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Research Article

The Relationship between Disease Diagnosis, Severity and Serum ADMA, SDMA and L-NMMA in Patients with Obsessive Sleep Apnoea Syndrome

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ABSTRACT

Background: Recurrent hypoxaemia and reoxygenation in patients with Obstructive Sleep Apnea Syndrome (OSAS) may trigger oxidative stress mechanisms. Oxidative stress alters the activities of enzymes involved in the production of Asymmetric Dimethylarginine (ADMA), Symmetric Dimethylarginine (SDMA) and N-Monomethyl L-arginine (L-NMMA), leading to changes in their amounts.

In previous studies, it was observed that intermittent hypoxia and hypoxia-re-oxygenation events increased Nitric Oxide (NO) synthesis by increasing endothelial Nitric Oxide Synthase (eNOS) synthase activity in OSAS patients. In our study, we examined the balance between L-arginine-nitric oxide and L-arginine-methylated arginine metabolites (ADMA, SDMA and L-NMMA) pathways due to intermittent hypoxia in newly diagnosed OSAS patients who have not yet developed OSAS-related complications and which pathways are activated. At the end of our study, we aimed to examine the effect of increased metabolites in predicting complications that may develop in the future due to OSAS.

Materials and methods: Male and female patients aged 18 years and older who voluntarily agreed to participate in the study were included in the study. Patients with an apnea-hypopnea index of 5 and above were included in the study. Patients with central apnea and laboratory abnormalities (Hg, AST, ALT, HbA1c, bilirubin, electrolytes, urea, homocysteine, folic acid and thyroid function tests) were excluded. Detailed anamnesis was obtained from the patients and those with comorbidities that would affect the study; cardiovascular system diseases (essential hypertension, hypercholesterolemia, hyperhomocysteinaemia, acute coronary events, congestive heart failure), diabetes, hyperthyroidism, chronic renal failure, insulin resistance and metabolic syndrome, low serum folic acid and high homocysteine levels were excluded from the study. Blood samples were obtained from the other patients with their informed consent for ADMA, SDMA, L-NMMA, arginine, complete blood count, renal function tests, lipid panel, thyroid function tests, fasting blood glucose, HbA1c, folic acid and homocysteine controls.

The study included 121 patients including 31 healthy and newly diagnosed mild ouas 30, moderate ouas 30 and severe ouas 31. The demographic characteristics, laboratory findings and clinical indices of the study participants according to the study groups were compared by One-Way Analysis of Variance, Welch F test or Kruskal Wallis tests. Tukey HSD, Games-Howell and Bonferroni corrected Dunn's tests were used for multiple comparisons for the parameters found to be significant as a result of these tests, respectively. The level of significance was taken as 5% in the evaluation of statistical hypotheses.

Results: As a result of our study, we found that arginine metabolites (ADMA and L-NMMA) levels were lower in patients with OSAS compared to the healthy group. SDMA values of healthy controls and OSAS patient groups were similar. When the laboratory findings of the study groups were analysed, TMAL value was significantly lower in OSAS patients compared to healthy controls.

Conclusion: The patients we included in the study were newly diagnosed with the polysomnography results and had not developed any complications related to the disease because of the early stage of the disease. Therefore, we did not observe an increase in arginine metabolites (ADMA and L-NMMA) due to the fact that the L-arginine pathway was registered to nitric oxide synthesis for compensation and therefore the methylated arginine pathway was not yet activated. There is a need for further controlled, prospective, prospective studies including NO measurement and nitric oxide synthase enzyme activities in terms of monitoring treatment efficacy, monitoring of complications and monitoring of disease progression in patients with OSAS.

Keywords: Disease diagnosis; Nitric oxide; Patients; Healthy controls; Intermittent hypoxia

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ABBREVIATIONS

ADMA: Asymmetric Dimethylarginine; SDMA: Symmetric Dimethylarginine; L-NMMA: N-Monomethyl L-Arginine; AHI: Apnoea-Hypopnoea Index; CPAP: Continuous Positive Airway Pressure; cGMP: Cyclic Guanazine Monophosphate; DDAH: Dimethylarginine Dimethylaminohydrolase; EDTA: Ethylene Diamine Tetra Acetic Acid; NADPH: Nicotinamide Adenine Dinucleotide Phosphate Oxidase; NOS: Nitric Oxide Synthase; NO: Nitric Oxide; OSAS: Obstructive Sleep Apnoea Syndrome; ODI: Oxygen Desaturation Index; EMG: Electromyography; EEG: Electroencephalography; ECG: Electrocardiography; EOG: Electrooculography; BMI: Body Mass Index; CAT: Catalase; EPO: Erythropoietin; TLC: Total Lung Capacity; CPAP: Continuous Positive Airway Pressure; MSLT: Multiple Sleep Latency Test; MWT: Maintenance of Wakefulness Test; APOE4: Apolipoprotein E4; TMAL: Total Methyl Arginine Load

INTRODUCTION

On average, we spend one third of the day asleep. OSAS, which occurs during this period in which we spend one third of our lives, is an important health problem that is examined in respiratory sleep disorders and concerns many systems in the body. OSAS is characterized by recurrent obstruction of the upper airway during sleep. It is the most common cause of insomnia in adults.

Today, the general prevalence of the disease is estimated to be around 5%. In the adult population, half of men and 30 per cent of women snore and 3%-5% of these snorers have the disease. The gold standard in the diagnosis of sleep apnea syndrome is polysomnography. Polysomnography (PSG) is a diagnostic method based on the simultaneous recording, analysis and interpretation of many physiological parameters during sleep during the night.

The most common nocturnal symptom is snoring and the most common daytime symptom is excessive sleepiness. Other symptoms reported in OSAS are shortness of breath at night, waking up tired in the morning, restless sleep, headache in the morning. Nocturia, enuresis, decreased libido and gastroesophageal reflux symptoms are less frequently reported complaints. OSAS is a source of serious morbidity and mortality with its social and neuropsychological consequences as well as cardiovascular consequences.

Repetitive hypoxaemia and re-oxygenation in patients with OSAS may trigger oxidative stress mechanisms. Oxidative stress alters the activities of enzymes involved in the production of ADMA, SDMA and L-NMMA leading to changes in their amounts.

In previous studies, it has been observed that intermittent hypoxia and hypoxia re-oxygenation events increase NO synthesis by increasing eNOS synthase activity in OSAS patients. In our study, we examined the balance between *L*-arginine-nitric oxide and *L*-arginine-methylated arginine metabolites (ADMA, SDMA and *L*-NMMA) pathways due to intermittent hypoxia in newly diagnosed OSAS patients who have not yet developed complications related to OSAS, which metabolites increase and which decrease. At the end of our study, we aimed to examine the effect of increased or decreased metabolites on predicting complications that may develop in the future due to OSAS.

Laboratory assessment

L-arginine forms methylated arginine metabolites (ADMA, SDMA and L-NMMA) by using S-adenosine methionine as a methyl donor through the Protein Arginine Methyl Transferase enzyme (PRMT). Among these metabolites, ADMA and L-NMMA have an inhibitory effect on nitric oxide synthase. SDMA has not been shown to have any effect on nitric oxide synthase [1,2].

NO has a vasodilator effect on many systems, especially the cardiovascular system [3]. *L*-arginine is formed *via* the PRMT using S-adenosine methionine as a methyl donor to form methylated arginine metabolites (ADMA, SDMA and L-NMMA) [2].

There are two types of PRMT enzymes. Type 1 is responsible for the synthesis of ADMA and L-NMMA and Type 2 SDMA [4]. ADMA and L-NMMA have inhibitory effects on nitric oxide synthase. SDMA has no effect on nitric oxide synthase [5]. ADMA and L-NMMA cause dysfunction in many organs (cardiovascular damage, atherosclerosis, diabetes, chronic renal failure, hypertension, etc.) by suppressing NO synthesis. [5-8]. Arginine metabolites are also thought to cause cell damage by directly increasing free oxygen radicals [9,10].

NO is produced from *L*-arginine (*L*-arg) and oxygen (O_2) in a reaction catalysed by Nitric Oxide Synthase (NOS). Three isoforms of NOS have been identified in humans: neuronal (nNOS), which is localised mainly in nervous system cells, inducible NOS (iNOS), whose expression is induced in various cell types by pro-inflammatory cytokines and endothelial NOS (eNOS), which is expressed almost exclusively from endothelial cells [11].

The major fraction of plasmatic and cellular arginine in adult humans comes from the physiological whole body protein cycle [12]. Due to widespread post-traclational modification, *L*-arg residues within proteins can be methylated by a family of enzymes termed protein arginine methyl transferases. Subsequent degradation of such proteins results in the release of free methylated arginine derivatives: L-NMMA, ADMA and SDMA [13,14]. ADMA and SDMA interfere with the cellular uptake of *L*-arginine by the y^+ transporter and thus potentially reduce *L*-arginine uptake [15,16].

In general, hypoxia can be divided into acute or chronic according to its duration or persistent or intermittent according to its nature. For example, chronic lung diseases result in persistent hypoxia, whereas Obstructive Sleep Apnoea (OSA) is associated with intermittent hypoxia consisting of cycles of hypoxia and re-oxygenation [17].

The hypoxic response differs slightly depending on the nature of hypoxia in cellular models of intermittent hypoxia, an enhanced proangiogenic and pro-inflammatory phenotype was observed [18]. Furthermore, severe hypoxia followed by re-oxygenation can cause ischaemia-reperfusion injury, a phenomenon of cellular damage observed after myocardial ischaemia, stroke or organ transplantation resulting from increased Reactive Oxygen Species (ROS) generation [19]. OSAS-associated intermittent hypoxia may also lead to ischaemia-reperfusion injury, which is recognised as an important contributor to the pathogenesis of OSAS comorbidities through increased ROS production [20,21].

In renal failure, all arginine metabolites, especially SDMA, are increased due to decreased clearance and endothelial insufficiency [22]. With the increase in ADMA level, cardiovascular risk increases in patients with renal failure, which is the most important cause of mortality [4,23]. Endothelial damage in the early stages of atherosclerosis increases ADMA and L-NMMA levels and elevated levels of these metabolites also indicate an increased risk of coronary artery disease [24]. Arginine metabolites are also elevated in Behçet's Disease, another disease characterized by endothelial damage [25,26].

Hyperinsulinemia or hyperlipidaemia increases ADMA levels by altering the activity of enzymes responsible for ADMA production or degradation. It has been shown that hyperglycaemia causes an increase in ADMA levels by decreasing DDAH levels which catalyses ADMA degradation [27]. Arginine metabolites are increased in cardiovascular diseases, peripheral vascular disease, lipid metabolism disorder, homocysteine elevation, renal failure, cerebrovascular events [28,29].

In psychiatric and neurological diseases, decreased NO level with the effect of increased serum ADMA level is thought to be the cause of loss of cognitive function [30]. Oxidative stress also decreases the activity of dimethyl arginine dimethylamine hydrolase enzyme, which is responsible for the hydrolysis of arginine metabolites, leading to an increase in its amount in serum [31]. The shift of production in *L*-arginine metabolism to the ADMA, SDMA and *L*-NMMA pathways decreases NO synthesis and causes a decrease in *L*-arginine levels. Nitric oxide synthesis increases with external administration of *L*-arginine and the negative effect of arginine metabolites decreases [32].

Our aim in this study was to investigate the effect of oxidative stress on methylated arginine metabolites (ADMA, SDMA and L-NMMA) in chronic hypoxic diseases. We compared the serum levels of methylated arginine metabolites in OSAS, the main mechanism of which is intermittent hypoxia, between the healthy group and patients diagnosed with OSAS by dividing the patients diagnosed with OSAS into groups according to disease severity (mild-moderate-severe).

MATERIALS AND METHODS

This study is derived from the thesis and was approved by the decision of Selçuk University Faculty of Medicine Clinical Research Local Ethics Committee dated 04.01.2022 and numbered 2022/20. This study is a case-cohort study. Patients participating in the study were informed about the study and signed informed consent forms. The study was conducted between January, 2022 and January, 2023.

In this study, polysomnography results of patients admitted to the Sleep Clinic of the Chest Diseases Clinic of Selçuk University Faculty of Medicine were analysed. Apnea was defined as apnea when 90% of the airflow was interrupted for at least 10 sec and hypopnea was defined as apnea when 50% of the airflow was interrupted and accompanied by 3% saturation decrease or 30% interrupted and accompanied by 4% saturation decrease. Male and female patients aged 18 years and older who voluntarily agreed to participate in the study were included in the study. Patients with an apnea-hypopnea index of 5 and above were included in the study.

Patients with central apnea and laboratory abnormalities (Hg, AST, ALT, HbA1c, bilirubin, electrolytes, urea, homocysteine, folic acid and thyroid function tests) were excluded. A total of 121 patients including 31 healthy, 30 with mild ouas, 30 with moderate ouas and 31 with severe ouas were included in the study.

Detailed anamnesis was obtained from the patients and those with comorbidities that would affect the study; cardiovascular system diseases (essential hypertension, hypercholesterolemia, hyperhomocysteinaemia, acute coronary events and congestive heart failure), diabetes, hyperthyroidism, chronic renal failure, insulin resistance and metabolic syndrome, low serum folic acid and high homocysteine levels were excluded from the study. Blood samples were obtained from the other patients with their informed consent for ADMA, SDMA, L-NMMA, arginine, complete blood count, renal function tests, lipid panel, thyroid function tests, fasting blood glucose, HbA1c, folic acid and homocysteine controls.

Statistical analysis and evaluation of sample size

All statistical analyses were performed using R version 3.6.0 (The R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org). Before the analyses, normality of the data was checked by Shapiro-Wilk normality test and Q-Q graphs and homogeneity of group variances was checked by Levene's test. Findings related to numerical variables were presented as mean ± standard deviation or median (quartiles) and findings related to categorical variables were presented as frequency (n). The demographic characteristics, laboratory findings and clinical indices of the study participants according to the study groups were compared by one-way analysis of variance, Welch F test or Kruskal Wallis tests. Tukey HSD, Games-Howell and Bonferroni corrected Dunn's tests were used for multiple comparisons for the parameters found to be significant as a result of these tests, respectively. In addition, since the age distribution differed between the study groups, the age variable was controlled (due to its possible confounding effect) and the comparison of laboratory findings and clinical indices between the groups was re-examined by analysis of covariance and generalized linear model. A significance level of 5% was taken in the evaluation of statistical hypotheses.

RESULTS

A total of 122 individuals aged between 19 and 77 years (43.34 ± 10.28), 86 males (70.5%) and 36 females (29.5%) were included in the study. Of these 122 participants, 31 were healthy controls (25.4%) and the remaining 91 OSAS patients were clinically classified as mild OSAS (n=30, 24.6%), moderate OSAS (n=30, 24.6%) and severe OSAS (n=31, 25.4%).

Demographic characteristics, laboratory findings and clinical indices of the participants according to the study groups are given in Tables 1 and 2. The mean age of patients with severe OSAS was significantly higher than healthy controls and mild OSAS patients, but similar to moderate OSAS patients. In addition, the mean age of moderate OSAS patients was also higher than healthy controls but similar to the mild OSAS group. On the other hand, the mean ages of the mild OSAS group and healthy controls were similar. The gender distributions of healthy controls and OSAS patient groups were similar (p=.169).

When the laboratory findings of the study groups were analysed, ADMA values were significantly lower in mild (0.19 ± 0.09), moderate (0.18 ± 0.05) and severe (0.21 ± 0.04) OSAS patients compared to healthy controls (0.31 ± 0.05). The results were similar when adjusted for age.

SDMA values of healthy controls and OSAS patient groups were similar and this similarity was also valid when analysed with adjustment for age.

LNMMA values in mild and moderate OSAS patients were significantly lower than those in severe OSAS patients and healthy controls and LNMMA values in severe OSAS patients were significantly lower than those in healthy controls. The results of the analyses performed by controlling the age of the groups were similar.

Arginine values were significantly lower in mild and severe OSAS patients than in healthy controls and moderate OSAS patients. Age-adjusted results were similar.

The arginine/ADMA ratio was significantly higher in moderate OSAS patients than in healthy controls and severe OSAS patients and similar in mild OSAS patients.

Total Methyl Arginine Load (TMAL) value was significantly lower in mild, moderate and severe OSAS patients compared to healthy controls.

When the differences of the study groups in terms of clinical indices were analyzed;

It was found that the mean O_2 level was lower in OSAS patients than in healthy controls and according to the severity of OSAS disease, the mean O_2 level in moderate OSAS patients was similar to that in mild and severe OSAS patients. The lowest mean O_2 level was found in the severe OSAS patient group.

It was found that the lowest O_2 level decreased as the severity of OSAS disease increased and the lowest O_2 level was lower in OSAS patients than in healthy controls.

It was found that DESAT (Desaturation) index increased as the severity of OSAS disease increased and DESAT index was higher in OSAS patients than in healthy controls.

When the laboratory findings of the study groups were analysed, the ADMA value was significantly lower in OSAS patients (0.19 ± 0.06) compared to healthy controls (0.31 ± 0.05). The results were similar when corrected for age.

When the laboratory findings of the study groups were analysed, the L-NMMA value was significantly lower in OSAS patients (0.013 ± 0.006) compared to healthy controls (0.024 ± 0.005). The results were similar when corrected for age.

When the laboratory findings of the study groups were analysed, the TMAL value was significantly lower in OSAS patients (0.49 ± 0.10) compared to healthy controls (0.62 ± 0.09). The results were similar when corrected for age (Tables 1 and 2).

Table 1: Demographic characteristics, laboratory findings and clinical indices of the participants with severe OSAS according to the study groups.

	Healthy control (n=31)	OSAS (n=91)	p-value	Adjusted p-value
Demographic characteristics				
Age (years)	38.39 ± 9.70	45.02 ± 9.97	.002[1]	
Gender (M/F)	17/14	69/22	.047[2]	
Laboratory findings				
ADMA	0.31 ± 0.05	0.19 ± 0.06	<.001[3]	<.001
SDMA	0.29 ± 0.05	0.29 ± 0.06	.798[1]	.257
LNMMA	0.024 ± 0.005	0.013 ± 0.006	<.001[1]	<.001
Arginine	89.85 ± 29.15	76.78 ± 33.14	.053[1]	.042
Arginine/ADMA	286.35 (252.52-361.35)	397.22 (252.32-602.07)	.007[4]	.004
TMAL	0.62 ± 0.09	0.49 ± 0.10	<.001[1]	<.001
Clinical indices				
AHI	2.50 (1.05-3.55)	21.50 (11.10-39.70)	<.001[4]	<.001
Average O_2	94.26 ± 1.48	91.03 ± 2.36	<.001[1]	<.001
Lowest O_2	89.48 ± 2.41	77.40 ± 8.80	<.001[3]	<.001
DESAT index	2.70 (1.40-3.80)	24.20 (13.10-43.90)	<.001[3]	<.001

Note: 1: Independent sample t-test; 2: Yates chi-square test with continuity correction; 3: Welch's t-test; 4: Mann-Whitney U test.

Table 2: Demographic characteristics, laboratory findings and clinical indices of the participants with the mean ages of the mild OSAS according to the study groups.

	Healthy control (n=31)	Mild(n=30)	Medium (n=30)	Heavy (n=31)	p-value	Adjusted p-value
Demographic characteristics						
Age (years)	38.39 ± 9.70[a]	40.9 ± 9.74[ac]	46.4 ± 10.25[bc]	47.68 ± 8.89[b]	<.0011	
Gender (M/F)	17/14	23/7	22/8	24/7	.1692	
Laboratory findings						
ADMA	0.31 ± 0.05[a]	0.19 ± 0.09[b]	0.18 ± 0.05[b]	0.21 ± 0.04[b]	<.0013	<.001
SDMA	0.29 ± 0.05	0.29 ± 0.05	0.28 ± 0.08	0.30 ± 0.05	.6211	.277
LNMMA	0.024 ± 0.005[a]	0.011 ± 0.006[b]	0.012 ± 0.007[b]	0.017 ± 0.004[c]	<.0013	<.001
Arginine	89.85 ± 29.15[a]	69.05 ± 18.68[b]	98.02 ± 28.26[a]	63.72 ± 38.70[b]	<.0013	<.001

Arginine/ADMA	286.35 (252.52-361.35)[a]	389.90 (251.29-601.30)	589.11(499.57-740.78)[b]	261.62 (162.40-391.98)[a]	<.0014	<.001
TMAL	0.62 ± 0.09[a]	0.49 ± 0.11[b]	0.47 ± 0.11[b]	0.52 ± 0.07[b]	<.0013	<.001
Clinical indices						
AHI	2.44 ± 1.36[a]	9.16 ± 2.52[b]	21.81 ± 4.08[c]	54.39 ± 18.90[d]	<.0013	<.001
Average O_2	94.26 ± 1.48[a]	92.20 ± 1.61[b]	90.87 ± 2.27[bc]	90.06 ± 2.63[c]	<.0011	<.001
Lowest O_2	89.48 ± 2.41[a]	83.63 ± 5.26[b]	77.40 ± 5.72[c]	71.35 ± 9.86[d]	<.0013	<.001
DESAT index	2.94 ± 2.05[a]	10.24 ± 3.88[b]	23.59 ± 4.97[c]	61.90 ± 23.07[d]	<.0013	<.001

Note: Data are presented as mean ± standard deviation or median (IQR); Different upper letters in each row indicate a statistically significant difference;1: One-way analysis of variance; 2: Pearson chi-square test; 3: Welch F test; 5: Kruskal-Wallis test.

DISCUSSION

Oxidative stress is a process that results in increased production of reactive oxygen and nitrogen products due to disruption of the oxidant and antioxidant balance in the body [33]. The effect of intermittent hypoxia on oxidative stress in the mechanism of OSAS is not clear [34].

NO is produced from *L*-arginine (*L*-arg) and Oxygen (O_2) in a reaction catalysed by Nitric Oxide Synthase (NOS). Three isoforms of NOS have been identified in humans: neuronal (nNOS), which is localized mainly in cells of the nervous system, inducible NOS (iNOS), whose expression is induced in various cell types by pro-inflammatory cytokines and endothelial NOS (eNOS), which is almost exclusively expressed in endothelial cells [11].

This tissue-specific expression of eNOS has been shown to be controlled through epigenetic mechanisms, including specific DNA methylation patterns and post-translational histone modifications [35,36]. Different NOS isoforms produce NO at different rates and NO concentration is an important determinant of its function. iNOS is the most potent NO donor and high concentrations of NO (e.g., produced by activated macrophages) have cell growth inhibitory and lethal effect. In contrast, eNOS produces the lowest levels of NO, which can activate soluble guanylate cyclase (sGC) to produce the second messenger cGMP resulting in vasorelaxation and inhibition of platelet aggregation, thus preventing atherogenesis [37,38]. Therefore, endothelium-derived NO plays a crucial role in maintaining vascular homeostasis and appropriate eNOS activity is critical for vascular health [39].

eNOS expression and activity are regulated at transcriptional, posttranscriptional and posttranslational levels [40]. Any disruption of this complex regulation is reflected by changes in NO bioavailability. Various stimuli such as oxidative stress, inflammation, as well as hypoxia affect eNOS expression and activity. Inadequate oxygen supply is a confirmed modulator of eNOS; however, research findings in this area are somewhat inconsistent. Hypoxic regulation of eNOS expression is complex and unclear depending on the species (human and rodents and others), endothelial heterogeneity in different vascular beds, experimental model (*in vitro* cell culture or animal studies) or developmental stage [41-45].

Interestingly, in bovine pulmonary artery endothelial cells, human saphenous vein endothelial cells, as well as in the lungs of patients with pulmonary hypertension *in vivo* or in the aortas and mesenteric arteries of mice exposed to chronic intermittent hypoxia, the effect of hypoxia on eNOS may vary depending on whether it is arterial or venous endothelium. In contrast to human umbilical vein endothelial cells, in human umbilical artery endothelial cells, hypoxia has been shown to up-regulate eNOS expression *in vitro* [46-50]. eNOS upregulation was observed in the pulmonary endothelium of mice or rats exposed to hypoxia and in hypoxic porcine aortic endothelial cells *in vitro* [51,52]. Chronic hypoxia has also been shown to up-regulate eNOS expression in the uterine endothelium of pregnant sheep, but not in the femoral or renal arteries or in the uterus of non-pregnant sheep [53]. Some studies show that hypoxia does not alter the expression of eNOS but affects its enzymatic activity [54,55].

Hypoxia can affect the availability of *L*-arg, the substrate for NO production by eNOS. The intracellular *L*-arg concentration depends on dietary intake, whole body protein turnover, endogenous synthesis, cellular uptake and metabolism and the major fraction of plasma *L*-arg comes from protein breakdown [12,56]. Under physiological conditions, intracellular *L*-arg levels far exceed the Km (concentration of substrate required to fill the active central half of NO synthase) and eNOS is theoretically saturated with substrate [57].

However, NO formation depends on extracellular *L*-arg concentrations, a phenomenon known as the *L*-arginine paradox. In this context, the actual uptake of *L*-arg by endothelial cells regulates eNOS activity and highlights the role and efficiency of the *L*-arg transporter. In endothelial cells, *L*-arg is taken up mainly *via* the cationic amino acid transporter CAT-1 [58-60]. Interestingly, CAT-1 was reported to co-localize with and interact with eNOS in plasma membrane caveolae [60].

This mutual proximity would facilitate the direct delivery of *L*-arg to eNOS and further emphasize the role of CAT-1 in the regulation of eNOS efficiency. Therefore, factors that alter the activity or expression of the CAT-1 transporter will affect nitric oxide synthesis. There are only a few reports on hypoxic regulation of CAT-1 activity; nevertheless, they are consistent: Hypoxia negatively regulates *L*-arg uptake by endothelial cells. Inadequate oxygen supply has been shown to inhibit *L*-arg uptake and its intracellular content in porcine pulmonary artery endothelial cells [61,62]. Consistently, overexpression of CAT-1 in hypoxic human pulmonary microvascular endothelial cells increased nitric oxide production [63].

The major fraction of plasmatic and cellular arginine in adult humans comes from the physiological whole body protein cycle [12]. Due to widespread post-traclational modification, L-arg residues within proteins can be methylated by a family of enzymes termed Protein Arginine Methyl Transferases (PRMTs). Subsequent degradation of such proteins results in the release of free methylated arginine derivatives: N-Monomethyl L-Arginine (L-NMMA), Asymmetric Dimethylarginine (ADMA) and Symmetric Dimethylarginine (SDMA) [13,14]. ADMA and SDMA interfere with the cellular

uptake of *L*-arg by the cationic amino acid transporter and thus potentially reduce *L*-arg uptake [15,16].

In general, hypoxia can be divided into acute or chronic according to its duration or persistent or intermittent according to its nature. For example, chronic lung diseases result in persistent hypoxia, whereas Obstructive Sleep Apnea (OSA) is associated with intermittent hypoxia consisting of cycles of hypoxia and re-oxygenation.

ADMA level was found to be higher in patients with (Chronic Obstructive Pulmonary Disease) COPD compared to the healthy group, but no statistically significant difference was found. In our study, arginine metabolites, especially ADMA, were significantly higher in the healthy group. We think that this difference is due to the fact that the hypoxia mechanism in COPD is chronic continuous and the hypoxia mechanism in OSAS is intermittent [64].

The hypoxic response differs slightly depending on the nature of hypoxia. In cellular models of intermittent hypoxia, an enhanced proangiogenic and pro-inflammatory phenotype was observed [18]. Furthermore, severe hypoxia followed by re-oxygenation can cause ischemia-reperfusion injury, a phenomenon of cellular damage observed after myocardial ischemia, stroke or organ transplantation resulting from increased generation of Reactive Oxygen Species (ROS). OSAS-associated intermittent hypoxia may also lead to ischemia-reperfusion injury, which is recognized as an important contributor to the pathogenesis of OSAS comorbidities through increased ROS production [20,21].

No significant difference was found between antioxidant metabolites in patients with OSAS and healthy individuals in the study by Christou et al. [65].

In the study by Liu et al., patients diagnosed with connective tissue disease were divided into two groups as those who developed pulmonary arterial hypertension and those who did not. ADMA levels were measured between these two groups and ADMA was found to be significantly higher in those who developed pulmonary arterial hypertension [66]. In this study, it was emphasized that methylated arginine metabolites (ADMA) were responsible for disease-related complications.

In the study conducted by Barcelo et al., ADMA levels tended to be higher in patients with severe OSAS, but no statistical difference was found. This result suggested that ADMA elevation in OSAS patients may be related to obesity and metabolic disorders [67].

Nural et al., compared serum levels of inflammatory mediators such as C-Reactive Protein (CRP), Tumor Necrosis Factor-α (TNF-α) and Asymmetric Dimethylarginine (ADMA) in Chronic Obstructive Pulmonary Disease (COPD), Obstructive Sleep Apnea Syndrome (OSAS) and Overlap Syndrome (OVS) patient groups [68]. In addition, changes in these mediators were examined with Continuous Positive Airway Pressure (CPAP) treatment in OSAS and OVS. CRP, TNF-α and ADMA levels were analysed in blood samples obtained from patients with COPD, OVS and moderate to advanced OSAS. First blood samples were taken in the morning after polysomnography and second blood samples were taken from OSAS and OVS patients receiving regular CPAP treatment. When the three groups were compared before CPAP treatment, ADMA level was significantly lower in OSAS compared to COPD, but CRP and TNF-α were similar between the groups. When we compared the parameters before and after CPAP treatment, CRP level decreased significantly in both OSAS and OVS, while no significant difference was observed in TNF-α and ADMA levels [68]. This study supports that the elevation of arginine metabolites in patients with OSAS is increased not in the early period but rather in the advanced period and after the development of complications related to OSAS. In our study, arginine metabolites (ADMA and L-NMMA) were significantly higher in the healthy group compared to the group diagnosed with OSAS. We think that this difference is due to the fact that our study was prospective and included patients who were newly diagnosed and therefore had not yet developed complications related to OSAS. It supports that the *L*-arginine pathway shifts more to nitric oxide synthesis to compensate for the vasoconstriction occurring in pulmonary vascular structures due to intermittent hypoxia in newly diagnosed patients who have not yet developed complications related to OSAS, so the synthesis of metabolites (ADMA and L-NMMA) formed by methylation of *L*-arginine has not yet been activated. The observation in the literature that nitric oxide synthase activity increased and accordingly the nitric oxide level in serum increased in measurements performed on animals exposed to intermittent hypoxia and without additional comorbidities suggests that the nitric oxide synthase pathway is particularly active in intermittent hypoxia and the arginine methylation pathway is activated in the progression of the disease.

In the literature, it is emphasized that arginine metabolites are responsible for the complications of many systemic diseases. In our study, we excluded patients with elevated arginine metabolites and additional comorbidities from the study and selected from the newly diagnosed patient group. Therefore, we think that the *L*-arginine pathway shifts to NO synthesis in the early period and reduces methylated arginine metabolites and is not yet actively synthesized. We think that in the later stages of the disease and with the development of complications, the arginine methylation pathway will be activated and arginine metabolites (ADMA, L-NMMA) will increase.

CONCLUSION

As a result of our study, we found that arginine metabolites (ADMA and L-NMMA) levels were lower in patients with OSAS compared to the healthy group. The patients we included in the study were newly diagnosed with the results of polysomnography and had not developed any complications related to the disease due to the early stage of the disease. Therefore, we did not observe an increase in arginine metabolites (ADMA, L-NMMA) due to the fact that the *L*-arginine pathway was registered to nitric oxide synthesis for compensation and therefore the methylated arginine pathway was not yet activated.

There is a need for further controlled, prospective, prospective studies including NO measurement and nitric oxide synthase enzyme activities in terms of monitoring treatment efficacy, monitoring of complications and monitoring of disease progression in patients with OSAS.

LIMITATIONS

The limitations of our study were that we could not measure the activity of nitric oxide synthase, which leads to nitric oxide synthesis from l-arginine activated by intermittent hypoxia and the level of NO in serum in the patient group. We could not measure the activity of nitric oxide synthase and enzymes involved in the formation of methylated arginine metabolite.

ETHICS APPROVAL

The study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Selcuk University Faculty of Medicine Clinical Research Local Ethics Committee with the decision numbered 2022/20 on 04.01.2022. Patients participating in the study were informed about the study and signed informed consent forms.

PATIENT CONSENT FOR PUBLICATION

Patients participating in the study were informed about the study and signed informed consent forms.

AVAILABILITY OF DATA AND MATERIALS

The data generated in the present study may be requested from the corresponding author.

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AUTHORS CONTRIBUTIONS

Emrah Bolca: Primary role in conducting the dissertation, wrote the main manuscript text, corresponding author.

Recai Ergun: Contributed to the sample size by providing patients who were followed and treated by the author. Evaluation and analysis of biochemical parameters.

Muslu Kazim Korez: Statistical analysis.

Ali Unlu and Duygu Eryavuz Onmaz: Evaluating the biochemical markers.

Dilek Ergun: Dissertation director (advisor), also contributed to the sample size by providing patients who were followed and treated by the author.

CONFLICT OF INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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