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Original Article BIOCHEMISTRY

Assessment of serum vascular endothelial growth factor, nitric oxide and asymmetric dimethyl arginine levels in non-small cell lung cancer

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ABSTRACT

OBJECTIVE: Lung cancer is one of the most prevalent malignancies worldwide, with 80–85% of cases diagnosed as non-small cell lung cancer (NSCLC). The majority of NSCLC patients present with advanced disease, contributing to high mortality and limited treatment options. Angiogenesis, a crucial process in cancer progression, is largely regulated by growth factors and cytokines. Vascular Endothelial Growth Factor (VEGF) is a key regulator of angiogenesis. Asymmetric Dimethyl Arginine (ADMA) inhibits endothelial nitric oxide synthase (eNOS), leading to reduced nitric oxide (NO) release and subsequent endothelial dysfunction. The aim of this study is to investigate the serum levels of ADMA, NO, VEGF and several tumor markers including Carcinoembryonic Antigen (CEA), Cancer Antigen 125 (CA 125), Neuron Specific Enolase (NSE), Lactate dehydrogenase (LDH) and Cyfra 21-1 in NSCLC patients to assess their potential role in early diagnosis, tumor invasion, and staging of the disease.

METHODS: Our study consisted of 56 newly diagnosed NSCLC patients and 32 controls with similar demographic characteristics. Patients with chronic diseases and inflammatory disorders were excluded. Statistical analysis was conducted using R Statistical Software.

RESULTS: In our study, compared to the control group, the serum VEGF, NO, ADMA, CA 125, CEA, Cyfra 21-1 and NSE levels were significantly higher in NSCLC group ($p=0.001$, $p=0.013$, $p=0.041$, $p<0.001$, $p<0.001$, $p<0.001$ and $p=0.001$, respectively). In the diagnosis of NSCLC, Cyfra 21-1 exhibited the highest diagnostic efficacy with a 71% sensitivity and 94% specificity. The combination of VEGF, CA125, and Cyfra 21-1 showed a 73% sensitivity and 100% specificity, while the combination of CA125, CEA, and Cyfra 21-1 achieved an 85% sensitivity and 91% specificity.

CONCLUSION: Our study revealed that the serum concentrations of VEGF, NO, ADMA, CA125, Cyfra 21-1, CEA, and NSE were significantly elevated in patients with NSCLC compared to the control group, and that levels of Cyfra 21-1, LDH, and NSE increased with advancing TNM stage. The combination of markers distinguished NSCLC with high sensitivity and specificity. Further studies involving larger populations, including those with benign lung diseases, are needed to validate and expand upon our findings.

Keywords: ADMA; CA125; Cyfra 21-1; lung cancer; NO; VEGF.

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Lung cancer is a significant public health issue, as evidenced by around 1.8 million deaths and nearly 2.2 million new cases worldwide [1]. Due to the limitations of current diagnostic methods and screening tests, as well as late clinical presentation and the lack of efficient biomarkers for early diagnosis, a mere 16% of individuals diagnosed with lung cancer are identified at an early stage with a chance for curative treatment [2]. Smoking represents the primary etiological factor contributing to the development of lung cancer, as nearly 90% of individuals with lung cancer are smokers [3].

The classification of lung cancer is predominantly divided into Non-Small Cell Lung Cancer (NSCLC), responsible for around 85% of all cases, and Small Cell Lung Cancer (SCLC), which represents the remaining 15% [4]. The distinction between SCLC and NSCLC is due to the more aggressive nature of SCLC, the frequent presence of metastases at diagnosis, and generally the lack of surgical options. Therefore, it has become important to develop reliable and noninvasive tools for lung cancer screening and early detection.

Vascular endothelial growth factor (VEGF), a homodimeric glycoprotein, is an essential factor in the regulation of angiogenesis [5]. The biological effects of VEGF are mediated through its binding to transmembrane tyrosine kinase receptors present on endothelial cells [6]. Nitric oxide (NO) is synthesized in mammals from L-arginine in the presence of O_2 via the nitric oxide synthase (NOS) enzyme [7]. It increases vascular permeability during angiogenesis, induces endothelial cell proliferation and migration, and stimulates the expression of VEGF, while mediating many of its angiogenic effects [8, 9]. Methylarginines are derivatives formed by post-translational modification of arginine residues in proteins. Asymmetric dimethylarginine (ADMA), a derivative of methylarginine, is a competitive endogenous inhibitor of the NOS enzyme [10].

Cyfra 21-1 is a fragment of cytokeratin 19, which is a structural protein and an intermediate filament protein essential for the stability of epithelial cells. Cancer antigen 125 (CA 125) is a mucin glycoprotein found in the structure of the ocular surface, the respiratory tract, and the epithelium of the reproductive system, creating a hydrophilic environment that serves as a lubricating barrier [11]. Carcinoembryonic antigen (CEA) is a cell surface glycoprotein, composed of approximately 60% carbohydrates. Although it is found in relatively high concentrations in carcinomas and fetal tissue, it is also present in low amounts in many tissues [12]. Enolase is a glycolytic enzyme that can be found in the cytoplasm of all cells. Neuron Specific Enolase (NSE) is a γ-enolase with a molecular weight of 39 kDa, that is found primarily in neurons and neuroendocrine cells. Lactate dehydrogenase (LDH) is a cytosolic enzyme that reversibly catalyzes the formation of lactate from pyruvate under anaerobic conditions. Serum biomarkers including CA 125, CEA and Cyfra 21-1 are implemented in clinical practice following the recommendations of the National Academy of Clinical Biochemistry [13, 14]. However, despite their high specificity (~90%), these biomarkers exhibit limited sensitivity (50–60%), which limits their effectiveness and suitability for clinical use [15, 16].

Angiogenesis is essential for tumor formation, with VEGF being one of the most critical factors, often elevated in various cancer types [17]. Increasing evidence suggests that NO can modulate angiogenesis via VEGF in lung cancer [18]. ADMA levels may rise due to reduced dimethylaminohydrolase (DDAH) enzyme activity, which, alongside NO dysregulation, contributes to inflammation and oxidative stress, key processes in cancer progression [19]. The purpose of this study was to assess the relationships between the levels of serum VEGF, NO, and ADMA, which could contribute to the pathogenesis of angiogenesis in NSCLC, a significant cause of cancer-related deaths, and the levels of tumor markers known for their efficacy in diagnosis and management, including Cyfra 21-1, CEA, CA 125, NSE, and LDH, with the extent of tumor spread, and their potential utility in diagnosis.

Highlight key points

- The combination of CA 125, CEA, and Cyfra 21-1 significantly improved diagnostic accuracy, achieving an AUC of 0.932 with 85% sensitivity and 91% specificity in diagnosing NSCLC.
- Lung cancer diagnosis is hindered by the limitations of existing methods, highlighting the urgent need for more accurate and accessible early detection tools.
- The increase in serum ADMA, NO, and VEGF in NSCLC provides novel insights into the pathophysiology of lung cancer, and targeting these pathways could offer new therapeutic opportunities in the management of NSCLC

MATERIALS METHODS

Study Population

The study included 56 NSCLC patients who were diagnosed with NSCLC through biopsy and presented to the Chest Diseases Outpatient Clinic of Selcuk University Faculty of Medicine, along with 32 healthy individuals who had similar distributions of sex, age, body mass index (BMI), and smoking status as the patient group. Individuals with systemic diseases such as hypertension, diabetes mellitus, coronary heart disease, chronic bronchitis, and liver disease; those with active infections or a history of malignancy; those taking medication; and those who had received any antitumor therapy were excluded from the study. Our study was conducted in compliance with the principles specified in the Declaration of Helsinki.

Initially, 151 NSCLC patients were included in the study. However, 2 patients were excluded due to a history of malignancy, 22 patients due to the inability to determine the stage of NSCLC, 14 patients due to incomplete data, 31 patients due to the presence of chronic diseases and ongoing medication use, and 26 patients due to active infectious diseases. Venous blood samples from the patients were collected into BD Vacutainer® SST™ II Advance tubes (Becton Dickinson, NJ, USA) between 08:00 and 10:00 am following at least 8 hours of fasting. Approximately 4–5 ml of blood was taken, and the tubes were transported to the laboratory and left upright for 20 minutes to allow for clotting before centrifugation. After centrifugation at 1500 × g for 10 minutes via a Sigma 3K30 centrifuge (Sigma, St. Louis, MO, USA), the samples were placed into Eppendorf tubes and stored at -80 °C.

The study received research approval from the Clinical Research Ethics Committee of Selcuk University with the decision number 2018/21 dated 07.11.2018.

Laboratory Analysis

Serum ADMA and arginine levels were analyzed using an API 3200 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex) with positive electrospray ionization, combined with a Shimadzu HPLC system (Kyoto, Japan) and a Phenomenex Luna C18 column (50 mm × 4.6 mm). The method for measuring ADMA concentrations was based on and modified from the study by Di Gangi et al. [20]. Briefly, 200 µL of the sample was pipetted into glass tubes, followed by the addition of 100 µL of internal standard (d7-ADMA), and the mixture was vortexed for 10 seconds. Then, 1 mL of 100% methanol was added to the samples and vortexed for 30 seconds to precipitate the proteins. Centrifugation of the samples was performed at 13,000 rpm for a period of 10 minutes, and the supernatant was carefully collected and placed into glass tubes and evaporated under nitrogen gas in a water bath at 60 °C. After evaporation, 200 µL of freshly prepared 5% (v/v) acetyl chloride/butanol solution was added for derivatization, and the mixture was incubated at 60°C for 20 minutes. The evaporation process was repeated under nitrogen gas at 60 °C. After evaporation, the residue was reconstituted with 200 µL of 0.1% (%v/v) formic acid in 100 µL of a water-methanol (90:10, v/v) solution. The reconstituted samples were transferred to vials and loaded onto the instrument.

The concentration of serum VEGF was determined via a sandwich enzyme-linked immunosorbent assay (ELISA) using a Quantikine® human VEGF ELISA kit (Catalog No: DVE00, R&D Systems, USA). Serum NO levels were determined using the R&D Systems Total NO/Nitrite/Nitrate ELISA kit (Catalog No: KGE001, Minneapolis, MN, USA).

Serum CEA, NSE, and CA 125 concentrations were measured using the Cobas e601 autoanalyzer (Roche Diagnostics, Mannheim, Germany), whereas the Cyfra 21-1 concentration was measured using the Abbott Architect i2000 autoanalyzer (Abbott Park, Illinois, USA). Serum LDH was determined spectrophotometrically using a Beckman Coulter AU5800 (Beckman Coulter Inc., Brea, CA, USA).

Statistical Analysis

Statistical analyses were performed utilizing the R version 4.2.1. Software. Normality was determined via the Shapiro-Wilk test, while variance homogeneity was evaluated with the Levene test. For data that followed a Gaussian distribution and had homogeneous variances between groups, the results are presented as the means±standard deviations (SDs). When the one-way analysis of variance (ANOVA) test indicated significant differences, a Tukey HSD post hoc test was employed. For data that did not follow a Gaussian distribution, the findings were reported as the median [minimum–maximum], and differences between groups were analyzed using the Mann-Whitney U test and the Kruskal-Wallis test. If the Kruskal-Wallis test indicated significant differences, the Conover-Iman test with Bonferroni correction was employed to identify the specific groups with

Table 1. Comparison of demographic characteristics and serum levels of VEGF, NO, ADMA, arginine, CA 125, CEA, NSE, LDH, and Cyfra 21-1 in patients with NSCLC and control groups

Parameters	Healthy (n=32)	NSCLC (n=56)	p
Age (years)	58.91±7.33	62.34±8.05	0.052
BMI (kg/m²)	26.39±3.55	24.39±3.91	**0.019**
Smoking status (yes) (%)	81.2	89.2	0.8
Smoking (pack/year)	30 (0–90)	40 (0–90)	0.22
Gender (%)			0.291
Male	81.25	89.29	
Female	18.75	10.71	
VEGF (ng/mL)	397.97 (150.59–779.81)	594.94 (169.48–1812.36)	**0.001**[b]
NO (µmol/L)	19.51 (4.80–39.10)	24 (10.06–78.56)	**0.013**[b]
ADMA (µmol/L)	0.70±0.14	0.77±0.16	**0.041**[a]
Arginine (µmol/L)	268 (186–453)	306 (192–605)	0.092[b]
Arginine/ADMA	419.51±107.85	423.56±106.03	0.892[a]
CA 125 (IU/mL)	13.44 (5.52–48.32)	26.84 (9.01–126.4)	<**0.001**[b]
CEA (ng/mL)	2.52 (0.69–6.61)	4.62 (1–64.33)	<**0.001**[b]
LDH (IU/L)	177 (132–346)	194.5 (93–280)	0.104[b]
Cyfra 21-1 (ng/mL)	1.28 (0.72–3.86)	6.48 (1.06–97.60)	<**0.001**[b]
NSE (ng/mL)	12.92 (6.39–21.24)	18.46 (6.58–41.49)	**0.001**[b]

a: Student t test; b: Mann Whitney U test; VEGF: Vascular endothelial growth factor; ADMA: Asymmetric dimethyl arginine; NO: Nitric oxide; CEA: Carcinoembryonic antigen; CA 125: Cancer antigen 125; NSE: Neuron specific enolase; LDH: Lactate dehydrogenase; NSCLC: Non-small cell lung cancer; BMI: Body mass index.

differences. For further analysis, we conducted receiver operating characteristic (ROC) curve analysis to identify the diagnostic performance and the area under the curve (AUC) was determined. A p-value below 0.05 was deemed to be statistically significant.

RESULTS

A total of 88 participants, including 56 NSCLC patients and 32 healthy controls who fulfilled the eligibility requirements were enrolled in this study. Patients were broadly comparable in terms of age (58.91±7.33 vs. 62.34±8.05, p=0.052) and sex (81.25% vs. 89.29% for females, p=0.291) distributions between the study groups. The mean BMI of the healthy control group (26.39±3.55) was found to be greater than that of the patient group (24.39±3.91) (p=0.019). The main clinical and demographic characteristics and laboratory findings of the patients with NSCLC and the control group are summarized in Table 1. When the control group and NSCLC patient group were compared, the serum levels of NO, VEGF, ADMA, NSE, CA 125, CEA, Cyfra 21-1, and NSE were found to be elevated in NSCLC (p=0.013, p=0.001, p=0.041, p=0.001, p<0.001, p<0.001, and p<0.001, respectively). The levels of arginine, arginine/ADMA ratio, and LDH were found to be similar between the control group and the NSCLC group (p=0.092, p=0.892 and p=0.104, respectively).

In our patient group, 18 individuals (32.1%) had adenocarcinoma, while 38 (67.9%) had squamous cell carcinoma as their histological subtype. As summarized in Table 2, the evaluation of VEGF levels in relation to the histological subtypes of NSCLC revealed a significant increase in patients with squamous cell carcinoma compared to healthy individuals (664.6 [min–max, 169.4–1812.3] vs. 397.9 [min–max, 150.5–779.8], p<0.001). However, although the serum VEGF level in the adenocarcinoma subgroup was greater than in the control group, this difference was not statistically significant (486.1 [min–max, 279.2–1578] vs. 397.9 [min–max, 150.5–779.8], p=0.051). Similarly, compared with those in the control group, serum NO levels were found to be elevated solely in the squamous cell carcinoma subgroup (24.18 [min–max, 10.06–78.5] vs. 19.51 [min–max, 4.8–39.1], p=0.009). The serum levels of ADMA, arginine, LDH, and the arginine/ADMA ratio were not significantly different between the histological subtypes and the controls. Compared with those in the control group, CA 125, CEA,

Table 2. Findings of serum levels of VEGF, NO, ADMA, arginine, CA 125, CEA, NSE, LDH, and Cyfra 21-1 in NSCLC and control groups according to histological subtype

	Control (a) (n=32)	Adenocarcinoma (b) (n=18)	Squamous cell carcinoma (c) (n=38)	p	a/b	b/c	a/c
VEGF (ng/mL)	397.9 (150.5–779.8)	486.1 (279.2–1578)	664.6 (169.4–1812.3)	**0.001**[b]	0.051	0.212	<**0.001**
NO (µmol/L)	19.51 (4.8–39.1)	23.31 (10.62–55.46)	24.18 (10.06–78.5)	**0.033**[b]	0.172	0.412	**0.009**
ADMA (µmol/L)	0.70±0.14	0.77±0.15	0.77±0.16	0.124[a]	–	–	–
Arginine (µmol/L)	268 (186–453)	309 (192–400)	305.5 (202–605)	0.231[b]	–	–	–
Arginine/ADMA	419.51±107.85	403.97±75.16	432.8±117.6	0.632[a]	–	–	–
CA 125 (IU/mL)	13.44 (5.52–48.32)	30.21 (11.81–126.40)	26.84 (9.01–107.5)	<**0.001**[b]	<**0.001**	0.223	<**0.001**
CEA (ng/mL)	2.52 (0.69–6.61)	6.57 (1.56–38.50)	3.89 (1–64.33)	<**0.001**[b]	<**0.001**	0.181	<**0.001**
LDH (IU/L)	177 (132–346)	214 (134–270)	188.50 (93–280)	0.184[b]	–	–	–
Cyfra 21-1 (ng/mL)	1.28 (0.72–3.86)	3.57 (1.06–73.75)	7.33 (1.18–97.60)	<**0.001**[b]	<**0.001**	**0.041**	<**0.001**
NSE (ng/mL)	13.48±3.99	20.60±9.71	19.34±6.84	<**0.001**[a]	**0.002**	0.787	**0.001**

Parametric data are presented as mean±standard deviation. while non-parametric data are presented as median (min–max). a: One-Way Analysis of Variance (ANOVA) b: Kruskal-Wallis test; VEGF: Vascular endothelial growth factor; ADMA: Asymmetric dimethyl arginine; NO: Nitric oxide; CEA: Carcinoembryonic antigen; CA 125: Cancer antigen 125; NSE: Neuron specific enolase; LDH: Lactate dehydrogenase; NSCLC: Non-small cell lung cancer.

Table 3. Findings of serum levels of VEGF, NO, ADMA, arginine, CA 125, CEA, NSE, LDH, and Cyfra 21-1 in early stage NSCLC (stages 1 and 2), advanced stage NSCLC (stages 3 and 4), and control groups

	Control (a) (n=32)	Early stage NSCLC (b) (n=16)	Advanced stage NSCLC (c) (n=40)	p	a/b	b/c	a/c
VEGF (ng/mL)	397.9 (150.5–779.8)	383.5 (169.4–1052.5)	639.09 (253.2–1812.3)	<0.001[b]*	0.218	**0.032**	<**0.001**
NO (µmol/L)	19.5 (4.8–39.1)	21.10 (10.62–78.56)	25.92 (10.06–78.56)	0.008[b]*	0.585	0.057	**0.002**
ADMA (µmol/L)	0.70±0.14	0.73±0.19	0.78±0.14	0.063[a]	–	–	–
Arginine (µmol/L)	268 (186–453)	323.5 (220–451)	304 (192–605)	0.144[b]	–	–	–
Arginine/ADMA	419.5±107.8	472.7±138.2	403.90±84.34	0.087[a]	–	–	–
CA 125 (IU/mL)	13.4 (5.5–48.3)	18.06 (10.4–117.9)	29.6 (9.01–126.4)	<0.001[b]*	**0.002**	0.229	<**0.001**
CEA (ng/mL)	2.52 (0.69–6.61)	4.73 (1–21.18)	4.36 (1.54–64.33)	<0.001[b]*	**0.002**	0.847	<**0.001**
LDH (IU/L)	177 (132–346)	179.50 (138–280)	205 (93–277)	0.017[b]*	0.646	**0.015**	**0.015**
Cyfra 21-1 (ng/mL)	1.2 (0.72–3.86)	2.4 (1.08–18.80)	12.2 (1.06–73.75)	<0.001[b]*	**0.001**	**0.002**	<**0.001**
NSE (ng/mL)	13.48±3.9	15.15±3.9	21.58±8.2	<0.001[a]*	0.661	**0.003**	<**0.001**

Parametric data are presented as mean±standard deviation. while non-parametric data are presented as median (min–max). a: One-Way Analysis of Variance (ANOVA); b: Kruskal-Wallis test; VEGF: Vascular endothelial growth factor; ADMA: Asymmetric dimethyl arginine; NO: Nitric oxide; CEA: Carcinoembryonic antigen; CA 125: Cancer antigen 125; NSE: Neuron specific enolase; LDH: Lactate dehydrogenase; NSCLC: Non-small cell lung cancer.

and NSE levels were significantly elevated in both the adenocarcinoma and squamous cell carcinoma subtypes, but no differences were detected between the histological subtypes. Cyfra 21-1 levels were found to be elevated in both the squamous cell carcinoma and adenocarcinoma subtypes compared to the control group ($p<0.001$ and $p<0.001$, respectively), and a significant difference was found between the histological subtypes ($p=0.04$).

As shown in Table 3, when patients were categorized into early-stage (stages-1 and 2) and advanced-stage (stages-3 and 4) NSCLC groups and compared with healthy individuals, serum levels of CA 125, CEA, and Cyfra 21-1 were found to be significantly elevated in the early-stage NSCLC group compared to controls ($p=0.002$, $p=0.002$, and $p=0.001$, respectively). No notable differences were

detected between early-stage NSCLC patients and the controls in terms of serum VEGF, NO, ADMA, arginine, LDH, NSE, and arginine/ADMA ratio. However, a comparison of the advanced-stage NSCLC group with the control group revealed that the serum levels of VEGF, NO, CA 125, CEA, LDH, Cyfra 21-1, and NSE were significantly increased ($p<0.001$, $p=0.002$, $p<0.001$, $p<0.001$, $p=0.015$, $p<0.001$, and $p<0.001$, respectively).

When examining our biochemical parameters across different groups based on primary tumor size (T), Cyfra 21-1 and NSE levels were different among the groups ($p<0.001$ and $p=0.034$, respectively). In terms of regional lymph node involvement (N), serum ADMA, LDH, and Cyfra 21-1 levels varied significantly among the groups ($p=0.002$, $p=0.022$, and $p=0.021$, respectively). Regarding the presence of metastasis (M), notable differences were observed in CA-125, LDH, and NSE levels ($p=0.042$, $p=0.048$, and $p=0.009$, respectively). Lastly, LDH, Cyfra 21-1, and NSE levels demonstrated significant differences when comparing the results among the stages of the TNM classification ($p=0.027$, $p=0.012$, and $p=0.005$, respectively). The results according to the staging systems are presented in Table 4.

In the ROC analysis detailed in Table 5, the diagnostic parameters intended to differentiate the NSCLC group from the control group indicated that VEGF, NO, CA 125, CEA, Cyfra 21-1, and NSE were effective in the diagnosis ($p<0.001$, $p=0.006$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively).

DISCUSSION

Although both invasive and non-invasive methods exist for the diagnosis of lung cancer, their application is limited. The limitations in early detection and screening methods for lung cancer, coupled with the insufficient diagnostic accuracy of laboratory tests and the lack of clinical symptoms until the disease has progressed to an advanced stage. These challenges underscore the need for more effective and accessible diagnostic tools to improve early detection rates of lung cancer. In the context of NSCLC, various markers have been assessed to increase diagnostic accuracy, assess disease progression, and monitor treatment response.

Angiogenesis plays a crucial role in the progression from a premalignant lesion to a malignant lesion and ultimately to metastasis. For tumor tissue to grow beyond a size of 2 mm^3, angiogenesis is essential; without vascular support, tumors may undergo necrosis or apoptosis [21]. Hypoxia triggers angiogenesis in tumor tissue, and as the tumors grow, hypoxia reoccurs, creating a vicious cycle that continually stimulates angiogenesis [22]. Among the various molecules involved in the pathogenesis of angiogenesis, VEGF stands out as the key molecule. Therefore, VEGF levels have been reported to increase in many types of cancer [17, 23]. VEGF increases not only in malignant conditions but also in various benign situations; therefore, patients with chronic diseases were excluded from our study [24, 25]. Lai et al. [14] found that serum VEGF exhibited sensitivities of 75% and 75%, with specificities of 93.3% and 95.6%, respectively, in distinguishing patients with NSCLC from healthy individuals and those with benign pulmonary nodules, and that combining VEGF with CA 125, CEA, and Cyfra 21-1 further increased the diagnostic accuracy. Tamura et al. [26] reported that plasma and serum VEGF levels were elevated in patients with primary lung cancer compared with healthy controls. They also reported positive correlations between plasma VEGF, intratumoral VEGF concentrations, and microvessel density. It has been reported that VEGF levels do not differ across the histological subtypes of NSCLC [27, 28]. In contrast to these studies, Shimanuki et al. [29] reported that in a cohort of 63 preoperative NSCLC patients, serum VEGF levels were elevated in the squamous cell carcinoma subtype compared with the adenocarcinoma subtype, and that serum VEGF levels correlated solely with disease TNM stage, with no significant association with T or N factors.

Increasing evidence indicates that NO, due to its lipophilic nature and ability to diffuse between cells, plays a significant role in the pathophysiology of various cancers by influencing tumor formation, progression, and characteristics [30, 31]. It has been reported that genetic disruption of inducible nitric oxide synthase (iNOS) significantly reduces lung tumor formation in mice by 80% along with a 54% reduction in the VEGF concentration in tumor tissue, suggesting that NO modulates angiogenesis in lung tumors [18]. Colakogullari et al. [32] reported that in their study of 31 patients with lung cancer, and 15 control patients, serum nitrate and VEGF levels were elevated in lung patients compared with controls, and increased serum nitrate levels were linked to poor prognosis. In our study, serum NO levels in NSCLC patients were found to be elevated, which is

Table 4. Evaluation of serum levels of VEGF, NO, ADMA, Cyfra 21-1, CEA, LDH, NSE, and CA-125 according to primary tumor size (T), regional lymph node involvement (N), distant metastasis (M), and TNM stage

	VEGF	NO	ADMA	CA 125	CEA	LDH	Cyfra 21-1	NSE
T								
T1	498.2±364.8	22.9 (17.5– 40.4)	0.65±0.12	17.7 (10.4–117.9)	4.1 (1–21.1)	188±27.3	2.31[a] (1.08–20.14)	13.7[a]±3.02
T2	554.3±242.8	21.4 (13.9–72.3)	0.82±0.21	27.9 (10.4–107.5)	4.5 (1.5–12.8)	194.1±37	2.55 (1.06–10.31)	17.2±6.9
T3	706.2±424.5	22.9 (10.6–60.6)	0.75±0.11	20.7 (9–95.6)	5.1 (1.5–64.3)	197.4±48	5.52 (1.06–51.02)	22.2[b]±9.4
T4	747.1±358.1	26.9 (10–78.5)	0.79±0.14	34.4 (15.9–126.4)	3.8 (1.6–38.5)	207.3±38.2	21.8[b] (1.93–73.75)	21.7[b]±7.04
p	0.249	0.401	0.092	0.317	0.804	0.637	<**0.001**	**0.034**
N								
N0	608 (169–1221)	22.9 (10.6–55.9)	0.7±0.15[a]	27.9 (10.4–117.9)	3.9 (1–21.1)	183.4±29.3[a]	3.26[a] (1.08–51.48)	16.3±5.1
N1	376 (323–849)	31.2 (17.1–55.7)	0.88±0.21[b]	18 (10.4–93.4)	4.5 (1.6–12.8)	198.8±41.7	2.4 (1.06–33.61)	18.5±7.01
N2	499 (253–1812)	22.9 (10–72.3)	0.74±0.12	22.2 (9.01–88.3)	3.9 (1.5–14.3)	195.6± 43	6.90 (1.06–25.99)	20.7±8.5
N3	682 (408–1406)	24.8 (15.1–78.5)	0.84±0.15[b]	50.9 (17.4–126.4)	6.5 (2.5–64.3)	226.1±33.3[b]	23.2[b] (1.18–73.75)	23.6±8.6
p	0.187	0.291	**0.020**	0.181	0.266	**0.022**	**0.021**	0.063
M								
M0	504 (169–1812)	24 (10.6–72.3)	0.76±0.17	21.3 (9–117.9)	4.1 (1–21.1)	192±38.9	5.99 (1.08–51.48)	15.8 (10–41.4)
M1	630 (334–1396)	23.3 (10–78.5)	0.77±0.12	37.3 (12.3–126.4)	5.8 (1.5–64.3)	214.1±36.2	12.9 (1.06–73.75)	25 (6.5–36.6)
p	0.265	0.833	0.823	**0.042**	0.184	**0.048**	0.316	**0.009**
TNM								
S-1	294 (169–1052)	22.1 (13.9–40.4)	0.64±0.11	22.2 (10.4–117.9)	4.1 (1–21.1)	178.5±27.4[a]	2.35 (1.08–3.91)	14.07±3.02[a]
S-2	450 (323–906)	18.9 (10.6–55.7)	0.82±0.21	18 (10.4–95.6)	4.9 (2.5–12.8)	184.6±41.8	3.01 (1.13–18.8)	16.2±4.61
S-3	571 (253–1812)	27.2 (14.5–72.3)	0.79±0.17	21.3 (9.01–58)	3.8 (1.5–12.3)	193.5±37.2	9.29 (1.18–51.48)	19.1±7.43
S-4	656 (334–1396)	24.6 (10–78.5)	0.78±0.11	44.1 (12.3–126.4)	5.8 (1.5–64.3)	218±37.6[b]	20.7 (1.06–73.75)	24.02±8.4[b]
p	0.225	0.215	0.075	0.067	0.280	**0.027**	**0.012**	**0.005**

VEGF: Vascular endothelial growth factor; ADMA: Asymmetric dimethyl arginine; NO: Nitric oxide; CEA: Carcinoembryonic antigen; CA 125: Cancer antigen 125; NSE: Neuron specific enolase; LDH: Lactate dehydrogenase.

Table 5. Effectiveness of VEGF, NO, CEA, CA 125, Cyfra 21-1, and their combinations in differentiating NSCLC patients from the control group according to ROC analysis

	ROC analysis			Diagnostic measures (%)			
	AUC (%95 CI)	p	Cut-off value	Sensitivity	Specificity	NPV	PPV
VEGF (ng/mL)	0.723 (0.618–0.829)	**<0.001**	≥557.43	54	88	81	65
NO (µmol/L)	0.660 (0.545–0.774)	**0.006**	≥16.03	86	44	60	75
ADMA (µmol/L)	0.606 (0.485–0.726)	0.084	≥0.71	66	53	58	61
Arginine (µmol/L)	0.609 (0.489–0.729)	0.076	≥247	84	47	61	74
CA 125 (IU/mL)	0.809 (0.718–0.901)	**<0.001**	≥16.36	82	72	74	80
CEA (ng/mL)	0.768 (0.668–0.868)	**<0.001**	≥4.62	51	94	89	66
LDH (IU/L)	0.605 (0.485–0.725)	0.087	≥179	73	53	61	66
Cyfra 21-1 (ng/mL)	0.883 (0.815–0.952)	**<0.001**	≥2.61	71	94	92	77
NSE (ng/mL)	0.750 (0.649–0.851)	**<0.001**	≥14.02	77	66	69	74
VEGF + CA 125 + Cyfra 21-1	0.926 (0.869–0.983)	**<0.001**	≥0.824	73	100	100	79
CEA + CA 125 + Cyfra 21-1	0.932 (0.877–0.987)	**<0.001**	≥0.486	85	91	90	86

ROC: Receiver operating characteristic; CI: Confidence interval; VEGF: Vascular endothelial growth factor; ADMA: Asymmetric dimethyl arginine; NO: Nitric oxide; CEA: Carcinoembryonic antigen; CA 125: Cancer antigen 125; NSE: Neuron specific enolase; LDH: Lactate dehydrogenase; NSCLC: Non-small cell lung cancer; PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under curve.

consistent with the literature; however, no relationship between VEGF and NO was observed.

The rise in ADMA is primarily due to increased protein methylation and metabolism, decreased dimethylarginine DDAH enzyme activity, and reduced urinary excretion of ADMA, with its concentration most commonly increasing alongside NO levels due to reduced DDAH activity in inflammation and oxidative stress, which is significant in cancer development [19]. Elevated plasma ADMA levels have been reported in various diseases, including hypertension, coronary artery disease, and cancer [33, 34]. However, studies on ADMA in relation to lung cancer are limited. Similar to our study, Bayraktutan et al. [35] demonstrated that both plasma NO and ADMA levels are elevated in patients with lung cancer. Increased ADMA in cancer may result from reduced DDAH activity due to abnormal oxidative stress associated with the downregulation of antioxidant enzymes or mitochondrial dysfunction in malignant cells [36].

In a study conducted in patients with NSCLC, the diagnostic sensitivity at presentation was 76% for Cyfra 21-1, 55% for CA 125, 52% for CEA, and 22% for NSE, with all tumor markers except for NSE showing a clear association with tumor histology and stage; Cyfra 21-1 was identified as the most sensitive marker, particularly when combined with CA 125 and CEA, raising the sensitivity to above 90% [37]. In another study involving 53 patients with NSCLC and 27 with benign lung diseases, the sensitivity of Cyfra 21-1 for diagnosis was found to be 50.9%, with a specificity of 81.4% and an AUC of 0.698 [38]. In the study by Chen et al. [39], involving 236 patients with early-stage NSCLC and 44 participants with benign lung diseases, the combined evaluation of CEA and Cyfra 21-1 resulted in a sensitivity of 47% and a specificity of 81%. It has been reported that using these markers in combination rather than individually may improve diagnostic accuracy [40]. In our study, CA 125 demonstrated a sensitivity of 72% and a specificity of 82%, CEA exhibited a sensitivity of 51% and a specificity of 94%, and Cyfra 21-1 exhibited a sensitivity of 71% and a specificity of 94% in the diagnosis of NSCLC. Additionally, when used in combination, they reached a sensitivity of 85% and a specificity of 91%. Additionally, we observed that CA 125, Cyfra 21-1, and CEA levels were elevated in early-stage NSCLC compared to the control group, demonstrating the utility of assessing these three tumor markers even in early-stage NSCLC.

One of the key strengths of our study is that the study cohort comprised individuals newly diagnosed with no comorbidities, who were not on any medications and had not received any antitumor treatment. Moreover, as far as we are aware, this is the first re-

search to simultaneously demonstrate the role of the VEGF, NO, and ADMA pathways in lung cancer.

The limitations of our study should be addressed. First, a separate group of patients with benign lung conditions could have provided a more comprehensive comparison. Second, we did not assess patient prognosis or survival, which restricts our ability to evaluate the long-term clinical significance of the biomarkers studied. Additionally, the inability to examine the expression of these molecules in tissue samples and DDAH activity limits the understanding of their role in lung cancer. Finally, the relatively small number of participants and the single-center design of our research may restrict the applicability of our results to broader populations.

Conclusion

Our study revealed that the serum concentrations of VEGF, NO, ADMA, CA 125, Cyfra 21-1, CEA, and NSE were elevated in patients with NSCLC compared with controls and that the levels of Cyfra 21-1, LDH, and NSE increased with advancing TNM stage. In the diagnosis of NSCLC, the diagnostic performance of Cyfra 21-1 was the most pronounced, recording an AUC of 0.883, a sensitivity rate of 71%, and a specificity rate of 94%. The combination of VEGF, CA 125, and Cyfra 21-1 showed an AUC of 0.925, 73% sensitivity, and 100% specificity, whereas the combination of CA 125, CEA, and Cyfra 21-1 achieved an AUC of 0.932, 85% sensitivity, and 91% specificity. Further studies involving larger populations, including those with benign lung diseases, are needed to validate and expand upon our findings.

Ethics Committee Approval: The Selcuk University Clinical Research Ethics Committee granted approval for this study (date: 07.11.2018, number: 2018/21).

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