Oxidative Stress in Obstructive Sleep Apnea*

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Study objectives: **To investigate the relationship between the severity of obstructive sleep apnea (OSA) and oxidative stress, which plays an important role in the pathogenesis of cardiovascular disease, and to elucidate the factors contributing to this relationship.**
Design: **Cross-sectional study.**
Participants: **A total of 128 consecutive subjects referred to the sleep laboratory of our hospital for screening or treatment of OSA.**
Interventions: **Not applicable.**
Measurements: **The severity of sleep-disordered breathing was evaluated by polysomnography. We measured urinary excretion of 8-hydroxy-2′-deoxyguanosine (8-OHdG) as an *in vivo* parameter of oxidative stress. Known risk factors for oxidative stress (age, obesity, smoking, hyperlipidemia, hypertension, and diabetes mellitus) were also investigated.**
Results: **Seventy subjects had nonsevere OSA (an apnea-hypopnea index [AHI] < 30), and 58 subjects had severe OSA (AHI ≥ 30). Urinary 8-OHdG excretion was significantly higher in the severe OSA group (p = 0.03). Furthermore, urinary 8-OHdG excretion was significantly correlated with parameters of sleep-disordered breathing, including AHI, the apnea index, the oxygen desaturation index (ODI), the duration of oxygen saturation < 90%, and the respiratory arousal index. However, only ODI was significantly correlated with urinary 8-OHdG excretion after adjustment for confounding factors that are considered to be related to oxidative stress.**
Conclusions: **The severity of OSA is independently associated with oxidative stress. Among various sleep-disordered breathing parameters, ODI is most closely related to oxidative stress.**

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Key words: 8-hydroxy-2′-deoxyguanosine; ischemia-reperfusion injury; obstructive sleep apnea syndrome; oxidative stress

Abbreviations: AHI = apnea-hypopnea index; AI = apnea index; BMI = body mass index; DBP = diastolic BP; ELISA = enzyme-linked immunosorbent assay; ESS = Epworth sleepiness scale; HbA_1c = glycosylated hemoglobin; ODI = oxygen desaturation index; 8-OHdG = 8-hydroxy-2′-deoxyguanosine; OSA = obstructive sleep apnea; ROS = reactive oxygen species; SBP = systolic BP; SDB = sleep-disordered breathing; T90 = the duration of an oxygen saturation below 90% and expressed it as a percentage of the total sleep time; T-chol = total cholesterol

In recent years, the obstructive sleep apnea (OSA) syndrome has emerged as an important risk factor for cardiovascular disease. Associations have been reported between sleep apnea and systemic hypertension, pulmonary hypertension, ischemic heart disease, and stroke.[1–6] However, the underlying mechanisms are not entirely understood. OSA is characterized by recurrent nocturnal obstruction of the upper airway. Each episode of airway obstruction is usually followed by a marked decrease of arterial oxygen saturation, which rapidly normalizes after ventilation resumes. These repeated changes of oxygen saturation could be considered analogous to recurrent episodes of ischemia-reperfusion injury, which causes damage after the restoration of blood flow to ischemic or hypoxic tissues. Although several mechanisms are involved, such damage is mainly attributed to the production of reactive oxygen species (ROS) during reoxygenation.[7,8] ROS are highly reactive molecules that interact with nucleic acids, lipids, and proteins, and are considered to have

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an important role in the development of cardiovascular disease. In patients with untreated OSA, episodes of hypoxia/reoxygenation occur frequently during each hour of sleep and may happen every night for several decades, so this cumulative oxidative stress may play a role in the onset of cardiovascular complications.

Studies have provided evidence that supports an increase of oxidative stress in OSA. Schulz et al[9] and Dyugovskaya et al[10] detected an increase in the production of ROS in OSA, while Barcelo et al[11] demonstrated an increase of plasma lipid peroxides. In addition, Christou et al[12] proved that patients with severe OSA have a reduced antioxidant capacity. Furthermore, Carpagnano et al[13] found an increase of the 8-isoprostane level in the exhaled breath condensate in OSA patients. All of these studies have indicated a significant relationship between OSA and oxidative stress. It is well known that the prevalence of asymptomatic sleep-disordered breathing (SDB) is several times higher than that of recognized SDB.[14] Therefore, oxidative stress in persons with SDB may also be a public health issue. Accordingly, we think that it is important to clarify the factor that most accurately predicts oxidative stress in persons with SDB. Recently, Lavie et al[15] demonstrated that the respiratory disturbance index was an independent predictor of lipid peroxidation according to stepwise regression analysis. Although the apnea-hypopnea index (AHI) or respiratory disturbance index are the common parameters used to assess the severity of SDB, these indexes reflect various components of SDB because the definition of hypopnea includes desaturation and/or arousal. Thus, it remains unclear which components of SDB contribute to the relationship with oxidative stress. Assuming that the desaturation-reoxygenation cycle causes oxidative stress, we hypothesized that the frequency of oxygen desaturation events, evaluated as an oxygen desaturation index (ODI), may be the best predictor of oxidative stress in SDB.

On the basis of these considerations, we performed the present study to confirm that the severity of OSA is correlated with oxidative stress, using a marker of DNA oxidation (8-hydroxy-2′-deoxyguanosine [8-OHdG]) to assess oxidative stress. We also evaluated the correlations between various parameters of SDB, including the ODI, and oxidative stress in a cross-sectional study.

Materials and Methods

Subjects

A total of 128 consecutive subjects (115 men and 13 women) referred to Tenri City Hospital with suspected OSA were enrolled between March 2003 and March 2004. Their primary symptoms were excessive daytime sleepiness and snoring. All of the subjects underwent polysomnography and completed the Epworth sleepiness scale (ESS) questionnaire.[16] Patients were also asked about their regular medications and smoking habits. We explained the purpose of this study and the procedures involved to the subjects and obtained written consent from all of them.

Anthropometric Measurements

The height and body weight were measured on the day when diagnostic polysomnography was performed. As an indicator of obesity, we used the body mass index (BMI). On the morning after polysomnography, the systolic BP (SBP) and the diastolic BP (DBP) were measured using an automated sphygmomanometer after the patient had rested in the supine position for at least 5 min.

Sleep Studies

Polysomnography was performed in all of the subjects. Data acquisition started from 9 PM and continued until 6 AM on the following morning. Nasal airflow was monitored by a thermistor (Nihon-Kohden; Tokyo, Japan), arterial oxygen saturation was measured with a pulse oximeter (Pulsox 7; Minolta; Tokyo, Japan), and thoracoabdominal wall motion was recorded by a respiratory inductance plethysmograph (Respitrace; Ambulatory Monitering; Ardsley, NY). Sleep patterns were monitored from the EEG (C3/A2 and O2/A1), electro-oculogram, and submental electromyogram, with the international 10–20 electrode system being used for EEG. Apnea was defined as cessation of airflow for $\geq$ 10 s, and hypopnea was defined as a decrease of respiratory inductance plethysmograph sum amplitude by at least 50% lasting for $\geq$ 10 s.[17,18] The apnea index (AI) was calculated as the number of apnea events per hour of the total sleeping time. AHI was calculated as the number of apnea-hypopnea events per hour of the total sleeping time. Arousals were defined according to the standard American Sleep Disorders Association criteria.[19] The respiratory arousal index was calculated as the number of arousals related to disordered-breathing events per hour of total sleeping time. To assess the severity of hypoxia induced by apnea-hypopnea events, we also measured the duration of an oxygen saturation $<$ 90% and expressed as a percentage of the total sleeping time (T90), as well as ODI, which is defined as the number of $>$ 4% dips in oxygen saturation per hour of sleep and the mean lowest saturation related to apnea-hypopnea events (mean nadir oxygen saturation).

Collection of Blood and Urine

On the morning after polysomnography, peripheral venous blood samples and urine samples were collected from the fasting subjects. The plasma levels of total cholesterol (T-chol) and glycosylated hemoglobin (HbA_1c) were measured by the hospital laboratory according to routine procedures.

As a parameter for *in vivo* estimation of oxidative stress, we measured the urinary excretion of 8-OHdG. The 8-OHdG is a modified DNA base that has recently been used for evaluation of oxidative DNA damage.[20,21]

The 8-OHdG concentration was determined using an enzyme-linked immunosorbent assay (ELISA) kit (Japan Institute for the Control of Aging; Nikken SEIL Corporation; Fukuroi, Shizuoka, Japan) and the urinary creatinine concentration was determined by a standard automated colorimetric assay. Then urinary 8-OHdG excretion was normalized for the urinary creatinine level and is presented as the urinary (8-OHdG [nanograms/milliliter]/creatinine [milligrams/milliliter]) ratio. A stable correlation between spot urine levels and 24-h excretion of 8-OHdG has already been established.[22]

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Statistical Analysis

The subjects were classified into two groups (nonsevere OSA and severe OSA) based on the results of polysomnography, using an AHI of 30 as the cut-off value. Comparison of continuous variables between the two groups was done by the unpaired *t* test, and categorical variables were compared by the χ^2 test. To investigate correlations between oxidative stress and SDB or the other confounding parameters, we performed a Pearson correlation analysis.

To determine whether the severity of OSA was independently associated with oxidative stress, multiple regression analysis was performed with 8-OHdG as the dependent variable. Before the analyses, log transformation of the data was performed (natural log [x + 0.1]) on T90 because of the skewed distribution of the data. Statistical analysis was done using software (SPSS Version 10.0 for Windows; SPSS; Chicago, IL).

Results

Based on the results of polysomnography, 70 subjects and 58 subjects were classified as having nonsevere OSA (including primary snoring and mild-to-moderate OSA) and severe OSA, respectively. The percentage of female subjects was significantly lower in the severe OSA group. Urinary 8-OHdG excretion was significantly higher in the severe OSA group (p = 0.03). The percentage of current smokers, BMI, and DBP were all significantly higher in the severe OSA group ($p < 0.01$). However, age, ESS score, and percentage of subjects receiving medications for hypertension, hyperlipidemia, or diabetes were similar between the two groups (Table 1). The difference in urinary 8-OHdG excretion remained even after excluding the female subjects and current smokers.

Figure 1 shows the correlations between urinary 8-OHdG excretion and the AHI or ODI. Urinary 8-OHdG excretion was significantly correlated with both the ODI ($r = 0.326$, p = 0.0002) and the AHI ($r = 0.273$, p = 0.0018). In addition, the AI ($r = 0.253$, p = 0.0039), the respiratory arousal index ($r = 0.263$, p = 0.0027), log-transformed T90 ($r = 0.235$, p = 0.0075), BMI ($r = 0.228$, p = 0.0096), and current smoking ($r = 0.277$, p = 0.0015) were significantly correlated with urinary 8-OHdG excretion. However, the age, ESS score, BP, T-chol, and HbA_1c were not correlated with urinary 8-OHdG excretion.

Multiple regression analysis was also performed to examine the independent relationship between SDB and urinary 8-OHdG excretion (Table 2), with 8-OHdG excretion as the dependent variable and the SDB parameters and confounding factors related to oxidative stress as independent variables. This analysis showed that only ODI had a significant independent relationship with urinary 8-OHdG excretion. The partial coefficient of determination (partial R^2) of ODI to the urinary 8-OHdG level after adjustment for confounding factors was 0.09. This means that ODI account for 9% of the total variance in the urinary 8-OHdG level.

Table 1—*Comparison of Characteristics in Patients With Nonsevere and Severe OSA*

Characteristics	Nonsevere OSA	Severe OSA	p Value
Subjects, No.	70	58	
Age, yr	49.3 ± 12.2	48.8 ± 11.0	0.83
Gender, No.			
Male	58	57	
Female	12	1	< 0.01
ESS	11.3 ± 5.7	12.9 ± 5.2	0.12
BMI	25.9 ± 3.8	29.5 ± 5.3	< 0.01
AHI	12.1 ± 9.3	60.7 ± 19.2	< 0.01
AI	7.0 ± 7.0	51.4 ± 20.3	< 0.01
ODI	10.5 ± 9.3	62.3 ± 24.2	< 0.01
Mean nadir oxygen saturation, %	90.9 ± 2.7	85.9 ± 5.6	< 0.01
T90, %	3.9 ± 7.3	31.4 ± 28.0	< 0.01
Respiratory arousal index	16.7 ± 9.9	60.6 ± 22.5	< 0.01
8-OHdG†	8.5 ± 2.4	9.5 ± 2.5	0.03
T-chol, mg/dL	198.2 ± 33.7	205.3 ± 32.5	0.23
HbA_1c, %	5.4 ± 0.7	5.5 ± 0.7	0.90
SBP, mm Hg	126.0 ± 14.5	131.7 ± 22.4	0.12
DBP, mm Hg	78.6 ± 11.9	86.7 ± 13.0	< 0.01
Hyperlipidemia, %‡	7.1	17.2	0.08
Hypertension, %‡	21.4	34.5	0.10
Diabetes mellitus, %‡	7.1	8.6	0.76
Current smoke, %‡	17.1	41.4	< 0.01

*Data are presented as mean ± SD unless otherwise indicated.

†8-OHdG is expressed as (8-OHdG [ng/mL]/creatinine [mg/mL]) ratio.

‡Percentage of patients receiving medication for hypertension, hyperlipidemia, or diabetes, and current smoke.

Discussion

The present study indicated that oxidative stress, as evaluated by urinary 8-OHdG excretion, was higher in severe OSA than in nonsevere OSA. Various parameters of the severity of OSA, including ODI, AHI, AI, respiratory arousal index, and T90 were significantly correlated with oxidative stress as expressed by 8-OHdG excretion. Among these parameters, ODI was most strongly correlated with oxidative stress, and the relationship survived adjustment for confounding factors that may contribute to oxidative stress.

Various methods for quantifying oxidative stress have been identified. We adopted the urinary level of 8-OHdG as a marker of oxidative stress; to our knowledge, this is the first study to assess the correlation between OSA and 8-OHdG. ROS induce several types of DNA damage, such as strand breaks, base modifications, and cross-linking between DNA and various proteins.[23] By inducing hydroxylation of

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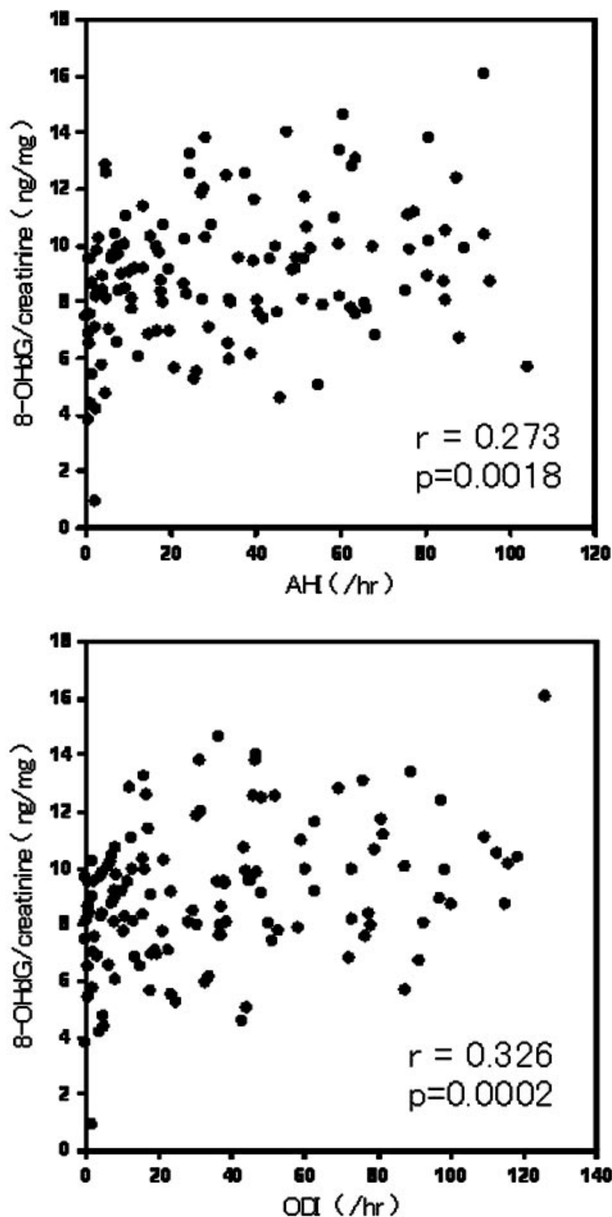

FIGURE 1. Scatterplots of 8-OHdG vs AHI (*upper panel*) and ODI (*lower panel*). 8-OHdG was significantly correlated with AHI (r = 0.273, p = 0.0018) and ODI (r = 0.326, p = 0.0002) by Pearson correlation analysis.

the C-8 position of 2′-deoxyguanosine, ROS produce 8-OHdG. This modified DNA base has recently been reported to be a reliable marker of oxidative DNA damage when measured in the tissues and urine.[20,21] Moreover, 8-OHdG is very stable and is excreted in the urine without being metabolized. Accordingly, measurement of urinary 8-OHdG is considered to be one of the most promising methods for quantifying *in vivo* oxidative damage. Measurement of 8-OHdG has been performed by several methods, among which high-performance liquid chromatography with electrochemical detection is well accepted. Recently, a commercial ELISA kit based on a monoclonal antibody was developed for the determination of 8-OHdG, and a good correlation has been demonstrated between 8-OHdG values measured by high-performance liquid chromatography with electrochemical detection and ELISA,[24,25] so we used this kit in the present study.

There is a growing body of evidence that OSA is associated with oxidative stress. Earlier studies[9–11] have demonstrated an increase of oxidative stress, which was assessed as plasma lipid peroxide levels or superoxide production, in patients with OSA compared to control subjects. A decrease of oxidative stress after continuous positive airway pressure treatment has also been demonstrated. More recent studies[12,13,15] have shown a correlation between oxidative stress (estimated by lipid peroxidation or antioxidant capacity) and AHI; these studies have revealed a relationship between the severity of OSA and the level of oxidative stress. However, the factors contributing to increased oxidative stress in OSA have not been fully elucidated. Although the AHI is a representative parameter of the severity of OSA, it usually includes factors other than disordered breathing alone, typically the severity of desaturation and/or arousal, depending on the definition of hypopnea that is used. In order to estimate these factors separately, we adopted a definition of hypopnea that did not include desaturation or arousal. As a result, although simple regression analysis showed a significant correlation between various SDB parameters (including AHI) and oxidative stress, the parameters other than ODI were not independent predictors of the urinary 8-OHdG level in multivariate analysis. It is interesting that ODI was the best predictor of the urinary 8-OHdG level, because ODI reflects the frequency of transient episodes of hypoxemia with subsequent rapid reoxygenation during which ROS are assumed to be produced. Another possible explanation for the tightest relationship between ODI and the oxidative stress is that ODI is thought to be a more reproducible variable than AHI. In either case, we think ODI is the most important parameter to predict the oxidative stress in SDB.

It is well established that oxidative stress is correlated with many factors, including obesity,[26,27] smoking,[28,29] age,[30,31] hypertension,[32,33] hyperlipidemia,[34,35] and diabetes.[36,37] A large percentage of patients with severe OSA also have one or more of these factors, so assessment of the relationship between OSA and oxidative stress could be influenced by such confounding variables. In our study, the urinary 8-OHdG level was correlated with BMI and current smoking, but was not correlated with age,

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Table 2—*Univariate and Multivariate Association Between Urinary 8-OHdG Excretion and SDB*

SDB Parameters	Crude				Adjusted*			
	B (β)‡	SE†	p Value	R^2	B (β)‡	SE†	p Value	Partial R^2
ODI	0.024 (0.326)	0.006	0.0002	0.106	0.020 (0.275)	0.010	0.0398	0.090
AHI	0.024 (0.273)	0.007	0.0018	0.074	0.019 (0.223)	0.010	0.0676	0.061
AI	0.023 (0.253)	0.008	0.0039	0.064	0.019 (0.211)	0.010	0.0716	0.053
Mean nadir oxygen saturation, %	− 0.084 (− 0.168)	0.044	0.0580	0.028	− 0.010 (− 0.021)	0.061	0.8648	0.004
T90, % (log transformed)	0.282 (0.235)	0.104	0.0075	0.055	0.167 (0.141)	0.150	0.2676	0.033
Respiratory arousal index	0.027 (0.263)	0.009	0.0027	0.069	0.021 (0.204)	0.013	0.1038	0.054

*BMI, current smoke, sex, DBP, age, T-chol, HbA_1c, medication adjusted.
†SE for unstandardized regression coefficient.
‡B = unstandardized regression coefficient; β = standardized regression coefficient.

BP, T-chol, and HbA_1c. Some of the medications used to treat hypertension, hyperlipidemia, and diabetes can reduce oxidative stress by an antioxidant action.[38–41] Therefore, we included all of these confounding factors as independent variables in the multiple regression model. As a result, we showed that only ODI was independently correlated with oxidative stress measured as urinary 8-OHdG excretion.

There are several potential limitations to our study. First, all of the subjects were referred to us with symptoms of OSA. They may have had more risk factors for oxidative stress than asymptomatic subjects, so we cannot exclude the possibility that our study population was biased. Second, there is no true control group in our study. We compared severe OSA group with nonsevere OSA group and we performed correlation analyses in these subjects. However, we think this limitation would have weakened rather than strengthened the estimated relationship between the oxidative stress and SDB. Third, the correlation coefficients between SDB and 8-OHdG, although statistically significant, are relatively low ($r = 0.326$ for ODI), which means approximately 11% of variance of 8-OHdG is explained by the ODI. Furthermore, after the influence of the confounding factors related to oxidative stress is excluded by multivariate analysis, ODI explains only 9% of variance of 8-OHdG. Therefore the clinical significance of this relationship is difficult to address in our study. Forth, this was a cross-sectional study, so we could not assess the causal relationship between OSA and oxidative stress. To clarify this point, a prospective study involving clinical intervention will be needed.

In summary, we conclude that oxidative stress is greater in patients with severe OSA than in those with nonsevere OSA. The severity of OSA (estimated as the ODI) was significantly correlated with oxidative stress (estimated as urinary 8-OHdG excretion) after adjustment for confounding factors. These findings may help to explain the pathogenesis of cardiovascular complications in patients with OSA, which is characterized by repeated episodes of desaturation.

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