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Values of Plasma Vascular Endothelial Growth Factors in Patients with Obstructive Sleep Apnea-Hypopnea Syndrome

Plazma Vasküler Endotelyal Büyüme Faktörünün Obstrüktif Uyku Apne Hipopne Sendromlu Olgulardaki Değerleri

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ABSTRACT

Background: Obstructive sleep apnea-hypopnea syndrome (OSAHS) is an important public health problem. Plasma levels of vascular endothelial growth factor (pVEGF), which is believed to play an active role in the pathogenesis of cardiac pathologies developing in OSAHS. In this study, the correlation between pVEGF levels in OSAHS patients and polysomnographic values was examined.

Materials and Methods: Patients who applied to our center for the first time and consecutively who were "informed or referred from another unit" were admitted to this study. First, a standard questionnaire for the diagnosis of OSAHS and the Epworth Sleep Test were administered to the selected cases. Polysomnography (PSG) was performed in the Sleep Disorders Laboratory of our hospital. On the morning of the PSG test, blood was drawn to measure pVEGF levels.

Results: The study was evaluated on 34 cases and 20 healthy individuals who were accepted as OSAHS with apnea-hypopnea index (AHI) value greater than 5. OSAHS patients who were admitted to the study, 5 were female and 29 were male. The pVEGF levels of the patients included in the study were higher than those of the control group. The AHI value and pVEGF levels were correlated. pVEGF levels were higher in patients with severe OSAHS were higher than mild OSAHS. There was a moderate correlation between desaturation and pVEGF levels. There was a moderate correlation between pVEGF levels and mean duration of apnea, number of total hypopneas, mean duration of hypopnea, apnea index, total apnea, and obstructive apnea.

Conclusion: We found that pVEGF levels were elevated in patients with OSAHS, which is considered to account for 1-5% of the population. pVEGF levels were directly correlated with the AHI value, which is linked to disease severity. In severe OSAHS cases, changes in pVEGF levels can be effective in the development of cardiovascular pathologies.

Keywords: OSAHS, apnea, VEGF

ÖZ

Amaç: Obstrüktif uyku apne hipopne sendromu (OSAHS) önemli bir halk sağlığı problemidir. Vascular endothelial growth factor (pVEGF), OSAHS'da gelişen kardiyak patolojilerin patogenezinde etkin olduğu düşünülen anjiogenetik bir sitokindir. Biz çalışmamızda, plazma VEGF düzeylerinin OSAHS'lı olgularda polisomnografik değerlerle korele olup olmadığını göstermeyi amaçladık.

Gereç ve Yöntemler: Bu çalışmaya, merkezimize "kendisi bilgilenerek gelen ya da başka bir birimden refere edilen" ilk kez ve ardarda başvuran hastalar kabul edildi. Seçilen olgulara öncelikle OSAHS tanısına yönelik standart bir anket formu ve Epworth Uyku Testi uygulanmıştır. Hastanemiz Uyku Bozuklukları Laboratuvarında Polisomnografik inceleme (PSG) uygulandı. Çalışmaya katılan olgulardan ve kontrol grubundan PSG incelemenin ertesi sabahı pVEGF incelemesi için kan alındı.

Bulgular: Çalışma, apne-hipopne indeksi (AHİ) değeri 5'den büyük bulunarak OSAHS olarak kabul edilen 34 olgu ve 20 sağlıklı birey üzerinden değerlendirildi. Çalışmaya kabul edilen OSAHS'lı olguların 5'i kadın ve 29'u erkekti. Çalışmaya dahil edilen hastaların pVEGF düzeyleri kontrol grubuna göre daha yüksekti. AHİ değeri ile pVEGF düzeyleri korele idi. Şiddetli OSAHS'li hastaların plazma VEGF düzeyleri hafif OSAHS'li hastalara göre anlamlı olarak yüksekti. Desatürasyon sayıları ile pVEGF düzeyleri arasında orta derecede korelasyon vardı. pVEGF düzeyleri ile ortalama apne süresi, total hipopne sayısı, ortalama hipopne süresi, apne indeksi, total apne ve obstrüktif apne arasında orta derecede korelasyon vardı.

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This study was produced from "Plasma Vascular Endothelial Growth Factor Levels in Patients With Obstructive Sleep Apnea Hypopnea Syndrome" titled theses.

ÖZ

Sonuç: Sonuç olarak; biz, toplumun %1-5'ini oluşturduğu kabul edilen OSAHS'lı olgularda plazma VEGF düzeylerini yükselmiş bulduk. Plazma VEGF düzeyleri direkt hastalığın ciddiyetiyle bağlantılı olan AHİ değeri ile koreleydi. Ağır OSAHS'lı olgularda, kan VEGF düzeyindeki değişiklikler, bu olgulardaki kardiyovasküler patolojilerin gelişmesine etkili olabilmektedir.

Anahtar Kelimeler: OSAHS, apne, VEGF

Introduction

About one-third of human life is spent asleep. For this reason, scientists have focused on the physioanatomy of sleep for centuries. In 8th century BC, a researcher named Hesiod described sleep as "the brother of death". In Shakespeare's Hamlet and Cervantes' Don Quixote, sleep is interpreted as "a temporary suspension of life and a chance to dream" (1). It is known today that these views are not true.

In 1929, Hans Berger recorded the electrical activity of the human brain and revealed the existence of different rhythms between sleep and wakefulness (2). This development; he accelerated sleep research and, for the first time in 1966, Gastaut added a new dimension to sleep research using polysomnography (3). Obstructive sleep apnea-hypopnea syndrome (OSAHS) is a condition that manifests as recurrent episodes of upper airway closure during sleep and a decrease in blood oxygen saturation (4).

OSAHS is observed in 5% of the population. Given its high prevalence, OSAHS is an important public health problem because it is not less common than diseases such as diabetes mellitus and allergic bronchial asthma (5,6). Although some negative problems are observed in sleep even in healthy individuals, the results of OSAHS, which we consider the most important picture of respiratory disorders during sleep, lead to various morbidities and an increase in mortality rates in these patients. The most severe effects of OSAHS are on the cardiovascular system, which may even result in myocardial infarction and sudden death during sleep (3,6).

Vascular Endothelial Growth Factor (VEGF) is an angiogenetic, heparin-dependent-soluble glycoprotein of 34-36 kilodalton. This cytokine regulates many endothelial cell responses, including apoptosis, mitogenesis, vascular permeability, and tone (7,8). Hypoxia is the main stimulant that controls VEGF synthesis, gene transcription, and mRNA stability. In addition to hypoxemia, synthesis is stimulated when cells are deprived of glucose and inflammation (7,9).

VEGF plays a major role in physiological and pathophysiological angiogenesis. The measurement of circulating VEGF levels is believed to have diagnostic and prognostic value in cardiovascilar diseases, inflammatory diseases, and malignancies (10,11).

VEGF is an angiogenetic cytokine that is believed to be effective in the pathogenesis of cardiac pathologies in OSAHS. The present study aimed to determine whether plasma VEGF (pVEGF) levels correlate with polysomnographic values in patients with OSAHS.

Materials and Methods

This study was conducted in the Sleep Disorders Laboratory of the Chest Diseases and Tuberculosis Clinic of University of Health Sciences Türkiye, Dışkapı Yıldırım Beyazıt Training and Research Hospital between January 2003 and May 2003.

Study Population

Patients who applied to our center for the first time and consecutively who were "informed or referred from another unit" were admitted to this study.

Patients aged between 25 and 70 (without distinction between men and women), common complaints of habitual snoring, witnessed apnea and/or daytime sleepiness, an apnea-hypopnea index (AHI) of 5> as a result of overnight polysomnographic examination (PSG), and sleep efficiency of at least 60% or more were included.

Those who do not meet these criteria; patients younger than 25 or older than 70 years, those with chronic respiratory diseases, those with hypothyroidism, those with class II (progeny) and class III (retrogenic) jaw occlusion abnormalities, patients who had previously undergone surgery for snoring, those with sleep efficiency below 60%, those with cancer or who were screened for suspected cancer, and individuals with a chronic inflammatory disease or with leukocytes-sedimentation elevation were excluded from the study.

First, a standard questionnaire for the diagnosis of OSAHS and the Epworth Sleep Test were administered to the selected cases. Then, whole system physical examinations, pulmonary function tests, laboratory tests (complete blood count, sedimentation level, total biochemistry, thyroid function tests, Antistreptolizin O-rheumatoid factor-C3-C4 tests), electrocardiography, arterial blood gases, and posteroanterior chest radiographs of the patients were completed before PSG. Neck circumference (NC) was measured at the cricothyroid membrane level. Body mass index (BMI) of the patients was calculated. In addition, Doppler Echocardiography was performed in all patients

included in the study, and pulmonary artery pressure (PAP) and left ventricular function were evaluated. All patients underwent endoscopic examination at the Ear-Nose-Throat clinic. Patients were informed that they should not consume alcohol on the day of PSG, should not sleep until the time of the test in the afternoon, and should not use any sedative drugs from 1 week before the day of PSG.

Cases with non-OSAHS sleep-disordered breathing disorders, such as obesity hypoventilation syndrome, Upper Respiratory Tract Resistance Syndrome, leukocytosis, thrombocytosis, hypothyroidism after PSG, laboratory tests, and patients with malignancies and inflammatory diseases were excluded from the study.

In the case group, a total of 49 patients (41 men and 8 women) were included in the study. A total of 20 healthy individuals were included in the control group; 12 women and 8 men without any complaints. Common complaints were habitual snoring, witnessed apnea, and/or daytime sleepiness, and patients with an AHI>5 as a result of PSG examination were accepted as OSAHS and were included in the study program. All patients and the control group were first informed about the study and its purpose, and their consent was obtained. A 5 cc volume of venous blood was taken from the patients participating in the study and the control group into the EDTA tube at 07:00 on the morning after the PSG examination. The blood was centrifuged at 3000 rpm for 10 minute in 1 hour. Two 1 cc plasma samples were separated from each patient and stored at 20 °C until the study was conducted.

Materials Used

Questionnaire Forms

A routine questionnaire prepared by the Sleep Disorders Laboratory of the Chest Diseases and Tuberculosis Clinic at SSK Dışkapı Ankara Training and Research Hospital was applied to all cases. Identity information, personal and family history information, symptoms and signs related to the diagnosis of OSAHS, history information, physical examination results, and laboratory findings of the cases were recorded on this form. Second, the "Epworth Sleepiness Scale", which is a subjective test that is currently the most commonly used method to determine sleepiness throughout the day, was applied.

Polysomnography

Sleep efficiency was assessed using the Oxford Medilog SAC-SRI device in the Sleep Disorders Laboratory of our Polysomnography Hospital (Sleep efficiency: the ratio of the time spent in sleep to the entire recording time). This rate was at least 60% or more than 60%. Electroencephalography, electrooculography, electromyography, electrocardiography, thoraco-abdominal movements, body position, oro-nasal airflow, tracheal microphone, pulse oximetry, and fingertip oxygen saturation were measured as standard measurement parameters.

Apnea in polysomnographic study; It was considered as the cessation of airflow for 10 second or more. The condition was interpreted as "obstructive" if there was apnea despite the presence of thoraco-abdominal movements, "central" if there was no respiratory effort with apnea, and "mixed apnea" if the apnea was central at the beginning and continued despite the onset of respiratory effort. Since it was decided at the ATS congress held in Boston in 1998 that mixed apneas should be evaluated as obstructive apnea; We evaluated mixed apneas as obstructive apnea. Hypopnea; It was interpreted as a 3% decrease in oxygen saturation or arousal development with at least a 50% decrease in airflow for 10 second or more (12).

After the sleep test recording, manual scoring was performed. Sleep, respiratory, and cardiac evaluations were performed by scoring. In this way, sleep staging, changes in breathing patterns (apnea, hypopnea, arousal, etc.), changes in heart rate, presence of arrhythmias, if any, and periodic limb movement scoring were recorded. As a result of the PSG study, patients with an AHI>5 and/or 5 and the total number of obstructive/mixed apneas was found to be >80% of the total number of apneas were diagnosed with OSAHS.

Measurement of pVEGF Levels

Human VEGF (hVEGF) levels; worked with hVEGF ELISA test (BioSource, Nivelles, Belgium) kit. In this method, microplaques coated with specific polyclonal antibodies for hVEGF are used. In the first incubation, hVEGF-specific monoclonal antibodies were added after the hVEGF antigens found in the patient and control samples bind to the antibodies. The amount of color formed by the added enzyme and substrate is then evaluated. The darkness of the resulting color is directly proportional to the hVEGF concentration in the sample. The lowest detected value by this method was 5 pg/mL. In measurements taken from 15 healthy individuals, pVEGF values ranged from 0 to 120 pg/mL (mean: 20 pg/mL).

Ethics Committee Approval

This study was conducted before the ULAKBİM-TR Dizin decision dated 25.02.2020; therefore, an ethics committee decision was not reached. This study was produced from "Values of pVEGF in patients with obstructive sleep apnea hypopnea syndrome" titled theses. A clinical suitability certificate was obtained for the study (Sleep Disorders Laboratory of the Chest Diseases and Tuberculosis Clinic

of University of Health Sciences Türkiye, Dışkapı Yıldırım Beyazıt Training and Research Hospital, approval number: 6, date: 07.01.2003).

Statistical Analysis

The data obtained from the cases were encoded and recorded on a computer in SPSS for Windows 10.0. The Kruskal-Wallis test and Mann-Whitney U analysis were used for statistical evaluation, $p<0.05$ was accepted as significant.

Results

As a result of PSG, a total of 15 cases (12 men and 3 women) whose sleep duration and quality were not sufficient and whose AHI value was less than 5 were excluded from the study. The study included 34 cases and 20 healthy individuals who were accepted as OSAHS with an AHI>5.

Of the patients with OSAHS who were admitted to the study, 5 were female (14.7%) and 29 were male (85.3%). The mean age was 48.7±10 years. BMI was 32.7±5.7. The NC was 43.8±3.7 cm. The mean age of the control group was 43.4±5.8 years, BMI was 23.2±3.6 and NC was 34.4±2.7 cm. The demographic characteristics of patients with OSAHS and controls are presented in Table 1. As can be seen in the table, there was a difference in the BMI and NC measurement values between the patients and the control groups. As expected, the Epworth Sleepiness Scale scores were high in patients with OSAHS who had excessive daytime sleepiness.

The average recording time of the PSG test performed on the patients was 7 hours, and sleep adequacy did not fall below 60% in any patient. The changes in sleep stage rates observed in patients with OSAHS were also observed in our study cohort. An increase in superficial sleep level and a decrease in deep sleep and rapid eye movement sleep were observed. Table 2 presents the PSG results of patients with OSAHS.

The first aim of this study was to investigate whether there was a difference between the pVEGF levels of the control group and patients with OSAHS. There was a significant difference between our patients and the control group included in the study ($p<0.001$) (Table 3).

Table 1. Demographic parameters of patients with OSAHS

	OSAHS	Control	
Number of cases (M/F)	34 (29/5)	20 (12/8)	
Age, year, mean ± SD (min.-max.)	48.7±10 (31-67)	43.4+5.8 (26-56)	p<0.05
BMI, mean ± SD (min.-max.)	32.7±5.7 (24.8-49.9)	23.2±3.6 (18.6-33.2)	p<0.05
NC (cm) , mean ± SD (min.-max.)	43.8±3.7(36-51)	34.4±2.7 (30-39)	p<0.05

Control: Healthy individual, OSAHS: Obstructive sleep apnea hypopnea syndrome, M: Male, F: Female, SD: Standard deviation, BMI: Body mass index, min.-max.: Minimum-maximum, NC: Neck circumference

Table 2. PSG results in patients with OSAHS

	OSAHS, mean ± SD	Control, mean ± SD
Total recording time (minute)	432.2±55.3	442.4±45.6
Sleep efficiency (%)	85.4±11.8	92.2±12.3
Phase 0 (%)	14.4±11.8	10.4±6.2
Phase 1 (%)	12.0±9.0	8.1±4.2
Phase 2 (%)	49.2±14.6	50.3±14.6
Phase 3 (%)	9.6±9.0	6.6±2.2
Phase 4 (%)	3.2±5.2	14.2±4.7
REM (%)	11.4±8.8	19.1±7.3
Non-REM (%)	74.2±12.1	79.5±11.1
Number of desaturation steps	198.4±140.0	10.8±3.1
During sleep, mean sO_2	89.26±7.19	93.9±2.6
During sleep, minimum sO_2	65.88±13.30	82.1±5.7
AHI	33.1±27.2	1.0±0.7

Control: Healthy individual, OSAHS: Obstructive sleep apnea hypopnea syndrome, SD: Standard deviation, REM: Rapid eye movement, AHI: Apnea-hypopnea index, sO_2: Oxygen saturation, PSG: Polysomnography

pVEGF levels of patients with OSAHS were compared with AHI values, which are considered to indicate disease severity. A moderate correlation was found between these two values (Table 4). Patients with OSAHS were divided into 3 groups according to AHI values (AHI: 5.0-14.9 mild OSAHS, AHI: 15-29.9 moderate OSAHS, ⩾30 severe OSAHS). There were 12 patients in the mild OSAHS group, 8 in the moderate OSAHS group, and 14 in the severe OSAHS group. pVEGF levels of these 3 groups were compared with each other. pVEGF levels were significantly higher in patients with severe OSAHS than in patients with mild OSAHS. ($p<0.05$); However, no difference was found between the other groups.

Again, the number of total apneas, obstructive apneas, and central apneas was compared with pVEGF. Mean apnea duration, longest apnea duration, apnea index (AI), total hypopnea count, mean hypopnea duration, hypopnea index, and pVEGF levels were compared. There was a moderate correlation between pVEGF levels and the mean duration of apnea, total number of hypopneas, mean duration of hypopnea, AI, total apnea, and number of obstructive apnea (Table 4).

The desaturation numbers, mean saturation levels, minimum saturation levels, and pVEGF levels of patients with OSAHS were compared. Although there was a moderate correlation between desaturation numbers and pVEGF levels, there was no correlation between mean saturation levels and minimum saturation levels and pVEGF values (Table 5).

We found elevated PAP in 14 (41.1%) of 34 patients diagnosed with OSAHS. Because PAP is directly related to quality of life and possible complications, we compared pVEGF levels and PAP values to determine whether elevated pVEGF levels in patients with OSAHS are significant in terms

Table 3. Group pVEGF levels

	Number of cases	pVEGF (pg/mL)	
Control (mean ± SD)	20	86.0±43.9	p<0.001
OSAHS (mean ± SD)	34	152.4±58.2	

OSAHS: Obstructive sleep apnea hypopnea syndrome, SD: Standard deviation,cpVEGF: Plasma vascular endothelial growth factor

Table 4. Correlation matrix analysis between AHI and pVEGF levels

	pVEGF (pg/mL)	
	r-value	p-value
AHI	0.454	0.007*
Total apnea count	0.474	0.005*
Obstructive apnea count	0.493	0.003*
Central apne count	0.259	0.139
Mean apnea duration (sec)	0.343	0.047*
Longest apnea duration (sec)	0.293	0.093
Apnea index	0.441	0.009*
Total hypopne count	0.354	0.040*
Mean hypopne duration (sec)	0.391	0.022*
Hypopne index	0.294	0.092

AHI: Apnea-hypopnea index, pVEGF: Plasma vascular endothelial growth factor

Table 5. Correlation matrix analysis of saturated VEGF and pVEGF levels

	pVEGF (pg/mL)	
	r-value	p-value
Number of desaturation steps	0.409	0.046*
During sleep, mean sO_2	-0.261	0.137
During sleep, minimum sO_2	-0.202	0.252

pVEGF: Plasma vascular endothelial growth factor, sO_2: Oxygen saturation

of prognosis. We did not find any difference in pVEGF levels between patients with and without high PAP. In addition, we did not find any correlation between PAP and pVEGF levels in patients with high PAP.

Discussion

OSAHS is an important public health problem because its prevalence is approaching 5%, similar to that of diabetes mellitus and allergic bronchial asthma (5,6). Even healthy individuals exhibit anomalies during sleep. OSAHS, the most important sleep disorder, leads to different morbidities and an increase in mortality rates in these patients (3,6).

OSAHS is a syndrome that manifests itself with recurrent episodes of upper airway closure during sleep and is often accompanied by a decrease in blood oxygen saturation (4). AHI is the most commonly used PSG test parameter in determining the diagnosis and severity of OSAHS. The AHI parameter's limit value in OSAHS patients is not clear and precise. In various studies, AHI ranges from 5 to 20. However, clinically important patients with AHI>20 have increased mortality risks (13). More than half of our patients had AHI values >20. It is known that there is a relationship between apnea and obesity, and weight loss may lead to improvement in OSAHS. Obesity, especially central obesity, increases the tendency to develop OSAHS at a remarkable rate by affecting the patency of the upper respiratory tract and the complicity of the region with fat storage and by affecting the respiratory pattern with abdominal fat accumulation. In individuals over the age of 40 years, the risk of OSAHS increased 8-12 times in individuals with a BMI>29 kg/m^2 compared with individuals who were not overweight. This risk is much higher, particularly in those with more pronounced fat accumulation in the upper body region and in morbidly obese patients with a BMI>40 kg/m2 (14,15). In recent studies, NC was shown to be a determining factor for OSAHS. More than 43 cm in male individuals and 38 cm in female individuals was considered significant (15). BMI and NC measurements were found to be significantly higher in our cases.

VEGF regulates multiple endothelial cell responses, including apoptosis, mitogenesis, vascular permeability, and tone (7,8). pVEGF levels increase mainly in hypoxia, inflammations and cancers. In our study, we showed that the pVEGF levels of patients with OSAHS were higher than those of healthy individuals ($p<0.001$).

Previous studies have shown that pVEGF levels are elevated due to hypoxia. Hypoxia. It is the main stimulant that controls VEGF synthesis, gene transcription, and mRNA stabilization (9). The relationship between VEGF concentration and nocturnal hypoxia in patients with OSAHS leads to increased hypoxia-sensitive gene expression and consequently increased protein production in recurrent intermittent nocturnal hypoxemic episodes (16). Apart from hypoxia, the most common conditions associated with an increase in VEGF level are; for example, disseminated cancer, chronic inflammatory, and autoimmune diseases were not present in our patients and, consequently, could not be responsible for changes in the VEGF concentration. Our findings were the same as those of Lavie et al. (16), Schulz et al. (17), Gozal et al. (18), Imagawa et al. (19), and Teramoto et al. (20).

Schulz et al. (17) observed a significant increase in serum VEGF levels in obstructive sleep apnea (OSA) patients with severe nocturnal hypoxemia compared with OSA patients with mild hypoxemia and the control group (17). Gozal et al. (18) found VEGF levels to be significantly higher in both children and adults with OSAHS compared with very few sick and non-sick individuals.

Imagawa et al. (19) measured hemoglobin, serum erythropoietin, and VEGF levels in 106 patients with severe OSAHS. Their results showed that transient hypoxemia increased hemoglobin. In addition, they found a small increase in erythropoietin levels and a large increase in VEGF levels (19). Teramoto et al. (20) showed that serum VEGF levels are elevated due to nocturnal hypoxia in patients with OSAHS. They observed that serum VEGF levels decreased in patients with OSAHS whose nocturnal hypoxemia was corrected by applying oxygen (O_2) at 2 L/min during the night. However, the administration of compressed air did not affect pVEGF levels and O_2 desaturation in patients with OSAHS (20).

Lavie et al. (16) found that patients with OSAHS had high circulating VEGF. They also found that VEGF levels were significantly higher in patients with OSAHS than in snoring and healthy young adult participants of the same age. Researchers have reported that VEGF levels also decreased in patients whose hypoxemia improved after non-invasive continuous positive airway pressure treatment. This result supports the idea that elevated VEGF levels in patients with OSAHS result from nocturnal hypoxemia.

In our study, although we established a relationship between pVEGF levels and apnea-related hypoxia; We did not find a linear relationship between the depth of nocturnal dephased and pVEGF levels. For this purpose, we investigated the number of desaturations, average saturation times, and minimum saturation values. Although there was a correlation between the number of desaturation and pVEGF levels ($p<0.05$), there was no correlation between the mean saturation levels and minimum saturation levels and pVEGF values. This result led us to suggest that although recurrent hypoxemic attacks are direct stimulants of VEGF release, deepening hypoxia does not act as an extra stimulator of

VEGF release. In contrast, Schulz et al. (17) reported that serum VEGF concentration was significantly correlated with the degree of oxygen desaturation in OSAHS (17). Gozal et al. (18) also found a correlation between serum VEGF and oxygen levels in their studies (18).

The second issue is whether there is a difference between plasma and serum samples in VEGF levels due to hypoxia in OSAHS. Schulz et al. (17), Gozal et al. (18), and Imagawa et al. (19) detected VEGF in serum and not plasma, and their values were higher than those of ours and Lavie et al. (16-19). Serum VEGF was released from platelets and other blood cells during blood clotting. And that is why they reported a 2-7 fold increase in VEGF. In addition, their values reflect blood platelet counts rather than VEGF synthesis by peripheral tissues; It may not directly reflect hypoxia. In Jelkmann's review study and two separate studies, the necessity of using plasma to measure VEGF levels was emphasized (11,21,22).

In their study, Lavie et al. (16) reported that some patients responded to hypoxia with high VEGF mRNA synthesis *in vitro*, whereas others had little or no response (16). In our study, we did not detect elevated pVEGF levels secondary to deep hypoxia in 3 patients. This personal answer difference; In the study conducted by Schultz et al. (17), it was reported that this was correlated with the height of the coronary collateral tree (23).

We compared pVEGF with AHI, apnea, and hypopnea parameters to evaluate its efficacy in patients with OSAHS. We found a correlation between pVEGF levels and AHI, which is considered to indicate the weight of OSAHS ($p<0.05$). When we divided our cases into 3 groups according to the AHI value recommended by the American Sleep Disorders Association and then compared them with the pVEGF level again; We found that there was a significant difference between the light and severe groups in terms of pVEGF levels. This may explain the high pVEGF levels caused by hypoxemia due to apnea and hypopnea, which are more common in patients with severe OSAHS. In addition, we found a correlation between pVEGF levels and obstructive apnea count, total apnea count, AI, mean apnea duration, total hypopnea number, and mean hypopnea duration. These correlations strengthen the link between recurrent apnea-related hypoxia and pVEGF. Lavie et al. (16) and Gozal et al. (18) found a significant correlation between pVEGF concentrations and respiratory distribution index (16,18).

While planning the study, we assessed the difference in pVEGF levels among 14 (41.2%) patients with high PAP, considering that elevated PAP changes may affect pVEGF levels. We did not find any correlation between these two values ($p>0.05$). Although the location of echocardiography for PAP measurement is discussed; We preferred echocardiographic imaging for PAP measurement because it is non-invasive (24). We could not detect daytime hypoxemia and concomitant lung disease, which are believed to cause high PAP in patients with OSAHS. Considering that VEGF levels are elevated due to nocturnal hypoxemic attacks in all our cases; PAP pressure elevation was observed in only 14 (41.2%) patients, indicating that PAP may have increased due to another physiopathological mechanism and not due to nocturnal hypoxemic attacks.

There are no accepted opinions on the physiological significance of elevated VEGF levels in the blood of patients with OSAHS. The tissues in which VEGF secretion due to hypoxia was observed were the beginning of theories for the researchers. VEGF has been shown to be upregulated in cardiac myocyte, vascular smooth muscle cells, and endothelial cells under hypoxic conditions, as well as in cardiac tissues following microvascular flow index (16). When we examine our own study, we cannot determine the main source of VEGF secretion observed in patients with OSAHS. Unlike endothelium, the site responsible for increased VEGF production is likely activated platelets in untreated patients with OSAHS. However, in vitro experiments have shown that platelets do not release significant amounts of VEGF against hypoxia (17).

As mentioned above, the fact that most cardiac responses are due to hypoxic conditions has led to two hypotheses in patients with OSAHS. The first of these; This is the view put forward by Lavie et al. (16) in their work. According to these findings, current studies suggest that VEGF may contribute to the atherogenic process on its own, not to mention its role in angiogenesis. VEGF-induced monocyte activation and migration regulate the growth of smooth muscle cells and are closely linked to the development of coronary atherosclerosis in humans (25). The second hypothesis was proposed by Schulz et al. (17). They are considered to be of the pathophysiological significance of their results; They hypothesized that increased VEGF production in OSAHS is an adaptive mechanism that offsets the urgency of OSAHS-related cardiovascular diseases. Theoretically, an increase in VEGF production in patients with OSAHS may facilitate the formation of new vessels in ischemic and atherosclerotic vascular areas. This prediction is supported by a study in which collateral vessel formation was correlated with hypoxic VEGF induction in coronary artery patients (23,26). Again, they are partial to the results of the study. They said it may be part of their review of the Sleep Heart Health Study, which explains that not all cardiovascular risk factors in OSAHS are directly linked to apnea severity (17).

In our opinion, VEGF, the most prominent function in the body of angiogenesis, is more likely to contribute to the development of atherosclerosis. As mentioned above and as

determined, pVEGF levels are higher in patients with severe OSAHS. It may not be a coincidence that cardiac and other complications are more common in these cases. Increased *in vivo* platelet activation was noted in patients with OSAHS, as shown in a study. In some studies, this event; It has been stated that OSAHS may increase cardiovascular outcomes (27,28). In addition, in one study, a decrease in plasma fibrinolytic activity was observed in patients with OSAHS, although the mechanism was unknown (29). However, Zakrzewski et al. (30) explained that patients with OSA had a severe risk of cardiovascular disorders due to increased pro-thrombotic activity. Risk factors such as elevated blood pressure, advanced age, obesity, and hyperlipidemia may contribute to the development of atherosclerosis along with increased VEGF levels.

Study Limitations

Because this was a prospective study, the number of participants was limited.

Conclusion

As a result; We found that pVEGF levels were elevated in patients with OSAHS, which is considered to constitute 1-5% of the population. pVEGF levels were correlated with AHI levels, which are directly related to disease severity. In patients with severe OSAHS, changes in blood VEGF levels may affect the development of cardiovascular pathologies.

Ethics

Ethics Committee Approval: This study was conducted before the ULAKBİM-TR Dizin decision dated 25.02.2020; therefore, an ethics committee decision was not reached.

A clinical suitability certificate was obtained for the study (Sleep Disorders Laboratory of the Chest Diseases and Tuberculosis Clinic of SSK Dışkapı Ankara Training and Research Hospital, decision no: 6/07.01.2003).

Informed Consent: All patients and the control group were first informed about the study and its purpose, and their consent was obtained.

Authorship Contributions

Surgical and Medical Practices: M.İ.B., S.K., Concept: M.İ.B., S.A., Design: M.İ.B., S.A., Data Collection or Processing: M.İ.B., S.K., Analysis or Interpretation: M.İ.B., S.A., S.K., Literature Search: M.İ.B., S.A., Writing: M.İ.B., S.K.

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