Clinical Implications for Vascular Endothelial Growth Factor Levels among Egyptians with Pulmonary Tuberculosis

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Abstract: Introduction: Vascular endothelial growth factor (VEGF) is a potent angiogenic factor. Intense angiogenesis has been found in active pulmonary tuberculosis. VEGF role in tuberculosis (TB) has not been fully elucidated. **Aim:** This study aimed to measure serum VEGF levels in active pulmonary tuberculosis and changes following chemotherapy. **Subjects and Methods:** Twenty five consecutive patients with active pulmonary tuberculosis and 15 healthy control subjects were enrolled in this prospective randomized controlled study. Complete medical history, full clinical examination, complete blood examination, erythrocyte sedimentation rate, liver function tests, kidney function tests, fasting blood sugar, radiological examination by plain X ray chest, tuberculin skin test by Mantoux method, sputum for acidfast bacilli by Zeil Neilsen stain and estimation of serum VEGF before treatment, 3 months and 6 months after treatment. **Results:** There was no statistical significant difference (p value >0.05) between patients and control group regarding age in years (with a mean of 36.75 ±8.95 for patients and 26.2±4.4 for control group) and smoking index in packs/year (with a mean of 239.65 ±215.40 for patients and 190.6 ±115.2 for control group). There was a highly statistical significant difference(p value<0.001) between patients pretreatment and control group as regarding serum VEGF (pg/ml)(with a mean of 596.02 ± 298.15 for patients and 336.61± 70.45 for control group). There was statistical significant difference(p value<0.05) between patients, 3 months after treatment and control group as regarding serum VEGF(pg/ml) (with a mean of 490.01 ±290.14 for patients and 336.60 ±70.45 for control group).There was a statistical significant difference(p value<0.05) between patients,6 months after treatment and control group as regarding serum VEGF(pg/m) (with a mean of 380.01 ±280.13 for patients and 336.6 ± 70.45 for control group). There was a statistical significant difference (p value<0.05) for serum VEGF levels(pg/ml) in patients pretreatment, 3 and 6 months after treatment(with a mean of 596.02 ± 218.15 pretreatment, 490.01±240.11 three months after treatment, 380.01±217.12 six months after treatment). **In conclusion:** our observations revealed that increased serum VEGF may be an indicator of active pulmonary tuberculosis, since levels were higher in patients with active pulmonary tuberculosis and were lower after successfull treatment. The role of VEGF medicated angiogenesis in pathogenesis and progression of pulmonary tuberculosis lesions should be further elucidated.

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1. Introduction:

Pulmonary tuberculosis is one of the granulomatous diseases, and its pathogenesis has been linked to monocytes and alveolar macrophages[1]. Yet there are few serological markers that keenly reflect its activity. Recently, vascular endothelial growth factor (VEGF), which induces angiogenesis in malignant tumors[2], was found to be associated with Crohn's disease[3]. Although detailed immunological mechanisms of pulmonary tuberculosis are still unclear, alveolar macrophages are reported to play an important role through the cytokine interaction with T-cells[4,5]. Additionally, intense angiogenesis has been found in active pulmonary tuberculosis lesions .VEGF is known to be associated with angiogenesis[6].In terms of incidence of tuberculosis, Egypt is ranked among the midlevel incidence countries. Tuberculosis in Egypt is considered the second most important public health problem after Bilharziasis. The annual risk of infection (ARI) in Egypt stands at 0.32%. As each one percent of ARI corresponds to approximately 50 new smear positive case annually, we can expect about 10.000 new smear positive patients each year in Egypt[7]. Vascular endothelial growth factor (VEGF) is a potent mediator of angiogenesis which has multiple effects in lung development and physiology. VEGF is expressed in several parts of the lung and the pleura while it has been shown that changes in its expression play a significant role in the pathophysiology of some of the most common respiratory disorders, such as acute lung injury, asthma, chronic obstructive pulmonary disease, obstructive sleep apnea, idiopathic pulmonary fibrosis, pulmonary hypertension, pleural disease, and lung cancer. However, the exact role of

VEGF in the lung is not clear yet, as there is contradictory evidence that suggests either a protective or a harmful role[8,9] .VEGF is 45-KD homodimeric glycoprotein stimulating normal and abnormal vessel growth[10]. VEGF is essential for neo-angiogenesis during embryonic development, wound healing, and tumor growth[11].

Its role in the pathophysiology of cardiovascular disease is less well established, however, preliminary data suggests a therapeutic benefit of VEGF application in patients with coronary artery and peripheral vascular disease[12]. A part from a number of cytokines, hormones, and growth factors, the expression of the VEGF gene is mainly stimulated by hypoxia through mediation of hypoxia-inducible factor (HIF)[13]**.** Recently,EGF induces angiogenesis in malignant tumors [14]**,** it was also found to be associated with Crohns disease[15]. Intense angiogenesis has been found in active pulmonary tuberculosis lesions[16]. Among the numerous angiogenic factors,VEGF is the most extensively studied and is significantly related to the severity of inflammatory lung diseases, such as active tuberculosis, chronic bronchitis, pulmonary aspergilloma, and pulmonary disease caused by cystic fibrosis[17-21].Alveolar macrophages are reported to play an important role through the cytokine interactions with T cells[22]. Some investigators have reported that VEGF levels were higher in sera of patients with active pulmonary tuberculosis than in sera of patients with inactive pulmonary tuberculosis and in acute bronchitis[18].

Aim of the work:

The aim of this study was to evaluate the levels of VEGF among Egyptian patients with active pulmonary tuberculosis and changes following antituberculosis chemotherapy.

2. Subjects and Methods:

This prospective, randomized, controlled study comprised consecutive twenty five patients within two years period. They were selected from the outpatient clinic of Internal Medicine and Chest Departments of Benha University Hospitals and they gave verbal consent after explanation of the study purposes and procedures.

Inclusion criteria:

Patients with newly diagnosed active pulmonary tuberculosis, new patients who have never had treatment for TB or who has taken anti-TB drugs for less than 4 weeks, sputum of patients were smear positive(smear positive pulmonary TB patients is defined as: patient with at least two sputum specimens positive for acidfast bacilli by microscopy or patient with at least one sputum specimen positive for acid fast bacilli by microscopy and radiographic abnormalities consistent with pulmonary TB), and decision by a physician to treat with a full course of anti-TB chemotherapy or, a patient with at least one sputum specimen positive for acid fast bacilli by microscopy, which is culture positive for *M. Tuberculosis*.

Exclusion criteria:

We excluded patients with rheumatoid arthritis, diabetes mellitus, acute or chronic liver disease, and immunological abnormalities that predispose to opportunistic infection and the presence of positive HIV, patients on corticosteroids or other immunesuppressive agents. Also, patients with co-existing connective tissue disorder, hematological malignancies, and pulmonary diseases such as asthma, COPD and lung cancer, were excluded. Fifteen apparently healthy subjects were taken as control group for comparison. The diagnosis of tuberculosis was made according to the 1990 edition of Diagnostic Standards and Classification of Tuberculosis published by the American Lung Association[23]. For all patients and the control group thorough history taking, full clinical examination, complete blood examination, Erythrocyte sedimentation rate(before, 3 and 6 months after the beginning of therapy) , fasting and 2 hrs post prandial blood sugar, plain X-ray chest PA-and lateral views (at diagnosis , 3 and 6 months after treatment), tuberculin skin testing by Mantaux methods, sputum for acid fast bacilli by Z.N stain (at diagnosis and every month) and estimation of vascular endothelial growth factor levels(before treatments, 3 and 6 months of treatments).All patients were treated with standard anti-TB drugs which consisted of : Isoniazide 300 mg once daily for 6 months, Rifampicine 600 mg once daily for six months, Ethambutol 1.5 gm once daily for two months and Pyrazinamide 1.5-2 gm once daily for two months.

Measurements of VEGF:

VEGF levels in sera of patients and control group were measured with a commercially available Human VEGF Quantikine Elisa Kit (R&D systems; Minneapolis, MN) according to manufacturer' s instructions. The sensitivity of the VEGF kit was 5 pg/ml.

Statistical analysis:

All data were recorded on an investigative report form. Data were expressed as mean ± standard deviation (SD). $P< 0.05$ level considered statistically significant. All data were transferred to IBM-card using IBM-PC with analysis of data by a statistical programme: SPSS (statistical package for social science). Software package, v g 0.05 (USA. Lyyb. Echo soft carporatem). Correlation between variables was evaluated by pearson's correlation coefficient.

3. Results:

Twenty five consecutive patients (20 males and 5 females) and 15 healthy control subjects (10 males and 5 females)were enrolled in this study (Table2). There was no statistical significant difference (*p* value >0.05) between patients and control group regarding age in years (with a mean of 36.75 ±8.95 for patients and 26.2±4.4 for control group) and smoking index in packs/year(with a mean of 239.65 ±215.40 for patients and 190.6 ±115.2 for control group) (Table 1). There was a highly statistical significant difference (*p* value<0.001) between patients pretreatment and control group as regarding serum VEGF (pg/ml) (with a mean of 596.02 ± 298.15 for patients and 336.61± 70.45 for control group) (Table 3). There was statistical significant difference (*p* value<0.05)between patients, 3 months after treatments and control group as regarding serum VEGF (pg/ml)(with a mean of 490.01 ±290.14 for patients and 336.60 ±70.45 for control group) (Table 4). There was a statistical significant difference(*p* value<0.05) between patients,6 months after treatments and control group as regarding serum VEGF(pg/m)(with a mean of 380.01 ±280.13 for patients and 336.6 ± 70.45 for control group) (Table5). There was a statistical significant difference (*p* value<0.05) of serum VEGF levels(pg/ml) in patients pretreatments, 3 and 6 months after treatments (with a mean of 596.02 ± 218.15 pretreatment, 490.01±240.11for three months after treatment, 380.01±217.12 for six months after treatment)(Table 6).

Table 1: Statistical comparison between patients and control group regarding age(years) and smoking index (SI)(packs/ year):

	Range	Mean	±SD	*P* value	Significant
Age: Patients Control	 20-52 19-39	 36.75 26.2	 8.95 4.4	>0.01	Non significant
SI:Patients Control	200-400 160-500	239.65 190.6	215.40 115.2	>0.01	Non significant

Table 2: Sex distribution in patients and control:

	Patients	Control
Male	20 (80) %	10 (66.6%)
Female	5 (20) %	5 (33.2%)
Total	25	15

Table 3: Statistical comparison between patients pretreatment active pulmonary tuberculosis and control group as regarding serum VEGF (pg/ml):

VEGF	Mean	±SD	*P* value	Significant
Patients	596.02	298.15	<0.001	Highly significant
Control	336.61	70.45		

Table 4: Statistical comparison between patients 3 months after treatments and control group as regarding serum VEGF(pg/ml):

VEGF	Mean	±SD	*P* value	Significant
Patients	490.01	290.14	<0.05	Significant
Control	336.60	70.45		

Table 5: Statistical comparison between patients 6 months after treatments and control group as regarding serum VEGF(pg/m):

VEGF	Mean	±SD	*P* value	Significant
Patients	380.01	280.13	<0.05	Significant
Control	336.6	70.45		

Table 6: Statistical comparison of serum VEGF levels(pg/ml) in patients pretreatments, 3 months and 6 months after treatments:

VEGF	Mean	±SD	*P* value	Significant
Pretreatment 3 months after treatment	596.02 490.01	218.15 240.11	<0.05	Significant
Pretreatment 6 months after treatment	596.02 380.01	218.15 217.12	<0.001	Highly significant
3 months after treatment 6 months after treatment	490.01 380.01	240.11 217.12	<0.05	Significant

4. Discussion:

Tuberculosis is a disease with high mortality and morbidity. Approximately one third of the world population is infected with tubercle bacillus, and there are 8 million deaths annually from tuberculosis in the world[24].Despite the new knowledge about the pathogenesis and activity of tuberculosis, it remains an important disease especially in developing countries[25]. VEGF is the major mediator of angiogenesis and vascular permeability. VEGF, also known as vascular permeability factor or vasculotropin, has potent angiogenic mitogenic, and vascular permeability enhancing activities that are specific for endothelial cells. But in healthy tissues,VEGF expression has been found in activated macrophages, neutrophils, Hepatocytes, smooth muscle cells, 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[26]. Although increased VEGF levels have been demonstrated in patients with malignancies[27], there are only few reports showing increased levels of VEGF in patients with infectious diseases, especially pulmonary tuberculosis[28].

In the present study, we demonstrated levels of VEGF in sera of patients with active pulmonary tuberculosis before treatments, 3 and 6 months after treatment and comparing these levels with levels in healthy volunteers.

We demonstrated that, there was no significant correlation between patients and control group regarding age and smoking index. Our study also demonstrated that, there was higher significant difference between levels of VEGF between patients with active pulmonary tuberculosis before treatments and control healthy volunteers. This finding is in agreement with other study[23] which reported that VEGF levels were higher in the sera of patients with active pulmonary tuberculosis than in patients with inactive tuberculosis or healthy subjects. Our study was also in agreement with another trial[28] which 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. They indicated that increased serum VEGF levels subdue cavity formation through local immunity in active pulmonary tuberculosis. Our study also demonstrated that there were a significant difference in levels of serum VEGF levels between patients with active pulmonary tuberculosis before treatment and the same patients 3 months after treatment, as patients with active pulmonary tuberculosis had higher levels of serum VEGF and these levels significantly decreased with treatment. These results are in agreement with that done by other investigators[23] who found that the serum VEGF levels of patients with active pulmonary tuberculosis were significantly decreased 3 months after beginning of therapy. Our study found also that there is highly significant difference regarding serum VEGF for those patients with active pulmonary tuberculosis before treatment and 6 months after treatment. Our results are also in agreement with the same study done by other investigators[23] who followed up ten active pulmonary tuberculosis patients and examined the serum level of VEGF at the end of tuberculosis treatment and they found that there is a significant decrease of VEGF levels between active pulmonary tuberculosis patients before and 6 months after treatment. According to these findings, serum VEGF levels of patients with active pulmonary tuberculosis were decreasing parallel to tuberculosis treatment.

In a study done by other investigators[16], they found that there is intense angiogenesis present in active pulmonary lesions. It is generally accepted that activated macrophages are the main cells that secrete VEGF in tuberculosis lesion. In the study done by Matsuyoma *et al.* [23], they 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 increased in our active pulmonary tuberculosis patients possibility due to increased production and secretion of VEFG, especially by the alveolar macrophages. So, circulating VEGF levels are increased in patients with active pulmonary tuberculosis compared to healthy controls and patients with old tuberculosis, and decrease after successful treatment[23,29]. The source of VEGF in

pulmonary tuberculosis is believed to be the alveolar macrophages and the CD4 T-lymphocytes[29,30]. Alveolar macrophages in pulmonary tuberculosis may release VEGF together with several cytokines and contribute to the recruitment of T-cells to the lesion[18]. Serum VEGF levels were found higher in TB patients without cavitary lesions compared to those with typical chest cavities, suggesting that increased serum VEGF levels may subdue cavity formation[28]. However, this finding was not replicated in subsequent studies[23]. Two studies had reported that VEGF levels may be used for the diagnosis of active tuberculosis, with great sensitivity (93% and 95.8% for cut-off values of 250 pg/mL and 458.5 pg/mL, respectively) but with relatively low specificity[23,29]. VEGF may serve as a marker of disease activity in tuberculosis; however, further studies are needed in this direction. The weak point in our trial is the relatively low number of patients recruited. Recommendations: Further studies, on larger scales, must be done to correlates serum VEGF levels to that expressed in alveolar macrophages around active tuberculosis lesions by immunohistochemistry. And also, to correlate and compare the changes that occurs in serum levels of VEGF and other new inflammatory markers of tuberculosis e.g. IL 2 receptors; also the role of VEGF mediated angiogenesis in the pathogenesis of pulmonary tuberculosis should be further elucidated. Additional studies with larger numbers of pulmonary tuberculosis patients are needed to clarify the point.

Conclusion:

Although the diagnosis and activities of tuberculosis is ultimately accomplished by isolation of TB bacilli, the increased serum VEGF levels in our patients with active pulmonary tuberculosis may be an important finding, VEGF may be a useful non invasive screening marker for active tuberculosis, as a negative result greatly reduces the likelihood of tuberculosis. However, a positive result requires confirmation.

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