The Relationship Among Oxidative and Anti-Oxidative Parameters and Myeloperoxidase in Subjects with Obstructive Sleep Apnea Syndrome

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BACKGROUND: Obstructive sleep apnea (OSA) is a highly prevalent breathing disorder in sleep. It is characterized by intermittent hypoxia leading to hypoxemia, hypercapnia, sleep fragmentation, and increased respiratory efforts. We evaluated the relationship between OSA and myeloperoxidase activity, the oxidative stress index (OSI), total anti-oxidative capacity (TAC), and total oxidative capacity (TOC).

METHODS: A total of 70 consecutive subjects (mean age ± SD: 51.7 ± 11.7 y) were diagnosed with OSA after a night polysomnography recording between January 2014 and June 2014. The subjects in the OSA group were divided according to the severity of the disease into three subgroups, consisting of 11 mild, 17 moderate OSA, and 22 severe OSA subjects. Twenty subjects with simple snoring were considered as the control group.

RESULTS: We included a total of 70 subjects: 50 with OSA (11 subjects 6.9% mild, 17 subjects 24.7% moderate, and 22 subjects 68.5% severe) and 20 subjects with simple snoring as control cases. The mean age of the mild OSA subjects was 44.5 ± 11.7 y, moderate OSA subjects’ mean age was 52.5 ± 11.9 y, and severe OSA subjects’ mean age was 52.1 ± 10.1 y; 54.2% were male. There were statistically significant differences among the 4 groups’ OSI, TAC, and TOC levels, but there was no statistically significant difference between the other values. The mean myeloperoxidase, TOC, OSI, and TAC levels were 55 ± 12, 61.2 ± 21.1, 3.04 ± 1.04, and 2.03 ± 0.4 in the mild OSA group; 58.7 ± 17.2, 60 ± 18.9, 3.05 ± 1, and 2 ± 0.33 in the moderate OSA group; 56.6 ± 17.9, 52.1 ± 17.9, 2.7 ± 0.76, and 1.94 ± 0.24 in the severe OSA group; and 49.8 ± 12.5, 54.3 ± 16.4, 3.08 ± 0.88, and 1.78 ± 0.26 in the control group, respectively.

CONCLUSIONS: In our study, there were no differences in studied parameters between control and OSA groups. Furthermore, our low number of cases was a restrictive factor. Further studies should be undertaken to clarify this relation. Key words: sleep apnea; obstructive; oxidative stress; peroxidase.

Introduction

Obstructive sleep apnea (OSA) is a highly prevalent sleep-related breathing disorder. It is characterized by intermittent hypoxia, leading to hypoxemia, hypercapnia, sleep fragmentation, increased respiratory efforts, and increased sympathetic activity. OSA is a common disorder of middle-aged adults, affecting 4% of men and 2% of women. However, the prevalence of sleep-disordered

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breathing in men and women who do not display daytime somnolence is approximately 24% and 10%, respectively. Moreover, in obese and elderly populations, this value increases to 60%. OSA is also an independent risk factor for cardiovascular morbidity. The incidence of cardiovascular morbidities such as hypertension, ischemic heart disease, chronic heart failure, arrhythmias, and strokes is higher than that in the general population, making OSA a major public health problem by affecting a patient’s health and quality of life.

Myeloperoxidase (MPO) is a heme-containing peroxidase expressed abundantly in neutrophils, and to a lesser extent, in monocytes. It is also one of the principal enzymes released from secondary granules following neutrophil activation. Although the generation of oxidants by MPO is beneficial in terms of the immune response to invading pathogens, there is considerable evidence that inappropriate stimulation of oxidant formation can result in host tissue damage. Several studies have indicated the presence of higher levels of oxidative stress or decreased activity of the anti-oxidant system in OSA subjects compared with non-OSA controls.

We evaluated the relationship between OSA and MPO activity, the oxidative stress index (OSI), total anti-oxidative capacity (TAC), and total oxidative capacity (TOC).

Methods

This study was approved by the local ethics committee in accordance with the Helsinki Declaration. Written informed consent was received from the OSA syndrome subjects and control subjects before enrollment in the study. The OSA and control cohorts were recruited from the Pulmonary Medicine Department, Medical Faculty, Yuzuncu Yil University, Van, Turkey. Blood samples were analyzed at the Biochemistry Laboratory of Harran University, Şanlıurfa, Turkey.

Subject Selection

A total of 70 consecutive subjects (male/female: 38/32; mean age ± SD: 51.7 ± 11.7 y) were diagnosed with OSA after night polysomnography recording between January 2014 and June 2014. They were enrolled in the study following receipt of their written informed consent. The exclusion criteria for the OSA subjects were as follows: history of ischemic cardiovascular diseases, chronic obstructive pulmonary diseases, ischemic cerebral diseases, chronic inflammatory diseases, or chronic and acute systemic infections at the time of the study. Sleep-disordered breathing events were scored manually by the same examiner, according to the 2012 American Academy of Sleep Medicine criteria. OSA was defined as a drop in peak oronasal thermal sensor excursion by ≥ 90% of baseline for at least 10 s. Hypopnea was defined as at least a 50% drop in air flow for at least 10 s despite respiratory efforts, and at least a 3% drop in oxyhemoglobin saturation. Subjects were diagnosed with OSA if the apnea-hypopnea index (AHI) was ≥ 5. The grading was scored as follows: mild OSA, AHI ≥ 5 and < 15; moderate OSA, AHI ≥ 15 and < 30; severe OSA, AHI ≥ 30. The subjects in the OSA group were divided into three subgroups according to the severity of the disease: 11 mild, 17 moderate OSA, and 22 severe OSA subjects. Twenty subjects with simple snoring were considered as control group.

Polysomnography

Overnight polysomnography was performed using a 16-channel Embla device (Medcare, Reykjavik, Iceland) under continuous monitoring by a sleep technician. The system consists of 4 electroencephalogram channels (with electrode placements at C4-A1, C3-A2, O2-A1, and O1-A2), 2 electro-oculogram channels, a submental electromyogram, nasal air flow using a nasal pressure cannula, thoracic and abdominal movements, pulse oximeter oxygen saturation, tibial electromyogram, body position, electrocardiogram readings, and tracheal sound. Apnea was defined as the complete cessation of air flow lasting more than 10 s. Hypopnea was defined as a reduction > 30% in air flow lasting more than 10 s accompanied by > 4% desaturation and/or arousal. The average number of episodes of apnea and hypopnea per hour of sleep was measured as AHI. The OSA diagnosis was made based on AHI ≥ 5. Sleep stages were scored according to standard

QUICK LOOK

Current knowledge

Obstructive sleep apnea (OSA) is a common sleep-related breathing disorder characterized by snoring, sleep fragmentation, hypoxemia, and daytime hypersomnolence. Significant cardiac complications including stroke, heart failure, and hypertension are associated with worsening sleep apnea. Obstructive sleep apnea is also linked to an increase in the inflammatory response.

What this paper contributes to our knowledge

In a comparison of subjects with and without sleep apnea, there were no differences in measured markers of oxidative stress or systemic inflammation. In this small cohort, a link between OSA and systemic inflammation as a possible explanation for cardiovascular disease was not identified.
criteria with 30-s epochs, and were reviewed and verified by a certified sleep physician.

Blood Withdrawal and Laboratory Analysis

Samples

Fasting blood samples were drawn into heparinized tubes and centrifuged at 3,000 rpm for 10 min to separate the plasma. The samples were stored at −80°C until analysis.

Measurement of the Total Oxidant and Anti-Oxidative Status

The TAC and TOC levels were measured using an automated measurement method developed by Erel. The TAC measurement method involves the production of a potent biological hydroxyl radical. Ferrous ion solution is mixed with hydrogen peroxide. Thus, it is possible to measure the anti-oxidative effect of the sample against the potent free radicals reactions initiated by the production of the hydroxyl radical. The TOC method is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in an acidic medium, and the measurement of the ferric ion by xylenol orange. The results were expressed in mmol Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) Eq/L, mmol H_{2}O_{2}/L, and mg/dL, and the assay was characterized by excellent precision values < 3%.20

Oxidative Stress Index

The percent ratio of TOC to TAC yields the OSI, an indicator of the degree of oxidative stress. For its calculation, the resulting unit of TAC was changed to mmol/L, and the OSI value was calculated according to the following formula: OSI (arbitrary units) = TOC (mmol H_{2}O_{2} Eq/L)/TAC (mmol Trolox Eq/L).21

Myeloperoxidase

MPO activity was determined spectrophotometrically using a modification of the O-dianisidine method. First, 290 µL (50 mM, pH 6) of phosphate buffer, 3 µL (20 mg/mL) of O-dianisidine hydrochloride substrate solution, and 3 µL of H_{2}O_{2} (20 mM) were added in sequence into each well of a 96-well plate. Next, samples (10 µL) were added to each well to start the reaction, and the change in absorbance at 450 nm was monitored for 10 min. One unit of MPO activity was defined as that degrading 1 µmol of H_{2}O_{2}/min at 25°C, and is expressed as units/L.

Statistical Analyses

Statistical analyses were performed using SPSS 16 software (SPSS, Chicago, Illinois). Continuous data are expressed as means ± SD. Statistical comparisons were performed using one-way analysis of variance. To determine the relationships between these variables in each group separately, Pearson correlation coefficients were calculated. AHI, TOC, MPO, body mass index (BMI), TAC, and MPO variables in the OSA and control groups were assessed. Results were considered statistically significant when the P value was < 0.05.

Results

We included a total of 70 subjects: 50 in the OSA group (mild disease, 11 subjects [6.9%]; moderate disease, 17 subjects [24.7%]; severe disease, 22 subjects [68.5%]) and 20 in the simple snoring group as control cases. The mean age of the mild OSA subjects was 44.5 ± 11.7 y, that of the moderate OSA subjects was 52.5 ± 11.9 y, that of the severe OSA subjects was 52.1 ± 10.1 y, and that of the control group was 55.9 ± 12.5 y; 54.3% of the subjects were male. There were statistically significant differences among the four groups between the OSI levels and TAC or TOC levels, but there were no statistically significant differences regarding the other values. The parameters in the OSA and control groups are shown in Table 1. There was a positive correlation between the OSI levels and TAC or TOC levels (P = .02 or P < .001, respectively). The mean values of age, MPO, BMI, TAC, TOC, and OSI were similar among the three OSA groups compared with those of the subjects with simple snoring in the control group (P > .05). The mean MPO levels were 55 ± 12 in the mild OSA group, 58.7 ± 17.2 in the moderate OSA group, 56.6 ± 17.9 in the severe OSA group, and 49.8 ± 12.5 in the control group. The mean TOC levels were 61.2 ± 21.1 in the mild OSA group, 60 ± 18.9 in the moderate OSA group, 52.1 ± 17.9 in the severe OSA group, and 54.3 ± 16.4 in the control group. The mean OSI levels were 3.04 ± 1.04 in the mild OSA group, 3.05 ± 1 in the moderate OSA group, 2.7 ± 0.76 in the severe OSA group, and 3.08 ± 0.88 in the control group. The mean TAC levels were 2.03 ± 0.4 in the mild OSA group, 2 ± 0.33 in the moderate OSA group, 1.94 ± 0.24 in the severe OSA group, and 1.78 ± 0.26 in the control group.

Discussion

We investigated the relationships among OSA, oxidative stress biomarkers, and MPO. We found a positive correlation between OSI levels and TAC or TOC levels.
However, we did not find a correlation between OSI levels and those of the other parameters.

The relationship between systemic inflammation and OSA has been investigated in previous studies, but to date, only a few studies have been performed to elucidate persistent systemic inflammation as indicated by MPO activity and the TAC, TOC, and OSI levels in the blood of OSA subjects. In our study, we measured MPO activity, which reflects the pro-inflammatory and pro-oxidative status as risk indicators for cardiovascular diseases in subjects with OSA compared with age- and gender-matched healthy subjects. However, we did not find a correlation between AHI and MPO levels.

The mechanism underlying the increased risk for cardiovascular diseases in OSA is unclear, but a multifactorial etiology is likely to be involved. Systemic inflammation is thought to be present in many conditions and comorbidities such as atherosclerosis, vascular inflammation, endothelial dysfunction, hypertension, hyperlipidemia, ischemic diseases, arterial hypertension, coronary artery disease, myocardial infarction, and stroke. Remarkably, all of these comorbidities are likely to be associated with OSA. To investigate whether intermittent hypoxia was related to the severity of systemic inflammation, we performed correlation tests for AHI and minimum oxygen saturation as the indicators of disease severity, and TAC, TOC, OSI, and MPO as predictors of inflammation.

Obesity is common in OSA patients. In our study, there was no difference in BMI scores between the control and OSA groups. The mean scores were 31.05 ± 5.66 in the control group and 32.98 ± 5.16 in the OSA group. Because our control group comprised individuals who had no OSA symptoms, we believe our work is strengthened because of the lack of difference between the groups. In addition, there were no statistically significant differences between the groups regarding age.

MPO is mainly released from activated neutrophils in the setting of inflammation, has pro-oxidant and inflammatory enzymatic activities, and catalyzes the synthesis of some oxidants, causing oxidative damage at the inflammation area. It plays a role in the activation of metalloproteinases, causing plaque destabilization and susceptibility to plaque rupture. Recent studies have shown that elevated MPO levels correlate with an increased risk for cardiovascular diseases as an independent predictor of mortality in subjects with acute coronary syndrome. Recent studies have also shown a significant correlation between MPO levels and AHI scores. However, in our study, we found no such correlation. In that previous study, the BMI scores of the OSA group was significantly higher than that of the control group. Thus, the differences in the results might be due to these demographic differences. Akpinar et al. found a correlation between control and patient groups in salivary MPO, but no statistically significant correlation was found in serum MPO, a finding that is similar to that of our study.

Previous studies have suggested a significant relationship between OSA and increased oxidative stress. Wali et al. and Grabska-Kobylecka et al. studied catalase and glutathione peroxidase, and found no evidence of the overproduction of oxidants by studying circulating phagocytes. We also found no difference in the oxidative and antioxidative status between the control and OSA groups. In addition, we found no significant differences in the TOC, TAC, and OSI levels between the OSA and control groups.

Conclusions

We found no differences in the studied parameters between the control and OSA groups. However, our low number of cases was a limiting factor. Further studies should be undertaken to clarify this trend.

REFERENCES


