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Original Article

Oxidative stress parameters and their correlation with clinical, metabolic and polysomnographic parameters in severe obstructive sleep apnea syndrome

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Abstract: The aim of the present study was to assess the levels of oxidative stress markers, catalase (CAT), glutathione peroxidase (GPX) and malondialdehyde (MDA) in severe OSAS and to investigate any correlation between oxidative stress markers and clinical, metabolic and polysomnographic parameters. A total of 30 patients with severe OSAS and 30 healthy controls were included in this cross-sectional, clinical study. Demographic data, polysomnographic, biochemical and clinical indices as well as serum levels of CAT, MDA and GPX were measured and compared in OSAS and control groups. Furthermore, OSAS patients with and without pulmonary hypertension (PHT) were evaluated in terms of levels of CAT, MDA and GPX. Patients with severe OSAS exhibited significantly lower serum levels of CAT (P<0.001) and GPX (P<0.001). Serum MDA levels were remarkably higher in OSAS group (P<0.001). Correlation analysis revealed that levels of CAT and GPX were correlated with apnea-hypopnea index and there was a correlation between serum levels of MDA and CRP. Severe OSAS patients with and without PHT did not reveal any differences for CAT (P=0.789), MDA (P=0.805) and GPX levels (P=0.281). Our results have shown that oxidative stress markers significantly changed in patients with severe OSAS. This information is noteworthy because documentation of the role of oxidative stress in OSAS may have important implications regarding diagnosis, monitoring, treatment and prognosis.

Keywords: Obstructive sleep apnea syndrome, hypoxia, oxidative stress, inflammation

Introduction

Obstructive sleep apnea syndrome (OSAS) is a common nocturnal disorder characterized by recurrent episodes of apnea and hypopnea accompanied with daytime sleepiness and cardiopulmonary dysfunction. Owing to the cessation of breathing, recurrent intermittent hypoxia and oxygen desaturation occur. These disturbances lead to arousals, imbalance of autonomic nervous system and hypoxia-reoxygenation resulting in ischemia-reperfusion injury. 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].

Oxidative stress is defined as an imbalance between oxidant and antioxidant mechanisms. The role of oxidative stress in OSAS has been studied in several publications [2-5]. Cardiovascular complications have been associated with oxidative stress and inflammatory reactions in OSAS [6, 7].

It is questionable whether OSAS itself causes in oxidative stress and inflammation or these changes occur as a result of accompanying metabolic comorbidities [1]. Studies focussing on the relationship between the oxidative stress and inflammation markers with metabolic and clinical parameters may aid not only in a better understanding of the underlying pathogenesis but also biomarkers can be developed for diagnostic, prognostic and therapeutic purposes. Malondialdehyde (MDA) is an indicator of oxidative stress induced lipid peroxidation [8], while catalase (CAT) and glutathione peroxidase

(GPX) are anti-oxidant enzymes that combat against oxidative stress injury [9].

The present study was performed in order to investigate the alterations of CAT, GPX and MDA in severe OSAS and to investigate whether alterations of these oxidative stress markers were linked with clinical, metabolic and polysomnographic parameters.

Materials and methods

Study design

This cross-sectional, controlled, clinical trial was carried out in the chest diseases department of our tertiary care center subsequent to the approval of the local Institutional Review Board. Written informed consent was obtained from all participants and adherence to the principles of the Helsinki Declaration of 1975, as revised in 2000, was provided.

The study population consisted of 60 adults consistent of 30 severe OSAS patients and 30 healthy controls. Variables under investigation were demographics (age, gender, smoking habit, neck circumference, body-mass index), serum levels of haemoglobin, haematocrit, low-density lipoproteins, triglycerides, high-density lipoproteins, cholesterol, C-reactive protein, D-dimer cardiac and polysomnographic indices include ejection fraction, apnea-hypopnea index, oxygen desaturation index, minimum and mean oxygen saturations, arousal index, mean and maximum durations of apnea and duration and percentage of periods with oxygen saturation <90%.

Inclusion criteria for OSAS patients consisted of apnea hypopnea index >30, medical history of no accompanying systemic diseases or active treatment likely to influence oxidant/antioxidant balance. Control group was composed of healthy adults with apnea hypopnea index <5 who were devoid of any systemic disorders or medications that may alter oxidant/antioxidant balance. Cases that are <18 or >65 years of age, presence of mild or moderate OSAS diagnosed by polysomnography were excluded from the study.

Levels of oxidative stress markers (MDA, CAT, GPX) were compared in OSAS and control groups. Moreover, correlation of with inflammatory, biochemical and metabolic variables were sought.

Pulmonary hypertension (PHT) was described as mean pulmonary artery pressure (PAP) >20 mmHg [10]. Patients with severe OSAS were subgrouped into two categories with respect to the presence of PHT. Seven of OSAS patients (23.3%) had PHT. Obstructive sleep apnea syndrome patients with and without PHT were compared in terms of CAT, GPX and MDA levels.

Sleep studies

Apnea is associated with ≥4% reduction of oxygen saturation from baseline, while hypopnea was diagnosed when flow was reduced by ≥50% in the presence of thoracic and abdominal breathing movement. Hypopneas were only included if they were linked with a subsequent reduction of oxygen saturation ≥4% from baseline [11]. A haemoglobin oxygenation-desaturation index (ODI) was termed as the number of desaturations (>4% from baseline) per hour of sleep time [11].

Grading of OSAS was made as follows: i) mild if 5≤ AHI <15; ii) moderate if 15≤ AHI <30; iii) severe if AHI ≥30 [11, 12]. All of the OSAS patients recruited in this study had been diagnosed with severe OSA with respect to these criteria after an overnight polysomnography in the sleep laboratory of a university hospital. The investigation was performed with EMBLA 4500 (Cogent Technologies, USA) monitoring system. According to the known diagnostic standards, the minimal time for examination was 6 h. For the documentation of the sleep, we used standard 14 channel polysomnography, including electroencephalogram (C3-A2, C4-A1, O1-A2, O2-A1), electro-oculograms, electromyograms (EMG) of the left/right extremity, electrocardiogram (ECG), heart rate, nasal and oral air flow, thoracic and abdominal movements, registration of snoring, position of the body, pulse oxymetry monitored oxygen saturation (SaO_2) and a polysomnography with video-watching.

Pulmonary hypertension was diagnosed with respect to internationally approved criteria [10]. Echocardiography was performed in all patients before polysomnography. M-mode, 2-D and colour Doppler options were used in left lateral

Table 1. Descriptive statistics for variables under investigation in OSAS and control groups

Variable	OSAS group	Control group
Catalase‡ (U/mg)	0.002-0.006	0.004-0.006
Glutathione peroxidase (μmol/mg)	0.04±0.03	0.159±0.47
Malondialdehyde (Hmol/L)	0.698-0.434	0.179±0.56
Haemoglobin (g/dl)	15.93±1.74	14.85±1.57
Haematocrit (%)	47.61±5.67	44.66±4.58
Low-density lipoprotein (mg/dl)	115.47±39.43	107.73±33.18
Triglycerides (mmol/l)	213.00-143.47	130.23±112.91
High-density lipoproteins (mmol/l)	42.00±10.64	43.30±11.22
Cholesterol (mmol/l)	203.93±41.98	173.27±41.03
C-reactive protein‡ (mg/l)	0.919-0.872	0.49-0.52
D-dimer (μg/l)	129.27-93.75	136.57±68.53
Ejection fraction (%)	61.87±5.34	64.30±4.76
Smoking habit‡	10.30-14.33	3.70-8.82
Apnea-hypopnea index	69.02±29.04	2.23±1.43
Oxygen desaturation index	37.19±21.20	1.73±1.60
Minimum O_2 saturation (%)	72.03±10.37	92.20±2.56
Mean O_2 saturation (%)	86.63±4.39	94.27±1.68
Arousal index	13.41±8.76	1.77±1.52
Body-mass index	34.35±6.23	25.48±2.29
Neck circumference	41.73±3.22	38.43±1.50
Duration of O_2 saturation <90%	179.61±130.06	3.47±3.19
Percentage of O_2 saturation <90% (%)	51.21±38.23	1.73±1.60
Mean duration of apnea	17.81±4.12	3.47±3.19
Maximum duration of apnea	79.41±28.09	1.73±1.60

Hint: ‡= expressed in median-interquartile range; the rest of variables are demonstrated as median ± standard deviation.

position and pulmonary arterial pressures, structure and functions of right heart were evaluated according to criteria of American Heart Association [13]. Transthoracic echocardiography was performed on all subjects, and Philips HD 11 XE echocardiography device (Philips Medical Systems, Bothell, WA) was used for this purpose.

Serum samples

Fasting blood samples were collected by peripheral venipuncture from brachial region early in the morning (07: 00 A.M-08: 00 A.M) and put in EDTA tubes. Samples were centrifuged at 5,000 rpm for 5 minutes and separated serum samples were maintained frozen (-20°C) until analysis. The level of MDA was measured spectrophotometrically on UV-VIS spectrophotometer with thiobarbituric acid method. The reference range in healthy individuals was 4.6 to 9.4 Hmol/L [8]. Analysis of CAT activity was made according to the method previously described by Aebi *et al.* the literature [14]. Measurement of GPX activity was measured according to the method previously described by Beutler *et al.* [15].

Statistical analyses

Data were analysed using the IBM SPSS Statistics 20 program (*SPSS Inc, Chicago, IL, USA*). The conformability of the data for normal distribution was tested with Kolmogorov-Smirnov test. Parameters with normal distribution were analysed with parametric methods, while variables without a normal distribution were evaluated with non-parametric methods. Comparison of two independent groups was performed with Independent Samples T-test and Mann-Whitney U test. Categorical variables were compared using exact method of Pearson Chi square tests. Correlation of variables with normal distribution was carried out using Pearson Correlation test, whereas Spearman's rho test was used for variables without normal distribution. Quantitative variables were expressed as either mean ± standard deviation or median-interquartile range. Confidence interval was set at 95% and a statistical difference was taken as $P<0.05$.

Results

The demographic, clinical, polysomnographic variables under investigation as well as the oxidative stress parameters are shown in **Table 1**.

Table 2 indicates a comparison of demographic parameters and oxidative stress markers in OSAS and control groups. Levels of CAT and GPX were significantly lower in the OSAS group ($P<0.001$), while MDA level was remarkably higher in OSAS patients ($P>0.001$). In terms of

Table 2. Comparison of demographic variables and anti-oxidant levels in OSAS and control groups

Variable	OSAS group	Control group	*P* value
Catalase (U/mg)	0.041±0.030	0.159±0.047	<0.001*
Glutathione peroxidase‡ (μmol/mg)	0.0004-0.0006	0.002-0.002	<0.001*
Malondialdehyde‡ (Hmol/L)	0.596-0.112	0.202-0.117	<0.001*
Gender (male/female)	21/9	16/14	0.184
Smoking habit (%)	40%	25%	0.091

Hint: ‡= expressed as median-interquartile range, *= statistically significant; OSAS = obstructive sleep apnea syndrome.

Table 3. Oxidative stress parameters in OSAS patients with pulmonary arterial pressure <20 mmHg and ≥20 mmHg

Variable	OSAS group		*P* value
	PAP <20 mmHg	PAP ≥20 mmHg	
Catalase‡ (U/mg)	0.004-0.007	0.003-0.007	0.789
Glutathione peroxidase‡ (μmol/mg)	0.040-0.059	0.022-0.045	0.281
Malondialdehyde‡ (Hmol/L)	0.596-0.112	0.202-0.117	0.805

Abbreviations: ‡= expressed as median-interquartile range, OSAS = obstructive sleep apnea syndrome; PAP = pulmonary arterial pressure.

Table 4. Correlation of oxidative stress parameters to metabolic and clinical variables under investigation

Variable	Correlate	*r* value	*P* value
Catalase	Haemoglobin	0.574	0.001*
	Haematocrit	0.550	0.002*
	Neck circumference	0.517	0.003*
	Low density lipoprotein	0.375	0.041*
	Apnea-hypopnea index	0.469	0.009*
	Arousal index	0.366	0.047*
	Mean oxygen saturation	-0.369	0.045*
Glutathione peroxidase	Apnea-hypopnea index	0.407	0.026*
	Mean duration of apnea	-0.365	0.048*
Malondialdehyde	C-reactive protein	0.461	0.010*

Hint: statistically significant.

gender distribution and frequency of smoking habit, no difference could be detected between groups (*P*=0.184 and *P*=0.091, respectively).

Table 3 demonstrates the levels of CAT, MDA and GPX in OSAS patients with pulmonary arterial pressure (PAP) <20 mmHg and PAP ≥20 mmHg. No significant difference was observed between two groups regarding the levels of CAT (*P*=0.789), MDA (*P*=0.805) and GPX (*P*=0.281).

Correlation of oxidative stress indicators to metabolic, polysomnographic and biochemical variables is shown in **Table 4**. Levels of CAT were correlated directly with apnea-hypopnea index, arousal index, as well as haemoglobin, haematocrit, low-density lipoprotein levels and neck circumference. There was an inverse relationship between mean oxygen saturation and CAT levels. Glutathione peroxidase levels were directly correlated with apnea-hypopnea index and inversely correlated with mean duration of apnea. Levels of MDA were directly correlated with those of CRP.

Discussion

In this study, we aimed to evaluate the levels of oxidative stress indicators, MDA, CAT and GPX in severe OSAS patients and to investigate whether levels and activities of these markers were correlated with demographic, biochemical, metabolic and polysomnographic parameters. Our results yielded that OSAS patients had higher levels of MDA and lower levels of CAT and GPX. A correlation was detected between CAT and GPX levels to polysomnographic indices. Moreover, serum levels of MDA were correlated well with CRP levels. Co-existence of PHT seemed not to affect CAT, MDA and GPX in OSAS patients.

The pathology of OSAS seems to involve repeated cycles of hypoxia during sleep. Cyclic changes in arterial oxygen saturation may increase the production of ROS [7, 16]. However, the exact role of oxidative stress in OSAS pathogenesis has not been completely clarified. Hypoxia-reoxygenation injury may enhance release of free radicals, which subsequently leads to injury of vascular endothelium [17]. From this point of view, oxidative stress may contribute to the

association between OSAS and cardiovascular morbidities [1, 17].

Studies investigating the oxidative stress in OSAS have yielded controversial results. Some publications have shown elevated levels of oxidative stress in OSAS patients in comparison to healthy controls [18-20], while other authors have suggested that levels of oxidative stress indicators were similar in OSAS and controls [9, 21]. These differences may be attributed to factors like heterogeneity of groups and co-existent morbidities [16].

Many studies have reported that OSAS is associated with reactive oxidative stress (ROS) [16, 17]. OSAS patients have increased levels of reactive oxygen metabolites in the blood that may result in cellular damage [20]. Oxidative stress and OSAS are also linked to endothelial dysfunction, which may predispose OSAS patients to cardiovascular and cerebrovascular diseases [22]. However, whether or not there are indeed increased levels of oxidative stress in OSAS patients is still uncertain [17].

Our findings support that abnormal levels of oxidative stress markers are detected in OSAS and these changes are accompanied by relevant findings of polysomnographic and inflammatory parameters. Increased oxidative stress may have important clinical implications in OSAS patients in terms of diagnostic, therapeutic or prognostic aspects.

Conflicting results on the role of oxidative stress in OSAS may be due to different pathways that may be influenced by metabolic, systemic, genetic or inflammatory factors [16, 17].

Our data was in conjunction with publications that suggest a change in levels of oxidative stress markers in OSAS [5, 16, 18, 19]. Levels of CAT and GPX were correlated with polysomnographic indices such as AHI, mean duration of apnea and mean oxygen saturation. Potential roles of these molecules as supportive measures for polysomnography remain to be investigated in further studies. Another important result was that MDA levels were well correlated with CRP and this denotes an inflammatory aspect of oxidative stress injury, which may have diagnostic and therapeutic implications. Complex interactions of multiple parameters in oxidative and inflammatory pathways constitute obvious challenges for clarification of roles of molecules in an isolated fashion.

Our results were contradictory to publications that deny any significant changes in markers of oxidative stress in OSAS [9, 17, 21]. Owing to the cyclic episodes of hypoxia-reoxygenation, indices of oxidative stress and DNA damage can be increased in OSAS patients. These intermittent episodes may enhance the production of free radical production that subsequently results in oxidative stress and DNA damage. Wali et al. [9] did not come across any differences in GPX or CAT activities in hypoxic and non-hypoxic patients. They explained the lack of increased oxidative stress and DNA damage in OSAS patients by the hypothesis that OSAS does not lead to the generation of oxidative stress obviously in the absence of significant comorbidities. Their hypothesis was supported by the finding that continuous positive airway pressure therapy does not alter levels of antioxidant enzymes [16].

Some limitations of the present study must be mentioned. First, our sample size is relatively small and the study design is cross-sectional. Moreover, possible metabolic, inflammatory or dietary confounding factors or methodological differences, instability of reactive oxygen species may predispose to conflicting results. Hence, extrapolations from our results must be made with caution.

To conclude, results of the current study indicate that oxidative stress including lipid peroxidation and antioxidants was significantly more notable in OSAS patients. This information is noteworthy because documentation of the role of oxidative stress in OSAS may have important implications regarding diagnosis, monitoring, treatment and prognosis.

Disclosure of conflict of interest

None.

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