Advanced Oxidation Protein Products in Obstructive Sleep Apnea

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Abstract: Obstructive sleep apnea (OSA) is a risk factor of hypertension, coronary artery disease and stroke. OSA is also considered a cause of accelerated atherogenesis. Advanced oxidation protein products (AOPP) are among the biochemical indicators of higher risk of atherogenesis as an independent risk factor for coronary artery disease. 20 men suffering from OSA were examined using night polygraphy, the AOPP were determined from their morning blood samples. The mean AOPP concentration in the patients group was 91.8 (SD=42.3) μmol/l, in the control group 76.2 (SD=35.3) μmol/l, the difference was not significant. The AOPP were found correlated with the AHI (apnoe/hypopnoe index) (R=0.485, P=0.030). The results support the hypothesis that OSA increases the oxidative stress and atherogenesis.

Introduction

Obstructive sleep apnea (OSA) results from recurrent episodes of increased upper airways resistance in the course of sleep, leading to spells of apnea or hypopnea which again are responsible for nocturnal sleep fragmentation. OSA is regarded as an independent risk factor of hypertension and coronary artery disease [1]. OSA significantly increases the risk of stroke or death from any cause, this increase being independent of other risk factors, including hypertension [2, 3, 4, 5].

The pathophysiology of a greater risk of stroke in OSA still awaits full elucidation. Potential explanations pertain to vascular supply or also parameters of blood-erythrocyte deformability and plasma viscosity [6, 7].

The hallmark of OSA is intermittent hypoxia and increased formation of reactive oxygen species, which may injure surrounding tissues and act as signalling molecules, activating hypoxia-adaptive and inflammatory pathways that affect cellular and molecular mechanisms. Activation of inflammatory responses results in increased expression of inflammatory cytokines and adhesion molecules, which facilitates endothelial cells/leukocytes/platelets interactions. These cell interactions, which promote endothelial dysfunction and are a component of the mechanisms underlying atherosclerosis, are probably amplified in patients who have OSA [8, 9]. OSA is, indeed, associated with a higher incidence of atherosclerosis *in vivo* – proved by greater carotid intima-media thickness [10, 11, 12].

Advanced oxidation protein products (AOPP) are one of the biochemical parameters indicative of oxidation stress. AOPP are proteins, predominantly albumin and its aggregates damaged by oxidative stress [13, 14]. They contain quantities of dityrosines which allow crosslinking, disulfide bridges and carbonyl groups, and are formed mainly by chlorinated oxidants-hypochloric acid and chloramines resulting from myeloperoxidase activity. AOPP have several characteristics similar to those of advanced glycation end products (AGE)-modified proteins. Apart from a common formation mechanism (oxidative stress) leading to protein damage, they share some biological effects as well, including interaction

with receptors for AGE (RAGE). Induction of proinflammatory activities, adhesive molecules and cytokines is even more intensive than that caused by AGEs. They are referred to as oxidative stress markers as well as markers of neutrophil activation. Protein oxidation products mediated by chlorinated species generated by the enzyme myeloperoxidase were found in the extracellular matrix of human atherosclerotic plaques [14], and increased levels of AOPP were described as an independent risk factor for coronary artery disease [15].

A recent study describes AOPP elevation in the plasma and cerebro-spinal fluid of patients with amyotrophic lateral sclerosis [16], thus suggesting conceivable AOPP connection with not only premature atherosclerosis but also nerve tissue damage.

Material and Methods

Enrolled in the study were adult men indicated for nocturnal polygraphy for suspected OSA. Enrolment was preceded by detailed examination with special respect to the subjects' history of neurological impairment and sleep disorders. Not included in the study were patients suffering from the following diseases and conditions, past and present: stroke, craniocerebral injury, major cardiological pathologies including cardiac insufficiency, pulmonary arterial hypertension and coronary artery disease, treatment with antipsychotics, drug or alcohol abuse, and abnormal neurological physical findings. The patients rated their sleepiness according to the Epworth sleepiness scale [17]. During the night (23:00 to 06:00 hours), they were examined using the method of nocturnal polygraphy (air flow, chest and abdomen movements, heart rate, hemoglobin oxygen saturation, respiratory noises and body position). At 07:00 the following day, each patient had blood samples taken from the cubital vein for assaying their serum levels of AOPP and for the estimation of the basic biochemical and hematological parameters.

The nocturnal polygraphic record was scored visually. The following parameters were processed: the apnoea/hypopnea index (AHI – average number of spells of apnea and hypopnea per hour of subjective sleep duration), and the oxygen desaturation index (ODI – average number of oxygen hemoglobin saturation drops of 3% and more per hour of subjective sleep duration).

Only patients meeting the current diagnostic criteria for OSA [18] were included in the study cohort. A total of 20 men aged 37–79 years (mean age 50.9; SD = ±11.5 years, mean BMI 30.1±3.5) were studied. 9 patients had well compensated hypertension, and one had satisfactorily treated diabetes type 2. Serum creatinine was below 110 mmol/l in all subjects.

The results were compared with those of 20 age-matched healthy men (mean age 50.3±11.8 years) using Student's T-test and other parametric tests, since the value in both groups showed normal distribution. Pearson's correlation was used to assess the relationship between measured parameters.

AOPP were determined with a spectrophotometric method based on their reaction with potassium iodide and acetic acid according to Witko-Sarsat et al. [13]. 200 μl of plasma diluted 1:5 with PBS, pH 7.4, 200 μl of chloramine T (0–100 μmol/l) for calibration; 200 μl of PBS as blank was applied onto a microtiter plate. 10 μl of 1.16 mol/l potassium iodide and 20 μl of acetic acid were added, and absorbance at 340 nm was measured (Multiscan Ascent, Labsystems, Finland). The intra-assay and inter-assay variations were less than 3.3% and 8.0%, respectively.

Table 1 – Mean values of biochemical (serum) and hematological test results (blood) in patients with OSA (SD=standard deviation)

	Mean value (±SD)	Normal range
Urea (mmol/l)	5.08 (±0.87)	2.8–8
Creatinine (mmol/l)	86.20 (±10.27)	44–110
Uric acid (mmol/l)	385.14 (±50.38)	220–420
Total bilirubin (mmol/l)	15.83 (±7.02)	2–17
ALT (μkat/l)	0.71 (±0.35)	0.1–0.78
AST (μkat/l)	0.51 (±0.81)	0.05–0.72
GGT (μkat/l)	0.87 (±0.65)	0.14–0.84
Total protein (g/l)	70.85 (±5.51)	65–85
C-reactive protein (mg/l)	3.76 (±2.83)	0–7
Cholesterol (mmol/l)	4.95 (±0.96)	3.83–5.8
Triacylglycerol (mmol/l)	1.96 (±1.32)	0.68–0.69
Thyroid stimulating hormone (mlU/l)	1.58 (±0.83)	0.37–5
Ferritin (μg/l)	275.10 (±185.4)	22–322
Glucose (mmol/l)	4.96 (±0.48)	4.2–6
Leukocytes	8.14 (±1.87)	4.1–10.2
Erythrocytes	4.91 (±0,30)	4.19–5.75
Hemoglobin (g/l)	147.00 (±39.9)	135–174
Hematocrit	0.75 (±1.19)	0.39–0.51

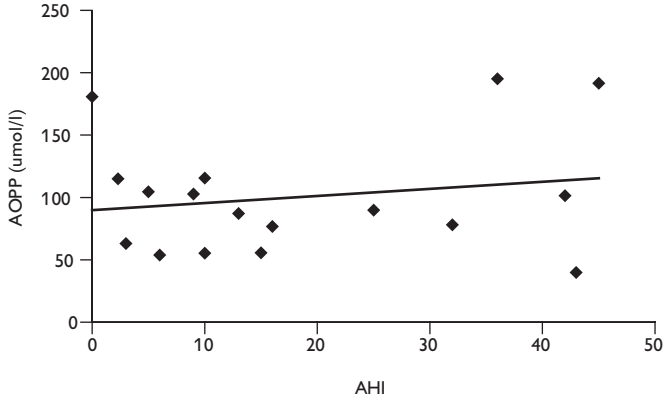

Figure 1 – Correlation analysis comparing morning AOPP level and AHI-expressed OSA severity (R = 0.485, P = 0.030)

The study was approved by the local ethics committee, and the patients gave their written consent each to being enrolled in the study.

Results

The average AHI was 21.9 (SD=14.0), the average ODI 23.1 (SD=17.0). The average ESS value in all of the patients cohort was 7.4±3.8.

The average values of the biochemical and haematological tests are shown in Table1. All but one (GGT) measured values were in the normal range.

The mean AOPP concentration in the patients group was 91.8 (SD=42.3) μmol/l, in the control group 76.2 (SD=35.3) μmol/l, the difference being of no statistical significance (Student's T-test).

The AOPP levels were found correlated with the AHI (R=0,485, P=0,030) (Figure 1) but not with the ODI and BMI (Pearson's correlation).

Discussion

The study showed that AHI, as a main parameter of rated OSA intensity, is correlated with the morning levels of AOPP. These parameters were found higher in patients with OSA, but the difference was not significant. With regard to our cohort's small size and age heterogeneity, we regard the results obtained as another indicator of higher risk of atherosclerosis in OSA. Despite the limited number of patients, the study suggests that atherogenesis in OSA patients does depend on its intensity. As follows from the measurements of carotid intima-media thickness, even moderate OSA leads to measurable atherosclerotic manifestations [19], quite in agreement with our results. Nevertheless, AOPP elevation is not specific of the atherosclerotic process; it is seen as a risk factor of several diseases [20]. It can be also connected with hypoxia [21] which is also typical of OSA. Our results do not support this hypothesis because there is no correlation with oxygen saturation parameters and AOPP.

Our cohort of patients falls short of providing sufficient data for comparing other clinical manifestations of OSA with AOPP levels as that would call for more research on a more compact group of patients. Nor can any statements be made as to the extent, to which oxidative stress affects the brain. However, more specific markers for the degree of oxidative stress, microinflammation and endothelial dysfunction should be used when focusing on vascular damage in OSA patients.

Conclusion

This study supports the hypothesis that OSA increases the oxidative stress and atherogenesis.

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