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Circulatory TGF- β1 is significantly higher in early stage of pulmonary sarcoidosis

Mehdi Mirsaeidi[1], Hesham R. Omar[2], Andrew Calzadilla[1], Ahmad El khatib[1], Philip Whitney[1], Nadera Sweiss[3], Daniel Culver[4], Michael Campos[1], Robert Baughman[5], Roberto Machado[2]

[1] Division of Pulmonary, Critical Care, Sleep and Allergy, Department of Medicine, University of Miami, FL, USA; [2] Internal Medicine Department, Mercy Medical Center, Iowa, USA; [3] Division of Pulmonary, Critical Care, Sleep and Allergy, Department of Medicine, University of Illinois at Chicago, IL, USA; [4] Department of Pulmonary, Allergy, and Critical Care Medicine, Cleveland Clinic, Cleveland, OH, USA; [5] Division of Pulmonary, Critical Care, Sleep and Allergy, Department of Medicine, University of Cincinnati, OH, USA

Abstract. *Introduction:* The pathogenesis of pulmonary fibrosis in sarcoidosis is not known. We hypothesized that higher levels of circulatory growth factors are present in early stages of pulmonary sarcoidosis and may be associated with pulmonary fibrosis. *Methods:* Age and sex-matched subjects with sarcoidosis stage 0-1 (n=18), stage 4-5 (n=13) and healthy controls (n=5) had their serum TGF-β1, FGF, and VEGF levels measured as well as their gene expressions determined in peripheral blood mononuclear cells. *Results:* TGF-β1 levels were significantly higher in patients with stage 0-1 sarcoidosis compared with normal healthy control patients (25,488 vs. 13800 pg/ml, P=0.05). Patients with sarcoidosis stage 4 had a 1.3-fold higher peripheral blood mononuclear cells (PBMC) gene expression of TGF-β1 compared with subjects at stage 0-1 (P= 0.041). The serum levels of FGF, and VEGF had a trend towards higher levels in sarcoidosis subjects compared to normal controls. *Conclusion:* These results suggest that cell growth factors levels are high in early stages of sarcoidosis. These findings should be validated in larger studies. *(Sarcoidosis Vasc Diffuse Lung Dis 2018; 35: 213-217)*

Key words: sarcoidosis, growth factor, TGF-β1, FGF, and VEGF

Introduction

Sarcoidosis is a granulomatous disease that involves the lungs in more than 90% of the cases (1). The incidence of sarcoidosis differs broadly throughout the world due to environmental and genetic factors. We and others reported that pulmonary fibrosis occurs in 10-20% of affected individuals and is the most common cause of sarcoidosis-related mortality (2). The pathogenesis of pulmonary fibrosis in sarcoidosis is not known. It is theorized that certain mediators like transforming growth factor (TGF-β1), Platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF) have a role in its pathogenesis (3). If confirmed, it opens the possibility of treatment with medications that antagonize these growth factor receptors to prevent further fibrosis formation.

We hypothesized that subjects with early stage pulmonary sarcoidosis (Stages 0-1) will have higher levels of PDGF, FGFs and VEGF proteins and higher gene expression in peripheral blood mononuclear cells (PBMC) compared with fibrotic pulmonary sarcoidosis patients and healthy controls.

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Correspondence: Mehdi Mirsaeidi MD, MPH
Division of Pulmonary, Critical Care, Sleep and Allergy,
Department of Medicine, Division of Pulmonary,
Critical Care, Sleep and Allergy, Department of Medicine
University of Miami, Miller School of Medicine
1600 NW 10th Ave # 7060A - Miami, FL 33136
E-mail: msm249@med.miami.edu

Methods

Patient population

This is an IRB-approved retrospective study of adult subjects >18 years at the University of Illinois in Chicago diagnosed with sarcoidosis according to the European Respiratory Society (ERS), American Thoracic Society (ATS) and World Association of Sarcoidosis and other Granulomatous Disorders (WASOG) criteria (4) between January 2010 and January 2015. The study included three groups of patients (all between 18-70 years): 1st group comprised 18 subjects with confirmed sarcoidosis stage 0 or 1, 2nd group comprised 13 subjects with confirmed sarcoidosis stage 4 or 5 and 3rd group comprised 5 healthy controls (without history of sarcoidosis or pulmonary fibrosis). All subjects were African American and matched for sex and age. All participants signed an informed consent. Sarcoidosis staging from 0-4 was based on pattern of chest radiographic findings. Exclusion criteria included patients who were taking full-dose anticoagulant therapy or high-dose antiplatelet therapy at screening, patients with non-cutaneous malignancy treated in the past two years or if hemoglobin was <7gm/dL.

Measurement of growth factors and gene expression

The concentration of serum TGF-β1, VEGF, acidic fibroblast growth factor (aFGF), bFGF and their genes expression in PBMC were measured and compared across the three groups. Subjects who satisfied the inclusion and exclusion criteria for the study were subjected to blood draw during routine laboratory blood tests. Serum was isolated and stored for PDGF, bFGF, and VEGF, TGF-β1 measurements via ELISA as previously described (5, 6). RNA was extracted from blood mononuclear cells for each sample. Growth factor genes expression was evaluated utilizing Affymetrix GeneChip based global expression profiling in the University of Illinois Genomic Center (7). Peripheral blood mononuclear cell (PBMC) growth factor gene expression was compared amongst 2 groups of subjects with sarcoidosis stage 0-1 and those with confirmed sarcoidosis stage 4.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) and compared using Student t-test and ANOVA (with Tukey's post-hoc test). Mean comparison was used for continues variables. Regression model was used to establish cause-and-effect relationships between low DLCO and circulatory growth factors levels.

A P-value ≤0.05 was considered statistically significant. All statistical significance was assessed using a 2-sided P values. Data were analyzed using IBM SPSS 22.0 statistical software (IBM SPSS Version 21.0. Armonk, NY) and GraphPad Prism.

Results

Compared with patients with sarcoidosis stages 0-1, those with sarcoidosis stage 4 had lower body mass index (P=0.014), higher frequency of cough (P=0.013), sputum production (P=0.019), and dyspnea (P=0.034), and lower mean FVC (P=0.043), FEV1 (P=0.03) and DLCO (P=0.002) by univariate analysis. There was no significant difference on medical therapy between two sarcoidosis groups. Table 1 compares baseline demographics, clinical and laboratory characteristics, and pulmonary function tests among patient with sarcoidosis stages 0-1 versus stage 4.

We found a statistically significant increase in the average serum levels of TGF-β1 in subjects with stage 0-1 sarcoidosis as compared with the normal healthy controls (25,488 vs. 13800 pg/ml, P=0.05). The average VEGF serum levels in stages 0-1 was non-significantly increased when compared to the normal healthy patient as well (9483 vs. 328 pg/ml, P=0.375). Figure 1 illustrates the mean levels of growth factors among the three study groups. We have also found that the PBMC gene expression levels of TGF-β1 in African American subjects with stage 4 pulmonary sarcoidosis were 1.30-fold higher compared with stage 0-1 (P= 0.041) (table 2). Table 2 illustrates the gene expression levels of growth factors in PBMC among the sarcoidosis subjects. We were not able to find any growth factor as an independent variable associated with low DLCO In the regression model (data not shown).

Table 1. Comparison of baseline demographics, clinical and laboratory characteristics, and pulmonary function tests among patient with sarcoidosis stages 0-1 versus stage 4

	Sarcoidosis Stage 0-1 (N=18)	Sarcoidosis Stage 4 (N=13)	P-value
Age (year, m±SD)	50.9±10.8	51.2±8.4	0.952
Male sex % (n)	16.7% (3/18)	7.7% (1/13)	0.462
BMI (Kg/m2, m±SD)	37.8±7.3	32.2±15.5	0.014
Sarcoidosis duration (year, m±SD)	9.3±6.9	12.2±7.6	0.236
Extrapulmonary sarcoidosis % (n)	64.7% (11/17)	61.5% (8/13)	0.858
Hypertension % (n)	72.2% (13/18)	53.8% (7/13)	0.291
DM % (n)	27.8% (5/18)	7.7% (1/13)	0.162
Cough % (n)	50% (9/18)	92.3% (12/13)	0.013
Sputum production % (n)	25% (4/16)	75% (6/8)	0.019
Dyspnea % (n)	43.8% (7/16)	83.3% (10/12)	0.034
Fatigue % (n)	27.8% (5/18)	53.8% (7/13)	0.141
FVC volume (L, m±SD)	2.7±0.5	2.2±0.5	0.043
FVC % (m±SD)	84.4±34.3	79.2±19.9	0.386
FEV1 volume (L, m±SD)	2.2±0.5	1.6±0.4	0.03
FEV1 % (m±SD)	91.1±21.6	69.8±23.5	0.047
VC volume (L, m±SD)	2.75±0.5	2.45±0.4	0.277
VC % (m±SD)	100.5±18	86.9±18.3	0.277
TLC volume (L, m±SD)	4.29±0.8	4.1±0.5	0.248
TLC % (m±SD)	89.8±15	80.3±11.5	0.178
RV (L, m±SD)	103.3±18.7	99.1±16.2	0.565
DLCO vol (ml/min/mmHg, m±SD)	17.6±2.4	11.1±3.3	0.002
DLCO % (m±SD)	74.6±11.5	60±13.8	0.058
ESR (mm/hr, m±SD)	54.1±47.5	57.8±35.6	0.382
CRP (mg/L, m±SD)	3.14±4.6	2.1±1.2	0.947
ACE (U/L, m±SD)	52±43.7	73±35.3	0.237
Oral steroid % (n)	82.4% (14/17)	92.3% (12/13)	0.427
DMARD % (n)	50% (9/18)	54.5% (6/11)	0.812
Methotrexate % (n)	41.2% (7/17)	33.3% (3/9)	0.696
Leflunomide % (n)	11.1% (2/18)	18.2% (2/11)	0.592
Hydroxychloroquine % (n)	11.1% (2/18)	27.3% (3/11)	0.264

BMI: body mass index, DM: Diabetes Mellitus, FVC: forced vital capacity, FEV: forced expiratory volume, VC: vital capacity, TLC: total lung capacity, RV: residual volume, DLCO: diffusion capacity of lungs for carbon monoxide, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, ACE: angiotensin converting enzyme, DMARD: disease modifying anti-rheumatic drugs

Discussion

TGF-β superfamily is a large group of proteins which are active in cell growth, differentiation signals, and regulation of extracellular matrix production (8). TGF-β is produced from different cell lines including macrophages, T-cells, bronchial epithelial cells and type II alveolar epithelial cells. TGF-β induces differentiation of fibroblasts and epithelial cells to myofibroblast-like cells. TGF-β also exhibits pro- and anti-inflammatory properties, and its increased production is associated with fibrotic diseases. Zissel and co-workers found that lung cells of sarcoidosis subjects with persistent active disease produced significantly lower levels of TGF-β compared with those whose disease spontaneously regressed within 6 months (9). Interestingly, in our study patients with Stage 4 sarcoidosis had a mean circulatory TGF-β level of 21,977 pg/mL that was lower than subjects with non-fibrotic stage 0-1 (25,488 pg/mL). Lower circulatory TGF-β in the fibrotic group supports previous study findings and suggests that low circulatory TGF-β may be a potential biomarker for a poorer prognosis in pulmonary sarcoidosis. The higher circulatory TGF-β levels in subjects with sarcoidosis compared with normal healthy subjects is expected due to the inflammatory nature of disease. The TGF-β mechanism of effect in pulmonary sarcoidosis still remained unclear and required further investigation.

Our study showed a non-significant trend towards increased values of circulatory VEGF for patients that had sarcoidosis when compared with normal patients. Sekiya et al., evaluated the relationship of serum VEGF with the clinical status of sarcoidosis (10). They measured the concentrations of VEGF in serum of 33 subjects with confirmed sarcoidosis and

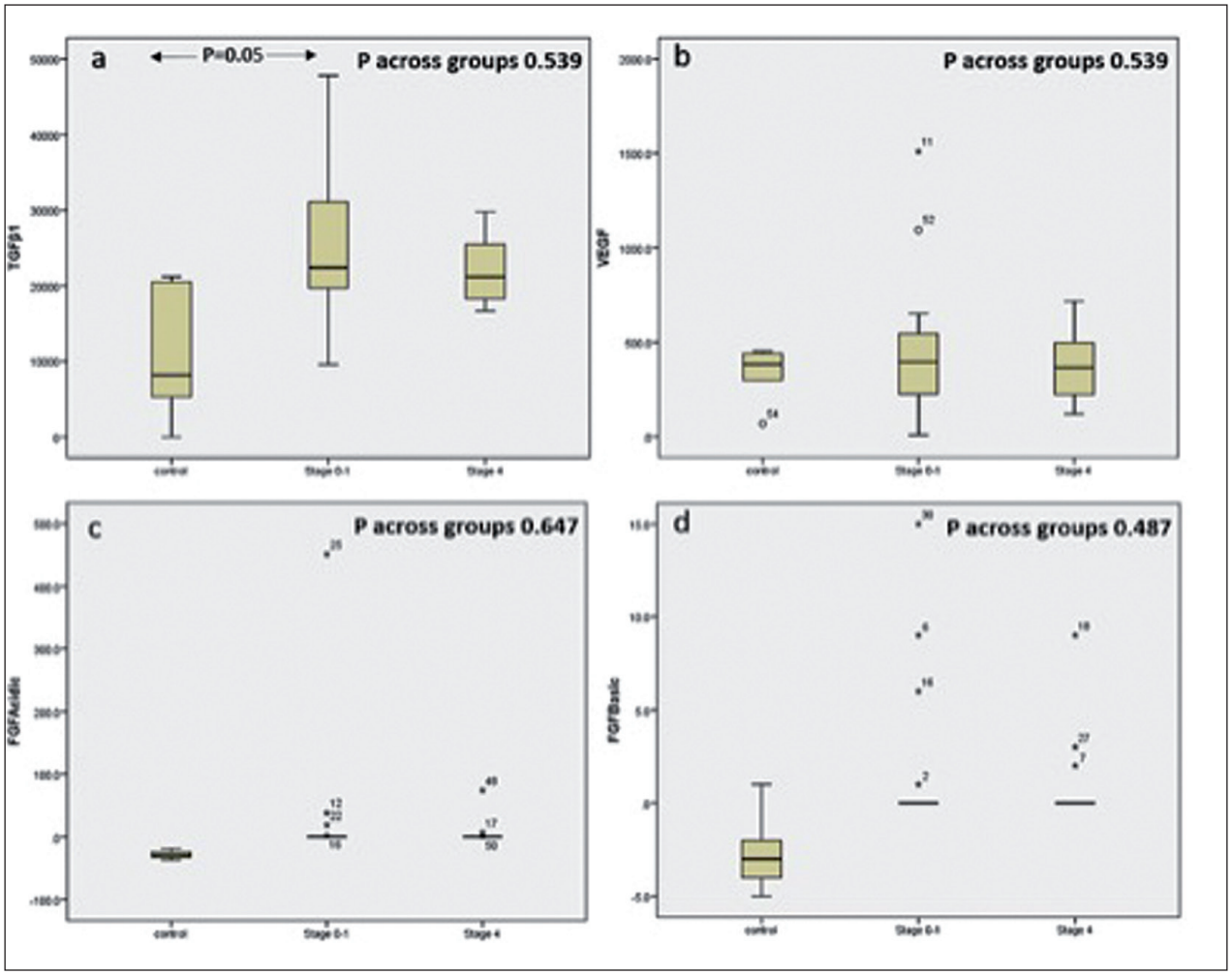

Fig. 1. The mean levels of serum growth factors among the three study groups (sarcoidosis stage 0-1, stage 4, control subjects).
TGF-β1: Transforming growth factor beta 1, VEGF: Vascular endothelial growth factor, FGF1: Fibroblast growth factor 1, FGF2: Fibroblast growth factor 1, M: Mean, SE: Standard error. All units per pg/ml.

Table 2. PBMC gene expression levels of growth factors in African American subjects with pulmonary sarcoidosis

Gene name	Sarcoidosis stage 0-1 M±SE	Sarcoidosis stage 4 M±SE	Fold-change (Stage 4 vs. 0-1)	P-value
TGF-β1-mRNA	4.609±0.0999	4.997±0.1509	1.307	0.041
VEGF-mRNA	6.564±0.02333	6.622±0.0448	1.041	0.260
FGF1 -mRNA	3.323±0.0347	3.330±0.0333	1.005	0.882
FGF2-mRNA	2.725±0.0518	2.820±0.0491	1.068	0.194

TGF-β1: Transforming growth factor beta 1, VEGF: Vascular endothelial growth factor, FGF1: Fibroblast growth factor 1, FGF2: Fibroblast growth factor 1, M: Mean, SE: Standard error

investigated VEGF values with extension of disease, and prognosis. They found that serum VEGF was higher among subjects who required treatment with steroids when compared to those with spontaneous remission (P<0.05). It was also found that patients with pulmonary sarcoidosis alone had lower values of average VEGF when compared to those with pulmonary and extrapulmonary sarcoidosis. They concluded that serum VGEF might be a novel marker for prognosis in sarcoidosis.

Antoniou et al. , compared VEGF and its receptor (fms-like tyrosine kinase 1,Flt-1) levels in BAL of subjects with idiopathic pulmonary fibrosis (IPF) and pulmonary sarcoidosis (11). They enrolled 3 groups of subjects in the study, 18 subjects with IPF, 16 subjects with sarcoidosis and 10 healthy volunteers. They found significant increases of *VEGF* gene expression in IPF comparing to pulmonary sarcoidosis (P=0.02) but no statistically significant difference was found in VEGF protein levels between sarcoidosis and IPF (mean levels 154 pg/mL versus 344 pg/mL, p=0.2). Comparing to normal healthy subjects, *VEGF* gene expression in BAL cells of sarcoidosis subjects was higher. In addition, there was no significant difference on the levels of *VEGF receptor* gene expression in BAL fluid of sarcoidosis compare to IPF or healthy subjects (p=0.4). This study did not address the sarcoidosis stages and fibrotic sarcoidosis.

Our study is limited in number of samples in each group and only showed a non-significant trend towards increased values of circulatory FGFs for patients with sarcoidosis when compared with normal patients. Recently, Sexton and coworkers investigated FGF-23 serum levels in 39 subjects with acute sarcoidosis (12). They found that serum level of FGF-23 was more than 9.9 pg/mL in 15% of sarcoidosis subjects; those had higher serum calcium (P=0.007) and lower serum iPTH (P<0.001). FGF-23 was below the level of detection in the majority of subjects. The role of FGF in the sarcoidosis needs more investigation.

Conclusion

Our preliminary data confirms that cell growth factors levels are higher in sarcoidosis subjects particularly those in early stages in comparison to normal healthy subjects. Our findings suggest that circulatory levels of serum growth factors may be novel markers for prognosis in sarcoidosis, although, they should be validated in a larger sample size. The protein and gene expression of cell growth factors in BAL cells (alveolar macrophages and fibroblasts) should be investigated to address local activity of growth factors. This approach will improve our risk stratification of sarcoidosis subjects and probably find novel therapeutic targets.

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