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Potential Aspirin Resistance in Patients with Obstructive Sleep Apnea Syndrome

Obstrüktif Uyku Apne Sendromlu Hastalarda Olası Aspirin Direnci

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Abstract

Objective: Obstructive sleep apnea syndrome (OSAS) is a common disorder among the public and it is associated with many cardio-cerebrovascular consequences. Aspirin is used as the first line of treatment regarding such complications; however, >20% of the patients have drug resistance which causes ongoing vulnerability to vascular accidents. The objective of this study is the evaluation of potential aspirin resistance in drug naïve severe OSAS patients.

Materials and Methods: Thirty patients with a diagnosis of severe OSAS and 30 age-matched non-OSAS controls enrolled in the study. Urine samples of both patients and controls were analyzed for 11-dehydrothromboxane B2 (11-DHTBXB2) levels and compared afterward. Pairwise comparisons were performed and a two-tailed p-value of <0.05 was considered statistically significant.

Results: Biochemical analysis of the urine samples revealed an 11-DHTBXB2 level of 26.7 pg/dL for the patient group, whereas the relative value was 12.7 pg/dL for the control group (p=0.005). The significant increase in urinary 11-DHTBXB2 levels among patients was independent of sex, age, and body mass index.

Conclusion: Patients with OSAS had higher levels of urine 11-DHTBXB2 levels, hence higher aspirin resistance than controls. Identification of aspirin resistance and prediction of potential unresponsiveness to first-line antiplatelet therapy is essential in OSAS to guide the primary or secondary prophylaxis in this population who have a marked propensity to vascular insult.

Keywords: Obstructive sleep apnea syndrome, OSAS, aspirin, acetilsalyciclic acid, resistance, aspirin unresponsiveness, cardiovascular morbidity

Öz

Amaç: Obstrüktif uyku apne sendromu (OSAS) toplumda oldukça yaygın görülmektedir ve istenmeyen pek çok kardiyo-serebrovasküler sonlanımla ilişkisi iyi tanımlanmıştır. Aspirin, tanımlanan komplikasyonların pek çoğunda primer ya da sekonder profilaksi olarak önerilmektedir ancak hastaların %20'sinden fazlasında yeterli tedavi uyumuna rağmen aspirin direnci ile karşılaşılmaktadır ve bu durum süregelen vasküler riski beraberinde getirmektedir. Bu çalışmada amaç, aspirin kullanımı olmayan ağır OSAS olgularında olası aspirin direncini araştırmaktır.

Gereç ve Yöntem: Ağır OSAS tanılı 30 hasta ve yaşça eşlenmiş 30 kontrol çalışmaya dahil edildi. Hastalar ve kontrollerin sabah ilk idrar numuneleri 11-dehidrotromboksan B2 düzeyleri (11-DHTBXB2) çalışılmak üzere toplandı. Biyokimyasal analiz sonrası iki grubun idrar 11-DHTBXB2 düzeyleri karşılaştırıldı. P<0,05 istatistiksel olarak anlamlı kabul edildi.

Bulgular: OSAS grubunda ortalama idrar 11-DHTBXB2 düzeyi 26,7 pg/dL iken, kontrol grubunda bu değer 12,7 pg/dL saptandı (p=0,005). Hastalarda üriner 11-DHTBXB2 atılımı, dolayısı ile aspirin direnci kontrollere göre anlamlı ölçüde yüksekti ve bu fark cinsiyet, yaş ve vücut-kitle indeksi gibi değişkenlerden bağımsızdı.

Sonuç: Bulgularımız, OSAS hastalarında üriner 11-DHTBXB2 atılımı ve dolayısı ile aspirin direncinin kontrollere göre daha yüksek olduğunu göstermektedir. OSAS hastalarında aspirin direncini; başka bir deyişle primer veya sekonder profilakside tercih edilen ilk sıra antiagregan ilaca karşı potansiyel yanıtsızlığı öngörmek önemlidir. Zira bu öngörü, halihazırda yüksek vasküler risk taşıyan bu grup hastalarda tedavi planını şekillendirebilir.

Anahtar Kelimeler: Obstrüktif uyku apne sendromu, OSAS, aspirin, asetilsalisilik asit, direnç, aspirin duyarsızlığı, kardiyovasküler morbidite

Introduction

Obstructive sleep apnea syndrome (OSAS) is a common disorder among the public, of which the prevalence approximates 2-4% and it is associated with many undesirable cardiometabolic and vascular consequences, including hypertension, coronary artery disease, and cerebrovascular accidents (1,2). Based on the 2013 updated Wisconsin Sleep Cohort longitudinal study, 34% of men and 17% of women aged 30-70 have at least mild OSA [i.e., at least apnea hypopnea index (AHI) 5/h of sleep], and estimations for moderate to severe OSA (AHI of 15/h) are 13% for men and 6% for women (3). Aspirin (acetilsalyciclic acid) has been demonstrated to be effective in both primary and secondary prophylaxis of such vascular complications

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(4,5). It binds irreversibly to Cytochrome-c oxidase I enzyme within the platelets and inhibits the critical step, which converts arachidonic acid to a highly prothrombotic compound called thromboxane A2 (6). In other words, it provides an antithrombotic effect by inhibiting arachidonic acid-mediated platelet aggregation. However, drug resistance could be present in up to 28% of users (6,7). Documentation of the presence and frequency of aspirin resistance in patients with OSAS is critical since the failure of antiaggregant prophylaxis among this population could result in the preservation of already existing vascular risk. Regarding the clinical context, aspirin resistance attributes to the occurrence of thromboembolic events despite appropriate treatment adherence, and this resistance could be demonstrated in several ways. Common methods include bleeding time, active coagulation time, optic aggregometry, whole blood aggregometry, flow cytometry, platelet aggregation rate, platelet surface proteins, and blood & urine thromboxane A2 levels (8). Measurement of urinary 11-dehydrothromboxane B2 (11-DHTBXB2) level -a stable metabolite of serum thromboxane A2- is a relatively simple and non-invasive method for demonstration of aspirin resistance and provides valuable information in drug naïve individuals either (6). In this study, we aimed to investigate aspirin resistance in drug-naïve severe patients with OSAS using urine analysis of 11-DHTBXB2 and discuss the potential link between resistance and the disease.

Materials and Methods

Study Population

Thirty patients were diagnosed with severe OSAS following overnight diagnostic polysomnography (PSG) in the Sleep Disorders Clinic of the Neurology Department in Aydın Adnan Menderes University Training and Research Hospital, and 30 age-matched controls participated in this study. Patients between 8-80 years of age, whose diagnosis of severe OSAS was confirmed by all-night diagnostic PSG and who did not use aspirin and non-steroidal anti-inflammatory drugs, who had no history of smoking, diabetes mellitus, hypertension, renal failure and who did not experience any surgery, ischemic events or infection within the two weeks before evaluation were included. Thirty separate subjects who matched the inclusion criteria, excluding the presence of OSAS-related symptoms (snoring, excessive daytime sleepiness, and witnessed apnea), did serve as the control group (non-OSAS). All participants were subjected to a structured interview regarding the presence of any sleep disorders and a complete neurological examination was performed for each. An assessment of routine hemogram and serum biochemistry studies, as well as consultations with pulmonary and ear-nose-throat departments, were completed for all of the patients. The Epworth Sleepiness Scale (ESS) scoring test, which has an appropriate validation of the Turkish version in terms of specificity and sensitivity (9), was applied to both patient and control groups. Informed consent was taken from all of the participants and the study protocol regarding this cross-sectional research was approved by the Ethics Committee of Adnan Menderes University (protocol no: 2023/89, date: 04.05.2023).

Polysomnography

All patients underwent all-night diagnostic PSG using [SOMNOscreen EEG10-20 (Somnomedics GmbH - Randersacker, Germany)], which recorded six channel electroencephalogram, electrooculogram, electrocardiogram, chin and leg electromyogram; together with nasal airflow and oronasal thermal flow, thoracic and abdominal respiratory effort, pulse oximetry and body position. The polysomnographic data were scored manually according to American Academy of Sleep Medicine guidelines by the same neurologist who has a national board certificate regarding sleep medicine. Patients with an AHI of ≥30/hour were classified as severe OSAS (10).

Laboratory Analysis

The urine samples were collected from the patients on the morning following diagnostic PSG and preserved at -80 °C until biochemical analysis. The control group also provided the first urine samples of the morning. The samples were dissolved and diluted in a 1:4 ratio just before the analysis and all were analyzed using a commercial kit [Cayman Chemical (Ann Arbor, Miami, USA) - catalog number: 519501- lot number: 206839]. The measurements were performed according to the standards of the kit manual. The results were multiplied by the dilution factor and given in pg/mL.

Statistical Analysis

IBM SPSS 16.0 (Statistical Package for Social Sciences) package program was used for statistical analysis. The normality of data was assessed by the Kolmogorov-Smirnov test. Regarding pairwise comparisons, independent samples t-test was used for normally distributed variables, and the Mann-Whitney U test was used for not normally distributed variables. Bivariate correlations were evaluated using Pearson or Spearman test according to the normality of data. The results were given in a 95% confidence interval, and a two-tailed p-value of <0.05 was considered statistically significant.

Results

Sixty participants, including 30 severe patients with OSAS and 30 controls, were evaluated in this study. Gender distribution [19 (63%) male vs 11 (37%) female for the patient group, and 15 (50%) male vs 15 (50%) female for the control group, p=0.29] and mean age were similar between the two groups (51±22 years vs 54±24 years for patients and controls, respectively, p=0.42). However, mean body mass index (BMI) (34.8±10.8 kg/m^2 for patients vs 29.3±7.6 kg/m^2 for controls, $p<0.001$) and average ESS scores (12±8 in the patient group vs 2±2 in the control group, $p<0.001$) were significantly higher in the patient group. Biochemical analysis of the urine samples revealed that the patient group had an 11-DHTBXB2 level of 26.7 pg/dL, whereas the relative value was 12.7 pg/dL for the control group (p=0.005) (Figure 1). There was a significant increase in terms of urine 11-DHTBXB2 levels among patients when compared to controls and this association was independent of sex, age, and

BMI. Further analysis regarding the patient group demonstrated no significant correlation between urine 11-DHTBXB2 levels and BMI, ESS, AHI, durations of apnea and hypopnea on PSG, and hypoxia indices, including oxygen desaturation index (ODI), time spent <90% spO_2, minimum spO_2, average spO_2 (Table 1). There was a moderate but significant correlation between ESS and ODI (p=0.02, rho=0.424).

Discussion

In the current study, our results revealed that morning urine samples of patients with OSAS contain significantly elevated levels of 11-DHTBXB2 -a reliable marker of aspirin resistance- when compared to age-matched non-OSAS controls. This indicates higher ASA resistance among patients with OSAS concerning non-OSAS subjects. Concerning the accumulating data from different patient groups, increased baseline platelet reactivity plays a vital role in developing aspirin resistance (11,12). OSAS, the severe form, in particular, is known to be associated with raised platelet distribution width and mean platelet volume values which indicate more active platelets in terms of metabolic and enzymatic processes (13,14). Larger platelets contain more granules, have elevated thromboxane A2 levels, express more glycoprotein IIb/IIIa receptors, and aggregate promptly with collagen (15,16). There is also a well-identified correlation between these two indicators of baseline platelet reactivity and AHI, as well as other breathing parameters in PSG. In addition to excessive sympathetic activity and chronic inflammation, hypoxia acts as the major contributor to this process here (13). It disrupts thrombocyte function, resulting in acquired prothrombotic features due to hypoxia/reoxygenation course that was already demonstrated in postischemic cerebral microcirculation (17). A similar case occurs in the context of OSAS and recurrent hypoxia throughout the night seems to exaggerate the activation and aggregation of thrombocytes (18). Impaired coagulation and thrombocyte aggregation give rise to a large extent to cardio-cerebrovascular morbidity/mortality in OSAS, and aspirin is currently the first-line treatment regarding primary or secondary prevention of such vascular events (18,4,5). Unfortunately, >20% of aspirin users experience recurrent cardio-cerebrovascular accidents, which likely occur due to drug resistance (19,7). The resistance could be investigated through several methods as discussed above; however, measurement of urinary 11-DHTBXB2 -as a stable metabolite of serum thromboxane A2 - has some advantages due to its non-invasive nature and easier applicability (6). In 2002, Bruno et al. (20) introduced a significant decrease in urine 11-DHTBXB2 levels in response to aspirin use among 71 stroke patients when compared to non-users. In the same year, Eikelboom et al. (19) identified the predictive role of elevated urine 11-DHTBXB2 levels in terms of adverse cardiovascular outcomes in a large population of aspirin users retrieved from the Heart Outcomes Prevention Evaluation study. The authors described 2 and 3.5 times increased risk of myocardial infarction and cardiovascular death, respectively, among patients in the upper quartile in comparison to the ones in the lower quartile (19).

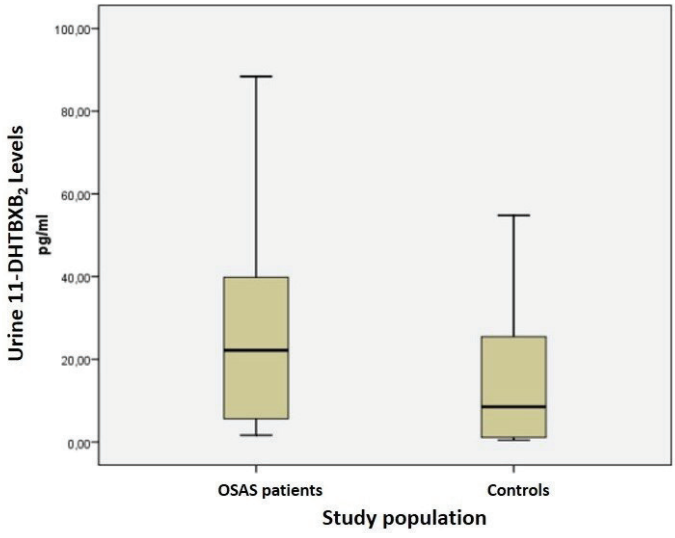

Figure 1. Urinary levels of 11-dehydrothromboxane B2 (11-DHTBXB2) in patients and controls
OSAS: Obstructive sleep apnea syndrome

This finding of higher risk regarding undesirable cardiovascular outcomes due to aspirin resistance has been confirmed in more recent studies also. For example, Pasala et al. (21) reported a 32.3% rate of cardio-cerebrovascular morbidity/mortality among their 31 aspirin-resistant patients with peripheral artery disease, whereas the relative rate was only 14.6% in the aspirin-responsive group (n=89). Among 275 patients taking aspirin with a diagnosis of acute ischemic stroke, Wang et al. (22) demonstrated that the patients in the third and fourth quartiles of the aspirin reaction unit had 145% and 317% increased adjusted risk of the unfavorable outcome as to the ones in

Table 1. Bivariate correlations between urinary 11-dehydrothromboxane B2 (11-DHTBXB2) levels and covariates

		BMI	ESS	AHI	Ap. dur.	Hyp. dur.	Avg. AH dur.	Tot. AH dur.	ODI	Dur. spO_2 <90%	Min. O_2	Avg. O_2	Mean desat.
Urine DHTB-2	rho	0.162	0.007	0.079	0.293	0.222	0.206	0.186	0.015	0.275	-0.200	-0.075	-0.218
	p	0.394	0.970	0.678	0.117	0.239	0.275	0.324	0.938	0.141	0.289	0.695	0.247
	n	30	30	30	30	30	30	30	30	30	30	30	30

Urine DHTB-2: Urinary 11-dehydrothromboxane B2, BMI: Body mass index, ESS: Epworth Sleepiness Scale, AHI: Apnea hypopnea index, Ap. dur.: Average apnea duration, Hyp. dur: Average hypopnea duration, Avg. AH dur: Average apnea hypopnea duration, Tot. AH dur: Total apnea hypopnea duration, ODI: Oxygen desaturation index, Dur. spO_2<90%: Mean duration of desaturation (time spent <90% spO_2), Min. O_2: Minimum spO_2, Avg. O_2: Average spO_2, Mean desat.: Mean desaturation, rho: Correlation coefficient, p<0.05 denotes statistical significance

the first quartile. Khan et al. (23) detected significantly higher rates of major cardiovascular and adverse limb events (hazard ratio 3.68) among aspirin-resistant patients in comparison to the aspirin-sensitive ones, in a cohort of 150 individuals with a diagnosis of peripheral arterial disease or carotid artery stenosis. Despite an indisputable association between aspirin resistance and cardio-cerebrovascular consequences, and the existence of plenty of studies about this issue, data from patients with OSAS are limited and somewhat contradictory. In 2018, Gong et al. (24) described that acute coronary syndrome patients with concomitant OSAS had a significantly lower inhibitory rate of the adenosine diphosphate receptor pathway compared to those without OSAS. More recently, Scinico et al. (25) reported a 17% prevalence of aspirin resistance verified by optical detection of residual platelet aggregation among 59 patients with OSAS on aspirin therapy. In the second phase of that study, which analyzed continuous positive airway pressure (CPAP) response in 18 aspirin naïve subjects regarding endothelial function and aspirin resistance, the authors observed a significant recovery in endothelial function following CPAP therapy; however, the trend towards improvement in aspirin responsiveness could not reach statistical significance (25).

Conclusion

In the current study, we aimed to disclose the potential '*de novo* aspirin resistance in drug naïve severe OSAS patients. Urinary 11-DHTBXB2 analysis was used to determine aspirin resistance since it is known to be a stable metabolite of serum thromboxane A2. Drug naïve individuals were given priority upon patient selection to exclude potential resistance mechanisms which could develop under continuous treatment and act as confounders during the interpretation of the results. To our knowledge, it is the first study in the literature evaluating *de novo* aspirin resistance among drug naïve severe patients with OSAS with this method. The results revealed that patients with OSAS have higher levels of urine 11-DHTBXB2 levels. Thus, higher aspirin resistance than age-matched controls and this association is independent of potential confounders, such as age, gender, and BMI. Identification of aspirin resistance, as well as prediction of potential unresponsiveness to first-line antiplatelet prophylaxis in OSAS, seems essential since these patients are highly vulnerable to vascular consequences, and the choice of alternative prophylaxis in such cases could prevent further treatment failure.

Ethics

Ethics Committee Approval: The study was approved by Ethics Committee of Adnan Menderes University (protocol no: 2023/89, date: 04.05.2023).
Informed Consent: Was taken from all of the participants and the study protocol regarding this cross-sectional research.
Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: U.O.A., A.A., Design: U.O.A., Data Collection or Processing: U.O.A., Analysis or Interpretation: U.O.A., Literature Search: U.O.A., Writing: U.O.A.

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