Increased Asymmetric Dimethylarginine and Ischemia-Modified Albumin Levels in Obstructive Sleep Apnea

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BACKGROUND: Asymmetric dimethylarginine and ischemia-modified albumin are new biomarkers that are used for evaluation of ischemia and oxidative stress. The present study aimed to investigate whether serum levels of asymmetric dimethylarginine and ischemia-modified albumin are altered in subjects with obstructive sleep apnea (OSA). METHODS: A cross-sectional, clinical study was implemented on data derived from 79 subjects who underwent polysomnography. Cases were allocated into 3 groups with respect to polysomnography results: Group 1 consisted of 22 subjects without apnea, whereas Group 2 comprised 29 subjects with mild to moderate OSA, and Group 3 included 28 subjects with severe OSA. These 3 groups were compared in terms of demographic datas and polysomnographic parameters, serum levels of asymmetric dimethylarginine and ischemia-modified albumin. RESULTS: Serum levels of ischemia-modified albumin were significantly higher in Groups 2 and 3 ($P = .001$). Mean S_{pO_2} of Group 3 was notably lower than that of Groups 1 and 2 ($P < .001$), whereas times for S_{pO_2} <90% were statistically significantly different from each other in all 3 groups ($P < .001$). Serum levels of asymmetric dimethylarginine in Group 3 were notably higher than those in Group 1 ($P = .027$). Levels of ischemia-modified albumin were correlated positively with AHI and time S_{pO_2} <90% values ($P = .008$ and $P < .001$, respectively). CONCLUSIONS: Ischemia-modified albumin and asymmetric dimethylarginine were significantly higher in subjects with OSA. Furthermore, ischemia-modified albumin was independently associated with severity of OSA defined by AHI and severity of oxygen desaturation. *Key words: obstructive sleep apnea; asymmetric dimethylarginine; ischemia modified albumin.*
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Introduction

Obstructive sleep apnea (OSA) is a common disorder characterized by recurrent episodes of upper airway obstruction during sleep.[1] Due to intermittent hypoxia, chronic sympathetic activation, and systemic inflammation seen in conjunction with OSA, the risk of cardiovascular morbidity was found to be increased.[2-8] However, the underlying pathophysiology for the link between OSA and cardiovascular disorders has not been precisely elucidated.[4,6,7]

Asymmetric dimethylarginine is the endogenous inhibitor of nitric-oxide synthase. Since asymmetric dimethylarginine inhibits nitric oxide synthase activity, decreased nitric oxide levels result in impaired endothelial function and decreased vasodilatation. Asymmetric dimethylarginine is now known to be a mediator molecule of the adverse vascular effects of many other factors and markers of cardiovascular risk. Asymmetric dimethylarginine levels increase under hypoxic conditions.[9] Increased asymmetric dimethylarginine concentration indicates endothelial dysfunction and is an important parameter in determining cardiovascular morbidity and mortality.

Ischemia-modified albumin has been regarded as a new and emerging marker of ischemia and oxidative stress.[2] During ischemia/reperfusion, the structure of the amino terminus of albumin is changed in a way that causes the

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decrease of its cobalt binding capacity, leading to the formation of an ischemia-modified albumin.[10] It increases within minutes after the onset of ischemia, and its levels remain elevated for several hours and return to normal within 24 h.[11]

Intermittent hypoxia, hypoxia/reoxygenation sequences, and high oxidative stress in patients with OSA are the major events causing endothelial dysfunction. Vascular tone and endothelial function are 2 important factors that may be involved in the association of OSA with cardiovascular morbidity. Impairment of endothelium-dependent vasodilatation had been reflected as changes in levels of nitric oxide and asymmetric dimethylarginine in OSA.[8,12] Similarly, one study[13] implied that serum ischemia-modified albumin, an indicator of oxidative stress and inflammation, may serve as a biomarker in OSA. Our hypothesis was that these levels would be elevated. The aim of the present study was to investigate the alteration of serum levels of asymmetric dimethylarginine and ischemia-modified albumin in OSA and to test their correlation with demographic and polysomnographic indices.

QUICK LOOK

Current knowledge

Intermittent hypoxia, hypoxia/reoxygenation sequences, and high oxidative stress in patients with obstructive sleep apnea (OSA) are the major events causing endothelial dysfunction. Asymmetric dimethylarginine and ischemia-modified albumin are produced in response to ischemic stresses, such as hypoxia. However, asymmetric dimethylarginine and ischemia-modified albumin in OSA causing endothelial dysfunction has not been precisely evaluated.

What this paper contributes to our knowledge

Ischemia-modified albumin and asymmetric dimethylarginine were significantly higher in subjects with OSA. Furthermore, ischemia-modified albumin levels were associated with severity of OSA defined by apnea-hypopnea index. Serum levels of ischemia-modified albumin and asymmetric dimethylarginine may be altered in OSA, reflecting the deterioration of vascular endothelial function.

Methods

Study Design

This cross-sectional, clinical study was conducted in the Pulmonology Department at the Faculty of Medicine at Yuzuncu Yil University Hospital (Van, Turkey) between January and June 2014. The study was approved by the institutional review board. Seventy-nine subjects with a complaint of excessive daytime sleepiness and snoring were recruited for polysomnography. OSA was defined as apnea-hypopnea index (AHI) $\geq$5 events/h. Cases with AHI $<$ 5 constituted Group 1 (n = 22), whereas cases with AHI values between 5 and 30 were termed as mild to moderate OSA (Group 2, n = 29), and subjects with an AHI of $\geq$30 (severe OSA) constituted Group 3 (n = 28).

Previous treatment for sleep disorder (oral appliances or positive airway pressure before the sleep study), history of uvulopharyngeal or orthognathic surgery, any neurological deficit or neuromuscular diseases, and diagnosis of another sleep disorder, including central apnea, were exclusion criteria for the present study. In addition, patients with hypertension, diabetes mellitus, hyperlipidemia, COPD, interstitial lung disease, or asthma were also excluded from the study.

Sleep Study

Diagnosis of OSA was accomplished with respect to the results of overnight polysomnography by means of a digital 16-channel Embla (Medcare, Reykjavik, Iceland) polygraph performed at our sleep laboratory. Generally accepted definitions and scoring methods were utilized, and diagnosis of OSA was established in the presence of chest and abdominal paradoxical movement during apnea events or flow limitation on the nasal pressure signals.[1]

Biochemical Assays

Blood samples were obtained early in the morning after an overnight fasting period. Peripheral venipuncture was made from brachial veins, and blood samples were collected into vacutainer tubes. Serum samples were centrifuged at 3,000 rpm for 5 min and were maintained at -80°C until analysis.

Reduced cobalt-to-albumin binding capacity (ischemia-modified albumin level) was measured using the rapid and colorimetric method developed by Bar-Or et al[14] Briefly, 200 μL of subject serum was transferred into glass tubes, and 50 μL of 0.1% $CoCl_2 \times 6H_2O$ (Sigma-Aldrich, St. Louis, Missouri) was added. After gentle shaking, the mixture was incubated for 10 min to ensure sufficient cobalt-albumin binding. Then 50 μL of 1.5 mg/mL dithiothreitol (Sigma-Aldrich) was added as a coloring agent. After 2 min, 1 mL of 0.9% NaCl was added to halt the binding between the cobalt and albumin. A blank was prepared for every specimen. At the dithiothreitol addition step, 50 μL of distilled water was used instead of 50 μL of 1.5 mg/mL

Table 1. Comparison of Demographic, Polysomnographic, and Biochemical Variables Under Investigation in the 3 Subject Groups

Variable	Group 1 (n = 22)	Group 2 (n = 29)	Group 3 (n = 28)	P
Male/female sex, n	14/8	22/7	21/7	.58
Age, mean ± SD y	35.8 ± 9.5	46.2 ± 10.0	47.0 ± 14.0	< .001
BMI, mean ± SD kg/m^2	27.8 ± 3.9	31.3 ± 5.7	33.1 ± 7.0	.008
AHI, mean ± SD h	2.2 ± 1.1	15.6 ± 6.2	71.3 ± 28.2	< .001
REM AHI, mean ± SD h	2.5 ± 2.4	2.9 ± 2.3	4.7 ± 2.5	.005
Non-REM AHI, mean ± SD h	2.0 ± 1.2	15.0 ± 9.1	72.4 ± 31.0	.001
ODI, mean ± SD	2.5 ± 1.7	15.3 ± 8.0	60.3 ± 27.9	.001
Number of apneas, mean ± SD	2.7 ± 2.5	20.5 ± 15.3	275.0 ± 242.4	< .001
Times for S_{pO_2} <90%/min, mean ± SD %	0.15 ± 2.55	11.40 ± 34.30	46.35 ± 41.30	< .001
Mean S_{pO_2}, mean ± SD %	92.60 ± 3.05	91.60 ± 2.10	87.10 ± 15.28	< .001
IMA, mean ± SD units/L	0.84 ± 0.49	1.98 ± 1.43	2.28 ± 1.00	.001
ADMA, mean ± SD μmol/L	1.54 ± 0.35	1.69 ± 0.15	1.72 ± 0.20	.027

BMI = body mass index
AHI = apnea-hypopnea index
REM = rapid eye movement
ODI = oxygen desaturation index
Times for S_{pO_2} <90% = time arterial oxygen saturation <90%
IMA = ischemia-modified albumin
ADMA = asymmetrical dimethylarginine

dithiothreitol to obtain a blank without dithiothreitol. The absorbances were recorded at 470 nm with a UV1601 spectrophotometer (Shimadzu, Kyoto, Japan). Color formation in specimens with dithiothreitol was compared with color formation in the blank tubes, and the results were expressed as absorbance units. Serum levels of asymmetric dimethylarginine were detected with a commercially available enzyme-linked immunosorbent assay kit. Measurement was performed using a computer-based enzyme-linked immunosorbent assay reader (ELx 800 Universal Microplate Reader, Biotek Instruments, Winooski, Vermont) with a wavelength of 405 nm.

Outcome Parameters

Demographic data (age, sex, body mass index), polysomnographic indices (AHI, mean oxygen saturation, time for arterial oxygen saturation [S_{pO_2}] <90%, number of apnea), and biochemical parameters (serum levels of asymmetric dimethylarginine and ischemia-modified albumin) were noted and compared in 3 groups. Moreover, correlations of biochemical parameters to demographic and polysomnographic variables were sought.

Statistical Analysis

Data were analyzed using SPSS Statistics 20.0 for Windows (IBM, Armonk, New York). Normal distribution of variables was assessed with the Kolmogorov-Smirnov test. Parametric methods were used in the analysis of the variations that have normal distribution, and nonparametric methods were used in the analysis of the variations that do not have normal distribution. Correlation between variables with normal distribution was evaluated with the Pearson correlation test, whereas Spearman's rho test was preferred if at least one of the variables did not exhibit normal distribution. Comparison of groups in terms of variables with normal distribution was implemented with one-way analysis of variance. Variables without normal distribution were evaluated with the Kruskal-Wallis test. Homogeneity of variances of groups was analyzed with the Levene test. For groups with variables with homogeneous variances, the Duncan test was used. However, the Tamhane test was preferred for variables of groups with non-homogeneous variances. Quantitative variables were expressed as mean, SD, minimum, and maximum values. CI was set at 95%, and all differences associated with a P value of <.05 were considered statistically significant.

Results

A comparison of variables under investigation in the 3 groups is shown in Table 1. Distribution of male/female subjects was similar in the 3 groups (P = .58). Those in Group 1 were younger than those in Groups 2 and 3 (P < .001), but there was no age difference between those in Groups 2 and 3 (P = .99). The body mass index of Group 1 was remarkably lower than those of Groups 2 and 3 (P = .008). Serum levels of ischemia-modified albumin were significantly higher in Groups 2 and 3 (P = .001). Levels of serum ischemia-modified albumin in the 3 groups are shown in Figure 1. Mean oxygen saturation of Group 3 was notably lower than that of Groups 1 and 2 (P < .001), whereas times for S_{pO_2} <90% were statistically signifi-

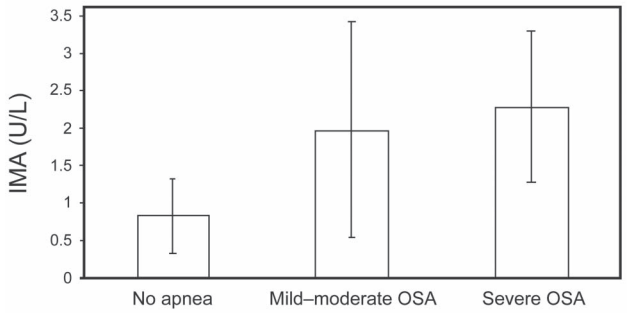

Fig. 1. Serum ischemia-modified albumin (IMA) levels in the 3 subject groups. $P = .001$.

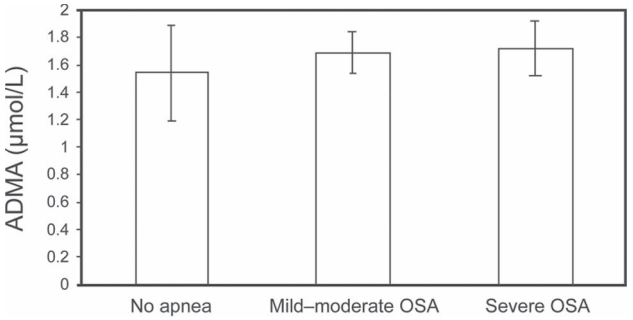

Fig. 2. Serum asymmetric dimethylarginine (ADMA) levels in the 3 subject groups. $P = .027$.

cantly different from each other in all 3 groups ($P < .001$). Serum levels of asymmetric dimethylarginine in Group 3 were notably higher than that in Group 1 ($P = .027$). Levels of serum asymmetric dimethylarginine in the 3 groups are demonstrated in Figure 2. Correlation analysis revealed that only serum levels of ischemia-modified albumin were correlated positively with AHI and time for S_{pO_2} <90% values ($r = 0.298$, $P = .008$ and $r = 0.458$, $P = .001$). In subjects with OSA, there was no correlation between ischemia-modified albumin and asymmetric dimethylarginine ($r = 0.75$, $P = .51$).

Discussion

The present study was carried out to investigate whether serum levels of asymmetric dimethylarginine and ischemia-modified albumin were altered in OSA and to determine whether levels of these biomarkers were correlated with demographic parameters or polysomnographic indices. Our results have shown that serum levels of ischemia-modified albumin and asymmetric dimethylarginine were significantly higher in subjects with OSA, and levels of ischemia-modified albumin were correlated positively with AHI and values for time S_{pO_2} <90%.

Obstructive sleep apnea is reported to be a risk factor for cardiovascular morbidity due to the hazardous outcomes of oxidative stress, vascular endothelial dysfunction, and inflammation.[15-18] Efforts made to clarify the link between OSA and cardiovascular disorders have pointed to endothelial dysfunction as a key step linking these 2 pathological processes.[16-18] Chronic intermittent hypoxia may have reverse effects on endothelial function, reducing endothelial nitric oxide bioavailability and amplifying the effects of oxidative stress and inflammation.[19,20] Increased levels of adhesion molecules and hypercoagulability enhance the vascular damage, leading to the occurrence of cardiovascular morbidity in OSA.[19,20]

Asymmetric dimethylarginine levels were increased under hypoxic conditions.[9] In addition to oxidative stress, elevated asymmetric dimethylarginine levels are substantial factors leading to impairment of nitric oxide availability and diminished endothelium-dependent vasodilatation, and their increased levels have been reported in cardiovascular disease.[21,22] Increased plasma concentration of asymmetric dimethylarginine is associated with hypertension, pulmonary hypertension, hypercholesterolemia, and severe peripheral artery occlusive diseases.[15,17,23,24] These findings suggest that asymmetric dimethylarginine is responsible for endothelial dysfunction. Barceló et al[25] assessed plasma asymmetric dimethylarginine in subjects with OSA and found it to be significantly higher in these subjects irrespective of the presence of further cardiovascular risk. Following CPAP therapy with significant reduction of intermittent hypoxemia, the levels of asymmetric dimethylarginine can be decreased.[15]

Ischemia-modified albumin may indicate underlying subclinical disease or vascular dysfunction.[26] Increased levels of ischemia-modified albumin may serve as a nonspecific risk marker for evaluation of the oxidative stress status and atherosclerotic burden.[13,27,28] Increased production of free radicals and pro-inflammatory cytokines in OSA are sources of the oxidative damage at the cellular and subcellular levels.[18] Yang et al[13] suggested that increased levels of ischemia-modified albumin in subjects with OSA may result from increased oxidative stress. Ischemia-modified albumin is produced in response to ischemic stresses, such as hypoxia and acidosis. The attenuation of ischemia-modified albumin frequently indicates an acute ischemic event like acute coronary syndrome, a pulmonary embolism, or acute ischemic stroke.[29-31] Similarly, our results demonstrated that serum ischemia-modified albumin levels were elevated significantly in all subjects with OSA. Correlation of ischemia-modified albumin levels with oxygen desaturation index and AHI is interesting, since it suggests that indicators of systemic inflammation may reflect the link between OSA and cardiovascular morbidity. Further trials must be carried out to investigate the predictive potential of ischemia-modified albumin for severities of oxygen desaturation and OSA.

A study done by Verma at al.[32] on the role of ischemia-modified albumin in pathogenesis of hyperthyroidism has indicated that elevated ischemia-modified albumin is associated with increased production of asymmetric dimethylarginine, which acts as an inhibitor of nitric-oxide synthase. This may account for ischemic injury caused by the reduction of nitric oxide synthesis. We could not find a correlation between ischemia-modified albumin and asymmetric dimethylarginine in the subjects with OSA.

Conclusions

Ischemia-modified albumin and asymmetric dimethylarginine were significantly higher in subjects with OSA. Furthermore, ischemia-modified albumin was associated with severity of OSA syndrome defined by AHI and severity of oxygen desaturation. Results of the current study have shown that serum levels of ischemia-modified albumin and asymmetric dimethylarginine may be altered in OSA, reflecting the deterioration of vascular endothelial function. Understanding the clinical importance of these potential biomarkers and their utility in terms of diagnosis, follow-up, and treatment will necessitate further clinical trials on larger series.

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