Advanced oxidation protein products and ischaemia-modified albumin in obstructive sleep apnea

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ABSTRACT

Background Several studies have shown that obstructive sleep apnea increases incidence of cardiovascular morbidity and mortality. The high systemic oxidative stress in obstructive sleep apnea has been considered as a major pathogenic mechanism leading to cardiovascular disease. Oxidative stress-related lipid and DNA oxidation in obstructive sleep apnea have been reported in the previous studies. In contrast, there is limited and contradictory information regarding protein oxidation in obstructive sleep apnea patients such as ischaemia-modified albumin and advanced oxidation protein products. Therefore, we aimed to investigate plasma ischaemia-modified albumin and advanced oxidation protein products and their correlation with total oxidative status and total antioxidative capacity in the obstructive sleep apnea patients.

Methods Plasma ischaemia-modified albumin, advanced oxidation protein products, total oxidative status and total antioxidative capacity were measured in 25 healthy volunteers and 59 obstructive sleep apnea patients diagnosed with polysomnography.

Results Plasma total antioxidative capacity was significantly lower ($P = 0.012$) and total oxidative status was significantly higher ($P < 0.001$) in the patients compared to the controls demonstrating increased oxidative stress in the patients. Plasma advanced oxidation protein products were significantly higher in the patients than the controls ($P = 0.024$). Plasma ischaemia-modified albumin levels were not statistically different between the obstructive sleep apnea patients and controls ($P = 0.74$).

Conclusions We conclude that high systemic oxidative stress in obstructive sleep apnea is reflected by increased advanced oxidation protein products without causing an increase in ischaemia-modified albumin.

Keywords Advanced oxidation protein products, ischaemia-modified albumin, obstructive sleep apnea, oxidative stress, total antioxidative capacity, total oxidative status.


Introduction

Obstructive sleep apnea (OSA) is a condition characterized by cessation of respiration due to obstruction of the upper airway during sleeping [1–4]. Its prevalence is approximately 4% in males and 2% in females in the adult population [1,2]. It is seen frequently in patients with morbid obesity, cardiac, neurological, renal and respiratory diseases [1,3].

In OSA, each episode of airway obstruction is followed by decreased arterial oxygen ($O_2$) saturation. Intermittent hypoxia and hypoxia/reoxygenation sequences causing increased production of ROS in OSA patients are associated with increased cardiovascular risk and mediated by several intermediary mechanisms such as oxidative stress and sympathetic activation [2–5].

Increased levels of ROS in the OSA patients may result in oxidative damage to proteins, lipids and DNA. Oxidative damage to proteins might lead more devastating results than damage to lipids and DNA. Protein oxidation is a relevant marker of oxidative stress as plasma proteins are critical targets for oxidants. Oxidative protein damage causes irreversible modifications in proteins. The structure and activity of oxidized proteins change profoundly in comparison with their native forms. Oxidative modification of proteins in vivo may affect a variety of cellular functions [6–9]. Advanced oxidation protein products (AOPP) are formed during oxidative stress and exert...
biological activities such as induction of proinflammatory cytokines and adhesive molecules. AOPP were described by Witko-Sarsat et al. [10] for the first time in 1996. AOPP are novel markers of oxidative stress in addition to total oxidative status (TOS) and total antioxidative capacity (TAC) levels, the well-known markers of oxidative stress in several diseases [4,11].

Ischaemia-modified albumin (IMA) is a new blood biomarker indicating increased risk for ischaemic cardiac disease. During ischaemic events, human serum albumin is modified and generates a variant called IMA in which N-terminal amino acid has a low capacity for binding transition metals such as copper, nickel and cobalt [12].

In several studies, increased oxidative stress was reported in sleep-disordered breathing using various markers of oxidative stress, but oxidative protein modifications related to increased oxidative stress in OSA remain controversial. Thus, reviewing MEDLINE, there are restricted and contradictory data about AOPP and only one publication regarding IMA levels in OSA patients and these data need to be confirmed. In contrast, a higher DNA damage in the OSA patients compared to the healthy controls has been reported clearly in the previous publications [13,14]. Therefore, we aimed to investigate blood levels of AOPP and IMA and their correlation with TOS and TAC in OSA patients.

Materials and methods

The study was approved by the local ethical committee and written informed consent was received from the patients and control subjects before being included into the study. The patient and control cohorts were recruited and examined at the Neurology and Cardiology Departments, Medical Faculty, Kafkas University. Blood samples were analysed at the Biochemistry Laboratory, Medical Faculty, Akdeniz University.

Patient selection

A total of 59 patients diagnosed with OSA after a night polysomnography recording between December 2011 and March 2012 were enrolled into the study. Patients having diagnosed coronary artery disease were excluded from the study. Sleep-disordered breathing events were scored manually by the same examiner according to the 2007 American Academy of Sleep Medicine criteria. Apnea was defined as a drop in the peak oronasal thermal sensor excursion by ≥ 90% of baseline for at least 10 s. Hypopnoea was defined as at least a 50% drop in airflow for at least 10 s despite respiratory efforts and at least a 3% drop in oxyhemoglobin saturation. Patients were diagnosed as OSA if the apnea–hypopnoea index (AHI) was ≥ 5 [15].

Blood samples were collected from 25 age- and gender-matched control subjects without a history of cardiac and cerebral ischaemic diseases, and OSA indicated by the absence of snoring, daytime sleepiness and witnessed apnea.

Blood withdrawal and analysis

Fasting morning venous blood samples were collected into EDTA tubes in the week following the polysomnography recording before starting continuous positive airway pressure therapy. After centrifugation, separated plasma samples were frozen at −70 °C until analysis.

Plasma IMA, AOPP, TOS and TAC levels were measured by the researchers blinded to the patient and control sample details.

Ischaemia-modified albumin (IMA) assay

This immunoassay is based on a quantitative sandwich enzyme immunoassay technique (Cusabio Biotech, Wuhan, Hubei, China) allowing in vitro quantitative determination of human IMA in serum and plasma. An antibody specific for IMA has been precoated onto a microplate. Standards and plasma samples are pipetted into the wells and any IMA present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IMA is added to the wells. After washing, avidin-conjugated horseradish peroxidase is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution containing 3, 3′, 5, 5′-tetramethylbenzidine is added to the wells. The colour development is stopped by addition of a sulphuric acid solution and the intensity of the colour developed in proportion to the amount of IMA bound in the initial step is measured at a wavelength of 450 nm. Concentration of IMA in the samples is determined comparing optical densities of the samples with those of the standards using a standard curve.

Advanced oxidation protein products (AOPP) assay

Plasma AOPP levels were measured on a microplate reader and are calibrated with chloramine-T solutions in the presence of potassium iodide using Witko-Sarsat V spectrophotometric method [10]. Briefly, 200 μL of sample diluted 1/5 in phosphate-buffered saline (PBS) is placed in the wells of a 96-well microtitre plate, and 20 μL of acetic acid is added. In standard wells, 10 μL of 1·16 M potassium iodide is added to 200 μL of chloramine-T solution (0–100 μmol/L) followed by addition of 20 μL of acetic acid. The absorbance of the reaction mixture is immediately read at 340 nm in the microplate reader against a blank containing 200 μL of PBS, 10 μL of potassium iodide and 20 μL of acetic acid. The chloramine-T absorbance at 340 nm is linear within the range from 0 to 100 μmol/L. AOPP concentrations are expressed as micromoles per litre of chloramine-T equivalents (μmol/L).
Plasma total antioxidative capacity (TAC) assay
TAC was determined using a novel automated colorimetric method developed by Erel [16]. In this assay, hydroxyl radical is produced by the reaction of ferrous ion solution, present in Reagent 1, with hydrogen peroxide, present in Reagent 2. Sequentially produced potent radicals such as the brown-coloured dianisidinyl radical cation are produced by hydroxyl radical. Further oxidation reactions increase the intensity of colour formation. Antioxidants present in the sample suppress oxidation reactions and colour formation. The suppression of colour formation is calibrated with Trolox, which is widely used as a traditional standard for TAC measurement assays. The assay has excellent precision values, > 97%. The results are expressed in terms of millimolar Trolox equivalent per litre (mmol Trolox equiv/L).

Total oxidative status (TOS) assay
TOS in plasma was determined using a novel automated colorimetric method, also developed by Erel [17]. Oxidants present in the sample oxidize ferrous ion-α-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundant in the reaction medium. The ferric ion forms a coloured complex with xylenetol orange in an acidic medium. The colour intensity measured spectrophotometrically is related to the quantity of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and results are expressed in terms of micromolar hydrogen peroxide equivalent per litre (μmol H₂O₂ equiv/L).

Statistical analysis
Statistical analysis was performed using SPSS Statistics Base 17.0 (SPSS Inc. Chicago, IL, USA). Quantitative data were given as mean ± SD and categorical data were given as percentages. Normal distribution and differences between variances were determined using Kolmogorov–Smirnov and Levene tests, respectively. For comparisons between the two groups, Student’s t-test and Mann–Whitney U-test for scalar variables and chi-squared test or Fisher’s exact test for categorical variables were used as appropriate. For subgroup analyses, Kruskal–Wallis test was used to determine significant differences. Mann–Whitney U-test was used to determine differences between the groups if a significant difference was found in Kruskal–Wallis test. A univariate analysis was performed to determine factors affecting IMA, AOPP and oxidative stress parameters. Spearman’s rho test was used to determine correlations between IMA, AOPP and oxidative stress parameters and AHII and minimum O₂ saturation. In all tests, a two-sided P value < 0.05 was considered statistically significant.

Independent predictors of TOS were searched using a linear regression analysis model (with standard entry method) with the following variables: age, body mass index (BMI), gender, the presence of OSA, the presence of diabetes mellitus (DM), the presence of hypertension (HT) and smoking habits. Categorical data were coded as dummy variables to include them within regression model. For all categorical data except gender, the absence of particular disease/condition was coded as 0 and the presence was coded as 1. For gender, females were coded with 0 and males were coded with 1. To test the validity and adequacy of linear regression model, normal distribution for regression standardized residuals of TOS was checked with a histogram, and standardized predicted values were plotted against studentized residuals.

Results
Demographical and laboratory findings of the OSA patients and control subjects were shown in Table 1. The mean AHI of the OSA patients was 52.48 ± 26.09 (mean ± SD) and range was 15.5–124. The mean minimum O₂ saturation (%) (mean ± SD) of the OSA patients was 71.41 ± 11.3 ranging from 50 to 84%. There was no significant difference in terms of age, gender and diabetes mellitus between the OSA patients and control subjects. BMI and incidence of HT were significantly higher (P < 0.001), and the presence of smoking habit and TAC levels were significantly lower in the OSA patients compared to the control group (P = 0.014, P = 0.012, respectively).

TOS levels were significantly higher in the OSA group compared to the control group (P < 0.001). AOPP levels were significantly higher in the OSA group compared to the control group (P = 0.024). Plasma IMA levels were not statistically different between the OSA patients and controls (P = 0.74). There was a very strong negative correlation (r = −0.987, P < 0.001) between TAC and AOPP levels (Fig. 1). AOPP levels correlated significantly with TOS levels (P < 0.001, r = 0.45) as shown in Fig. 2. A negative significant correlation (P < 0.0001, r = −0.6) was found between the AHI and minimum O₂ saturation (Fig. 3). There was a weak linear correlation between AHII and TOS level (r = 0.308, P = 0.018).

Our linear regression analysis demonstrated that the presence of OSA [β = 0.611, P < 0.05, 95% CI (22.97–54.10)] and female gender [β = −0.259, P = 0.022, 95% CI (−28.35 to −2.22)] had an independent association with TOS. The linear regression model had a R² value of 0.3, with an ANOVA F value of 4.63 (P = 0.01), indicating a significant, but weak correlation between the independent variables and TOS. Diagnostic statistics showed that the model was valid and adequate and was free of significant interactions between independent variables.
Discussion

Oxidative stress in OSA has been investigated in several studies [4,18–20]. Earlier oxidative stress studies in OSA reported inconsistent results, largely due to the use of different oxidative stress markers. In some studies, reactive oxygen metabolites such as superoxide, were reported to be raised in the OSA patients, in contrast plasma protein carbonyls, erythrocyte catalase activity, lipid peroxidation, osmotic fragility of blood cells and low density lipoprotein susceptibility to oxidation were found similar when compared to the healthy controls [3,11,13,20–26]. The discrepancies observed are indeed due to the use of different indicators of oxidative stress. The discrepancies in the previous data might be interpreted with the measurement of different indicators of oxidative stress. An increase in one parameter of oxidative stress might be compensated by a decrease in another parameter. Therefore, we measured total antioxidant capacity (TAC) and total oxidative status (TOS) to demonstrate the oxidative stress status in our

Table 1  Demographical and laboratory findings of the OSA patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 25</td>
<td>N = 59</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>48.68 ± 5.03</td>
<td>51.02 ± 9.37</td>
</tr>
<tr>
<td>Range (year)</td>
<td>39–58</td>
<td>27–68</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>16 (64%) / 9 (36%)</td>
<td>33 (56%) / 26 (44%)</td>
</tr>
<tr>
<td>DM (–/+)</td>
<td>22 (88%) / 3 (12%)</td>
<td>50 (84.7%) / 9 (15.3%)</td>
</tr>
<tr>
<td>BMI (mean ± SD)</td>
<td>25.84 ± 3.29</td>
<td>33.58 ± 5.47</td>
</tr>
<tr>
<td>Range (kg/m²)</td>
<td>21.2–33.2</td>
<td>22.8–46.6</td>
</tr>
<tr>
<td>HT (–/+)</td>
<td>23 (92%) / 2 (8%)</td>
<td>31 (52.5%) / 28 (47.5%)</td>
</tr>
<tr>
<td>Smoking (–/+)</td>
<td>13 (52%) / 12 (48%)</td>
<td>46 (78%) / 13 (22%)</td>
</tr>
<tr>
<td>TAC (mean ± SD)</td>
<td>2.131 ± 0.287</td>
<td>1.84 ± 0.54</td>
</tr>
<tr>
<td>Range (mmol/L)</td>
<td>0.93–2.48</td>
<td>0.05–2.47</td>
</tr>
<tr>
<td>TOS (mean ± SD)</td>
<td>33.95 ± 5.813</td>
<td>63.21 ± 30.62</td>
</tr>
<tr>
<td>Range (μmol/L)</td>
<td>26.62–49.89</td>
<td>26.91–172.63</td>
</tr>
<tr>
<td>IMA (mean ± SD)</td>
<td>31.50 ± 48.43</td>
<td>30.30 ± 29.02</td>
</tr>
<tr>
<td>Range (IU/mL)</td>
<td>0.78–246.43</td>
<td>0.43–127.10</td>
</tr>
<tr>
<td>AOPPs (mean ± SD)</td>
<td>37.19 ± 22.22</td>
<td>59.42 ± 45.88</td>
</tr>
<tr>
<td>Range (μmol/L)</td>
<td>8.67–82.68</td>
<td>12.11–200</td>
</tr>
</tbody>
</table>

BMI, body mass index; DM, diabetes mellitus; HT, Hypertension; IMA, ischaemia-modified albumin; AOPP, advanced oxidation protein product; TOS, total oxidative status; TAC, total antioxidative capacity.

![Figure 1](image1.png)  Correlation between TAC and AOPP levels.

![Figure 2](image2.png)  Correlation between TOS and AOPP levels.
patients and control subjects as better indicators of oxidative stress.

There is restricted and contradictory information regarding protein oxidation in blood of OSA patients as indicated by AOPP and IMA levels. Therefore, we measured AOPP, IMA, TOS and TAC in patients with OSA in comparison with the age- and gender-matched healthy subjects.

Presence of significantly higher hypertension (HT) in our OSA patients was associated with increased oxidative stress as indicated by increased TOS and decreased TAC levels compared to the healthy controls. Intermittent hypoxia, hypoxia/reoxygenation sequences and high oxidative stress in the OSA patients are the major events causing endothelial dysfunction. Oxidative stress and endothelial dysfunction initiate several mechanisms leading to the development of HT observed in the majority of the OSA patients [21,22] (Fig. 4).

We found significantly higher TOS, AOPP and significantly lower TAC levels in the OSA patients compared to the controls ($P < 0.001, 0.024$ and $0.012$, respectively). Lower TAC and higher TOS levels indicated that OSA patients had a decreased ability to cope with increased oxidative stress.

While there are numerous studies reporting increased oxidative stress in the OSA patients, only a limited number of studies demonstrated the level of antioxidant capacities in the OSA patients [23,27]. Faure et al. [27] reported impairment and reduction of serum albumin antioxidant properties in the OSA syndrome.

In the previous studies, no correlation between oxidative stress biomarkers and OSA severity indicated by AHI and $O_2$ saturation was demonstrated [3,11,20,23,24,26]. Mancuso et al. [3] found no correlation between AOPP levels and OSA disease severity.

To search whether intermittent hypoxia is related to oxidative stress, we performed correlation tests between oxidative stress and AHI and minimum $O_2$ saturation as the severity indicators of OSA. We found a negative significant correlation ($P < 0.0001, r = -0.6$) between AHI and minimum $O_2$ saturation (Fig. 3). In contrast, we found only a weak linear correlation between AHI as the disease severity index and TOS level ($r = 0.308, P = 0.018$). Our data demonstrated that other factors such as AHI as the severity indicator of OSA contribute to the higher TOS levels in OSA patients. Simiakakis et al. [26] reported that systemic oxidative stress in patients with OSA is not associated with the severity of sleep apnea. They found the level of oxidative stress lower in sleep apnea subjects despite similar degree of obesity in the control group. They explained this finding with the higher percentage of smokers and females in the control group. They concluded that obesity and smoking habit had a greater influence on the development of oxidative stress in OSA patients. They reported that the minimal oxygen saturation and smoking habit were the most important predicting factors for TAC levels [26]. In our study, the presence of smoking habit was significantly higher in the control group compared to the OSA group ($P = 0.014$). In contrast, BMI level was significantly higher in the OSA patients compared to the controls ($P < 0.001$).
Oxidative stress cumulatively causes cellular damage by oxidizing crucial biological molecules, such as DNA, lipid and proteins. Protein oxidation may lead to impairment of a variety of cellular functions and contribute to secondary damage to other biomolecules and enzymes, for instance, inactivation of DNA repair enzymes, loss of fidelity of DNA polymerases in replicating DNA. Thus, oxidative damage to proteins might lead more devastating results than damage to lipids and DNA [9,13,14,28]. There is limited and contradictory information regarding protein oxidation in OSA patients such as ischaemia-modified albumin (IMA) and advanced oxidation protein products (AOPP). Therefore, we aimed to investigate blood levels of AOPP and IMA and their correlation with total oxidative status (TOS) and total antioxidative capacity (TAC) in the OSA patients. Although we found an increased oxidative stress in the OSA patients compared to the controls, IMA levels were not significantly different between the control and patient groups. In contrast, AOPP levels were significantly higher in the OSA patients compared to the controls (59.42 ± 45.8 vs. 37.19 ± 22.22 µmol/L) (P = 0.024).

Advanced oxidation protein products (AOPP) are recognized as a marker of oxidative protein damage, an indicator demonstrating the intensity of oxidative stress. Oxidation of proteins leads to protein damage due to oxidation of amino acid residues such as tyrosine, formation of dityrosine, protein aggregation, cross-linking and fragmentation. The dityrosine-containing protein cross-linking products were designated as AOPP [29–31]. Our finding of a very strong negative relation (r = −0.987, P < 0.001) between TAC and AOPP indicates a prominent role of total antioxidant capacity in prevention of protein oxidation. In contrast, AOPP levels correlated positively with TOS levels in our patients (P < 0.001, r = 0.45) as shown in Fig. 2. In accordance with the previous studies and based on our data, we conclude that the systemic high oxidative stress in OSA increases protein oxidation to some extent as indicated by significantly higher AOPP levels [32,33].

Ischaemia-modified albumin (IMA) is a relatively new biomarker which is under research in several ischaemic and/or hypoxic conditions [12,34–39]. In a recent study performed in the obese subjects compared to the normal-weight controls, IMA and TOS were found to be elevated, whereas TAC significantly decreased and BMI was reported to be an independent determinant for IMA [34]. However, obese subjects in their study were not evaluated for coexisting OSA or other ischaemia-inducing conditions that might contribute to their positive finding. In some previous studies, serum IMA has been proposed as a sensitive and early marker of acute ischaemia and was found to increase within a few minutes after the onset of myocardial ischaemia [35,36]. Sinha et al. demonstrated that IMA values increased just after transient balloon inflation during percutaneous coronary intervention, even in the absence of detectable levels of cardiac troponin. The rapid increase in IMA levels after balloon inflation was followed by a subsequent decrease in 6 h and returned to normal values in 24 h [36]. Their findings indicate that IMA values decrease to baseline rapidly after an ischaemic cardiac event. We found similar IMA levels in the OSA patients and control subjects. The rapid change in IMA level in acute ischaemic cardiovascular events is not expected to be seen in chronic ischaemic diseases such as OSA. This might explain why we could not detect a higher IMA level in our OSA patients. Unlike AOPP, IMA is a biomarker for acute cardiovascular ischaemic diseases.

There is only one study published in November 2013 about IMA in the OSA syndrome [37]. To our knowledge, this is the first published study examining IMA in OSA [37]. However, there are some concerns regarding the design of this study [38]. Presence of ischaemic diseases, even stable coronary artery disease which is associated with an elevated IMA level, was not taken into consideration among the baseline characteristics of the two study groups in this study [39]. The probability of the presence of coronary artery disease might have a role in the high IMA results of this study. To avoid the drawback of the study by Yang et al., we investigated the clinical conditions of our OSA patients carefully and those having coronary artery disease were not enrolled into our study. We could not find a correlation between IMA and severity of OSA. Zhong et al. [38] report that there is dynamic ischaemic damage in the OSA patients; mild ischaemia and anoxia appear during daytime, and when the patients sleep, apnea occurs leading to more serious ischaemia and anoxia.

In conclusion, a high systemic oxidative stress in OSA as indicated by increased TOS and decreased TAC levels is reflected by increased AOPP without causing an increase in IMA.

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**Author contributions**
Serkan Ozben, Nergiz Huseyinoglu, Tolga Sinan Guvenc and Binnaz Zeynep Yildirim reviewed the literature, designed the study, recruited and examined the patients and controls, received informed consent and ethical approval and collected the blood samples; Ferhat Hanikoglu and Aysegul Cort performed the experiments; Serkan Ozben, Ferhat Hanikoglu, Sebahat Ozdem and Tomris Ozben analysed data and interpreted results of the experiments; Serkan Ozben, Ferhat Hanikoglu and Tomris Ozben drafted the manuscript; All authors edited, revised and approved the final version of the manuscript.

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**S. OZBEN ET AL.**

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Conflict of interest
The authors who have taken part in this study declare that they do not have anything to disclose regarding funding or conflict of interests with respect to this manuscript.

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