ENDOTHELIUM-DERIVED MARKERS AND ANTIOXIDANT STATUS IN THE BLOOD OF OBSTRUCTIVE SLEEP APNEA MALES

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Abstract

Objective: The relationship between obstructive sleep apnea (OSA) and cardiovascular disease is intensively discussed. Endothelial leukocyte adhesion molecule (E-selectin) is one of factors facilitating leukocyte migration to the subendothelial layer which could be considered proatherogenic. The aim of the study was to determine E-selectin levels and total plasma antioxidant status (TAS) in the blood of different stage OSA patients.

Methods: Non-smoking, OSA-suspected males, aged 30-63, were selected for the study. An EMBLA polysomnographic system was used to establish the severity of apneic episodes. The results of apnea/hypopnea index (AHI) allowed dividing patients into the following groups: OSA-0 with AHI 0-4.9 (n=14), OSA-1 with AHI 5-15 (n=14), OSA-2 with AHI 16-30 (n=13), OSA-3 with AHI ≥30 (n=13). Complete blood count (CBC), glycemia during oral glucose tolerance test, fasting plasma lipid profile, uric acid, and high sensitivity C-reactive protein (hsCRP) were estimated among routine parameters. We determined plasma concentrations of E-selectin and total antioxidant status.

Results: We found progressively decreasing concentrations of TAS (P=0.03) and increased concentrations of E-selectin (P=0.0001) from OSA-0 to OSA-3 subjects. No correlation between E-selectin and metabolic parameters was noted.

Conclusion: In the studied OSA groups, E-selectin appeared an independent proatherogenic factor.

Key words: obstructive sleep apnea, total antioxidant status, endothelium, E-selectin

INTRODUCTION

The relationship between obstructive sleep apnea syndrome (OSA) and cardiovascular risk factors is under debate. OSA is associated with increased cardiovascular and cerebrovascular morbidity and mortality [1-6]. Endothelial cells are thought to play an important role due to multifactor definition of atherosclerosis. These cells are related to intravascular environment and can react to metabolic and physical factors in the blood [7]. Leukocyte migration to the subendothelial layer is one of the earliest stages in the development of an atherosclerotic plaque, which consists of cell rolling on the endothelium, activation, adherence and diapedesis [8]. Specific adhesive molecules are involved in these processes: selectins, immunoglobulins, and integrins [9]. Normal function of the endothelium is characterized by nitric oxide (NO)-induced decreasing permeability for monocytes and granulocytes and a decreasing quantity of adhesive molecules on the endothelial surface [10].

Adhesive proteins contribute to platelet and leukocyte adhesion to the endothelium. L-selectin, present on the leukocyte surface, P-selectin, from platelet granules, and E-selectin, specific for endothelium, are considered to facilitate rolling and activation of leukocytes [11]. Endothelium dysfunction may be provoked by oxidative-antioxidant disturbances, e.g., by hyperglycemia or hypoxia [12, 13]. The course of oxidative stress depends, in a large measure, on the antioxidant state. The aim of the present study was to determine the level of the endothelial leukocyte adhesion molecule, E-selectin, and the total antioxidant status (TAS) in plasma of different stage OSA patients.

MATERIAL AND METHODS

STUDY GROUPS

The study protocol was approved by the Bioethics Committee of the University of Medical Sciences in Poznan, Poland. Informed consent to the procedures was obtained from the patients participating in the study.

Non-smoking male Caucasians, aged 30-63, suspected of OSAS, without any other acute or severe chronic disease, using no special diet or supplementation, were selected for the study from September 2008 to April 2009. After complete physical examination, they were subjected to polysomnographic and biochemical procedures. The exclusion criterion was newly diagnosed type II diabetes on the basis of an oral glucose tolerance test (OGTT). Complete blood count (CBC), differential white blood cell (WBC) count, and high sensitivity C-reactive protein (hsCRP) were also measured. The study group was divided according the apnea/hypopnea index (AHI) into four groups as follows: OSA-0 with AHI 0-4.9 (n=14), OSA-1 with AHI 5-15 (n=14), OSA-2 with AHI 16-30 (n=13), OSA-3 with AHI ≥31 (n=13).

MEASUREMENTS

The evaluation of OSA-suspected males was performed in the Sleep Laboratory of the Department of Respiratory Medicine of the University of Medical Sciences in Poznan, Poland, using a full-night polysomnographic monitoring system (EMBLA S4000, Remlogic, Denver, Colorado). Airflow was monitored by a nasal flow cannula. Abdominal and thoracic movements were assessed by respiratory inductive plethysmography. Night recordings of hemoglobin oxygen saturation were obtained by finger pulsoximetry. Snoring sounds, heart rate, and sleep position also were recorded. Apnea was defined as a cessation of airflow lasting for more than 10 s, and hypopnea as a discrete reduction (two thirds) of airflow and/or abdominal ribcage movements lasting for more than 10 s and associated with a decrease of more than 4% in oxygen saturation. In addition, systolic (SBP) and diastolic (DBP) blood pressure, and body mass index (BMI) were measured.

In all subjects, blood samples were collected from an ulnar vein twice: fasting, 0 min and at 120 min of OGTT. Fasting blood samples were used to determine complete blood count (Cell-Dyn Ruby, Abbott Laboratories, Abbott Park, Illinois). Plasma glucose concentrations, lipid profile: total cholesterol (T-C), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), triglycerides (TG) and serum uric acid (UA) concentrations as well as serum high sensitivity C-reactive protein (hsCRP) were measured (Dimension Xpand Plus Systems, Siemens Healthcare Diagnostics, Deerfield, Illinois). Plasma TAS was determined spectrophotometrically (Randox Laboratories, Crumlin, Antrim, UK) using Statfax™ 1904 Plus (Awareness Technolo-

gy, Palm City, Florida). Plasma samples were stored at -80°C until measurement of E-selectin using ELISA method (R&D Systems, Minneapolis, Minnesota) and microplater reader BioTek Elx800 (BioTek Instruments, Winooski, Vermont).

STATISTICAL ANALYSIS

All results were expressed as means ±SD and median. Shapiro-Wilka's test was used to check the normality of distributions. The significant differences among OSA-0, OSA-1, OSA-2 and OSA-3 subjects were assessed with a non-parametric Kruskal-Wallis test and subsequently Mann Whitney-U test. Statistical calculation was performed using Statistica 6.0 for Windows program.

RESULTS

The OSA-0, OSA-1, OSA-2, and OSA-3 groups did not differ in age, BMI, blood pressure, WBC differential, hsCRP, lipid profile, and glucose levels. Inflammation-characterizing parameters as well as basic metabolic factors affecting atherosclerosis and other details are shown in Table 1.

We found progressively decreasing concentrations of plasma TAS (1.56 ±0.19 vs. 1.34 ±0.13 vs. 1.49 ±0.59 vs. 1.36 ±0.13 mmol/l; P=0.03) (Table 1, Fig. 1) and increasing concentrations of E-Selectin (14.0 ±7.6 vs. 22.5 ± 6.9 vs. 30.0 ± 13.7 vs. 26.8 ± 15.7 ng/ml; P=0.0001) (Table 1, Fig. 2) from OSA-0 to OSA-3 subjects, respectively. There were no relationships between E-selectin and metabolic parameters in the studied groups.

Table 1. Clinical and biochemical characteristics of the studied groups.

|	OSA 0 (n=14)		OSA 1 (n=14)		OSA 2 (n=13)		OSA 3 (n=13)	
	Mean ±SD	Median	Mean ±SD	Median	Mean ±SD	Median	Mean ±SD	Median
Age (years)	50 ±10	53	50 ±12	55	51 ±8	53	51 ±11	54
AHI (#/h)	2.2 ±1.2	2.5	7.85 ±2.5	7.7	17.8 ±9.5	21.4	51.5 ±12.6	49.6
BMI (kg/m^2)	30.2 ±5.4	31.2	29.3 ±5.4	29.0	30.4 ±3.8	30.3	30.7 ±3.5	30.4
SBP (mmHg)	119 ±13	120	142 ±16	130	138 ±9	140	130 ±7	130
DBP (mmHg)	81 ±11	80	86 ±13	85	86 ±14	80	80 ±7	80
G 0 (mmol/l)	5.55 ±0.65	5.63	5.55 ±0.59	5.53	5.47 ±0.63	5.45	5.51 ±0.30	5.54
G 120 (mmol/l)	6.79 ±1.61	7.23	6.25 ±1.45	6.26	5.71 ±1.42	5.62	6.29 ±1.89	5.89
T-C (mmol/l)	5.31 ±1.70	5.01	5.41 ±1.01	5.39	5.96 ±1.00	5.90	5.55 ±1.36	5.428
HDL-C (mmol/l)	1.08 ± 0.25	1.30	1.20 ±0.19	1.19	1.30 ±0.19	1.23	1.14 ±0.23	1.13
TAG (mmol/l)	2.27 ±1.37	1.78	1.77 ±0.50	1.97	1.65 ±0.88	1.45	1.99 ±1.11	1.64
LDL-C (mmol/l)	3.50 ±1.53	3.50	3.52 ±0.71	3.56	3.94 ±0.97	4.04	3.57 ±1.21	3.48
UA (µmol/l)	461 ±143	449	342 ±66	344	325 ±25	325	336 ±69	342
WBC (T/l)	6.6 ±1.3	6.3	6.5 ±0.8	6.5	7.6 ±2.9	7.5	7.5 ±1.7	7.3
hsCRP (mg/l)	2.00 ±1.02	2.05	1.92 ±1.15	1.50	2.52 ±1.00	2.25	2.50 ±1.34	2.23
TAS (mmol/l)	1.56 ±0.19	1.58	1.34 ±0.13	1.34	1.49 ±0.59	1.46	1.36 ±0.13	1.38
E-selectin (ng/ml)	14.0 ±7.6	16.24	22.5 ±6.9	19.16	30.0 ±13.7	31.0	26.8 ±15.7	27.1
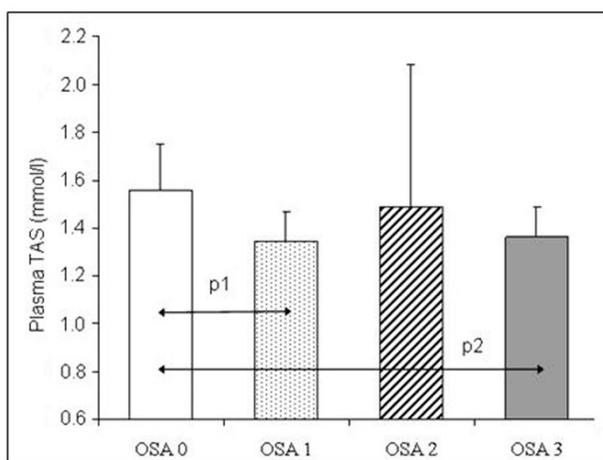
Fig. 1. Comparison of plasma total antioxidant status (TAS) among patients with different stages of obstructive sleep apnea (OSA). Significant differences: P1=0.009 and P2=0.006.
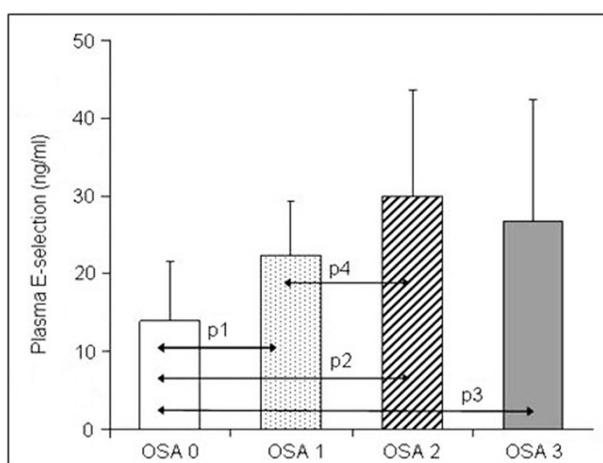

Fig. 2. Comparison of plasma E-selectin concentration among patients with different stages of obstructive sleep apnea (OSA). Significant differences: P1=0.03, P2=0.0005, P3=0.015, and P4=0.04.

Discussion

In the present study, we found that E-selectin serum levels were significantly higher in patients with more intensive obstructive sleep disorder. E-selectin could thus be one of importance between other reported parameters describing OSA pathophysiological pathways [14-17]. El-Solh et al. [18] found increased levels of adhesion molecules (E-selectin among others) in the blood of OSA subjects compared with control subjects; the molecules were positively related to AHI and oxygen desaturation index, but not to hypoxemia. The investigators suggested OSA elevate circulating adhesion molecules independently of the severity of coronary artery disease. A study by Zamaron-Sanz et al. [19] confirmed that the levels of E-selectin and intracellular cell adhesion molecule-1 (ICAM-1) significantly correlated with total oxygen desaturation. The authors concluded that OSA could be associated with changes in the levels of adhesion molecules,

possibly as a result of OSA- induced hypoxia [19].

Other selectins also seem to be important determinants of future cardiovascular events [20]. Minoguchi K et al. [21] found that P-selectin levels were significantly higher in patients with moderate-to-severe OSA, where they dropped significantly after 3-month CPAP treatment, than in patients with mild OSA or obese control subjects without evident sleep-breathing disorders. There was a significant correlation between the AHI or duration of nocturnal hypoxia and the serum levels of P-selectin in patients with OSA.

Our previous study documented an increased oxidative stress in OSA patients, with decreased plasma total antioxidant status [22]. Several studies suggesting a pathogenetic link between oxidative stress and endothelial dysfunction [23-25] and the lack of studies on this association in OSA subjects encouraged to perform the present study to investigate another group of OSA-suspected males and to determine the indicators of both antioxidant status and endothelial function. In the present study, we reported a decreased plasma TAS in obstructive sleep apnea subjects, but no relationship between TAS and increasing E-selectin levels was found. We conclude that E-selectin appears to be an independent proatherogenic factor and endothelial dysfunction is pathophysiologically plausible in obstructive sleep apnea.

Conflicts of interest: The authors declared no conflicts of interest in relation to this work.

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