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Impact of severity of obstructive sleep apnea (OSA) and body composition on redox status in OSA patients

Uticaj težine opstruktivne *sleep* apneje (OSA) i telesnog sastava na redoks status kod pacijenata sa OSA

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Abstract

Background/Aim. There is an increasing number of studies on the existence of systemic oxidative stress in patients with obstructive sleep apnea (OSA) which is an important mechanism linking OSA and endothelial dysfunction with increased risk for cardiovascular diseases. Comorbidities must be also considered, especially obesity, as the most important source of oxidative stress independently of OSA. The aim of this paper was to show if OSA severity increases the level of markers of systemic oxidative stress and reduces antioxidant capacity, independently from body mass index (BMI) and nutritional status. **Methods.** One hundred and twenty-eight patients with OSA were included in the trial. Based on the results of a sleep study-polysomnography, the examinees were divided into two groups according to apnea-hypopnea index (AHI < 15 and AHI ≥ 15). Nutritional status was estimated by using the BMI and body composition. Body composition was determined by dual X-ray absorptiometry (DXA) whole-body scan. Redox status of patients with OSA was determined by measuring the concentration of NO in plasma and antioxidant capacity was evaluated using the plasma level of reduced glutathione (GSH) as a marker of antioxidant capacity. **Results.** Significantly higher mean values of NO were found in the group with AHI ≥ 15 in comparison to AHI < 15 group (1.269 ± 0.789 vs. 0.462 ± 0.373 nmol/mL, respectively; $p = 0.001$), while significantly higher levels of GSH were found in the group with AHI < 15 in comparison to AHI ≥ 15 group (238.08 ± 84.37 vs. 172.77 ± 83.88 mg/mL, respectively; $p = 0.04$). Independent predictors of plasma GSH level (multivariate regression analysis) were: desaturation index (ODI) [B = -0.157; 95% confidence intervals (CI): -0.262–0.053], mean $SatO_2$ (B = -4.76; 95% CI: -9.21–0.306) and min $SatO_2$ (B = 0.118; 95% CI: 0.03–0.206). ODI was singled out as an independent predictor of NO concentration in plasma (B = 0.038; 95% CI: 0.011–0.065). No significant statistical difference was found in mean values of BMI and body composition parameters in patients with AHI < 15 and AHI ≥ 15. None of the markers of systemic oxidative stress was associated with BMI and body composition assessment parameters. **Conclusion.** OSA severity is significantly associated with reduced antioxidant capacity and increased level of systemic oxidative stress. The degree of desaturation during sleep considerably affects systemic oxidative stress in patients with OSA independently from nutritional status and body composition.

Key words:
sleep apnea, obstructive; oxidative stress; nutritional status; body composition.

Apstrakt

Uvod/Cilj. Sve je više istraživanja o postojanju sistemskog oksidativnog stresa kod pacijenata sa opstruktivnom apnejom u snu (OSA), kao mogućoj vezi OSA sa endotelnom disfunkcijom i bolestima kardiovaskularnog sistema. Mora se uzeti u obzir i postojanje komorbiditeta, pre svega gojaznosti, koji mogu doprineti oksidativnom stresu nezavisno od OSA. Cilj ovog rada bio je da pokaže da li težina OSA doprinosi povećanom nivou sistemskog oksidativnog stresa i smanjenju antioksidativnog kapaciteta, nezavisno od indeksa telesne mase (BMI) i telesnog sastava kod pacijenata sa OSA. **Metode.** Istraživanjem je bilo obuhvaćeno 128 pacijenata sa OSA. Na osnovu *sleep* studije – polisomnografije, ispitanici su bili podeljeni u dve grupe prema *apnea-hypopnea* indeksu (AHI): AHI < 15 i AHI ≥ 15. Stanje uhranjenosti je procenjeno pomoću BMI i telesnog sastava. Telesni sasatav je određen pomoću *whole-body* DEXA sken-

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era (Hologic QDR-4000). Redoks status pacijenata sa OSA je određivan pomoću koncentracije NO u plazmi i nivoa redukovanog glutationa (GSH) u plazmi kao markera antioksidativnog kapaciteta. **Rezultati.** U grupi sa AHI ≥ 15 bila je nađena značajno veća srednja vrednost koncentracije NO u plazmi u odnosu na pacijente sa OSA i AHI < 15 (1,269 ± 0.789 vs 0.462 ± 0.373 nmol/mL, p = 0,001), dok je značajno veći nivo GSH u plazmi bio u grupi sa AHI < 15 u odnosu na grupu sa AHI ≥ 15 (238,08 ± 84,37 vs 172,77 ± 83,88 mg/mL, p = 0,04). Nezavisni prediktori nivoa GSH u plazmi (multivarijantna regresiona analiza) bili su: index desaturacije (ODI) [B = -0.157; 95% granice poverenja (CI): -0.262–0.053], srednja vrednost SatO2 (B = - 4.76; 95% CI: -9.21–0.306) i minimalna SatO2 (B = 0.118; 95% CI: 0.03–0.206). Kao nezavisni prediktor koncentracije NO u plazmi izdvojio se ODI (B = 0,038; 95% CI: 0,011–0,065). Nije postojala statistički značajna razlika između srednjih vrednosti BMI i parametra za procenu telesnog sastava u grupi sa AHI < 15 i AHI ≥ 15. Nijedan od parametara za procenu stanja uhranjenosti i telesnog sastava nije značajno uticao na ispitivane parametre sistemskog oksidativnog stresa. **Zaključak.** Sa povećanjem težine OSA smanjuje se antioksidacijski kapacitet i raste nivo sistemskog oksidativnog stresa. Stepen desaturacije tokom spavanja ima značajan uticaj na redoks status pacijenata sa OSA, nezavisno od stanja uhranjenosti i telesnog sastava.

Ključne reči:
apneja u snu, opstruktivna; stres, oksidativni; nutritivni status; telo, sastojci.

Introduction

Obstructive sleep apnea (OSA) is defined by recurrent episodes of breathing interruptions lasting more than 10 seconds (apnea) or reduction in airflow (hypopnea) during sleep associated with sleep fragmentation, awakenings and decreased oxygen saturation. Since the respiratory control is considered to be imperfect, up to five respiratory events during 60 minutes of sleep are tolerated [1]. According to the mechanism of breathing interruptions, different entities of this disorder are: OSA, central sleep apnea (CSA) and mixed sleep apnea (MSA).

In OSA, the airflow is interrupted, but respiratory effort continues. Apnea-hypopnea index (the number of apnea and hypopnea per hour of sleep – AHI) represents a standard for determining the severity of OSA. There are several ways to show the severity of desaturation during sleep as a consequence of OSA. Desaturation index (ODI) is most commonly used and it represents the number of desaturations ($SatO_2$ reduction ≥ 3%) per hour of sleep, the mean oxygen saturation, minimal saturation and percentage of sleep hours with hemoglobin oxygen saturation less than 90% (Sat < 90%). It is assumed that cyclical occurrence of hypoxia-reoxygenation (intermittent hypoxia) that characterizes OSA is the most important mechanism in formation of free radicals, similar to ischemia-reperfusion damages in coronary disease. It is believed that intermittent hypoxia leads to mitochondrial dysfunction, activation of enzymes that use oxygen, such as xanthine oxidase or nicotinamide adenine dinucleatide phosphate (NADPH), activation of leukocytes and endothelial cell dysfunction which leads to synthesis of free radicals and increased inflammation [2]. Oxidative stress can lead to activation of redox-sensitive transcription factors which regulate the synthesis of inflammatory cytokines, chemokines and adhesion molecules [3]. When considering oxidative stress in OSA, comorbidities must also be taken into account, especially obesity, as the most important source of oxidative stress independently from OSA. In a large population study, it was shown that body mass index (BMI) alongside with smoking and diabetes is the most important independent factor associated with systemic oxidative stress, although the contribution of accompanied OSA cannot be excluded [4].

The aim of this paper was to show if the OSA severity increases the level of markers of systemic oxidative stress and reduces antioxidant capacity independently of nutritional status.

Methods

The study included 128 patients with OSA. Patients with concomitant diseases that are characterized by the existence of systemic inflammation were excluded, except for obesity. All examinees underwent the sleep study-polysomnography using Philips STARDUST sleep recorder. AHI, AHI in the supine position (AHI supinatio), AHI in other positions of sleep (AHI non supinatio), desaturation index (ODI), minimum saturation ($minSatO_2$), medium saturation (mean $SatO_2$) and the percentage of time with saturation less than 90 % ($SatO_2$ < 90%) were determined. On the basis of the results of the sleep study based on AHI, examinees were divided into two groups: with mild OSA (AHI ≤ 15) and moderate and severe OSA (AHI > 15). All examinees underwent assessment of nutritional status using the BMI = body mass (BM)/body height (kg/m^2). Body compositions were determined by dual X-ray absorptiometry – (DXA) whole-body scan (Hologic QDR-4000). The absolute value of body fat mass (FM) in kg, body fat percentage (FM%), lean body mass (FFM) in kg and lean body mass index [FFMI = FFM/BM^2 (kg/m^2)] were examined. The data from the National Health and Nutrition Examination Survey (NHANES) for FM% reference values using DXA measuring were used [5].

Determination of nitric oxide (NO): NO decomposes rapidly to form stable metabolite nitrite/nitrate products. Nitrite (NO_2^-) was determined as an index of nitric oxide production by using Griess reagent (1). 0.1 mL 3N PCA, 0.4 mL 20 mM EDTA and 0.2 mL plasma were kept on ice for 15 min, then centrifuged for 15 min at 6000 rpm. After the supernatant was poured off, 220 µl of K_2CO_3 was added. Nitrites were measured at 550 nm. Bidistilled water was used as a blank probe [6].

Detemination of reduced glutathione (GSH): Plasma level of (GSH) was determined according to Beutler [7] and is based on GSH oxidation via 5,5 dithiobis-6,2-nitrobenzoic acid (DTNB). Bidistilled water was used as a blank probe. Measurement was done at 420 nm.

Method of descriptive statistics was used for description of general characteristics of the examinees in observed groups, as well as results obtained on the basis of the completed test. Independent *t*-test was used for comparison of arithmetic means of characteristics of two populations. Correlation was used to test the relationship between the parameters, and its presentation and interpretation of the significance was performed by using the linear correlation coefficient. The influence of individual variables as independent predictors for observed parameters was examined by using the multivariate regression analysis.

Results

Patients were divided into two groups according to AHI (cut off = 15). In the group with AHI < 15 there were 56 (43.8%) patients (44 males) and in the group with AHI ≥ 15, there were 72 (52.2%) patients (48 males). Demographic characteristics of examinees, mean values of parameters for assessing nutritional status, body composition and the sleep study in examined groups (*t*-test) are presented in Table 1.

A risk predictors for AHI ≥ 15 in comparison to AHI < 15 were analyzed by using the univariate logistic regression analysis (Table 2).

The influence of individual variables as predictors of GSH value was analyzed by using the univariate linear regression analysis (Table 2).

Table 1

Patients' characteristics

Observed Parameters	AHI < 15 (n = 56) mean ± SD (min–max)	AHI ≥ 15 (n = 72) mean ± SD (min–max)	*p*
Age (years)	42.57 ± 13.63	52.44 ± 11.38	0.033
Smokers (%)	32 (57.1)	40 (55.5)	0.565
AHI	10.2 (5.1–14.8)	24.3 (15.1–82.0)	< 0.001
ODI	4.63 ± 7.06 (0–23.5)	51.66 ± 23.26 (13.5–84.8)	< 0.001
Mean SatO2	93.54 ± 2.90 (88–97)	85.63 ± 12.52 (43–96)	0.035
Min SatO2	86.08 ± 4.80 (80–94)	67.75 ± 13.26 (39–85)	< 0.001
SatO2 < 90%	4.90 ± 10.04 (0–32.6)	41.86 ± 38.43 (1–99)	0.001
GSH (mmol/mL)	238.08 ± 84.37 (80.79–352.38)	172.77 ± 83.88 (27.15–285.16)	0.04*
NO (nmol/mL)	0.462 ± 0.373(0.42–1.25)	1.269 ± 0.789 (0.125–3.46)	0.001*
BMI (kg/m^2)	32.29 ± 5.58 (23.5–42.2)	36.55 ± 6.74 (26.8–52.6)	0.156
FM%	31.97 ± 4.13 (26.8–40.3)	34.25 ± 9.36 (24.3–49.1)	0.234
FM (kg)	31.68 ± 7.78	34.89 ± 9.64	0.483
FFM (kg)	64.01 ± 12.14 (40.75–82.72)	59.0 ± 10.16 (40.26–72.33)	0.929
FFMI	20.80 ± 3.46 (14.1–27.38)	20.8 (17.89–23.81)	0.172

AHI – apnea-hypopnea index; ODI – desaturation index; Mean SatO2 – medium saturation; Min SatO2 – minimum saturation; SatO2 < 90% – time with saturation less than 90%; GSH – reduced glutathione; NO – nitric oxide; BMI – body mass index; FM% – body fat percentage; FM – body fat mass; FFM – lean body mass; FFMI – lean body mass index; SD – standard deviation.

Table 2

Influence of individual variables on reduced glutathione (GSH) value in obstructive sleep apnea (OSA) patients

The observed risk factors	Univariate analysis	
	#B (95% CI)	*p*
The age	0.089 (0.021–0.199)	0.109
AHI	0.418 (0.088–0.748)	0.017*
ODI	-1.555 (-3.063–0.048)	0.046*
MeanSatO2	0.194 (0.028–0.36)	0.037*
MinSatO2	3.988 (-0.02–7.995)	0.047*
SatO2 < 90%	-0.192 (-0.284–0.10)	0.01*
Smoking	1.192 (-1.844–4.229)	0.198
BMI	0.141 (-0.415–0.697)	0.737
FM%	0.033 (-0.019–0.084)	0.220
FMkg	-0.184 (-0.386–0.018)	0.303
FFM kg	0.068 (-0.098–0.233)	0.437
FFMI	0.022 (-0.138–0.122)	0.703

#Unstandardized coefficient B; *statistically significant;

AHI – apnea-hypopnea index; ODI – desaturation index; Mean SatO2 – medium saturation; Min SatO2 – minimum saturation; SatO2 < 90% – time with saturation less than 90%; GSH – reduced glutathione; NO – nitric oxide; BMI – body mass index; FM% – body fat percentage; FM – body fat mass; FFM – lean body mass; FFMI – lean body mass index; CI – confidence interval.

The multivariate regression analysis determined independent predictors of GSH as follows: ODI, mean $SatO_2$, min $SatO_2$ (Table 3).

Table 3

Independent predictors of reduced glutathione (GSH) value in obstructive sleep apnea (OSA) patients

The observed risk factors	Multivariate analysis, $R^2 = 0.32$	
	B (95% CI)	*p*
ODI	-0.157 (-0.262 -0.053)	0.004*
MeanSatO2	-4.76 (-9.21 -0.306)	0.001*
MinSatO2	0.118 (0.03–0.206)	0.033*
SatO2 < 90%	-0.131(-0.80–0.537)	0.231
AHI	0.024 (-0.036–0.085)	0.687

[#]Unstandardized coefficient B; *statistically significant

AHI – apnea-hypopnea index; ODI – desaturation index; Mean SatO2 – medium saturation; Min SatO2 – minimum saturation; SatO2 < 90% – time with saturation less than 90%; CI – confidence interval.

The influence of the individual variables as predictors of NO value was analyzed by using the univariate linear regression analysis (Table 4).

Table 4

Influence of individual variables on nitric oxide (NO) value in obstructive sleep apnea (OSA) patients

The observed risk factors	Univariate analysis	
	[#]B (95%CI)	*p*
The age	0.016 (-0.005–0.036)	0.129
AHI	0.010 (0.001–0.019)	0.029*
AHI supinatio	0.016 (0.007–0.025)	0.003*
AHI nonsupinatio	0.016 (0.023–0.028)	0.017*
ODI	0.011 (0.002–0.02)	0.019*
MeanSatO2	0.009 (-0.02–0.039)	0.514
MinSatO2	-0.020 (-0.040–0.000)	0.052
SatO2 < 90%	0.001 (-0.008–0.01)	0.811
Smoking	-0.320 (-0.873–0.233)	0.247
BMI	0.014 (-0.029–0.058)	0.509
FM%	0.018 (-0.023–0.058)	0.372
FMkg	-0.004 (-0.041–0.032)	0.800
FFM kg	-0.025 (-0.052–0.002)	0.067
FFMI	-0.026 (-0.149–0.097)	0.664

[#]Unstandardized coefficient B; *statistically significant;

AHI – apnea-hypopnea index; ODI – desaturation index; Mean SatO2 – medium saturation; Min SatO2 – minimum saturation; SatO2 < 90% – time with saturation less than 90%; GSH – reduced glutathione; BMI – body mass index; FM% – body fat percentage; FM – body fat mass; FFM – lean body mass; FFMI – lean body mass index.

Multivariate regression analysis determined ODI as independent predictor of NO concentration in plasma (B = 0.038; 95% CI: 0.011–0.065) (Table 5).

Table 5

Multivariate linear regression analysis for nitric oxide (NO)

The observed risk factors	Multivariate analysis, $R^2 = 0.45$	
	B (95%CI)	*p*
ODI	0.038 (0.011–0.065)	0.011*
AHI	-1.221 (-2.558–0.117)	0.069
AHI supination	0.507 (-0.222–1.237)	0.150
AHI nonsupinatio	0.170 (-0.629–0.290)	0.425

[#]Unstandardized coefficient B; *statistically significant;

AHI – apnea-hypopnea index; ODI – desaturation.

Discussion

In our study, significantly lower plasma level of GSH and significantly higher mean values of NO concentration were found in plasma in the group with AHI ≥ 15 in comparison to subjects with AHI < 15. As independent predictors of a GSH level in plasma in OSA patients were found to be ODI, mean SatO2, min SatO2, while an independent predictor of NO concentration in plasma was ODI. We found no statistically significant difference in mean values of BMI and body composition parameters in patients with AHI < 15 and AHI ≥ 15. None of the markers of systemic oxidative stress in our research was significantly associated with BMI and body composition assessment parameters.

Obstructive sleep apnea is a major health problem due to the high prevalence, association with obesity (60%–90%), and there is an increasing amount of evidence suggesting that OSA is an important risk factor for cardiovascular diseases. GSH is an important antioxidant which, by using free radicals, prevents cell damage. Once oxidized, it can be reduced back by glutathione reductase, using NADPH as an electron donor. The ratio of reduced/oxidized glutathione (GSH/GSSG) is often used as a parameter for cell toxicity. Makris et al. [8] demonstrated that the level of GSH decreased during the night for about 15% in patients with OSA, and increased averagely for about 63% in patients without OSA. In a study done by Ntalapascha et al. [9] it was shown that GSH concentrations in plasma during night (morning-night), differed significantly between the patients with severe OSA and the control group (AHI < 5). This prospective research among the population with OSA, without significant comorbidities, provides evidence that OSA can independently be the cause of an increase in the level of systemic oxidative stress. In our study, as independent predictors of GSH in plasma were determined to be ODI, mean $SatO_2$, min $SatO_2$, while an independent predictor of NO concentration in plasma was ODI. This may indicate that the severity of desaturation during sleep, especially intermittent hypoxia (ODI) has a substantial impact on systemic oxidative stress in patients with OSA. In a study done by Simiakakis et al. [10], in the group with AHI > 15, antioxidant capacity was notably lower than in the control group (AHI < 5). Minimum desaturation was the most important predictor of levels of the biological antioxidant potential (BAP). Results of this study showed that OSA may affect the oxidative stress by reducing antioxidant capacity due to nocturnal hypoxia.

However, there are also studies with opposite results that did not show increased oxidative stress in OSA. In a study by Svatikova et al. [11], in patients with OSA without other chronic diseases, there was no increase in level of markers of systemic oxidative stress (thiobarbituric acid reactive substances – TBARS, oxidized low-density lipoproteins – LDL, isoprostanes) in comparison to the control group without OSA.

A source of oxidative stress in overweight is similar to that in patients with OSA, so the independent role of OSA in the occurrence of systemic oxidative stress is not yet completely clear. In a large population study, it was shown that

BMI, alongside with smoking and diabetes, is the most important independent factor associated with systemic oxidative stress, although the contribution of accompanied OSA cannot be excluded [4]. In our study, no statistically significant difference in mean values of BMI and body composition parameters in the patients with AHI < 15 and AHI ≥ 15 were found. None of the markers of systemic oxidative stress in our research were significantly associated with BMI and body composition assessment parameters. In the paper by Fujita et al. [12] no substantial association was found between markers of oxidative stress and parameters for OSA severity assessment. However, it has been shown that the waist/hip ratio (WHR) is an independent predictor of glutathione peroxidase (GPX) concentration and a total antioxidant status (TAS) (r = -0.317). The results of this study indicate that oxidative stress in OSA is more expressively associated with central obesity than with intermittent hypoxia and AHI [12].

Further studies are required to confirm whether the severity of OSA is an independent predictor of systemic oxidative stress which can significantly contribute to cardiovascular morbidity and mortality.

Conclusion

The results of our study are in favor of that severity of OSA is significantly associated with reduced antioxidant capacity and more expressed systemic oxidative stress. The degree of desaturation during sleep significantly affects systemic oxidative stress in patients with OSA aside from nutritional status and body composition.

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