

The levels of pro-fibrotic cytokines in pulmonary tuberculosis with minimal and extensive lesions

Triwahju Astuti, Iin Noor Chozin, Nevy Shinta Damayanti, Dian Nugrahenny¹

Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Brawijaya, ¹Department of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Malang 65145, Indonesia

ABSTRACT

Background: There are very few studies about the mechanism of fibrosis in tuberculosis (TB). This study aimed to determine the levels of tumor necrosis factor- α (TNF- α), insulin-like growth factor-1 (IGF-1), and transforming growth factor- β 1 (TGF- β 1) in pulmonary TB patients with minimal and extensive lesions. **Materials and Methods:** Cross-sectional observational study design was used to observe the pulmonary TB patients with minimal and extensive lesions, and also healthy controls, each consisting of ten patients. **Results:** The plasma levels of TNF- α , IGF-1, and TGF- β 1 in pulmonary TB groups were higher compared to the healthy controls. The TNF- α level in the minimal lesion of TB group was higher than the level in the extensive lesion but not significant ($P = 0.741$). The IGF-1 level in the minimal lesion of TB group was significantly ($P = 0.007$) increased compared to the extensive lesion. While the TGF- β 1 level in the minimal lesion of TB group was significantly ($P = 0.005$) lower than the level in the extensive lesion. **Conclusion:** In extensive lesion of TB group, there are differences in the levels of TNF- α , IGF-1, and TGF- β 1 compared to the minimal lesion of TB group as well as the healthy controls. The extent of lesions on chest radiograph also describes the state of ongoing pulmonary fibrosis which can be shown by the differences in the levels of pro-fibrotic cytokines.

KEY WORDS: Fibrosis, insulin-like growth factor-1, transforming growth factor- β 1, tuberculosis, tumor necrosis factor- α

Address for correspondence: Dr. Dian Nugrahenny, Department of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Jl. Veteran, Malang 65145, Indonesia. E-mail: diannugrahenny.fk@ub.ac.id, diannugr2407@gmail.com

INTRODUCTION

Tuberculosis (TB) is a major public health problem both regionally and internationally. The World Health Organization (WHO) stated that an estimated 10.4 million people suffered from TB in 2016: 90% were adults, 65% were male, 10% were people suffering from HIV (74% in Africa), and 56% were in five countries: India, Indonesia, China, the Philippines, and Pakistan.^[1]

Inflammation is the key pathogenesis of TB. The inflammatory response as a result of infection is needed to control infection, but on the other hand, also causes damage to the lung tissue. The local response to

Mycobacterium tuberculosis (Mtb) infection is initiated by an intense pro-inflammatory response followed by the production of anti-inflammatory mediators that regulate the tissue damage. In response to Mtb interaction with alveolar macrophages and dendritic cells, there is a release of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-(IL)-12 along with a variety of chemokines, which are considered to be key regulatory factors in the formation and maintenance of the granuloma structure. In addition, TNF- α also acts as a pro-fibrotic cytokine.^[2-4]

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Astuti T, Chozin IN, Damayanti NS, Nugrahenny D. The levels of pro-fibrotic cytokines in pulmonary tuberculosis with minimal and extensive lesions. Lung India 2018;35:204-8.

Access this article online	
Quick Response Code: 	Website: www.lungindia.com
	DOI: 10.4103/lungindia.lungindia_396_17

In the process of pulmonary fibrosis, insulin-like growth factor-1 (IGF-1) together with platelet-derived growth factor (PDGF) and fibroblast growth factor-2 (FGF)-2 are involved in fibroblast proliferation. It has been proven that IGF regulates the survival and migration of fibrogenic cells in the lungs. IGF pathway blockage promotes fibroblast apoptosis and pulmonary fibrosis resolution.^[5,6]

The final stage in the process of pulmonary fibrosis is the differentiation of fibroblasts into myofibrocytes. This stage occurs under the influence of transforming growth factor- β 1 (TGF- β 1), tenascin-C, N-terminal domain of connective tissue growth factor, and phosphate and tensin homolog deleted on chromosome 10 (PTEN).^[5,7,8] TGF- β is a pluripotent cytokine that modulates the immune response by downregulating acquired immunity and deactivating macrophages. TGF- β reduces the harmful inflammatory reactions related with T-cell immunity to Mtb infection. Studies have shown that TGF- β is upregulated in monocytes and macrophages in granuloma from active TB patients, and high levels of TGF- β is related with the severe stage of TB.^[4]

Clinicians use radiologic imaging of the chest to assess the severity of lung parenchyma damage in pulmonary TB. Based on the chest X-ray, pulmonary TB can be divided into minimal lesion and extensive lesion (moderately advanced and far advanced lesions).^[9] This study aimed to evaluate the profile of TNF- α , IGF-1, and TGF- β 1 in pulmonary TB patients with minimal and extensive lesions to explain the process of pulmonary fibrosis in TB.

MATERIALS AND METHODS

This study was a cross-sectional observational study aimed to determine the plasma levels of TNF- α , IGF-1, and TGF- β 1 in pulmonary TB patients with minimal and extensive lesions, and healthy controls, ten patients each. Participants were patients of Outpatient Department of Dr. Saiful Anwar Hospital, Malang, Indonesia, in July to August 2013.

Written informed consent was obtained from all the participants for being included in the study. All procedures were approved by the Institutional Ethics Committee of Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.

The inclusion criteria were men and women aged between 15 and 55 years old, patients diagnosed with pulmonary TB with a minimal lesion or extensive lesion, did not receive anti-TB drugs yet, have agreed to join the study and signed the informed consent. Whereas, the exclusion criteria were patients with pulmonary TB accompanied by other comorbidities (bacterial pneumonia, HIV-AIDS, heart diseases, diabetes mellitus, impaired liver function, kidney diseases, fibrotic diseases, and malignant

diseases), as well as pregnant patients. Healthy control was the one who did not have respiratory complaints and had a normal chest radiograph.

Pulmonary TB with minimal lesion includes fibroinfiltrates on the part of one or two lungs with an area of no more than lung volume located at the chondrosternal junction of the second ribs and spinous processes of the thoracic vertebrae IV or the thoracic vertebral body V (intercostal space II), and there are no cavities as well as no clinically and radiologically changes after 2 weeks of nonspecific treatment.^[10] Whereas, pulmonary TB with extensive lesion includes moderately advanced lesion and far advanced lesion. Moderately advanced lesion involves fibroinfiltrates which are wider than the minimal lesion and can be spread with a moderate density, but the area is not wider than one lung, or the total area of all process is as wide as one lung at most; or if the process is denser and thicker, the total area of all process should not take more than one-third of one lung, and this process may be or not accompanied by cavities; or if accompanied by cavity, then the total area of all cavities (diameter) of not more than 4 cm. The far advanced lesion is defined if the abnormalities are more extensive than the moderately advanced lesion.^[11]

The plasma levels of TNF- α (with units of pg/ml), IGF-1 (ng/ml), and TGF- β 1 (ng/ml) in pulmonary TB patients were measured by enzyme-linked immunosorbent assay using Quantikine kits (R&D System). Data are presented as mean $\pm$ standard deviation, and the differences between groups were analyzed using *t*-test, also one-way analysis of variance and least significant difference *post hoc* test with SPSS Statistics for Windows, Version 17.0. (SPSS Inc., Chicago). Only probability values of $P < 0.05$ were considered statistically significant.

RESULTS

Clinical characteristics of patients

The clinical characteristics of patients are presented in Table 1. In this study, there was no significant difference in terms of gender of the patients suffering from pulmonary TB with minimal lesion and extensive lesion. The patients with pulmonary TB based on age group were dominated by age group ≤ 30 years (60%), followed by age group 41–50 years (25%) and age group 31–40 years (15%). The patients with a minimal lesion and an extensive lesion were dominated by age group ≤ 30 years, that is, 70% and 50%, respectively.

The results of sputum smear examination were mostly negative in both groups of minimal (90%) and extensive lesions of pulmonary TB (70%). The average of erythrocyte sedimentation rate (ESR) was 39.22 mm/h in patients with a minimal lesion and 69.19 mm/h in patients with an extensive lesion of pulmonary TB. However, normal ESR was found in one patient with a minimal

lesion (ESR = 6 mm/h) and one patient with an extensive lesion of pulmonary TB (ESR = 10 mm/h).

Tumor necrosis factor- α levels

As shown in Table 2, the mean plasma TNF- α levels in the pulmonary TB groups with minimal lesion (21.276 ± 7.728 pg/ml, $n = 10$) and extensive lesion (19.746 ± 15.196 pg/ml, $n = 10$) were higher than that of the healthy control group (14.567 ± 4.993 pg/ml, $n = 10$), although not statistically significant ($P = 0.155$ and 0.269 , respectively). The mean plasma TNF- α levels in the group with minimal lesion was higher than that of the group with extensive lesion, although the result was not statistically significant ($P = 0.741$).

Insulin-like growth factor-1 levels

As shown in Table 2, the mean plasma IGF-1 levels in the pulmonary TB groups with minimal lesion (49.544 ± 30.778 ng/ml, $n = 10$) and extensive lesion (22.703 ± 17.511 ng/ml, $n = 10$) were significantly ($P = 0.000$ and 0.046 , respectively) higher than that of the healthy control group (3.493 ± 0.870 ng/ml, $n = 10$). The mean plasma IGF-1 level in the group with minimal lesion was significantly ($P = 0.007$) higher than that of the group with extensive lesion.

Transforming growth factor- β 1 levels

As shown in Table 2, the mean plasma level of TGF- β 1 in the pulmonary TB group with minimal lesion (5.484 ± 5.437 ng/ml, $n = 10$) was higher than that of the healthy control group (4.665 ± 1.390 ng/ml, $n = 10$), although not statistically significant ($P = 0.942$). The mean plasma

level of TGF- β 1 in the pulmonary TB group with extensive lesion (14.316 ± 10.113 ng/ml, $n = 10$) was significantly higher than that of the healthy control group ($P = 0.004$) and the group with minimal lesion ($P = 0.005$).

DISCUSSION

Our data suggest that cytokines TNF- α , Insulin like growth factor-1 and TGF- β 1 levels are elevated in patients with TB with minimal and extensive lesion on radiology as compared to healthy individuals but the trend was not statistically significant in case of the TNF- α and IGF-1 but TGF- β 1 levels were significantly higher in extensive lesion TB cases compared to healthy controls and those with minimal lesions. The number of female pulmonary TB patients who participated in this study was higher than that of male patients. While in some previous studies, the results are quite varied where there are the same number and even more men than women. Time limits, location, inclusion and exclusion criteria, and other factors may cause the distribution of patients by gender have not been able to represent the distribution of disease by gender in general. However, the WHO stated that the male: female ratio is 1.7 globally.^[1] In addition, the Indonesian Health Profile 2014 reported that the male: female ratio in Indonesia is 1.5.^[12]

The distribution of patients by age group indicated that the pulmonary TB patients in this study were dominated by the age group of <30 years old. This is in line with the Indonesian Health Profile 2014 reported that new cases of TB are most commonly found in the age group 25–34 years (20.76%).^[12]

The sputum smear examination in our patients showed positive results in only 10% of patients with a minimal lesion and 30% of patients with an extensive lesion of pulmonary TB. The WHO report in 2002 put the number of smear-positive patients in various countries ranges from 25% to 50% of all cases. Sputum smear will show a positive result when there are more than 1000 bacilli in each mL sputum. Therefore, sputum which contains caseous necrosis leaving the cavity tends to produce a positive result.^[13]

The mean ESR in patients with a minimal lesion (39.22 mm/h) and an extensive lesion of pulmonary TB (69.19 mm/h) were higher than the normal reference value (<20 mm/h). ESR is still used by clinicians as a parameter of therapeutic response. The 1st- and 2nd-h ESR

Table 1: Clinical characteristics of patients

Characteristics	Pulmonary tuberculosis with minimal lesions ($n=10$), n (%)	Pulmonary tuberculosis with extensive lesions ($n=10$), n (%)	Total ($n=20$), n (%)
Gender			
Male	4 (40)	5 (50)	9 (45)
Female	6 (60)	5 (50)	11 (55)
Age (years old)			
≤30	7 (70)	5 (50)	12 (60)
31-40	0	3 (30)	3 (15)
41-50	3 (30)	2 (20)	5 (25)
AFB smear			
Positive	1 (10)	3 (30)	4 (20)
Negative	9 (90)	7 (70)	16 (80)
ESR			
Increase	9 (90)	9 (90)	18 (90)
Normal	1 (10)	1 (10)	2 (10)

AFB: Acid-fast bacilli, ESR: Erythrocyte sedimentation rate

Table 2: Plasma levels of tumor necrosis factor- α , insulin-like growth factor-1, and transforming growth factor- β 1

Pro-fibrotic cytokines	Healthy controls ($n=10$)	Total pulmonary tuberculosis patients ($n=20$)	Pulmonary tuberculosis with minimal lesions ($n=10$)	Pulmonary tuberculosis with extensive lesions ($n=10$)
TNF- α (pg/ml)	14.567 ± 4.993	20.511 ± 11.759	21.276 ± 7.728	19.746 ± 15.196
IGF-1 (ng/ml)	3.493 ± 0.870	36.124 ± 27.992	49.544 ± 30.778	$22.703 \pm 17.511^*$
TGF- β 1 (ng/ml)	4.665 ± 1.390	9.900 ± 9.108	5.484 ± 5.437	$14.316 \pm 10.113^*$

Data are presented as mean $\pm$ SD. * $P < 0.05$ compared to the minimal lesion. TNF- α : Tumor necrosis factor- α , IGF-1: Insulin-like growth factor-1, TGF- β 1: Transforming growth factor- β 1, SD: Standard deviation

can be used as an indicator of patient healing. The ESR is often elevated in the active process, but a normal ESR does not exclude the diagnosis of pulmonary TB.^[10]

Fibrosis is the permanent cause of respiratory dysfunction in TB. Fibrosis occurs as a result of the responses which are not supposed to the lung injury. As the proper response, granuloma formation is continued with the granuloma destruction, dissolution of extracellular matrix, and then returns to the normal tissue. Pulmonary fibrosis can occur in the interstitial tissue, the capsule around the cavity, as ribbons with distortion of the lung architecture or can be a combination of those forms. The inhaled mycobacteria are first arrested by the alveolar macrophage, and then phagocytosis occurs mediated by various host receptors. T-cells, natural killer T-cells, and granulocytes are important cells of the body's defense, which are associated with increased production of interferon- γ (IFN- γ) and TNF- α . Collectively, these cells initiate a cascade of chemokines and cytokines that stimulate the arrival of other macrophages and T-cells to the site of infection. In the histological analysis of granuloma formation in mice, macrophage activation is regulated by cellular immunity (cell-mediated immunity), followed by the destruction of the macrophages, which is then accumulated as caseous necrotic and is often associated with the death of bacilli in it.^[14,15] In granuloma, both T-cells and macrophages secrete TNF- α and lymphotoxin- α 3. The TNF- α is not only crucial for the body's defense but also this factor and TGF- β 1 facilitate the formation of the fibrous capsule as granuloma wall that maintains the granuloma integrity. Macrophages also secrete IGF-1, fibroblast growth factor, fibronectin, and PDGF.^[16,17]

There are several cytokines and growth factors that play a role in the process of intrapulmonary fibroblast migration, including TNF- α , PDGF, IL-1 β , KL6, fibronectin, nerve growth factor, and tenascin-C. In this study, although there was no significant difference among the groups, the mean plasma level of TNF- α tended to increase in the pulmonary TB group than that of the healthy control group, also in the group with minimal lesion compared with extensive lesion.

The study by Deveci *et al.* showed that TNF- α levels are increased in patients with pulmonary TB compared to the normal group. TNF- α levels are then decreased in a row after treatment for 2 months, 4 months, and the lowest after 6 months of treatment. It was further reported that TNF- α levels are increased in the early stage of TB and also increased in the person in contact with TB patients.^[18] This report is in line with our study that TNF- α level in minimal lesion was higher than that of the extensive lesion.

Mononuclear cells and alveolar macrophages produce large amounts of TNF- α in response to Mtb infection. Physiologically, the level of TNF- α is important for immunity. The local production of TNF- α at the site of infection plays a role in the formation of granulomas, infection control, and elimination of the disease. Excessive

production of TNF- α at the site of infection can lead to local necrosis which is a hallmark of the disease progress. This causes TNF- α to enter the systemic circulation and contribute to the emergence of systemic manifestations such as hot and cachexia.^[3] In the process of pulmonary fibrosis, TNF- α stimulates fibroblast proliferation and induces fibroblast to produce PDGF.^[5] Although TNF- α plays a vital role in the inflammatory response, which is associated with progression of the disease, various studies illustrate the complex relationships in the immune system and effectors. Among the factors that also affect is the bacterial virulence.^[3]

In this study, measurement of IGF-1 levels was performed to represent the process of pulmonary fibrosis. The mean plasma level of IGF-1 was significantly higher in the pulmonary TB group than that of the healthy control group. Moreover, the mean plasma level of IGF-1 was significantly elevated in the group with minimal lesion compared with the group with extensive lesion.

In line with the results obtained in the measurement of the TNF- α levels, in fibroblast proliferation phase, the highest level of IGF-1 is achieved at an early stage in the disease process, that is, at the damage with minimal lesion. In theory, the process of pulmonary fibrosis begins with the release of growth factors such as IGF-1 and PDGF, which induce migration or proliferation of resident mesenchymal cells. Furthermore, the signal from the injury site in the lungs stimulates the recruitment of circulating fibrocyte (CCL2 and CXCL12) or bone marrow-derived stem cells. Then, under the influence of TGF- β 1, alveolar epithelial cells will change into fibroblast and myofibroblast.^[5,19]

In this study, TGF- β 1 was measured to represent the cytokines that are involved in the final stage of pulmonary fibrosis. The results of this study showed that the mean plasma level of TGF- β 1 tended to increase in the pulmonary TB group than that of the healthy control group. Moreover, the mean plasma level of TGF- β 1 was significantly increased in the group with extensive lesion compared with the group with minimal lesion. These results suggest that TGF- β 1 level is most dominant in patients with extensive fibrosis lesion of the lung.

TGF- β 1 and IL-10 reach the high levels on progressive TB. It is further mentioned that along with another cytokine, TGF- β 1 increases fibrosis and scarring around tuberculous lesion leading to reduced function of the lung parenchyma.^[13] TGF- β 1 is a pleiotropic cytokine that can modulate inflammatory and immune responses and also is involved in the process of fibrosis and tissue repair. TGF- β 1 is chemotactic for fibroblasts and indirectly induces proliferation through the expression also the autocrine and paracrine activities of PDGF-B.^[5]

Although TGF- β 1 has pro-inflammatory effects such as increases the monocyte chemotactic and the expression

of Fc receptors, it also has substantial anti-inflammatory effects, including the deactivation of macrophages to produce reactive oxygen and nitrogen intermediates, inhibition of T-cell proliferation, interfere with the function of natural killer cells and cytotoxic T lymphocytes, also downregulation of the release of IFN- γ , TNF- α , and IL-1. Moreover, TGF- β 1 increases the intracellular growth of Mtb. Otherwise, neutralizing antibodies against TGF- β 1 will reduce the intracellular development of Mtb. The fact above indicates that TGF- β 1 inhibits the antibacterial and facilitates the survival of Mtb.^[3]

CONCLUSION

In pulmonary TB with extensive lesion, there are differences in the levels of TNF- α , IGF-1, and TGF- β 1 compared to the minimal lesion as well as the healthy controls. The extent of lesion on chest radiograph also describes the state of ongoing pulmonary fibrosis which can be shown by the differences in the levels of pro-fibrotic cytokines.

The results of the study emphasize that the treatment of pulmonary TB should not distinguish between minimal and extensive lesions. The regimen used is INH, rifampicin, pyrazinamide, and ethambutol.^[9] The results of the study are in line with changes in TB treatment guideline of the WHO 2003–2008, which does not distinguish minimal and extensive lesions on chest X-ray.

Based on the development of TB immunology, immunotherapy is currently being explored as a treatment in addition to anti-TB drugs. It may take an agent to reduce nonspecific inflammatory response, such as TNF- α inhibitors such as pentoxifylline.^[13] Moreover, the possible use of a specific inhibitor of TGF- β receptor kinase is currently being developed.

Financial support and sponsorship
Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. World Health Organization. Global Tuberculosis Report 2017. Geneva: World Health Organization; 2017. p. 1-2.
2. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. 8th ed. Philadelphia: Elsevier/Saunders; 2015.
3. Rom WN, Garay SM. Tuberculosis. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2004.
4. Sasindran SJ, Torrelles JB. Mycobacterium tuberculosis infection and inflammation: What is beneficial for the host and for the bacterium? Front Microbiol 2011;2:2.
5. Todd NW, Luzina IG, Atamas SP. Molecular and cellular mechanisms of pulmonary fibrosis. Fibrogenesis Tissue Repair 2012;5:11.
6. Choi JE, Lee SS, Sunde DA, Huizar I, Haugk KL, Thannickal VJ, *et al.* Insulin-like growth factor-I receptor blockade improves outcome in mouse model of lung injury. Am J Respir Crit Care Med 2009;179:212-9.
7. Geng J, Huang X, Li Y, Xu X, Li S, Jiang D, *et al.* Phosphatase and tensin homolog deleted on chromosome 10 contributes to phenotype transformation of fibroblasts in idiopathic pulmonary fibrosis via multiple pathways. Exp Biol Med (Maywood) 2016;241:157-65.
8. Yang Z, Sun Z, Liu H, Ren Y, Shao D, Zhang W, *et al.* Connective tissue growth factor stimulates the proliferation, migration and differentiation of lung fibroblasts during paraquat-induced pulmonary fibrosis. Mol Med Rep 2015;12:1091-7.
9. World Health Organization. Treatment of Tuberculosis: Guidelines for National Programmes. 3rd ed. Geneva: WHO Press; 2003. p. 37-8.
10. Perhimpunan Dokter Paru Indonesia. Tuberkulosis: Pedoman Diagnosis Dan Penatalaksanaan Di Indonesia. Jakarta: Perhimpunan Dokter Paru Indonesia; 2011. p. 9-17.
11. National Tuberculosis Association of the USA. Diagnostic Standards and Classification of Tuberculosis. 11th ed. New York: National Tuberculosis Association; 1961.
12. Ministry of Health, the Republic of Indonesia. Indonesian Health Profile Year 2014. Jakarta: Ministry of Health, the Republic of Indonesia; 2015. p. 133.
13. Schaaf HS, Reuter H. Practical approaches to ordering diagnostic tests. In: Schaaf HS, editor. Tuberculosis: A Comprehensive Clinical Reference. London: Elsevier Publishers; 2009. p. 216-9.
14. Kuwano K, Hagimoto N, Nakanishi Y. The role of apoptosis in pulmonary fibrosis. Histo Histopathol 2004;19:867-81.
15. Wynn TA. Integrating mechanisms of pulmonary fibrosis. J Exp Med 2011;208:1339-50.
16. Chua F, Gaudie J, Laurent GJ. Pulmonary fibrosis: Searching for model answers. Am J Respir Cell Mol Biol 2005;33:9-13.
17. Harari S, Caminati A. IPF: New insight on pathogenesis and treatment. Allergy 2010;65:537-53.
18. Deveci F, Akbulut HH, Turgut T, Muz MH. Changes in serum cytokine levels in active tuberculosis with treatment. Mediators Inflamm 2005;2005:256-62.
19. Strieter RM, Keeley EC, Hughes MA, Burdick MD, Mehrad B. The role of circulating mesenchymal progenitor cells (fibrocytes) in the pathogenesis of pulmonary fibrosis. J Leukoc Biol 2009;86:1111-8.