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The evaluation of inflammatory process, endothelial dysfunction and oxidative stress in sleep apnea

Uyku apnesinde inflamatuar süreç, endotelial fonksiyon bozukluğu ve oksidatif stresin incelenmesi

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Abstract

Objective: To investigate the correlation of inflammatory process, endothelial dysfunction and oxidative stress with obstructive sleep apnea (OSA).

Methods: In our prospective cross-sectional clinical study in a tertiary referral hospital, we evaluated 63 patients with newly diagnosed OSA and 9 simple snorers. Each patient was evaluated in terms of additional systemic diseases and laboratory tests. In addition to routine blood analysis; oxidative stress markers (leptine, RBP), vascular endothelial markers (ICAM-I, VCAM-I) and inflammatory markers (Crp, IL-6, TNF-alpha, isoprostane) were analyzed. Polysomnography test was performed and study population was divided into four groups depending on their AHI values. The levels of markers were analyzed and compared between the four groups.

Results: There was a weak correlation between the isoprostane levels and mean apnea duration and also a mild correlation to the maximum apnea duration. A weak correlation was detected between leptine and VCAM levels to age and also a weak negative correlation was detected between CRP levels to age. The leptin levels were found to be mildly correlated to BMI and abdominal circumference. The ICAM levels were found to have a weak correlation to BMI and abdominal circumference.

Conclusion: Our results indicate a correlation between sleep apnea and oxidative stress. These results may help to explain the association of comorbid diseases with OSAS. Further investigators should aim to explain key steps of inflammatory response in sleep apnea.

Keywords: Sleep apnea, inflammation, oxidative stress, vascular endothelia.

Özet

Amaç: Uyku apnesinde inflamatuar süreç, endotelial fonksiyon bozukluğu ve oksidatif stresin ilişkisinin incelenmesi.

Yöntem: Üçüncü basamak bir referans hastanesinde planlanan çalışmaya 63 yeni tanı almış uyku apnesi hastası ve 9 basit horlama hastası dahil edildi. Hastalar ek sistemik hastalıklar ve laboratuvar testleri açısından değerlendirildi, fizik muayeneleri yapıldı. Rutin laboratuvar testlerine ek olarak oksidatif stres belirteçleri (leptin, RBP), endotelial fonksiyon bozukluğu belirteçleri (ICAM-I, VCAM-I) ve inflamatuar süreç belirteçleri (CRP, IL-6, TNF-alfa, izoprostan) açısından analiz edildi. Hastalar apne hipopne indekslerine göre 4 alt gruba ayrıldı. Gruplar arasında çapraz analizler yapılarak gruplar arasındaki farklılıklar test edildi.

Bulgular: İzoprostan seviyesi ile ortalama apne süresi arasında hafif, maksimum apne süresi arasında orta seviyede korrelasyon gözlendi. Hastaların yaş ortalaması ile leptin ve VCAM-I arasında zayıf pozitif korrelasyon gözlenirken, CRP seviyesi ile ortalama yaş arasında zayıf negatif korelasyon izlendi. Leptin seviyesi ile vücut kitle indeksi ve abdominal çevre arasında orta seviyeli korelasyon izlendi. ICAM-I seviyesi ile vücut kitle endeksi ve karın çevresi arasında zayıf korelasyon mevcuttu.

Sonuç: Çalışmamız sonucunda oksidatif stress ile uyku apnesi arasında ilişki olduğunu tespit ettik. Bu ilişki uyku apnesine eşlik eden komorbid hastalıkların açıklanmasında önemli bir basamak olabilir. Bu konuda yapılacak olan yeni çalışmalarda bu inflamatuar sürecin basamakları ve klinik yansımaları açıklanmaya çalışılmalıdır.

Anahtar sözcükler: Uyku apnesi, inflamasyon, oksidatif stress, vasküler endotel.

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Obstructive sleep apnea (OSA) is an important health problem with an incidence of 4% in men and 2% in women of middle-aged population.[1] OSA is characterized by complete or partial obstruction of breathing due to collapse of upper airway during sleep. It is associated with increased cardiovascular and cerebrovascular morbidity along with increased atherosclerosis and oxidative stress. Inflammatory response may play an important role in the pathogenesis of atherosclerosis.[2]

Obstructive sleep apnea associated with chronic inflammatory process of vessels has been described in recent studies.[3] Chronic inflammation has a potential risk of permanent vascular damage which may lead to stroke or myocardial infarction. The inflammatory process in this disease is most probably induced by chronic intermittent hypoxemia. An inflammatory response starts with TNF and continues with induction of other acute-phase proteins such as CRP, IL-6, retinol-binding protein (RBP), isoprostane. It was proposed that endothelial dysfunction and resulting vascular damage in OSA may lead to vasoconstriction, hypercoagulability-thrombosis and eventually cardiovascular/cerebrovascular complications.[4] Circulating levels of soluble adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cellular adhesion molecule-1 (VCAM-1) were detected to be elevated in OSA patients.[5]

Circulating adipokine levels were found to be related with insulin resistance and reduced sleep time.[6] Retinol-binding protein is one of the adipokines and found to be in elevated levels in OSA. The RBP level reduces with CPAP therapy. Leptin is a protein, derived from adiposite tissue. Leptin is found to be in elevated levels in OSA. Endothelial tissue has the leptine receptors. Elevated leptin levels may play a role in atherosclerotic process and comorbid diseases in OSA.

In the present study, our aim was to investigate the correlation of inflammatory process, endothelial dysfunction and oxidative stress with OSA.

Materials and Methods

Study Design

The study was approved by the ethical committee of Dışkapı Yıldırım Beyazıt Training and Research Hospital. In this cohort study 63 patients with newly diagnosed OSA and 9 simple snorers were evaluated. The subjects were examined by polysomnography (PSG) and blood tests, and they were stratified to 4 groups depending on their AHI values. The first group had 9 simple snorers with AHI<5. The second group had 28 subjects with mild OSA (5<AHI<15); the third group had 18 subjects with moderate OSA (15<AHI<30); the fourth group had 17 subjects with severe OSA (AHI>30).

After obtaining detailed medical history and performing physical examination blood tests were done. For each subject body mass index (BMI), neck circumference (NC), abdominal circumference (AC), Epworth sleepiness scale (ESS) were determined. Then all subjects underwent PSG (Compumedics, E serial, PSG 3, Victoria, Australia) with 16 channel sleep test at the sleep laboratory of our hospital. The sleep data obtained from each patient was assessed by the same clinician. Apnea-hypopnea index (AHI), maximum apnea and hypopnea durations, mean apnea and hypopnea durations, lowest O_2 level, oxygen desaturation index were determined.

Blood Collection and Biochemical Analysis

Blood samples were taken from anterior cubital vein after minimum 8 hours of fasting between 09:00 and 10:00 a.m. Blood samples were kept at -4 °C until centrifugation, and after centrifugation plasma was stored at -70 °C until assaying. Ultrasensitive CRP concentration analysis was carried out by using ultrasensitive latex-enhanced immunoassay. Assay of TNF-α, IL-6, isoprostane and RBP parameters were done by quantitative sandwich enzyme immunoassay technique. Leptine levels were analyzed by radioimmunoassay technique and circulating levels of ICAM and VCAM were investigated by enzyme immunoassay technique.

Statistical Analysis

The clinical and PSG parameters, and levels of inflammatory markers were analysed with respect to the severity of OSA. The comparison of four groups was performed by SPSS v.11.5 (SPSS Inc., Chicago, IL, USA). Parametric test assumptions were controlled for choosing the appropriate statistical analysis. Multivariate analysis of variance (MANOVA) test was used for comparing BMI, neck circumference and abdominal circumference variables. One way ANOVA test was used for comparing Epworth Sleepness Scale. Kruskal-Wallis test was used for comparing inflammatory markers and oxidative stress markers. Vascular endothelial markers were analyzed with MANOVA test. Association between the continuous variables was determined by Pearson correlation coefficient. Significance value was considered as $p<0.05$.

Results

There were 48 male and 24 female patients, and mean age was 47.873 (range: 24-75) years. The clinical findings of

Table 1. Characteristics of study population.

	Group I (n=9)	Group II (n=28)	Group III (n=18)	Group IV (n=17)	p value
BMI	27.82±4.46	31.82±4.72	31.00±5.56	30.00±7.01	0.276
Neck circumference	38.11±4.45	40.96±3.69	40.88±3.15	43.75±3.19	0.003*
Abdominal circumference	102.33±19.08	109.53±11.37	107.41±12.10	101.50 ±27.50	0.445
Epworth	13.55±12.36	8.85±5.67	8.50±4.25	12.52±5.03	0.076

*p<0.05 for Group I - Group IV.

the study population are given in Table 1. There was no statistically significant difference between the groups with respect to age, BMI, abdominal circumference and ESS. Neck circumference was significantly different between Group I to Group IV.

Median areas of neochondrogenesis in the control and the mean levels of inflammatory parameters and their correlation to OSA severity are given in Table 2. The mean leptine level seems to be elevated in the second group, the mean isoprostane, mean RBP and mean VCAM levels seem to be elevated in the third group and the mean IL-6 level seems to be elevated in fourth group; but we could not find any statistically significant difference between the groups.

There was a weak correlation between the isoprostane levels and mean apnea duration and also a mild correlation to the maximum apnea duration. A weak correlation was detected between leptine and VCAM levels to age. A weak negative correlation was detected between CRP levels and age. The leptin levels were found to be mildly correlated to BMI and abdominal circumference. The ICAM levels were found to have a weak correlation to BMI and abdominal circumference. We could not find any statistically significant correlation between IL-6 and RBP levels, TNF levels and other OSA related parameters. There was no association between AHI severity and the analyzed parameters.

All data were analyzed and compared simple snoring group (Group 1) and newly diagnosed sleep apnea groups (Group 2 + Group 3 + Group 4). We could not find any statistical significant correlation between the first group and the other newly diagnosed sleep apnea groups.

Discussion

Obstructive sleep apnea is a common and important sleep disorder. However, its ethiopathologic process is not clear yet. OSA has a potential risk of cardiovascular and cerebrovascular morbidity. Many investigators reported that the association between OSA and cardiovascular/cerebrovascular morbidity is independent of obesity. The cessation of airflow during sleep leads to intermittent hypoxia and re-oxygenation. Intermittent hypoxia may lead to oxidative stress and increased reactive oxygen species (ROS).[7] ROS may damage endothelial tissue that is related to atherosclerosis. Atherosclerotic process may play a serious role in acute myocardial infarction and stroke. Long term intermittent hypoxia may be responsible for neuronal changes via pro-inflammatory activation, increased oxidative stress eventually apoptosis and gliosis.[8]

Intermittent hypoxia in OSA resembles ischemia-reperfusion cycles due to decreasing blood oxygen content during apnea/hypopnea. An increase in the production of ROS was reported with ischemia-reperfusion. Depending on these findings, OSA may cause an increase in the ROS levels.[7] An increased production of superoxide from neutrophils and monocytes in OSA patients was also reported.[9] TNF-α has a critical role in inflammatory process,[10] which

Table 2. Mean values of parameters for each study group.

	Group I (n=9)	Group II (n=28)	Group III (n=18)	Group IV (n=17)	p value
Isoprostane	8.82±1.32	9.30±3.84	14.26 +/ 16.32	9.08±3.78	0.216
CRP	4.55±3.08	7.56±7.76	7.40±7.75	6.00±3.61	0.681
IL-6	1.41±0.79	3.07±3.12	3.34±4.47	8.29±24.96	0.323
Leptine	45.62 ±37.01	61.87±38.80	38.45±35.53	43.48±33.59	0.159
RBP	39.65 ±12.88	36.19±14.59	44.15±11.85	37.69±12.46	0.214
I-CAM	29.57±6.66	30.19±8.31	29.87±7.05	31.74±5.95	0.848
V-CAM	18.92±6.28	20.02±5.24	24.54±8.07	21.71±6.40	0.084
TNF	2.61±1.99	2.15±1.96	2.05±1.17	3.04±3.54	0.570

Table 3. Correlations of parameters.

	Isoprostane	CRP	IL-6	Leptine	RBP	I-CAM	V-CAM	TNF
BMI	-0.012	0.034	0.058	0.614†	-0.075	0.232	-0.020	-0.121
AHI	-0.006	0.102	0.023	-0.112	0.010	0.105	0.032	-0.012
Epworth	-0.055	-0.111	-0.195	-0.213	-0.058	0.026	-0.102	-0.064
Neck circumference	0.120	0.128	0.205	-0.019	0.113	0.199	0.048	0.093
Abdominal circumference	0.107	0.090	0.101	0.443†	0.006	0.263*	0.127	-0.056
Apnea-hypopnea index	0.030	-0.001	0.028	-0.077	-0.036	0.170	0.092	-0.005
Mean apnea duration	0.276*	-0.039	0.042	-0.085	0.018	0.063	-0.036	0.060
Maximum apnea duration	0.392†	-0.002	0.026	-0.012	0.056	0.105	-0.068	-0.031
Mean hypopnea duration	0.024	-0.032	0.068	-0.231	-0.016	0.002	0.114	0.178
Maximum hypopnea duration	0.223	0.068	0.079	-0.143	0.143	0.153	0.125	-0.010
Lowest SO_2	0.076	0.039	0.003	0.071	-0.034	-0005	-0.006	0.084
Mean desaturation	-0.082	0.048	-0.012	0.156	-0.069	-0.012	-0.071	-0.058
Age	-0.023	-0.249*	-0.017	0.245*	-0.141	0.092	0.273*	-0.044

*Correlation is significant at the 0.05 level, †Correlation is significant at the 0.01 level.

regulates the inflammatory gene expression.[11] Eventually TNF-α induces the other inflammatory mediators such as IL-6, CRP. Many clinical studies support the correlation of CRP levels with atherosclerosis and stroke.[12,13] Furthermore, reduction of CRP levels after an adequate continuous positive airway pressure (CPAP) therapy has been reported by Steiropoulos et al.[14] This result also supports the association between OSA and inflammation.

Oxidative stress in OSA may help to explain cardiovascular/neurovascular complications. A hypoxia and re-oxygenation cycle can induce the oxidative stress which leads to increased generation of ROS followed by damage to the endothelial tissues. Isoprostanes are produced by ROS induced peroxidation from arachidonic acid, and 8-isoprostane is one of the earliest oxidative stress markers.[15] Its blood levels are found to be elevated with OSA and decreased after the CPAP treatment.[16]

Current data demonstrates that adipose tissue acts as an active endocrine organ and releases many bioactive mediators which modulate blood pressure, lipid metabolism, atherosclerosis and inflammation.[17] Leptin is the one of adipocyte derived proteins which is induced by ob gene. Leptin level is related with body fat content. Recent data has shown that, leptin is an independent risk factor for cardiovascular diseases.[18] Leptin levels were found to be elevated in OSA[19] and decreased with CPAP therapy.[20] Endothelial tissue has the leptin receptors; it may play a role in atherosclerosis pathogenesis.

Retinol-binding protein is an adipokine, which is related to insulin resistance.[21] RBP modulates the insulin sensitivity in peripheral tissue. Harsch et al. reported the association of OSA and RBP.[22] They found increased RBP levels in moderate/severe OSA patients than those of the control groups; but no correlation between RBP and apnea related parameters. Interestingly, reduction of serum RBP levels with CPAP therapy was reported by Evagelia et al.[23] Diabetes mellitus (DM) and related complications in OSA may explain this correlation.

Cardiovascular complications may be related with endothelial dysfunctions. Endothelial dysfunction is described with imbalance of inflammatory mediators, vascular smooth muscle proliferation, hypercoagulobility, thrombosis and eventually adverse cardiovascular events.[4] Impaired endothelial functions were reported.[4] The potential mechanism depends on hypoxemia due to ROS generation and systemic inflammation. The association of obesity, hypertension, and hyperlipidemia with OSA was reported. These co-morbid diseases may contribute to adverse effects on endothelium. The circulating levels of ICAM and VCAM were reported as higher in OSA patients than those of the control groups.[24] Adhesion molecules have an important role in atherosclerosis.[25]

The limitations of our study include the small number of subjects and non-homogen patient groups. Further investigation should include larger subject groups so that association of co-morbid diseases and apnea related parameters may be deeply analyzed. The increased risk of co-morbid diseases can be predicted depending on such findings.

Conclusion

We have evaluated the inflammatory markers, endothelial dysfunction markers and oxidative stress markers in OSA. Current data support the association of cardiovascular/cerebrovascular complications and OSA. The strongest patho-

physiologic process depends on oxidative stress and chronic inflammatory response. Determining all the key steps of inflammatory response and endothelial dysfunction may be helpful in sleep apnea treatment. Our results indicate an association between oxidative stress and apnea duration during sleep. Further studies in larger series may explain all the pathophysiologic processes of co-morbid diseases in OSA.

Conflict of Interest: No conflicts declared.

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