Serum vascular endothelial growth factor levels in patients with non-small cell lung cancer

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Accepted for publication:
May 2004

Ann Saudi Med 2004;24(6):473-475

Pretreatment stage, performance status and weight loss are the most important prognostic factors in advanced stage NSCLC, while the major factors in early stage disease are the status of lymph node metastasis and tumor size.[1-2] However, response to treatment may be different in patients who have similar prognostic factors. New prognostic factors may have a role in treatment choices. Newer approaches to determine prognosis include epidermal growth factor receptors on lung cancer cells, expression of p53, type IV collagenase, platelet-derived endothelial cell growth factor, thrombospondin I and II, genetic markers such as K-ras oncogene and angiogenic factors.[3]

The formation of new microvessels from preexisting vessels (angiogenesis) is necessary not only for tumor growth, but also for metastasis. Normally, angiogenesis is controlled by interactions among growth factors, vascular cells and the extracellular matrix. This interaction is out of balance in malignancy because of tumor-associated angiogenic factors that can be produced directly by tumor cells or indirectly by inflammatory cells that infiltrate tumor. Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) are the angiogenic factors, but VEGF has emerged as the most important single growth factor in tumor angiogenesis.[4] Several studies have shown that VEGF is commonly expressed in a variety of human solid tumors, including breast and prostate. VEGF expression in tumor tissue is also a significant prognostic factor in patients with NSCLC.[5]

Although the importance of VEGF expression in tumor tissues is known, the clinical and prognostic significance of serum VEGF in patients with cancer is not defined. Clinical utility of serum VEGF in predicting clinical course and prognosis in NSCSL is under investigation as well as in the other cancers. There is a possible implication that serum VEGF level may be an independent prognostic factor among the known clinical factors if they are consistently elevated in tumors as opposed to other conditions, including normal conditions. Therefore, the aim of this study was to investigate the relationship between serum VEGF levels and the stage of the disease or nodal status in patients with NSCLC.

Patients and Method

Forty-two newly diagnosed patients with NSCLC were included in this study, which was conducted between March 1997 and April 1998. Patients were selected consecutively from the departments of chest disease, thoracic surgery and medical oncology. Patients were randomly allocated in each stage group, with the objective to have a similar number of patients in each group. After verbal informed consent was obtained, serum samples were obtained at the time of diagnosis and stored at -20°C. Staging was done by using the new International TNM staging system. Histopathologic and/or cytologic NSCLC diagnosis was the 'must' criteria to include a patient. Patients who had no definitive primary NSCLC diagnosis were excluded. There were no more exclusion criteria (comorbidity, performance status or life expectancy, etc.) since this trial was not a clinical treatment trial. Staging procedures included clinical history, physical examination, complete blood count, serum chemistry, chest x-ray, bronchoscopic examination, computed tomography of the thorax and abdomen, and bone scan, if indicated.

Levels of serum VEGF were measured by ELISA using "VEGF165 Kit" (Quantakine, human VEGF immune assay, R&D system). The analysis was performed according to the assay procedure described in the kit. The relationship between the levels of serum VEGF and disease stage or nodal status was evaluated by ANOVA. Student's t-test was also used to compare the serum VEGF levels and distant metastasis.

Results

All patients were male with a median age of 57 years (42-73 years). Primary tumors were T1 for 1 patient, T2 for half of

Table 1. Patients characteristics by clinical stages.

Parameter	No. of pts (n=42)	%
Tumor size		
T1	1	2.3
T2	20	47.9
T3	11	26.1
T4	10	23.7
Nodal status		
N0	10	23.8
N1	9	21.5
N2	15	35.7
N3	8	19.0
Metastasis		
M0	34	80.9
M1	8	19.1
Histopathology		
Squamous cell	30	71.5
Adenocancer	9	21.5
Large cell	1	2.3
Adenosquamous	2	4.7

the patients, T3 and T4 for the remaining patients (Table 1). Intrathoracic lymph node metastasis was not found in 10 patients. Eight (19.1%) patients had distant metastasis at the time of diagnosis. Squamous cell cancer was the prominent histopathologic type (71.5%).

Mean serum VEGF levels in advanced stages (stage IIIB and IV) were found to be lower than in early stages, although the results were not significant (Table 2). There was no correlation between serum VEGF levels and nodal status or distant metastasis ($P>0.05$).

Discussion

In the present study, serum VEGF levels were not different in terms of disease stage parameters. Although some trials reported a positive correlation with tumor load and VEGF expression in tumor tissue,[3,6-8] serum VEGF trials failed to show a definitive relationship.[9,10] But a few new trials have shown a survival advantage in favor of low serum VEGF levels of patients.[11,12] Brattstrom et al found elevated circulating levels of bFGF and VEGF in serum samples of 38% and 39% of 68 NSCLC patients, respectively. They showed that elevated bFGF values in sera was a statistically significant prognostic factor. However, no significant correlations could be demonstrated for elevated levels of VEGF in serum.[9] Takigawa et al measured serum VEGF levels in 75 patients with lung cancer (45 of them were NSCLC). Their control group was composed of 30 patients with benign lung diseases and 13 healthy subjects. Although mean serum level of VEGF in

Table 2. Serum VEGF levels by clinical stages.

Parameter	Number of patients	Serum level of VEGF Mean	Standard deviation	*P*
Stage				
I	10	431.72	± 295.81	>0.05
II	7	455.17	± 284.75	
IIIA	9	441.61	± 210.86	
IIIB	8	342.38	± 169.65	
IV	8	376.07	± 323.771	
Nodal status				
N0	10	431.72	± 295.81	>0.05
N1	9	420.87	± 269.31	
N2	15	409.16	± 210.76	
N3	8	372.87	± 300.48	
Distant metastasis				
M0	34	418.14	± 239.54	>0.05
M1	8	376.07	± 323.71	

patients with lung cancer was higher than in the group with benign lung diseases and healthy controls, no significant difference was observed between groups categorized according to histological type, stage of disease, or distant metastasis, for lung cancer patients.[10] VEGF expression in the patients with NSCLC who had lymph node metastasis was reported to be higher than those without lymph node metastasis.[13] But in the present trial we found no relationship between serum VEGF levels and lymph node status of patients.

Survival analysis could not be made in our trial, since more than 25% of patients were lost to follow-up. But lack of survival evaluation is not an important pitfall for this trial since serum VEGF levels in patients with NSCLC did not always correlate with survival. A randomized clinical trial in patients with NSCLC comparing chemotherapy versus chemotherapy plus anti-VEGF was presented in ASCO 2000 meeting in New Orleans.[14] Although, response rate and time to progression favored high dose anti-VEGF when it was first presented, long-term results are not known because is still not published.

In conclusion, we observed no relationship between serum VEGF level and disease stage or nodal status. The possible hypothesis might be insufficient VEGF release in soluble state into the serum, while VEGF is expressed in tumor tissue. In this respect, the serum level of VEGF in patients with NSCLC does not seem to be a hopeful prognostic factor. New ongoing studies may shed light on the prognostic significance of serum levels of VEGF in NSCLC patients in extended series.

This trial was presented at the European Cancer Conference in Vienna in 1999. The study was supported by a grant (code number: 98-09-00-049) from Ankara University Research Fund.

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