Clinical Significance of Serum Vascular Endothelial Growth Factor Levels in Patients with Advanced Non-Small Cell Lung cancer

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Abstract: *Objective:* Due to poor prognosis in advanced non-small cell lung cancer (NSCLC), new markers and more effective treatment methods are needed in the monitoring of the disease. The purpose of our study is to evaluate the clinical significance of serum vascular endothelial growth factor (VEGF) levels in patients with advanced NSCLC on prognosis and survival.

Materials and method: Sixty seven patients (62 men and 5 women) and 20 healthy volunteers (16 men and 4 women) were included in our study. The demographic and laboratory data and serum VEGF levels of two groups were compared.

Results: A statistically significantly high level of VEGF (p=0.0001) was detected in patients compared to the control group. The high level of serum VEGF has a statistically significant relationship with the short disease-free survival time (p=0.05). While the median progression free survival (PFS) time in patients with high VEGF levels was 157 days, the median PFS time in those with normal VEGF levels reached up to 340 days. This difference was statistically significant (p=0.003). When overall survival (OS) times was evaluated, it was 472 days in patients with low VEGF levels and 180 days in those with high levels and the difference was statistically significant (p=0.001).

Conclusion: In our study, the serum VEGF levels were determined to be statistically significant increased in the NSCLC patient group compared to the control group. In addition, a significant inverse relationship was discovered between the serum VEGF level and all survival times in the NSCLC patient group.

Keywords: Lung cancer, VEGF, Prognosis, Survival, Diagnosis.

INTRODUCTION

Lung cancer constitutes an important health problem throughout the whole world due to its ever-increasing incidence and mortality rate. Lung cancer comes in the lead among deaths associated with cancer in both genders. Even though significant developments have been achieved over the recent years in multimodal therapies, 5-year survival could only reach 15%. This situation has directed researchers towards studies aiming to develop efficient and targeted therapies by revealing and in accordance with the genetic, molecular, and biological characteristics of the disease [1].

Today, targeted therapies have started to be used gradually more frequently in the field of cancer treatment. Potential targets have focused on tumor angiogenesis and proliferation, because angiogenesis is an essential basic mechanism for tumor proliferation. Tumor cells increase the secretion of growth factors that are required for the differentiation and migration of endothelial cells that constitute the building blocks of the vein. The vascular endothelial growth factor (VEGF) is the most efficient growth factor for endothelial cells [2].

Development of high angiogenic activity associated with excessive VEGF excretion at a rate of 30% - 40% is reported in non-small cell lung cancer (NSCLC) patients. Many studies have shown that the existence of angiogenesis and micro vessel density constituted negative prognostic factors for survival [3, 4].

Due to poor prognosis in advanced (NSCLC) patients, new markers and more effective treatment methods are needed in the monitoring of the disease. Serum VEGF is the most potent angiogenetic factor defined today. The purpose of our study is to evaluate the impacts of serum VEGF levels in advanced NSCLC patients in diagnosis and prognosis.

MATERIAL AND METHOD

1. Patients and Control Group

With the approval of the Medical Ethics Committee of the Medical Faculty of Pamukkale University, 67 patients, who were examined by the Medical Oncology Department of our hospital for the first time, who had not previously received chemotherapy, and who were pathologically diagnosed with NSCLC, were evaluated in our study. Patients at advanced stage (stage IIIB and

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stage IV) with a performance of 0, 1, and 2 according to the World Health Organization (WHO) classification were included. Voluntariness was taken as basis for participation in the study. Those with a performance of 3 or worse at the time of application, with brain metastasis or suspected brain metastasis, aged 80 or over, with early stage (I, II, and IIIA) disease, and who did not sign the informed consent form were not included in the study.

A control group was formed from 20 healthy subjects between the ages of 53-79 (average 63.1± 6.3), who did not use any medicine and who did not have any known chronic disease. Attention was paid to ensure that the individuals constituting the control group were comparable with the patient group in terms of age and gender. 16 persons within the healthy control group were men (80%) and 4 of them were women (20%).

The age and gender of the patients and the control group, the tumor types, treatments, and pre-treatment performances of patients were recorded. An informative meeting was organized to explain the purpose and scope of the study to the individuals constituting the control and patient groups and written informed consent forms were received from the individuals.

2. Biochemical Analysis

Venous blood samples obtained for VEGF from the patient and control group on an empty stomach in the morning following a night time fasting were taken in vacuum tubes, centrifuged for 10 minutes at 15,000 rpm to separate the serum portion, and preserved in deep-freezer at -70^0 C. VEGF was measured with the ELISA (Enzyme-Linked Immunosorbent Assay) method (Digital and analog system, DAS, Palombara Sabina Italy).

The VEGF cut-off value was calculated with the automatic program included in the packaged software named Statistical Package for Social Sciences version 15.0 (SPSS-15.0, for windows). The cut-off value for VEGF was found to be 934 ng/ml. Those equal to or lower than these values were considered as low and those above were evaluated as high. Different cut-off values are included in NSCLC studies evaluating the VEGF level with the ELISA method in literature, and 500 pg/ml, 630 pg/ml, 500 pg/ml, and 119 pg/ml values were selected as cut-off in four different studies, by which the existence of a significant relationship between prognosis and VEGF was shown, respectively [4].

3. Statistical Analysis

The statistical analysis was conducted with the SPSS-15.0, for windows packaged software. The results were evaluated within the confidence interval of 95%. $P<0.05$ was accepted as statistically significant. The chi-square and the Mann Whitney-U tests were applied in the comparison of the characteristics of the control group and the patients. The Spearman and Pearson correlation test was applied for correlation analyses. The Kaplan Meier method was used for OS and PFS times and time-survival curves. Logistic regression analysis was applied for the analyses of the factors that affected survival and progression.

RESULTS

The main clinical and demographic characteristics of patient and control groups have been shown in Table **1**.

Table 1: Demographic and Clinical Characteristics of Patient and Control Groups

Characteristics	Patient Group (n=67) (%)	Control Group (n=20) (%)
Age (years)	62.9 ± 8.7	63.1±6.3
Gender (female/male)	5 (7.5) / 62 (92.5)	4 (20) / 16 (80)
Performance state 0-1	58 (86.6)	20 (100)
2	9 (13.4)	0
Additional disease (yes)	24 (35.8)	0
Smoking (yes)	54 (80.6)	0

The serum VEGF levels in patient and control groups have been shown in Table **2**.

Table 2: Serum VEGF Distributions of Patient and Control Groups

	Patient (n=67)	Control (n=20)	p Value
VEGF (ng/ml)	1089.7 ± 509	508.6±451.8	0.0001*

*p < 0.05 was considered to be significant.

The patients were divided into two groups as the group with high VEGF level (above 934 ng/ml) and the group with low VEGF levels (934 ng/ml and lower), and evaluated according to their clinical and pathological characteristics in Table **3**.

Table 3: Relationship of Serum VEGF Levels and Clinical Characteristics in the Patient Group

Clinicopathological Characteristics	VEGF Low	VEGF High	P Value
Histology (n=67)			0.757
Squamous	17	27	
Non-squamous	8	15	
Histology (n=67)			0.772
Adenocarcinoma	5	10	
Non-adenocarcinoma	20	32	
Progression (n=67)			0.085
No	8	6	
Yes	17	36	
Final state of the patient (n=67)			0.006*
Alive	11	5	
Exitus	14	37	
Age (n=67)			0.085
Below 55 years	8	6	
55 years and older	17	36	
Existence of metastasis (n=67)			0.068
Yes	5	18	
No	20	24	
Albumin (n=67)			0.077
Above 3 g/dl	25	36	
3 g/dl and lower	0	6	
Performance state (n=67)			0.466
0-1	23	35	
2-4	2	7	

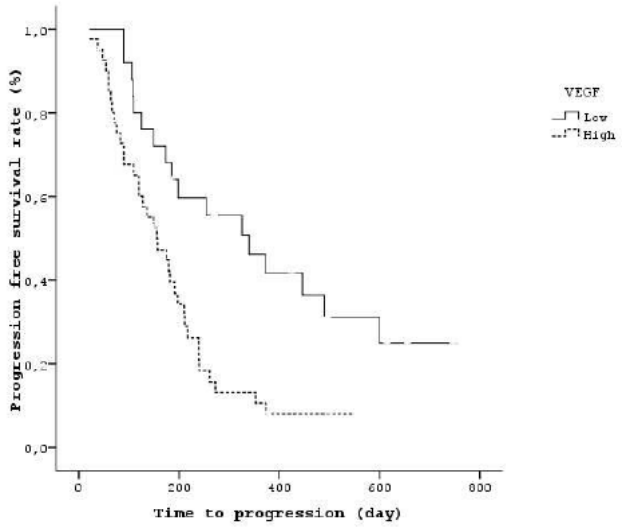

Figure 1: Progression free survival curve according to VEGF level.

While the median PFS time in patients with high VEGF levels was 157 days, the median PFS time in those with normal VEGF levels reached up to 340 days. This difference was statistically significant (p=0.003) (Figure **1**).

When the median OS times was evaluated, this was found to be 472 days in patients with low VEGF levels and 180 days in those with high VEGF levels and the difference in between was statistically significant (p=0.001) (Figure **2**).

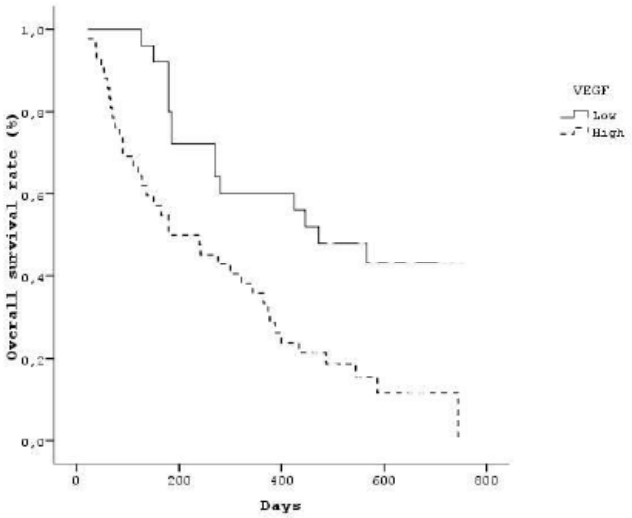

Figure 2: Overall survival curve according to VEGF level.

When evaluated with multivariate analysis, the high levels of VEGF was shown to have significantly affected all OS (8.8 times, p=0.003).

DISCUSSION

VEGF levels, which are known to play an important role in angiogenesis, were found to be high in NSCLC patients compared to healthy control groups [5, 6]. Taş *et al*. [5] compared the serum VEGF levels of 52 NSCLC patients and 16 healthy persons in a control group and found the VEGF level to be higher in the patient group. In another study, a statistically significant difference was found between the serum VEGF levels of 21 advanced NSCLC patients and those of 46 healthy volunteers [6]. We also found the serum VEGF levels to be statistically significantly high in the NSCLC patient group compared to our control group (p=0.0001), which was consistent with the literature.

Since the evaluations of the circulatory quantity of angiogenic factors have certain advantages over IHC (immunohistochemical) evaluation, we examined the

relationship between the serum VEGF value and survival and prognosis. These advantages are that it is more economic, easier, less time consuming, and easily repeatable, it does not require a tumor tissue, it is suitable for preoperative examination, and involves lower possibility of partiality. Delmotte *et al*. [7] examined the serum VEGF values in a total of 1549 NSCLC patients in 15 studies in their meta analyses and reported that micro vessels density and VEGF expression constituted a poor prognostic factor for survival at a statistically significant level (HR 1.8-2.0 and HR 1.5, respectively). Kim *et al*. [8] showed a statistically significant relationship between high serum VEGF levels and short OS time in 140 advanced stage NSCLC patients in their study. We also determined a significant relationship between serum VEGF levels and NSCLC prognosis and survival, which was consistent with the literature.

Fontanini *et al*. [9] immunohistochemically evaluated VEGF expression and density and number of micro vessels in tissue samples surgically resected from 105 NSCLC patients in their study. A statistically significant relationship was reported between the excessive excretion of VEGF and lymph node involvement, between the micro vessel density, and between survival and the period until progression. Yuan *et al*. [10] evaluated a total of 72 resected stage I, II, and III a NSCLC patients in their study. 4 VEGF mRNA isoforms were measured and micro vessel density was examined with the reverse transcriptase (RT) PCR method. As a result, they concluded that the patients with high VEGF mRNA expression were at a more advance stage and they had more lymph node involvement, higher micro vessel density in their tumor tissues, shorter survival times, and faster relapse rates. In the study conducted by Song *et al*. [11] serum VEGF levels in 48 newly diagnosed NSCLC patients were found to be significantly higher compared to those of the control group and furthermore, the serum VEGF levels increased with the increase in stages in NSCLC patients. Bieniasz *et al*. [12] showed in their study that serum VEGF levels also increased in a statistically significant manner with the increase of T stage according to the TNM system in NSCLC cases. It was found in the study conducted by Zhang *et al*. [13] that serum VEGF levels were significantly higher in the existence of lymph node involvement, which constituted an important part of staging, compared to node negative patients. In our study as well, results close to a statistically significant difference were found between the prevalence of the disease and the increase in the serum VEGF level as consistent with the literature (p=0.068). The low number of our patients may constitute a factor in the non-significance of the difference.

Imaging methods used for evaluation after cytotoxic therapies can only examine macroscopic changes after receiving at least 2 to 3 cycles of chemotherapy, in other words, after a long period of time passes. In addition, these methods involve radiation effects that are harmful to the patient. Therefore, the need for a simpler and more practical method that would evaluate the efficiency of the treatment at an earlier stage and show the contribution of the treatment on the response is ever increasing. For this reason, it is possible for tumor markers to evaluate a series of changes. Even though CEA, CYFRA 21-1, and nucleosomes are beneficial in predicting the diagnosis for NSCLC, they have not yet entered in clinical practice [14]. Changes in serum VEGF levels can also be used for the purpose of evaluating the treatment response. In a study performed with this aim, serum VEGF levels were examined 3 times in 42 newly diagnosed advanced NSCLC patients before platinum-based chemotherapy and before the second and third cycles of chemotherapy. VEGF dropped by a statistically significant rate compared to the basal level in patients in remission following the first cycle of chemotherapy. The VEGF level changes compared to the basal level prior to the second cycle displayed 71.4% sensitivity and 71.4% specify in the early prediction of the progression of the disease. However, as different from our study, a relationship was not found between the basal serum VEGF levels and survival in this study [14].

CONCLUSION

The prognostic role of VEGF for advanced NSCLC was shown in our study. VEGF can be used as a potential marker in NSCLC diagnosis. The aggressivity of treatment may be decided based on the serum VEGF levels. Likewise, it is possible to benefit from serum VEGF levels in evaluating response following treatment. However, many studies including more patients are needed in order to reveal the potential value of VEGF levels in NSCLC diagnosis and prognosis.

FINANCIAL OR PROPRIETARY INTEREST

None

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