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Changes in glycocalyx related biochemical parameters during lung resection in non-small cell carcinoma cases: A pilot study

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ABSTRACT

Objective: Non-small cell lung cancer (NSCLC) is one of the most common neoplasms with high mortality rates, and new studies are needed to understand its characteristics better. This study aimed to determine the changes in the glycocalyx structure related to surgery regarding histopathologic subtypes and to evaluate the correlation of these changes on the development of metastasis and mortality.

Material and methods: Serum levels of hyaluronan, VEGF-A, FGF-10, BMP-2, and BMP-4 were measured before and after surgery in 42 patients with NSCLC. The alterations in serum levels of studied markers were evaluated as related to metastasis status and mortality in post-operative 18-24 months.

Results: Our study included 15 adenocarcinoma and 27 squamous cell carcinoma cases. Pre- and post-operative values of serum hyaluronan, VEGF-A, FGF-10, and BMP-2 showed significant differences for the whole group (p=0.006, p=0.001, p=0.002, and p=0.004, respectively). Post-operative BMP-2 values also correlated with hyaluronan and VEGF-A values. Post-operative values of hyaluronan and VEGF-A values found incorrelation with metastasis and mortality, while BMP-2 with metastasis and FGF-10 with mortality. Serum values of hyaluronan, VEGF-A, FGF-10 and BMP-2 differed significantly in-between the pre- and post-operative measurements in adenocarcinoma cases (p=0.020, p=0.009, p=0.003, and p=0.011, respectively) , but not that of squamous cell carcinoma.

Conclusion: Pre- and post-operative changes in serum hyaluronan, VEGF-A, FGF-10 and BMP-2 values may be associated with metastasis and/or mortality in NSCLC. These findings were also more prominent in adenocarcinoma cases, though further extended studies are needed for a better conclusion.

Keywords: Non-small cell lung cancer, Surgery, Glycocalyx, Hyaluronan, VEGF-A, FGF-10, BMP-2, BMP-4

INTRODUCTION

Lung cancer is one of the most common and fatal neoplasms in the World. Unfortunately, most of the lung cancer patients are still diagnosed in advanced stages. The 5-year survival rate of patients with stage I non-small cell lung cancer (NSCLC) is 54.8%, while that of patients with stage IV is 4.2% 1. Due to the high mortality rates in lung cancer despite advanced treatment modalities, new scientific strategies are being developed to determine the prognosis (1, 2) .

Glycocalyx is a gel-like layer that covers the luminal surface of vascular endothelial cells, and maintains the homeostasis of the blood in the vascular system interacting with mechanotransduction, signaling, and blood cell–vessel wall interactions. Glycocalyx also composed of glycoproteins and proteoglycans in structure, and glycosaminoglycans (heparan sulfate, chondroitin sulfate, hyaluronan) are also closely related to this structure (3). Interestingly, hyaluronan, fibroblast growth factor (FGF) and VEGF (vascular endothelial growth factor), which are included in the functional structure of the glycocalyx, are among the biochemical markers studied in prognosis of lung cancer (4-6). Bone morphogenetic proteins (BMPs) play a role not only in embryonic and postnatal development, but also in tumor development and spread. Several experimental and clinical studies have also demonstrated the correlation between serum levels of BMP-2 or BMP-4 with poor prognosis or metastasis in NSCLC (7).

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Some studies have shown that glycocalyx damage may occur during the operations in patients with acute care surgeries as well as pulmonary resections (8, 9). In our literature review, we could not find out a prospective study on the possible relationship in-between the glycocalyx injury and the metastasis and mortality of cancer, in patients with NSCLC undergoing pulmonary resection.

The aim of this study is to determine the changes in serum markers (hyaluronan, VEGF-A, FGF-10) and molecular prognostic factors (BMP-2, BMP-4) of endothelial glycocalyx damage during surgery in NSCLC. In addition, we aimed to examine the relationship between the studied markers and post-operative mortality and metastasis.

MATERIAL and METHODs

This prospective cohort study was conducted in the Thoracic Surgery Department of Aydın Adnan Menderes University Medical Faculty Hospital between 2018-2021 after the approval of the ethics committee (2018/1444). Patients with NSCLC and scheduled for elective segmentectomy, lobectomy or pneumonectomy were included in the study after obtaining the informed consent. The exclusion criteria were; patients with primary lung cancers other than NSCLC, metastatic cancers, body mass index > 35 kg/m2, heart failure (>New York Heart Association class II), pulmonary hypertension, clinically severe obstructive or restrictive lung diseases or existence of coagulation disorders.

Pre-operative Evaluations, Anesthesia and Surgical Technique

In all cases, pulmonary function tests, blood gas analysis, and if necessary, V/Q (ventilation/perfusion) scan were performed to evaluate respiratory physiology in the pre-operative period. A 6-minute walk test was performed for cardiac evaluation, and in high-risk patients, a cardiology consultation with additional examinations was performed. All patients underwent thoracic computed tomography (CT scan) and positron emission tomography (PET) scanning to evaluate their AJCC designated staging by TNM (tumor, node, metastasis) classification to define NSCLC and the resectability of pulmonary malignancies before surgery (10) . For histopathological diagnosis flexible bronchoscopy was performed in all cases and also transthoracic needle aspiration biopsy under radiological guidance was achieved in appropriate cases.

All patients received 0.02 mg/kg midazolam for premedication. For general anesthesia induction, 1.5-2.5 mg/kg propofol, 1 µg/kg fentanyl and 0.8 mg/kg rocuronium were used. Tracheal intubation was performed with a double-lumen endotracheal tube, and the correct position of the tube was confirmed by fiberoptic bronchoscopy. Maintenance of anesthesia was provided by 0.1-0.4 µg/kg/min infusion of remifentanil and 0.5-2% sevoflurane. The patients were placed in the lateral decubitus position, and single lung ventilation (OLV) was started. After the resection was completed, double lung ventilation was started again. During the operation all fluid and blood treatments, hemodynamic data, vasoactive drug usage, and urine quantities of the patients were recorded.

Open thoracotomy was performed in our patients with a standard muscle-sparing posterolateral approach. During surgery, pulmonary resection (segmentectomy, lobectomy or pneumonectomy) with radical mediastinal lymph node dissection was performed in all cases after the malignancy was confirmed by examination of the frozen sections.

Biochemical Analysis of Specific Serum Proteins

All blood samples were obtained from the central vein catheter; first 15 minutes after anesthesia induction, just before the operation and then in the first hour after the operation and serum levels of hyaluronan, VEGF-A, FGF-10, BMP-2 and BMP-4 were measured. The correlation between pre- and post-operative changes in serum levels of these markers and the development of metastasis and mortality were evaluated.

An enzyme-linked immunosorbent assay (ELISA) was used to detect hyaluronan, VEGF-A, FGF-10, BMP-2 and, BMP4 levels in the collected blood serum samples. After centrifugation at 3500 rpm for 5 min at room temperature, the serum samples were stored at -80˚C until the tests were studied. All samples were thawed only once before use.

Serum hyaluronan levels were analyzed by using Human Hyaluronic Acid (HA) ELISA Kit-Sunred Catalogue NO: 201-12-1375 with inter-assay CV:<12%, and intra-assay CV:<10%, respectively. The mean detectable dose (MDD) of human HA was 2.113 ng/mL.

Serum VEGF-A levels were analyzed by using Human Vascular Endothelial Cell Growth Factor A (VEGF-A) ELISA Kit-Sunred Catalogue NO: 201-12-0051 with inter-assay CV: <12% and intra-assay CV: <10%, respectively. The MDD of human VEGF-A was 2.677 pg/mL.

Serum FGF-10 levels were analyzed by using Human Fibroblast Growth Factor 10 (FGF-10) ELISA Kit-Sunred Catalogue NO: SRB-T-81339 with inter-assay CV: <12% and intra-assay CV: <10%, respectively. The MDD of human FGF-10 was 4.451 ng/mL.

Serum BMP-2 levels were analyzed by using Human Bone Morphogenetic Protein 2 (BMP-2) ELISA Kit-Sunred Catalogue NO: 201-12-1990 with inter-assay CV: <12% and intra-assay CV: <10%, respectively. The MDD of human BMP-2 was 1.233 ng/L.

Serum BMP4 levels were analyzed by using Human Bone Morphogenetic Protein 4 (BMP4) ELISA Kit-Sunred Catalogue NO: 201-12-1991 with inter-assay CV:<11%, and intra-assay CV:<8%, respectively. The MDD of human BMP was 0.927 ng/L.

Clinical Follow-up & Detection of Metastases

Before and after surgical resections, all patients' physical examinations and follow-ups were performed by both the thoracic surgeon and a clinical oncologist. The patients were followed up with clinical examinations as well as radiological evaluations (chest x-ray, CT scans, PET) in the post-operative third month and then by three to four months intervals in the first two years. Only the results of the first post-operative two years of follow-up periods were evaluated in this study. If suspected lesions were detected, further evaluations and histopathological confirmation were performed as necessary.

Statistical Analysis

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The Kolmogorov-Smirnov test was used to assess the normality of numeric variables. For variables that weren't normally distributed, Wilcoxon T or Mann–Whitney U tests made comparisons between paired or independent two groups. Descriptive statistics are presented as mean ± standard deviation, median (25-75 percentiles) and frequency. Spearman correlation test was used to determine the relationship between biochemical markers and long-term metastasis and mortality. The p values below 0.05 were considered as statistically significant.

RESULTS

Our study included 15 adenocarcinoma and 27 squamous cell carcinoma cases. The demographical and clinical characteristics of the patients are summarized in Table 1. During the post-operative 18-24 months of follow-up, distant organ metastases (in the brain, liver, bones and opposite lung) were observed in 9 (21.4%) patients and death was observed in 7 (16.7%). The gender and age of the patients, histopathologic subtype, and stage of the cancer were not associated with the development of metastasis or mortality in the first two years of the post-operative follow-up (p>0.05 for all). Also the type of operation, operation time and the length of stay in the ICU in the post-operative period were not associated with the development of metastasis or mortality (p>0.05 for all).

Pre- and postoperative serum hyaluronan, VEGF-A, BMP-2, and FGF-10 values had differed significantly (p=0.006, p=0.001, p=0.004, and p=0.002, respectively) (Table 2). These differences between the measured values were not found to be related with either the gender or ages of the patients and the stage of the cancer (p>0.05). Also, the type of operation, operation time and length of stay in the ICU were not associated with these measurements significantly (p>0.05). According to the pre-operative values hyaluronan, VEGF-A, FGF-10, and BMP-2 values changed significantly in post-operative measurements in adenocarcinoma cases (p=0.020, p=0.009, p=0.003, and p=0.011, respectively), while there was no difference in squamous cell carcinoma (Table 3).

A significant difference was found between metastasis development and post-operative hyaluronan, VEGF-A and BMP-2 values (p= 0.001, p=0.002, and p=0.009, respectively) (Fig. 1A, B and C). Mortality was also found to be associated with post-operative hyaluronan, VEGF-A and FGF-10 values (p=0.001, p=0.001, and p=0.049, respectively) (Fig. 2A, B, and C). After the operation, VEGF-A values were correlated with hyaluronan and BMP-2 values (p=0.0001, r= 0.687 and p=0.0001, r=0.528, respectively), while hyaluronan values were also correlated with BMP-2 values (p=0.0001, r= 0.543).

Table 1. Demographical and Clinical Characteristics of Patients

Cancer Histopathology (n / %)	**SCC*** 27/62.8	**AC**** 15/37.2	**All** 42/100	**P value**
Age; (Mean±SD) (years)	64.18±7.24	65.46±8.5	64.64±7.6	0.503
Gender (n/%)				
Female	4/14.8	1/6.7	5/11.9	0.425
Male	23/85.2	14/93.3	37/88.1	
Stage[Ψ] (n/%)				
Ia	13/ 30.1	5/ 11.9	18/ 42	
Ib	2/ 4.7	1/ 2.3	3/ 7	
IIa	3/ 7.2	2/ 4.7	5/ 11.9	0.355
IIb	3/ 7.2	2/ 4.7	5/ 11.9	
IIIa	5/ 11.9	5/ 11.9	10/ 23.8	
IIIb	1/ 2.3	0/ 0	1/ 2.3	
Operation type (n/%)				
Segmentectomy	2/ 4.7	1/ 2.3	3/ 7	0.09
Lobectomy	18/ 42.8	14/ 33.3	32/ 77.1	
Pneomonectomy	7/ 16.6	0/ 0	7/ 16.6	
Mortality (n/%)	5/ 11.9	2/ 4.7	7/ 16.6	0.669
Metastasis (n/%)	6/ 14.2	3/ 7.1	9/ 21.3	0.868
Duration of Operation (Mean± SD) (minutes)	294±12.6	298±18.82	295.83±17.53	0.094
Duration of ICU[Ω] **stay**				
Median (Min-Max) (days)	2.5 (0-114)	2.2 (2-103)	2.3 (0-114)	0.124
Per centile 25-75	2-3	2-3	2-3	

* Squamous Cell Carcinoma, ** Adenocarcinoma, [Ψ] The AJCC, TNM (tumor, node, metastasis) classification to define NSCLC (10), [Ω] Intensive Care Unit, n=number, %= percentage of cases of the same histopathology

Table 2. Pre- and Post-Operative Marker Levels of All Patients

Marker	Pre-operative	Post-operative	P values
Hyaluronan (ng/mL)			
Median	65.21	77.71	0.006
Min-Max	38.59-188.14	44.30-432.87	
Percentile	59.03	61.20	
25 75	91.82	168.99	
VEGF-A (pg/mL)			
Median	45.91	52.56	0.001
Min-Max	8.99 – 98.58	22.83 - 625.72	
Percentile	29.90	95.02	
25 75	52.96	170.59	
BMP-2 (pg/mL)			
Median	60.02	69.51	0.004
Min-Max	28,22- 199.97	27.50-420.20	
Percentile	45.72	49.31	
25 75	67.57	147.81	
BMP-4 (pg/mL)			
Median	76.76	72.20	0.069
Min-Max	14.07-292.67	18.75-273.37	
Percentile	64.81	65.38	
25 75	103.38	84.74	
FGF-10 (pg/mL)			
Median	445.74	324.49	0.002
Min-Max	103.75-1351.86	70.68-991.44	
Percentile	217.01	163.56	
25 75	602.28	539.91	

Table 3. Pre- and Post-Operative Marker Levels and Histopathologic Subtypes

	Pre-operative Median (percentile 25%-75%)	**Post-operative** Median (percentile 25%-75%)	P values
	Adenocarcinoma		
Hyaluronan (ng/mL)	67,71 (59,19-93,55)	75,19 (62,67-171,89)	**0,020**
VEGFA (pg/mL)	42,59 (17,85-57,07)	61,89 (22,83-135,50)	**0,009**
BMP2 (pg/mL)	59,63 (46,7-67,52)	72,36 (50,68-148,31)	**0,011**
BMP4 (pg/mL)	76,22 (68,09-103,17)	73,12 (66,13-91,04)	0,061
FGF10 (pg/mL)	465,59 (313,4-607,45)	335,2 (220,39-542,48)	**0,003**
	Squamous Cell Carcinoma		
Hyaluronan (pg/mL)	62,65 (58,57-91,25)	80,24 (59,19-168,54)	0,156
VEGFA (pg/mL)	48,35 (11,78-74,99)	75,31 (16,68-99,53)	0,069
BMP2(pg/mL)	61,41 (42,69-67,72)	67,31 (45,99-143,2)	0,112
BMP (pg/mL)	80,92 (63,29-104,02)	71,74 (59,9-82,66)	0,609
FGF10 (pg/mL)	322,05 (157,11-557,98)	244,45 (132,22-477,29)	0,256

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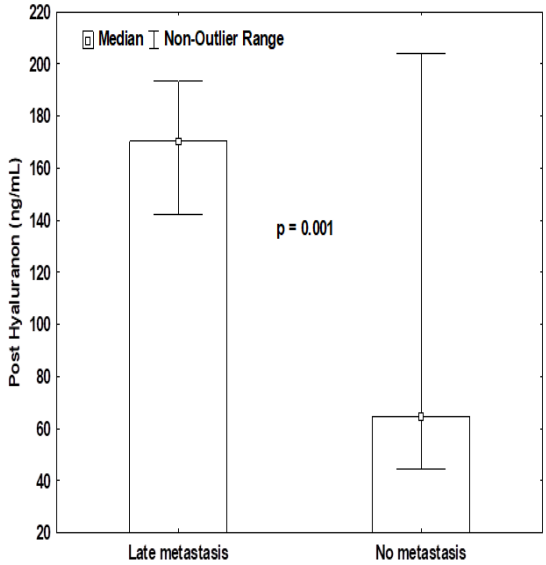

Figure 1A. Postoperative Hyaluronan Levels and Metastasis

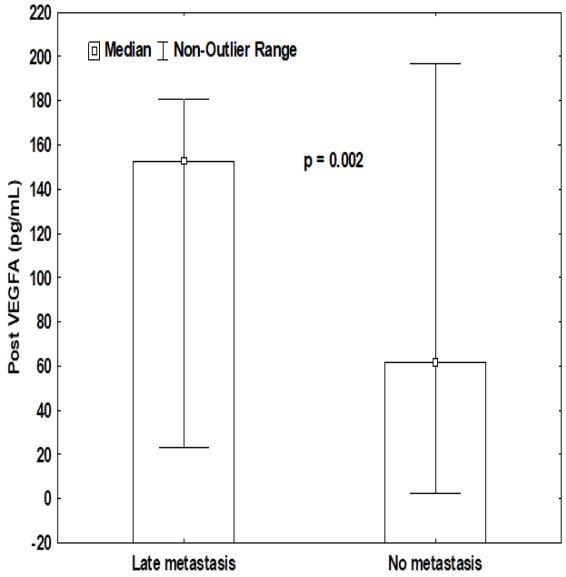

Figure 1B. Postoperative VEGF-A Levels and Metastasis

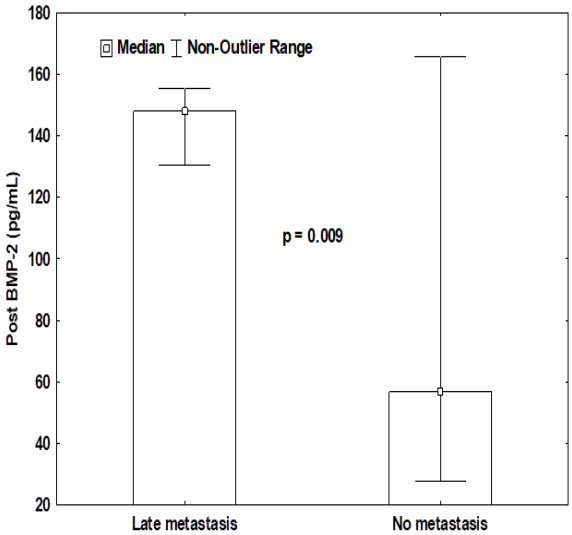

Figure 1C. Postoperative BMP-2 Levels and Metastasis

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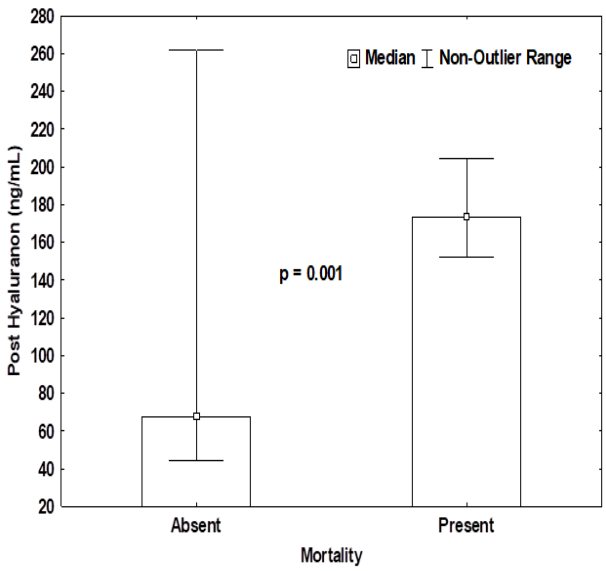

Figure 2A. Post-operative Hyaluronan Levels and Mortality

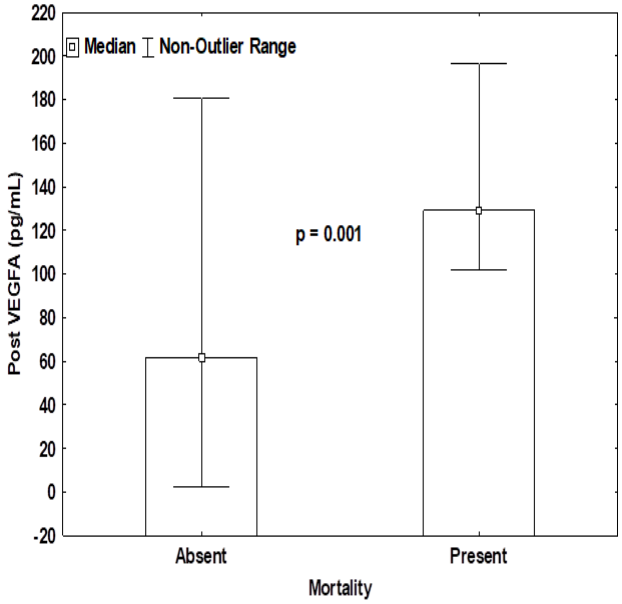

Figure 2B. Postoperative VEGF-A Levels and Mortality

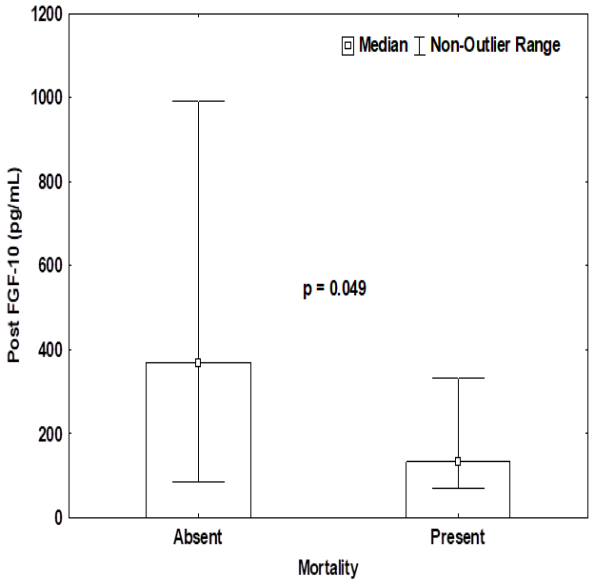

Figure 2C. Postoperative FGF-10 Levels and Mortality

DISCUSSION

In this study, significant changes were observed in serum levels of glycocalyx products (hyaluronan, VEGF-A and FGF-10) and molecular prognostic factors (BMP-2) during pulmonary resection operations in NSCLC patients. The differences were more prominent in adenocarcinoma cases, but not in squamous cell carcinoma. Another important finding was found in relationship between the studied biochemical parameters and metastasis development (hyaluronan, VEGF-A and BMP-2) as well as the mortality (hyaluronan, VEGF and FGF-10).

The endothelial glycocalyx plays an important role in homeostasis by regulating vascular permeability and reducing inflammation and coagulation. The endothelial glycocalyx structure includes membrane-bound (proteoglycan-fixed) sulfated glycosaminoglycans such as heparan sulfate and chondroitin sulfate, and non-sulfated (non-proteoglycan-bound) glycosaminoglycan hyaluronan (3) . In some vascular diseases such as atherosclerosis, stroke, hypertension, kidney diseases and sepsis, the glycocalyx is damaged and this causes increased level of glycocalyx components in the blood circulation (11) . Acute care surgery may also result with glycocalyx damage during the operation (8,9) . Inflammatory reactions caused by ischemia-reperfusion injury, hypoxia-reoxygenation and tissue trauma during the single lung ventilation for pulmonary resection surgeries may cause damage of the alveolocapillary glycocalyx (9,12) . Similarly, in this study, an increase was observed in the hyaluronan serum levels as an endothelial glycocalyx breakdown biomarker in the post-operative period. While hyaluronan has beneficial effects during normal tissue and wound healing, some forms can lead to an increase in metastasis and poor prognosis as a result of the changes in both production and disintegration processes in the presence of tumor cells (13). Several studies have shown the importance of hyaluronan in the progression of various human cancers such as breast, ovarian, colorectal, and lung cancers (4,14-16).

Increased hyaluronan levels in the stroma of NSCLC cases; especially in adenocarcinomas were shown to have significant prognostic value (17). In accordance with the literature, hyaluronan levels increased in all cases in the post-operative period in our study. Furthermore, the increase in serum hyaluronan level in the post-operative period was found to be associated with metastasis and mortality in our study. Similarly, Gong et al. (18) demonstrated that tumor hyaluronan level could be used as a novel biomarker for survival and metastasis in NSCLC in their cohort study involving 174 patients. In this study, the increase in serum VEGF-A level in the post-operative period was found to be associated with metastasis and mortality. Platelets and macrophages secrete VEGF-A in response to tissue damage in early wound healing. While VEGF promotes the growth, development and permeability of endothelial cells under normal conditions, it also increases the repair of the vascular wall in hypoxic and ischemic conditions (19). VEGF, one of the proangiogenic factors secreted by tumor cells, enables the formation of new blood vessels that feed the tumor and performs angiogenesis, a prerequisite for metastasis (20). Hyaluronan and VEGF-A are important biomarkers for the development of angiogenesis in malignancy cases (14). In our study, we found a positive correlation between hyaluronan and VEGF-A serum levels in accordance with the literature. Zhan et.al. (21) also concluded that VEGF overexpression indicates a poor prognosis for patients with NSCLC and small cell lung cancer (SCLC). In our study, we found a significant relationship in-between the rising serum VEGF levels in the post-operative period and mortality. The increment in the levels of angiogenic factors such as VEGF following oncological surgeries is important in residual tumor cell growth and metastasis. VEGF also plays a key role in wound healing, and so the increase in post-operative serum VEGF levels may reflect the extent of surgical trauma (21, 22).

Similarly, Ng et al. (22) investigated the influence of open and video-assisted thoracic surgery (VATS) lung resections for early stage NSCLC on post-operative circulating VEGF (as a circulating angiogenic factor) in their prospective study. The authors concluded that VATS might attenuate the angiogenic response by leading to lower circulating VEGF release compared to open surgery (22). However, they did not investigate the post-operative late metastasis development in NSCLC cases in their study. In our study, open surgery was performed, and a significant relationship was found between VEGF-A increments and late metastasis. In recent years, novel anticancer drugs related to hyaluronan and VEGF-A get promising results in clinical studies, though more randomized controlled studies are needed (18, 21) .

Fibroblast growth factors (FGFs) regulate many cellular functions, including migration, proliferation, differentiation, and survival. Malfunction of the FGF / FGF receptor (FGFR) signal axis can cause many diseases in the lung system, such as chronic obstructive pulmonary diseases, respiratory distress syndrome (RDS) and malignity (23). Twenty-two FGF ligands have been identified for the FGF family in humans. Of these, FGF10 can improve the lung repair and increase the epithelial survival after injury or reduce the inflammatory response after acute lung injury (ALI). It also has roles in alveolar repair and resolution in ALI or acute RDS (23, 24). In our study, serum FGF10 levels were significantly decreased in the post-operative period compared to pre-operative values, and this was also found to be associated with mortality. As in the literature mentioned above, the decrease in serum level of FGF10 during the operation may cause a decrease in its protective effect on the lung, and this may cause a risk in terms of cancer mortality.

In our study, serum BMP-2 levels increased in the post-operative period and were found to be associated with metastasis. However, no significant change was observed in BMP-4 level. BMP2 and BMP4 have been described to mediate pro-angiogenic effects and also promote tumor (ovarian, stomach, lung, colon, breast) angiogenesis through different mechanisms (24). Bieniasz et al. (7) suggested that two angiogenic factors such as VEGF and BMP-2 are interrelated and that they are important for metastasis in lung malignancy.

Our study also found a positive correlation between BMP-2 and VEGF serum levels. The weaknesses of our study can be summarized in four headings. Firstly, biochemical values could not be analyzed simultaneously in both blood samples and tumor tissues. Secondly, cases of open thoracic surgery were included in our study, and no comparison was made with video-assisted thoracoscopic surgery (VATS) cases.

CONCLUSION

In conclusion, pre-and post-operative changes in serum hyaluronan, VEGF-A, FGF-10, and BMP-2 values may be associated with metastasis and/or mortality in NSCLC. These findings were also more prominent in adenocarcinoma cases. Further extended studies are needed for a better conclusion.

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Author Contributions: SC, SS, IKO, AK, SS, SS: Study design, Literature review, Data collection and processing, **SC:** Writing, Revisions

Ethical approval: All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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