

Vascular Endothelial Growth Factor Levels in Active Pulmonary Tuberculosis*

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Background: Vascular endothelial growth factor (VEGF) is a mediator with potent angiogenic, mitogenic, and vascular permeability-enhancing activities that are specific for endothelial cells. Intense angiogenesis has been found in active pulmonary tuberculosis lesions.

Objectives: To determine whether active pulmonary tuberculosis is associated with increased serum levels of VEGF compared with inactive tuberculosis and VEGF levels in healthy subjects, and to assess the changes in serum VEGF levels before and after therapy.

Design: Prospective clinical study.

Setting: Chest clinic of a university hospital, Eskisehir, Turkey.

Patients and measurements: Serum VEGF levels of 44 patients with active pulmonary tuberculosis, 24 patients with inactive pulmonary tuberculosis, and 20 healthy subjects were determined.

Results: VEGF levels were increased in active pulmonary tuberculosis patients (mean \pm SD) VEGF level, 598.03 ± 298.25 pg/mL) compared to both inactive pulmonary tuberculosis patients (mean VEGF level, 296.98 ± 115.31 pg/mL) and control subjects (mean VEGF level, 339.67 ± 74.65 pg/mL). The increase in VEGF level observed in patients with active tuberculosis was statistically significant when compared with levels in two other groups ($p < 0.001$ for both). Serum VEGF levels were statistically different before treatment and after treatment in 10 patients who were observed from diagnosis to the end of treatment ($p < 0.01$).

Conclusions: Increased serum VEGF levels may be an indicator of active pulmonary tuberculosis, since levels were higher in patients with active pulmonary tuberculosis and were lower after successful treatment. The role of VEGF-mediated angiogenesis in the pathogenesis and progression of pulmonary tuberculosis lesions should be further elucidated.

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Key words: lung; tuberculosis; vascular endothelial growth factor

Abbreviations: PPD = purified protein derivative; ROC = receiver operating characteristic; VEGF = vascular endothelial growth factor

Tuberculosis is a disease with high mortality and morbidity. Approximately one third of the world's population is infected with the tubercle bacillus, and there are 8 million deaths annually from tuberculosis in the world.¹ Despite the new knowledge about the pathogenesis and activity of tuberculosis, it remains an important disease especially in developing countries.

Vascular endothelial growth factor (VEGF), a

homodimeric, heparin-binding glycoprotein of 34 to 42 kd is one of the major mediators of angiogenesis and vascular permeability.^{2,3} A few reports^{4,5} have shown the increase of VEGF in patients with tuberculosis. The aim of this study was to determine the serum concentration of VEGF in patients with active pulmonary tuberculosis, and to compare the changes in VEGF levels before and after therapy.

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MATERIALS AND METHODS

Forty-four patients (13 women and 31 men) with active pulmonary tuberculosis, 24 patients (11 women and 3 men) with inactive pulmonary tuberculosis who were admitted to our clinic, and 20 healthy persons from the hospital staff who served as a control group were included in this study.

The symptoms and purified protein derivative (PPD) skin test results of active pulmonary tuberculosis patients were noted. These patients underwent standard chest radiograph and high-resolution CT scan analysis if a typical chest cavity was not seen on the chest radiograph. No patients included in the study had a history of hypoxemia or an accompanying disease, such as diabetes mellitus, malignancy, and COPD.

Active pulmonary tuberculosis was diagnosed by positive sputum culture in 37 patients, by examination of bronchial biopsy specimens in 3 patients, and by clinical and radiologic evidence in 4 patients who did not recover with nonspecific treatment and after excluding all other possible diagnosis. The patients who had three sequential negative sputum culture results and were radiologically stable for at least 6 months were accepted as having inactive pulmonary tuberculosis.

Blood samples were collected from all subjects and were placed into disposable tubes. Serum was separated by centrifugation at 3000 revolutions per minute for 10 min and were stored at -80°C until assayed. In 10 patients with active pulmonary tuberculosis, blood samples were drawn in the same manner at the end of tuberculosis treatment. The mean (\pm SD) time between obtaining pretreatment and posttreatment VEGF specimens was 180 ± 5 days.

VEGF levels in sera were measured with a commercially available enzyme-linked immunosorbent assay kit that is highly specific for human VEGF (Quantikine VEGF Immunoassay; R&D Systems; Minneapolis, MN). The sensitivity of the VEGF kit was 5 pg/mL. The correlations among VEGF levels and symptoms, radiologic findings, and PPD skin test results also were determined in active pulmonary tuberculosis patients.

The results were expressed as the mean \pm SD. Statistical comparisons were made with analysis of variance, Spearman correlation test, and paired t test using a statistical software package (SPSS, version 10.0; SPSS Inc; Chicago, IL). p Values of < 0.05 were considered to be statistically significant. The receiver operating characteristic (ROC) curve was drawn based on the ratio of true-positive results to false-positive results. The best cutoff point was chosen, and based on that point the sensitivity and specificity were calculated.

RESULTS

The mean ages for persons in the active pulmonary tuberculosis group, the inactive pulmonary tuberculosis group, and the healthy control group were 43.22 years, 45.12 years, and 36.6 years, respectively. The most common symptoms in patients with active pulmonary tuberculosis were cough (100%) and sputum (93%). In this group, 33 patients (75%) had a PPD skin test result wheal of > 15 mm. However, six patients were anergic. Of the 44 patients with active pulmonary tuberculosis, 2 had a typical chest cavity, 19 had infiltration, and 23 had both a typical chest cavity and infiltration apparent on their radiographs.

Serum VEGF levels were significantly increased in

Table 1—Serum VEGF Levels in All Groups*

Participants	Serum VEGF, pg/mL
Active pulmonary tuberculosis patients	598.03 ± 298.25
Inactive pulmonary tuberculosis patients	$296.98 \pm 115.31^{\dagger}$
Healthy control subjects	$339.67 \pm 74.65^{\dagger}$

*Values given as mean \pm SD.

$^{\dagger}p < 0.001$ compared with active pulmonary tuberculosis patients.

patients with active pulmonary tuberculosis. The mean \pm SD values of all groups are given in Table 1, and the scatter diagram of serum VEGF levels are shown in Figure 1.

A significant correlation ($p < 0.05$) was observed between VEGF levels and fever, which was evaluated as a continuous variable in active pulmonary tuberculosis patients. There was no correlation between VEGF levels and radiologic findings or PPD skin test results in this group.

When we consider the usefulness of serum VEGF levels to differentiate between active tuberculosis patients and inactive tuberculosis patients, VEGF levels yielded a sensitivity of 68.2% and a specificity of 95.8%, with a cutoff value 458.5 pg/mL. The ROC graph of this evaluation is shown in Figure 2.

We were able to estimate the serum VEGF levels at the end of tuberculosis treatment in 10 patients with active pulmonary tuberculosis. The mean VEGF levels of these patients were 588.91 ± 283.22 pg/mL before the therapy and 287.61 ± 115.81 pg/mL after therapy. The difference was significant ($p < 0.01$). The VEGF levels of these patients are shown in Figure 3.

DISCUSSION

VEGF is the major mediator of angiogenesis and vascular permeability. VEGF, also known as vascular

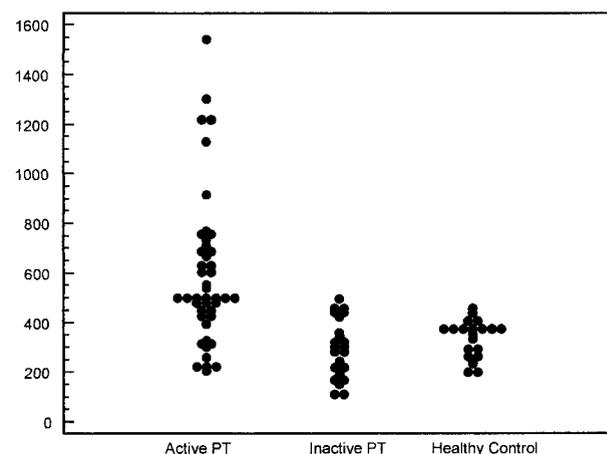


FIGURE 1. A scatter diagram of serum VEGF levels (in pg/mL) in all three groups. PT = pulmonary tuberculosis.

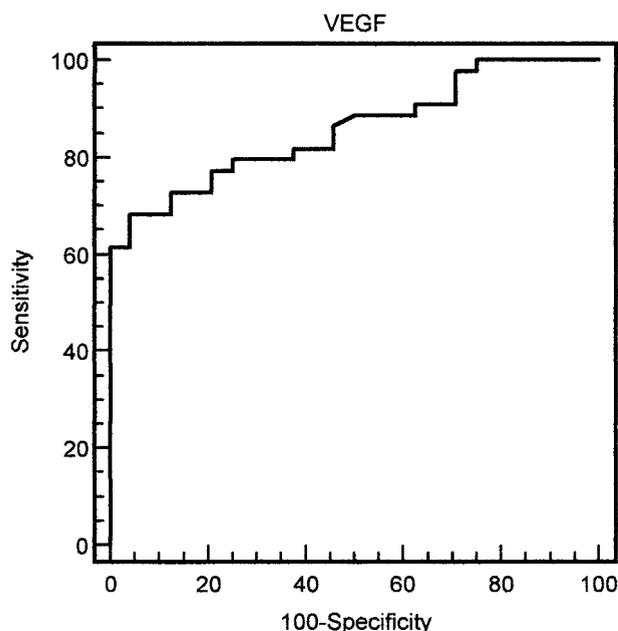


FIGURE 2. ROC curve for VEGF in pulmonary tuberculosis patients for the detection of disease activity.

permeability factor or vasculotropin, has potent angiogenic, mitogenic, and vascular permeability-enhancing activities that are specific for endothelial cells. But in healthy tissue, VEGF expression has been found in activated macrophages, neutrophils, hepatocytes, smooth muscle cells, and Leydig cells, and in the bronchial epithelium. The most important factors for increasing VEGF expression are tissue inflammation, hypoxia, and transforming growth factor- β levels.⁶⁻⁸ Although increased VEGF levels have been demonstrated in patients with malignancies,⁹⁻¹¹ there are only a few reports^{4,5,12} showing increased levels of VEGF in patients with infectious diseases, especially pulmonary tuberculosis.

Matsuyama et al⁵ have reported that VEGF levels were higher in the sera of patients with active

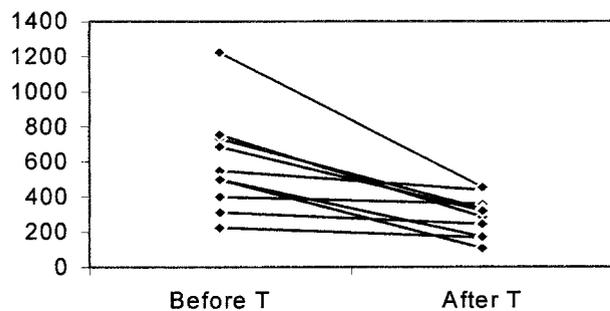


FIGURE 3. Serum VEGF levels (in pg/mL) before and after treatment in 10 follow-up active pulmonary tuberculosis patients. T = treatment.

pulmonary tuberculosis than in the sera of patients with inactive tuberculosis and in acute bronchitis patients. In the same study, the serum VEGF levels of seven patients with active pulmonary tuberculosis were significantly decreased 3 months after the beginning of therapy. However, there were no significant differences between the VEGF levels at 3 months and 6 months after therapy. Our results are compatible with those in the study by Matsuyama et al.⁵ We observed the highest VEGF levels in the sera of patients with active pulmonary tuberculosis. They were nearly twofold that of patients with inactive pulmonary tuberculosis. Moreover, 10 active pulmonary tuberculosis patients in whom we were able to examine the serum VEGF levels at the end of tuberculosis treatment showed a significant decrease. According to these findings, the serum VEGF levels of patients with active pulmonary tuberculosis were decreasing parallel to the improvement of tuberculosis. In future studies, with a larger number of tuberculosis patients, multiple measurements of VEGF would have elucidated the time course of the VEGF decrease in response to therapy.

Abe et al⁴ showed that patients with active pulmonary tuberculosis who did not have typical chest cavities had significantly higher serum VEGF levels when compared with healthy individuals and patients with typical chest cavities. They indicated that increased serum VEGF levels subdue cavity formation through local immunity in active pulmonary tuberculosis.⁴ Our results were not compatible with this study, since the VEGF levels of active pulmonary tuberculosis patients with typical chest cavities were not different from patients without typical chest cavities.

In a previous study,¹³ intense angiogenesis has been found in active pulmonary lesions. It is generally accepted that activated macrophages are the main cells that secrete VEGF in tuberculosis lesions. Matsuyama et al⁵ showed by immunohistochemistry that the expression of VEGF occurred in the alveolar macrophages around active tuberculosis lesions. Based on these findings, we suggest that serum VEGF levels were increased in our active pulmonary tuberculosis patients possibly due to the increased production and secretion of VEGF, especially by the alveolar macrophages. Additionally, serum VEGF levels decreased after tuberculosis treatment in 10 patients that we were able to examine both before and after treatment.

In conclusion, although the diagnosis and activity of tuberculosis is ultimately accomplished by the isolation of tubercle bacillus, the increased serum VEGF levels in our patients with active pulmonary tuberculosis may be an important finding, with increased VEGF levels even having been demon-

strated in patients with malignancies and inflammatory conditions. Abe et al⁴ suggested that the increased serum VEGF levels might have a suppressive effect on chest cavity formation. But there may be an alternative explanation for the relationship between high serum VEGF levels and disease activity in patients with tuberculosis. The increasing inflammatory mass stimulates the production of VEGF in order to supplant the needed blood supply by the formation of new blood vessels. The inability of the body to produce additional VEGF for increasing mass could lead to destruction and necrosis due to the lack of nutrition through poor angiogenesis. Today, our knowledge is inadequate to determine which hypothesis is true. Therefore, the role of VEGF-mediated angiogenesis in the pathogenesis of pulmonary tuberculosis should be further elucidated. Additional studies with a larger number of pulmonary tuberculosis patients are needed to clarify this point.

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