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Serum VEGF and TNF-α Correlate Bacterial Burden in Pulmonary Tuberculosis

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ABSTRACT

Background: Pulmonary tuberculosis (PTB) is one of the predominant causes of death worldwide. The tissue hypoxic condition seen in PTB induces the increased expression of vascular endothelial growth factor (VEGF) and the inflammatory cytokine tumor necrosis factor-alpha (TNF-α). In the present study we aimed to evaluate the role of serum VEGF, TNF-α and their correlation with bacterial burden measured in terms of sputum acid fast bacilli (AFB) grading in PTB.

Method: The study included 120 newly diagnosed PTB cases and 60 healthy controls. Sputum samples from cases were subjected to microscopy for the detection of AFB. Demographic and anthropometric characteristics were recorded. Serum VEGF and TNF-α were estimated by ELISA method.

Results: Serum levels of VEGF and TNF-α in PTB patients were significantly higher compared to controls ($p<0.001$ & $p<0.001$ respectively). BMI of PTB cases was lower than controls ($p<0.001$).We observed significant positive correlation between serum VEGF and sputum AFB grade ($r=0.773$, $p<0.001$) and serum TNF-α and sputum AFB grade ($r=0.662$, $p<0.001$). We also noted a significant positive correlation between serum VEGF and serum TNF-α ($r=0.763$, $p<0.001$) in PTB patients.

Conclusions: The increased serum levels of VEGF and TNF-α were associated with bacterial burden in PTB. Hence, positive sputum smear for AFB, low BMI and increased serum VEGF and TNF-α could be early diagnostic markers and may help immediate treatment regimen for PTB.

Keywords: *Angiogenic biomarker; Body mass index; Hypoxia; Inflammatory cytokine; Pulmonary tuberculosis; Sputum acid fast bacilli grading.*

INTRODUCTION

Pulmonary tuberculosis (PTB) is one of the predominant causes of death worldwide. Among the estimated global incidence of 10.4 million new tuberculosis (TB) cases, India alone accounted for approximately 2.2 million cases[1].

Several socioeconomic factors contribute towards the occurrence of TB like poverty, poor nutrition, illiteracy, poor housing, overcrowding, immigration and poor access to the health[2, 3]. The increased risk of progression from TB infection to active disease is associated with deficiencies of essential macro and micronutrients leading to a negative impact on cell mediated immunity[4]. A low body mass index (BMI) is another individual risk factor for the development of active TB[5].

The microenvironment is a characteristic feature of tissue hypoxia during inflammation and is associated with bacterial infection such as PTB. The increased

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oxygen demand and its decreased supply may result in hypoxia, which is observed during bacterial infection[6]. In mammalian cells response to hypoxia is mediated by hypoxia inducible factor-1α (HIF-1α), a transcription factor which is found to be a potent stimulant to express vascular endothelial growth factor (VEGF), erythropoietin, basic fibroblast growth factor, glycolytic enzymes and glucose transporters synthesizing genes involved in the regulation of metabolism, angiogenesis, apoptosis and cellular stress[7, 8].

VEGF is also considered as one of the major mediators of angiogenesis and vascular permeability. VEGF has multiple roles in lung development and is expressed in many parts of lungs and pleura[9]. Low oxygen microenvironment which can alter expression of VEGF and inflammatory marker like tumor necrosis factor-alpha (TNF-α) has been interest of research in TB. It has also been observed that stimulation of HIF-1α by hypoxia and bacterial exposure can also induce the production of TNF-α[8, 10].

The objective of the study is to evaluate the role of oxygen sensing cellular biomarkers like VEGF and TNF-α in the progression of PTB in a semi-urban backward area of North Karnataka, India.

MATERIALS AND METHOD

Study design and population: 120 newly diagnosed sputum positive PTB patients were enrolled prospectively in this cross sectional study as cases. The study was conducted at Navodaya Medical College Hospital and Research Centre Raichur, Karnataka, India from Jan 2016 to Jan 2017. All the cases were clinically diagnosed by pulmonologist and PTB was confirmed by microscopic examination of sputum specimen for the detection of acid fast bacilli (AFB). Age and sex matched 60 healthy individuals were included in the study as controls. Ethical clearance from the Institutional Ethics Committee was obtained. The participants enrolled as cases and controls in this study were explained in detail about the study procedure. The written informed consent was taken from all the study participants

Inclusion criteria: Cases diagnosed as "new case" of TB, possessing at least two sputum smear test positive for AFB were included as cases in the study. Healthy individuals with no previous history of any major diseases were included as control.

Exclusion criteria: Patients with extra pulmonary TB and/or patients requiring surgical intervention, chronic PTB (receiving at least two courses of anti TB treatment for more than six months), patients with other lung disorders, such as chronic obstructive pulmonary disease asthma, bronchitis and lung cancer, HIV, with organ transplantation, treatment with corticosteroids, chronic renal disease, liver failure and recent myocardial infarction were excluded from the study.

Physical anthropometry: Body mass index (BMI): All the study participants were weighed barefoot with minimum clothing using an electronic weighing machine. Body weight was recorded to the nearest of 0.1kg. Height was measured to the nearest of 0.1cm using standard measuring tape. BMI was calculated using the formula BMI=Weight (kg)/Height^2 (m). The cases and controls were classified based on BMI (kg/m^2) as per WHO criteria[11].

Waist to hip ratio: Was obtained by dividing the waist circumference by hip circumference

Molecular markers: From all the study subjects about 5 ml of venous blood samples were collected in plain vial from median cubital vein under aseptic condition which were allowed to clot for 30 minutes and then centrifuged at 3000 rpm for 10 minutes. Separated serum samples were stored at -80°C until assayed. Serum VEGF levels were measured by using commercially available VEGF ELISA kit (RayBiotech, Norcross, GA) and serum levels of TNF-α were measured by using commercially available TNF-α ELISA kit (Diaclone, France) according to manufacturers' instructions.

STATISTICAL ANALYSIS

The results were analyzed with descriptive statistics, wherever appropriate. The student unpaired "t" test, Spearman's rank correlation coefficient test, chi-square test and Pearson's correlation coefficient test were used to evaluate the statistical significance in the results. The *p value* of <0.05 were considered statistically significant. Statistical analysis was performed by using SPSS version 16.0 software.

RESULTS

In the present study, demographic and anthropometric characteristics of PTB cases and controls were shown in Table-1. About 81 (67.5%) PTB cases were from age groups between 25 and 54 years. Among these 81 cases, 33 (27%) cases constituted age group between 35 and 44 years.

Among 120 PTB cases, BMI of 110 PTB cases (91.67%) were lower (<18.5kg/m^2) than the controls. Further it was observed that, out of 110 PTB cases, 32 PTB cases (26.67%) were having <16.0kg/m^2 BMI. Waist to hip ratio of PTB cases was also found to be lower as compared to controls (p<0.001).

Table 1: Demographic and Anthropometric characteristics of PTB cases and controls

Characteristics	Controls n (%)	PTB Cases n (%)	Chi square (x^2)/t value	*p* value
Age (years) mean ± SD	**44.8 ± 16.9**	**42.5 ± 15.3**	**t = 0.7704**	**0.451***
Age groups (years)				
15-24	7(11.67)	14(11.66)	x^2=5.534	0.345*
25-34	8(13.33)	19(15.83)		
35-44	10(16.67)	33(27.50)		
45-54	14(23.33)	29(15.83)		
56-64	13(21.67)	18(15)		
≥65	8(13.33)	17(14.16)		
Sex				
Male	32(53.33)	75(62.50)	x^2=3.069	0.080*
Female	28(46.67)	45(37.50)		
BMI (Kg/m^2)				
Severely under weight (<16)	00	32(26.67)	x^2=115.3	<0.001**
Moderate under weight (16-16.9)	00	34(28.33)		
Mild under weight (17-18.49)	00	44(36.67)		
Normal (18.5-24.9)	57(95)	10(8.33)		
Over weight (≥25)	03(05)	00(00)		
Waist hip ratio				
Male (mean ± SD)	0.91 ± 0.03	0.82 ± 0.05	t=6.9452	<0.001**
Female (mean ± SD)	0.86 ± 0.04	0.78 ± 0.03	t=8.6684	<0.001**

**p* value (>0.05) not significant. ***p* value (<0.001) highly significant.

Serum levels of VEGF and TNF-α in PTB patients were significantly higher compared to controls (p<0.001) as shown in Table-2.

Table 2: Statistical comparison between PTB patients and control regarding serum VEGF (pg/ml) and TNF-α (pg/ml)

Parameters	Control	PTB Cases	t value	p value
VEGF (Mean ± SD)	26.36 ± 10.59	604.89 ± 323.43	10.557	<0.001**
TNF-α (Mean ± SD)	14.01 ± 3.59	92.79 ± 38.57	12.045	<0.001**

***p* value (<0.001) highly significant

Distributions of PTB cases based on bacterial load were illustrated in Fig.1.

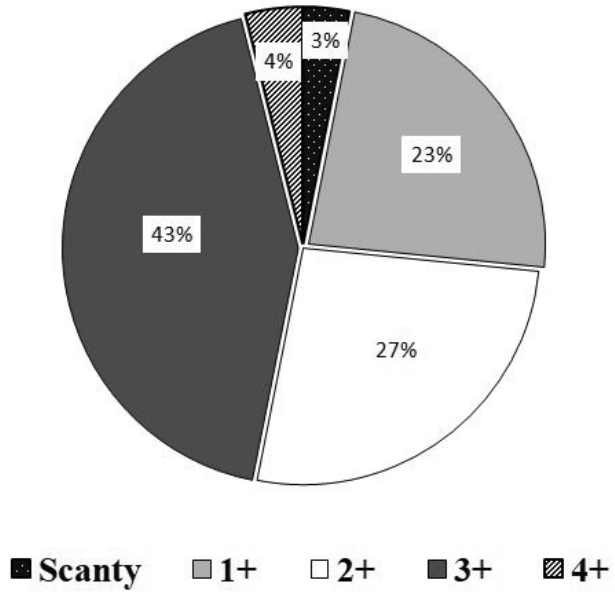

Fig. 1: Distribution of Pulmonary Tuberculosis cases based on bacterial load.
Scanty, 1+, 2+, 3+ and 4+ are AFB grading.

We observed significant positive correlation between serum VEGF and sputum AFB grade (r=0.773, p<0.001) in PTB (Fig. 2) and also between serum TNF-α and sputum AFB grade (r=0.662, p<0.001) in PTB (Fig. 3). Serum VEGF levels were more pronounced than serum TNF-α levels. Further, we have also found a significant positive correlation between serum VEGF and serum TNF-α (r=0.763, p<0.001) in PTB cases (Fig. 4).

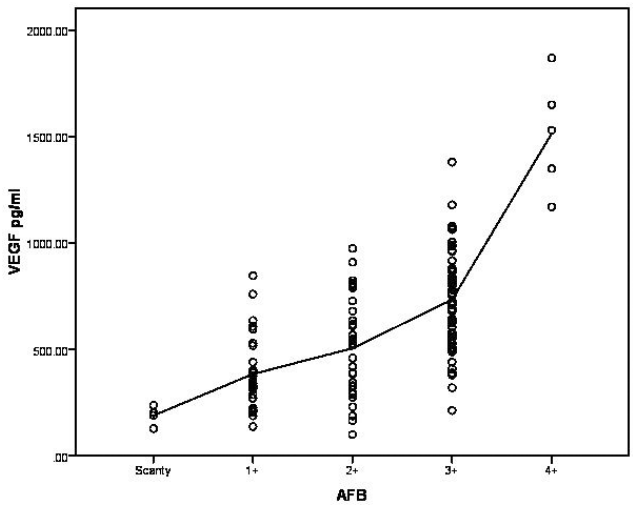

Fig. 2: Correlation between VEGF and sputum AFB grading in PTB cases, (r=0.773, p<0.001).

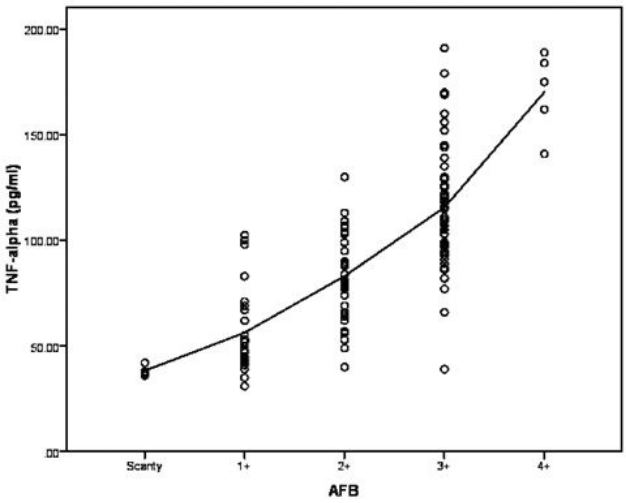

Fig. 3: Correlation between TNF-α and sputum AFB grading in PTB cases, (r=0.662, p<0.001).

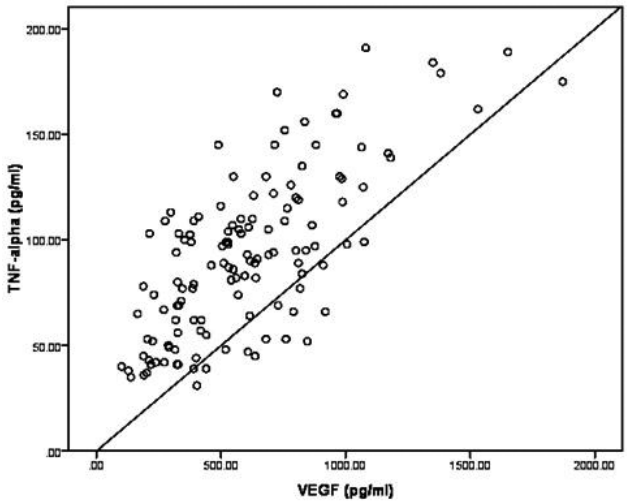

Fig. 4: Correlation between VEGF and TNF-α in PTB cases, (r=0.763, p<0.001).

DISCUSSION

Results from our study on BMI indicate a poor nutritional status in PTB. Previous studies showed that reduced BMI is a significant risk factor for PTB due to the induced impairment of cellular immunity[4, 12]. Further BMI was an important marker for the assessment of nutritional status in PTB patients[13]. In the present study waist to hip ratio of PTB patients was also found to be low when compared to control, which was in agreement with the study conducted by Tungdim *et al*[14].

Our study showed that serum levels of VEGF and TNF-α in PTB patient were significantly higher compared to controls. There was an increasing trend of hypoxia markers (VEGF and TNF-α) in agreement with severity

of PTB. As the bacterial burden increased the expression of VEGF and TNF-α were also increased. Tissue Hypoxia is a common feature in bacterial infection which induces increased expression of HIF-1α transcription factor which in turn increases the expression of VEGF gene by up regulating NF-κB[15]. Several factors regulate VEGF gene expression; among these hypoxia plays a key role in PTB. The activated alveolar macrophages are the main cells which secrets VEGF in PTB[16]. Few studies have indicated higher levels serum VEGF in PTB patients [17-20]. Our findings on serum VEGF corroborate with Alatas *et al*, who concluded increased activities of serum VEGF in PTB patients may be an indicator of active PTB[17]. VEGF might associate with pathogenesis of PTB and measurement of serum VEGF may be a useful screening marker of active PTB and also useful for the prognosis and monitoring the clinical effect of anti tuberculoses therapy[18]. VEGF could be used to indicate bacterial burden in addition to know the disease severity, extent of disease and therapeutic monitoring. Our study was in accordance with previous studies by researchers wherein we have showed, increased bacterial burden measured in terms of sputum AFB grade resulted in increased activity of VEGF in PTB cases[19]. It can be further stated that increased expression of VEGF probably increased the supply of blood and oxygen to affected lung tissues and develop angiogenesis to protect PTB patients from low oxygen sensing microenvironment[20]. It has been reported that circulating levels of angiogenic biomarker VEGF in individual with PTB, latent TB (LTB) or no TB infection (NTB) confirmed that VEGF was an important biomarker to distinguish PTB from LTB and NTB[19].

In the present study along with VEGF, the serum levels of inflammatory cytokine TNF-α were also increased in proportion with bacterial load suggesting a possible pathophysiological role of both VEGF and TNF-α in PTB. The low BMI observed in our study was probably due to the increased production of TNF-α. In PTB, TNF-α induces muscle and fat tissue catabolism resulting in weight loss[21]. This suggests that increased bacterial load increases TNF-α which may further lower BMI in PTB patients. The TNF-α increases early in TB and performs complex role in the host response to *Mycobacterium tuberculosis* by exhibiting antimycobacterial activity and promoting the formation of granuloma in PTB patients[22]. The high levels of TNF-α observed in PTB patients in our study were in agreement with other studies[23-26]. Few studies have indicated hypoxia can also induce TNF-α production in diseases[8, 10]. A significant positive correlation between VEGF and TNF-α in the present study indicate their association with bacterial burden and also shows their important pathophysiological role in PTB.

CONCLUSIONS

The present study concludes that bacterial burden increases concurrently with induction of hypoxia resulting in the increased expression of both VEGF and TNF-α. However among low oxygen sensing microenvironment markers, serum VEGF found to be relatively more pronounced when compared to serum TNF-α in PTB. It may be further concluded that positive sputum smear for AFB, low BMI along with increased serum levels of VEGF and TNF-α could be useful in the early diagnosis of active PTB and also it could be used as marker of early detection of tissue damage in PTB which could help in the immediate initiation of treatment regimen and propagation of PTB.

Conflict of Interest: The authors have none to declare.

Source of Funding: Self funded study.

Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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