ELSEVIER

Sleep Medicine 4 (2003) 225–228

SLEEP MEDICINE

www.elsevier.com/locate/sleep

Original article

Antioxidant capacity in obstructive sleep apnea patients

Kostas Christou, Anargyros N. Moulas, Chaido Pastaka, Kostantinos I. Gourgoulianis*

Pulmonary Department, Medical School, University of Thessaly, 22 Papakyriazi, 41222 Larissa, Greece

Received 16 April 2002; received in revised form 22 November 2002; accepted 25 November 2002

Abstract

Objectives: Obstructive sleep apnea syndrome (OSA) results in oxygen desaturation and arousal from sleep. Free oxygen radicals are highly reactive molecules, which can be produced by the OSA phenomenon known as hypoxia/reoxygenation. Hypoxic conditions, such as OSA, may also result in transient depletion of cellular reductants, which constitute a main line of antioxidant defense. Both apneas and hypopneas usually end in arousal, where reoxygenation causes the production of reactive oxygen species (free radicals). Living organisms have developed complex antioxidant systems to counteract reactive oxygen species and to reduce their damage. We evaluated the antioxidant capacity in serum from OSA patients and healthy people in order to confirm the hypothesis that there is a relationship between oxidative stress and OSA.

Materials and methods: A physician interviewed 25 participants, determining age, smoking habits and symptoms such as excessive daytime sleepiness and snoring. Physical examination and polysomnography were performed during patients' hospitalization. Antioxidant capacity was measured in blood samples by Trolox Equivalent Antioxidant Capacity assay.

Results: Seventeen out of 25 subjects had an apnea/hypopnea index (AHI) greater than 10 (OSA group). The measurement of antioxidant capacity did not differ between the OSA patients and our healthy sample (of 25 subjects, seven with an AHI less than 10). Furthermore, patients with severe OSA (AHI >20, $N = 14$) had linearly negative correlation between antioxidant capacity in their blood samples and AHI ($R = -0.551$, $P = 0.041$).

Conclusions: Reduced antioxidant capacity in serum is an index of excessive oxidative stress. Patients with severe OSA have reduced values of antioxidant capacity.

Keywords: Sleep apnea; Antioxidant capacity; Oxidative stress

1. Introduction

Obstructive sleep apnea syndrome (OSA) is a condition characterized by repetitive obstruction of the upper airway often resulting in oxygen desaturation and arousal from sleep [1]. OSA is linked to oxidative stress in the respiratory system due to a phenomenon known as hypoxia/reoxygenation: cyclical alterations of arterial oxygen saturation are observed, with oxygen desaturation developing in response to apneas followed by resumption of oxygen saturation during hyperventilation [2].

Repeated apneas and hypopneas are followed by reoxygenation in a patient with sleep apnea syndrome, such that hypoxic conditions may be present for 50% of a night. Both apneas and hypopneas usually end in arousal (caused by gradually increased effort against an occluded upper airway), during which reoxygenation causes the production of reactive oxygen species (free radicals). Microsomal radical generation is maximal under hypoxic conditions, which may also be associated with loss of cellular energy metabolites, and hence reductants that defend against radical fluxes. Hypoxic conditions may also result in transient depletion of cellular reductants, which constitute a main line of antioxidant defense. Thus, hypoxia/reoxygenation could be expected to enhance oxidative stress [2].

Free oxygen radicals are also thought to contribute to the development of cardiovascular disease [3,4]. OSA is associated with cardiovascular morbidity such as arterial hypertension, coronary artery disease and cerebrovascular disease [5].

Living organisms have developed complex antioxidant systems to counteract reactive oxygen species and to reduce

* Corresponding author. Tel.: +30-410-682897; fax: +30-410-670240.
E-mail address: kgourg@med.uth.gr (K.I. Gourgoulianis).

1389-9457/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S1389-9457(02)00253-8

their damage [6]. The processes are both enzymatic and non-enzymatic [7]. Several methods have been developed and used to measure the total antioxidant capacities of various biological samples. All methods measure the inhibition of an artificially generated oxidative process by antioxidants in plasma or serum [8–10].

The purpose of our study was to evaluate the antioxidant capacity in serum from OSA patients and our healthy sample in order to confirm the link between oxidative stress and OSA.

2. Materials and methods

A physician interviewed 25 subjects, determining age, smoking habits and symptoms such as excessive daytime sleepiness and snoring. Physical examination and polysomnography were performed during patients' hospitalization in the University Hospital of Larissa. Electroencephalogram, electrooculogram and electromyogram of the submandibular and pretibial muscles were simultaneously recorded. Ventilatory airflow at the nose and mouth was registered with thermistors. Breathing movements of the chest and abdomen were monitored by inductive plethysmography. Arterial oxygen saturation (Sa_{O2}) was measured transcutaneously by pulse oximetry at the fingertip and an electrocardiogram was obtained. All data were registered on a computerized polysomnograph with capability for analog registration (Alice 4 Diagnostic Device OBS/G7829, Respironics). Analysis of sleep stages was performed manually at 30 s intervals, according to the criteria of Rechtschaffen and Kales [11].

An obstructive apnea was diagnosed if complete cessation of oronasal flow occurred in the presence of thoracoabdominal breathing movements. If neither oronasal flow nor breathing efforts of the chest and abdomen could be detected, a central apnea was scored. Hypopnea was defined as a reduction of the respiratory amplitude by greater than 50% with regard to the preceding effort signals. If a clear amplitude reduction of a validated measure of breathing during sleep did not reach the above criterion, other criteria for hypopnea were considered: association of amplitude reduction with either an oxygen desaturation of $>3\%$ or an arousal [1].

All apneas and hypopneas were required to have a duration of at least 10 s. The apnea/hypopnea index (AHI) was obtained by dividing events into the total sleep time. An AHI of more than 10/hours was considered OSA.

Peripheral venous blood samples were obtained at 08:00 h following the night a diagnostic study was performed. The blood samples were centrifuged at 3500 rev./min for 5 min and the serum was immediately stored at -70 °C until measurement of antioxidant capacity by TEAC assay. The TEAC (Trolox Equivalent Antioxidant Capacity) assay was reported first by Miller et al. [12,13] and then modified by Re et al. [14]. This assay is based on the inhibition by antioxidants of the absorbance of the radical cation of 2,2-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS), which has a characteristic long-wavelength absorption spectrum showing maxima at 660, 734 and 820 nm. The ABTS radical in the original version of the method, which has been commercialized by Randox Laboratories Ltd. (Crumlin, Co. Antrim, UK), is formed by the incubation and interaction of ABTS with the ferrymyoglobin radical species, generated by the activation of metmyoglobin with H_2O_2. This original TEAC assay measures the ability of a compound in reducing the ABTS radicals (Fig. 1). Antioxidants present in subjects' serum samples inhibit the reaction and develop a blue-green color, which, in the test that we used, can be detected at 600 nm. The degree of inhibition is proportional to the concentration of antioxidants in the subjects' samples. The values were expressed as mmol/l, equivalent Trolox (a synthetic proportion of vitamin E).

All data were analyzed by SPSS. For comparisons between OSA patients and healthy people the *t*-test was employed. The Pearson correlation coefficient (*R* test) was used for the correlation between values of antioxidant capacity and AHI.

3. Results

All participants had a mean age of 51.5 ± 1.9 years. Seventeen out of 25 people had an AHI greater than 10 (OSA group), while seven had an AHI of 10 or less (our healthy group). Table 1 shows patient characteristics. There were no statistically significant differences in age, height, weight or body mass index. In addition, smokers and ex-smokers among patients with OSA were eight and eight, respectively, with a mean value in pack × years (PYS) of 47.6. The similar values for our healthy group were three smokers and two ex-smokers, with 41.8 PYS. The peripheral white blood cells were within the normal range

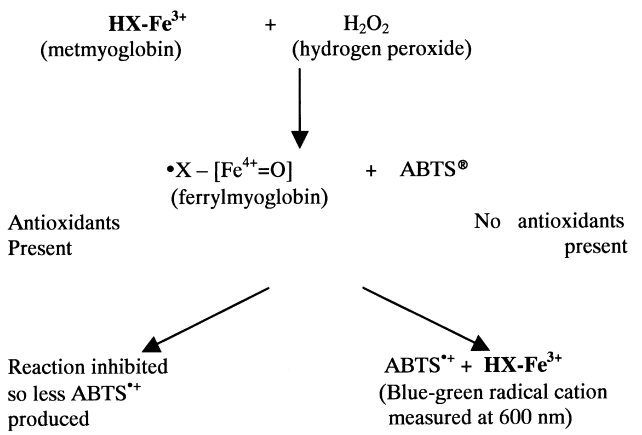

Fig. 1. The TEAC (Trolox Equivalent Antioxidant Capacity) assay (Randox Laboratories Ltd.; Crumlin, Co. Antrim, UK). This original TEAC assay measures the ability of a compound in reducing ABTS radical.

Table 1
Patient characteristics of the OSA group and the healthy group

	OSA group	Healthy group
N	17	8
Female/male	1/16	4/4
Age (years)	51.9 ± 8.4	50.9 ± 11.8
Height (cm)	177.2 ± 7.7	166.2 ± 11.7
Weight (kg)	107.1 ± 22	97.6 ± 22.5
BMI	34 ± 6.2	35.4 ± 10.2
Total sleep time (min)	386.8 ± 95.7	421.5 ± 60.3
Perimeter of neck (cm)	43.06 ± 2.76	40.94 ± 2.14
AHI*	44.1 ± 23.1	4.3 ± 3
Sa_{O2} <90 (%)*	43.8 ± 32.1	12 ± 4.3
Mean Sa_{O2} (%)*	87.3 ± 5.9	92.9 ± 3.2
Lowest Sa_{O2} (%)*	69.8 ± 12.5	83.2 ± 13.1
Antioxidant capacity (mmol/l, Trolox Equivalent)	2.21 ± 0.6	1.97 ± 0.52
Antioxidant capacity >2 (mmol/l, Trolox Equivalent)	10	3
Antioxidant capacity <1.5 (mmol/l, Trolox Equivalent)	1	1

AHI, apnea/hypopnea index; BMI, body mass index; Sa_{O2}, nocturnal oxygen saturation. Values are means ± SEM. *$P < 0.05$.

for all patients. The measurement of antioxidant capacity did not differ between the OSA patients (mean value 2.21 mmol/l) and our healthy sample (mean value 1.97 mmol/l). Ten out of 17 OSA patients had antioxidant capacity greater than 2 mmol/l, while three out of seven healthy people had a value greater than 2. In addition, one OSA patient had antioxidant capacity of less than 1.5 mmol/l, while one healthy person had a value less than 1.5.

Table 1 shows that our patients with OSA had moderate to severe sleep-disordered breathing, with marked nocturnal oxygen desaturation and disturbed sleep architecture.

Patients with severe OSA (AHI >20, $N = 14$) had linearly negative correlation between antioxidant capacity in their blood samples and AHI ($R = -0.551$, $P = 0.041$) (Fig. 2). The correlation was stronger for more severe syndrome (AHI >30, $N = 12$, $P = 0.016$, $R = -0.676$; AHI >40, $N = 10$, $P = 0.027$, $R = -0.692$). Patients with severe OSA also had linearly negative correlation between antioxidant capacity in their blood samples and number of desaturations/hour (AHI >30, $N = 12$, $P = 0.024$, $R = -0.643$).

4. Discussion

Antioxidant capacity, measured in peripheral blood samples, was not different between OSA patients and the healthy group. Patients with severe OSA syndrome had less

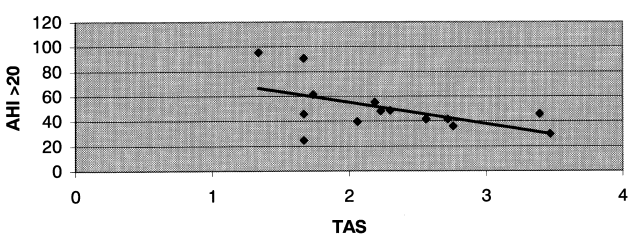

Fig. 2. Negative relationship between severe OSA and antioxidant capacity.

antioxidant capacity, indicating the presence of systemic oxidative stress. Free oxygen radicals have been implicated in the pathogenesis of cardiovascular disorders. OSA is associated with increased cardiovascular morbidity and mortality. Nasal continuos positive airway pressure (nCPAP) therapy has been shown to have beneficial effects on long-term survival of patients with OSA [15]. Thus, CPAP therapy can prevent hypoxia/reoxygenation and the resulting consequences.

Richard Schulz et al. found that among subjects with untreated OSA the release of superoxide radical ($O_2^{\cdot -}$) from circulating neutrophils was markedly enhanced when compared with control subjects [16]. The underlying mechanism of oxidative respiratory burst is not known. It is believed that the hypoxia/reoxygenation phenomenon plays a key role in the OSA pathophysiology [2]. Recently, our research group also found increased values of reactive oxygen metabolites (by d-ROM test) in blood samples of OSA patients, indicating the important role of oxidative stress in OSA (unpublished data).

Living organisms have developed complex antioxidant systems to counteract reactive species and to reduce their damage. These antioxidant systems include enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, macromolecules such as albumin, ceruloplsmin and ferritin, and an array of small molecules, including ascorbic acid, a-tocopherol, β-carotene, ubiquinol-10, reduced glutathione methionine, uric acid and bilirubin [6]. Intracellular antioxidants and membrane radical scavengers protect parenchymal cells, especially in the lungs. In addition, the fluid lining the epithelial surface contains a catalase-like antioxidant that protects the epithelial cells from oxidants. This protective mechanism took place in the pathogenesis of emphysema [17].

The patients with severe OSA syndrome had reduced antioxidant capacity. A possible explanation is that microsomal radical generation is maximal under hypoxic

conditions. Hypoxic conditions may also result in transient depletion of cellular reductants, which constitute a main line of antioxidant defense [18,19].

The plasma antioxidant concentration in TEAC assay is an index of antioxidant status, but may not necessarily reflect concentration in target tissue (lungs), where oxidative stress is greater [20]. Hence, it would be wise to use antioxidant capacity status with caution. However, it could be used as a marker of oxidative stress, which probably participates in the pathogenesis for cardiovascular morbidity among OSA patients. Recent study has also shown that cardiovascular abnormalities in OSA patients can be explained by the severe hypoxia-elevated values of vascular endothelial growth factor (VEGF) [21].

Furthermore, some lifestyle activities such as smoking and excessive exercise have effects on serum antioxidant status. The production of reactive intermediates increases during exhaustive exercise, while regular exercise increases antioxidant defenses [22]. Nia et al. demonstrated that serum antioxidant capacity measured by TEAC assay was slightly higher in smokers compared to non-smokers [23]. We did not find such a difference among smokers, ex-smokers and non-smokers in our study. Furthermore, no participant referred to recent consumption of antioxidant-rich food or drink that might have caused transient increases in serum antioxidant concentrations [24,25].

In conclusion, reduced antioxidant capacity in serum is an index of excessive oxidative stress. Antioxidant status is an indicator of redox homeostasis and is under strong genetic control, especially among smokers [26]. Patients with severe OSA have reduced values of antioxidant capacity. Hence, imbalance between oxidative stress and antioxidant status may play an important role in the pathophysiological relationship in OSA patients between hypoxia and cardiovascular disease.

References

[1] American Academy of Sleep Medicine Task Force, Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. Sleep 1999;22:667–89.

[2] Dean RT, Wilcox I. Possible atherogenic effects of hypoxia during obstructive sleep apnea. Sleep 1993;16:S15–S22.

[3] Halliwel B. The role of oxygen radical in human disease, with particular reference to the vascular system. Haemostasis 1993;23:118–26.

[4] Muge A. The role of reactive oxygen species in atherosclerosis. Z Kardiol 1998;87:851–4.

[5] Shepard JW. Hypertension, cardiac arrhythmia, myocardial infarction and stroke in relation to obstructive sleep apnea. Clin Chest Med 1992;13:437–58.

[6] Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev 1994;74:139–62.

[7] Storey KB. Oxidative stress: animal adaptations in nature. Braz J Med Biol Res 1996;29:1715–33.

[8] Mercuri F, Quagliaro L, Ceriello A. Oxidative stress evaluation in diabetes. Diabetes Technol Ther 2000;2:589–600.

[9] Prior RL, Cao G. In vivo total antioxidant capacity: comparison of different analytical methods. Free Rad Biol Med 1999;27:1173–81.

[10] Cao G, Prior RL. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. Clin Chem 1998;44:1309–15.

[11] Rechtschaffen A, Kales A. A manual of standardized terminology: techniques and scoring system for sleep stages of human subjects. Los Angeles, CA: UCLA Brain Information Service/Brain Research Institute, 1968.

[12] Miller NJ, Rice-Evans C, Davies MJ, et al. A novel method for measuring antioxidant capacity and its application to monitoring antioxidant status in premature neonates. Clin Sci 1993;84:407–12.

[13] Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. Methods Enzymol 1994;234:279–93.

[14] Re R, Pellegrini N, Proteggente A, et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med 1999;26:1231–7.

[15] He J, Kryger MH, Zorick FJ, et al. Mortality and apnea index in obstructive sleep apnea (experience in 385 male patients). Chest 1998;94:9–14.

[16] Schulz R, Mahmoudi S, Hattar K, et al. Enhanced release of superoxide from polymorphonuclear neutrophils in obstructive sleep apnea. Am J Respir Crit Care Med 2000;162:566–70.

[17] Cantin A, Crystal RG. Oxidants, antioxidants and the pathogenesis of emphysema. Eur J Respir Dis Suppl 1985;139:7–17.

[18] Richards DCH, Jessup W, Dean RT. Cell surface proteins may be the primary target during free radical-mediated cytolysis. Biochim Biophys Acta 1988;946:281–8.

[19] Vince GS, Dean RT. Is enhanced free radical flux associated with increased intracellular proteolysis? FEBS Lett 1987;216:253–6.

[20] Duthie C. Determination of activity of antioxidants in human subjects. Proc Nutr Soc 1999;58:1015–24.

[21] Schulz R, Hummel C, Heinemann S, et al. Serum levels of vascular endothelial growth factor are elevated in patients with obstructive sleep apnea and severe nighttime hypoxia. Am J Respir Crit Care Med 2002;165(1):67–70.

[22] Leeuwenburgh C, Heinecke JW. Oxidative stress and antioxidants in exercise. Curr Med Chem 2001;8:829–38.

[23] Nia AB, Van Schooten FJ, Schilderman PA, et al. A multi-biomarker approach to study the effects of smoking on oxidative DNA damage and repair and antioxidant defense mechanisms. Carcinogenesis 2001;22:395–401.

[24] Hollman PCH, van Trijp JM, Buysman MN, et al. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. FEBS Lett 1997;418:152–6.

[25] Duthie G, Pedersen MW, Gardner PT, et al. The effect of whisky and wine consumption on total phenol content and antioxidant capacity of plasma from healthy volunteers. Eur J Clin Nutr 1998;52:733–6.

[26] Wang XL, Rainwater DL, VandeBerg JF, et al. Genetic contributions to plasma total antioxidant activity. Arterioscler Thromb Vasc Biol 2001;21:1102–3.