be acknowledged. Firstly, it was a preliminary study, with a relatively small sample. Secondly, the presence of T2DM and hypertension was assessed based only on the medical history collected from the patients, with no objective tests performed. Thirdly, the patient characteristics only comprised basic variables. The analyzed data did not include the smoking status, comorbidities other than T2DM and hypertension, inflammatory markers, basic laboratory tests, or the use of medications, all of which may affect the level of oxidative stress. Moreover, the lack of information on some factors influencing the pro-oxidant–antioxidant balance, such as supplement or vitamin intake, diet, or physical activity, may be considered a limitation. These factors should be evaluated in future studies. Therefore, the obtained results should be interpreted with caution.

To conclude, in accordance with previous studies, we showed that patients with OSA had an elevated oxidative stress level, which correlated with OSA severity. For the first time, the total number of desaturations was shown to be a potential predictive factor of increased oxidative stress level. Therefore, this parameter could serve as an unexpensive marker to assess the risk for the development of OSA comorbidities. Further research is needed to understand the crucial role of oxidative stress in OSA and its possible clinical usefulness in the prediction of certain OSA complications.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

ACKNOWLEDGMENTS None.

FUNDING The study was supported by a grant from the Medical University of Lodz (564/5-000-00/564-20-054; to ST).

CONFLICT OF INTEREST None declared.

HOW TO CITE Turkiewicz S, Sochal M, Ditmer M, Gabryelska A. Evaluation of oxidative stress in obstructive sleep apnea and its comorbidities using serum concentrations of oxidized guanine species. Pol Arch Intern Med. 2023; 133: 16542. doi:10.20452/pamw.16542

REFERENCES

1 Turkiewicz S, Ditmer M, Sochal M, et al. Obstructive sleep apnea as an acceleration trigger of cellular senescence processes through telomere shortening. Int J Mol Sci. 2021; 22: 12536.

2 Wysocka E, Cofta S, Cymerys M, et al. The impact of the sleep apnea syndrome on oxidant-antioxidant balance in the blood of overweight and obese patients. J Physiol Pharmacol. 2008; 59: 761-769.

3 Yamauchi M, Nakano H, Maekawa J, et al. Oxidative stress in obstructive sleep apnea. Chest. 2005; 127: 1674-1679.

4 Yağmur AR, Çetin MA, Karakurt SE, et al. The levels of advanced oxidation protein products in patients with obstructive sleep apnea syndrome. Ir J Med Sci. 2020; 189: 1403-1409.

5 Hopps E, Canino B, Calandrino V, et al. Lipid peroxidation and protein oxidation are related to the severity of OSAS. Eur Rev Med Pharmacol Sci. 2014; 18: 3773-3778.

6 Koike S, Sudo H, Turudome S, et al. Hyperoxidized peroxiredoxin 2 is a possible biomarker for the diagnosis of obstructive sleep apnea. Antioxidants. 2022; 11: 2486.

7 Kapur VK, Auckley DH, Chowdhuri S, et al. Clinical practice guideline for diagnostic testing for adult obstructive sleep apnea: an American Academy of Sleep Medicine clinical practice guideline. J Clin Sleep Med. 2017; 13: 479-504.

8 Reichert E, Mosiewicz J, Myśliński W, et al. Level of serum fetuin-A correlates with heart rate in obstructive sleep apnea patients without metabolic and cardiovascular comorbidities. Int J Environ Res Public Health. 2022; 19: 6422.

9 Hu Y, Mai L, Luo J, et al. Peripheral blood oxidative stress markers for obstructive sleep apnea—a meta-analysis. Sleep Breath. 2022; 26: 2045-2057.

10 Lankin VZ, Tikhaze AK, Melkumyants AM. Malondialdehyde as an important key factor of molecular mechanisms of vascular wall damage under heart diseases development. Int J Mol Sci. 2022; 24: 128.

11 Peres BU, Allen AJH, Shah A, et al. Obstructive sleep apnea and circulating biomarkers of oxidative stress: a cross-sectional study. Antioxidants. 2020; 9: 476.

12 Wan W, Wu Z, Lu J, et al. Obstructive sleep apnea is related with the risk of retinal vein occlusion. Nat Sci Sleep. 2021; 13: 273-281.

13 Jurado-Gámez B, Fernandez-Marin MC, Gómez-Chaparro JL, et al. Relationship of oxidative stress and endothelial dysfunction in sleep apnoea. Eur Respir J. 2011; 37: 873-879.

14 Ekin S, Yildiz H, Alp HH. NOX4, MDA, IMA and oxidative DNA damage: can these parameters be used to estimate the presence and severity of OSA? Sleep Breath. 2021; 25: 529-536.

15 Wonisch W, Falk A, Sundl I, et al. Oxidative stress increases continuously with BMI and age with unfavourable profiles in males. Aging Male. 2012; 15: 159-165.

16 Stanek A, Brożyna-Tkaczyk K, Myśliński W. Oxidative stress markers among obstructive sleep apnea patients. Oxid Med Cell Longev. 2021; 2021: 9681595.

17 Cifuentes-Mendiola SE, Moreno-Fierros L, González-Alva P, García-Hernández AL. Docosahexaenoic acid improves altered mineralization proteins, the decreased quality of hydroxyapatite crystals and suppresses oxidative stress induced by high glucose. Exp Ther Med. 2022; 23: 235.

18 Black HS. A synopsis of the associations of oxidative stress, ROS, and antioxidants with diabetes mellitus. Antioxidants. 2022; 11: 2003.

19 Wang F, Liu Y, Xu H, et al. Association between upper-airway surgery and ameliorative risk markers of endothelial function in obstructive sleep apnea. Sci Rep. 2019; 9: 20157.

20 O'Donnell C, O'Mahony AM, McNicholas WT, Ryan S. Cardiovascular manifestations in obstructive sleep apnea: current evidence and potential mechanisms. Pol Arch Intern Med. 2021; 131: 550-560.