ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jul 15; 7(13):2075-2078.
https://doi.org/10.3889/oamjms.2019.544
eISSN: 1857-9655
Basic Science

The Correlation between Levels of Transforming Growth Factor-β with Pulmonary Fibrosis in Post Pulmonary Tuberculosis in Medan, North Sumatera – Indonesia

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Citation: Christine T, Tarigan AP, Ananda FR. The Correlation between Levels of Transforming Growth Factor-β with Pulmonary Fibrosis in Post Pulmonary Tuberculosis in Medan, North Sumatera – Indonesia. Open Access Maced J Med Sci. 2019 Jul 15; 7(13):2075-2078. https://doi.org/10.3889/oamjms.2019.544

Keywords: TGF-β; Post-pulmonary tuberculosis; Lung fibrosis

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Received: 25-Apr-2019; **Revised:** 09-Jun-2019; **Accepted:** 10-Jun-2019; **Online first:** 13-Jul-2019

Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Untreated or undertreated, pulmonary tuberculosis could cause severe complications until death. After treatment, residual lesions might occur. The presence of residual lesions is varied, including fibrosis, cavity, bronchiectasis and calcification. Transforming growth factor-β (TGF-β) is a cytokine associated with lung inflammation, which plays a role in lung fibrosis. However, only a few studies have assessed the serum level of TGF-β in post-treatment tuberculosis patients.

AIM: The main objective of this study was to determine the correlation between TGF-β levels and pulmonary fibrosis in patients with pulmonary tuberculosis.

DESIGN: A group of 51 patients that had undergone anti-tuberculosis treatment were observed, consisting of 31 men, 20 women. Of all patients, there were 26 people with a smoking history, including 25 men and 1 woman. All patients had been recovered, confirmed by the clinical state, laboratory and radiology examination. The ELISA test was performed to measure TGF-β level, while the chest X-ray was used to look for the occurrence of pulmonary fibrosis.

RESULTS: The mean level of TGF-β in patients with a lesion (+) was 7628.02 (SD: ± 4928.38) while the mean level of TGF-β in patients with a lesion (-) was 2315.11 (SD: ± 505.83). The statistical test showed a significant relationship between TGF-β level and fibrosis lesion ($p < 0.001$).

CONCLUSION: TGF-β level was significantly higher in post-tuberculosis patients with pulmonary fibrosis.

Introduction

Tuberculosis (TB) is an infectious disease caused by germs from the *Mycobacterium tuberculosis*. Pulmonary TB is a type of TB infections which invade the pulmonary parenchyma, including respiratory bronchiole and alveolus [1]. According to the Global Tuberculosis Report 2016, Indonesia is the second country with the highest incidence of pulmonary TB after India and followed by China [2].

TB patients who are recovering from TB usually have residual lesions and sign and symptoms similar to chronic obstructive pulmonary disease (COPD) such as shortness of breath during activity [3], [4]. In a case series study, it showed that in patients with post pulmonary TB, some of them develop a reduction of lung functions, with the majority of patients had a moderate obstruction and mild restriction [3]. TB sequelae is a condition of which there is permanent deformity after TB treatment. TB sequelae may appear in lung and extra lung. Lung fibrosis is one of the variations in residual lesions that occur in the lung parenchyma [5], [7].

To find out the process of fibrosis formation, recent studies had discussed the molecular level of lung fibrosis of which several inflammatory cytokines were found to be involved in defence mechanisms against bacterial infections, including pro-inflammatory cytokines such as interferon-γ (IFN-γ), and anti-inflammatory cytokines such as Tumor Necrosis Factor Alpha (TNF-α), and Transforming Growth Factor – β (TGF – β) [4], [6], [7]. TGF – β is a cytokine that plays an important role in cell proliferation and differentiation. TGF-β has been recognised as the suppressor in the immune system and stimulator in

the pathogenesis of pulmonary fibrosis. Thus excessive production of TGF-β can cause the excessive deposit of scar tissue and fibrosis [7], [8], [9].

Smoking is one of the aggravating factors in forming lung fibrosis. Cigarette smoke can induce pro-inflammatory and immunosuppressive cytokines that stimulate fibrogenesis in post-pulmonary TB. A study showed the increased level of TNF-α could decrease the lung functions in post-pulmonary TB [4].

The studies of TGF-β level in post-TB patients have not been widely conducted in Indonesia. Figen Devici et al. found that inactive TB patients, inactive TB and healthy people, TGF-β level were 258.1 ± 306.6, 185.1 ± 216.5, 110.3 ± 83.2 [10]. A Study about the correlation between TGF-β level and pulmonary fibrosis in post-TB patients has never been conducted in one of the provinces in Sumatera. These facts interest the authors to conduct a study about the relationship between TGF-β level and pulmonary fibrosis in post-TB-patients.

Material and Methods

Study population

This study was an analytical study with cross-sectional design and conducted in Haji Adam Malik General Hospital, Medan, North Sumatera.

Inclusion Criteria

1. Patients with pulmonary TB who had been recovered, confirmed by negative smear examination in the sixth month of treatment.

2. Patients aged over 17 years.

3. Willing to follow the study after receiving information about this study (written informed consent).

Exclusion criteria

1. Patients diagnosed with asthma; and/or

2. Patients suffer from diseases that can interfere with lung function.

Procedures

After had been contacted via mobile phone, all participants were informed about the content of the study, and if they were willing to participate, they were asked to come to the hospital. Then, they needed to sign the informed consent and were interviewed about the history of TB. TGF-β examination was conducted from a vein blood sample, processed with Enzyme Link Immunosorbent Assay (ELISA) by using TGF-β kit. Pulmonary fibrosis was confirmed by Chest X-Ray.

Statistical Analysis

All data were analysed using SPSS version 23. Then, they were analysed if they were normally distributed by Kolmogoroff-Smirnov. In this data, Independent T-test was used to determine the significant correlation between TGF-β levels and pulmonary fibrosis. P-value < 0.05 considered to be significant.

Results

This study involving 51 subjects who matched inclusion criteria and did not have any exclusion criteria. The study samples were post-TB patients and were grouped based on gender, age, smoking history, Brinkman-Index, level of education, occupational, and fibrosis lesson (Table 1).

Table 1: Characteristics of study subjects based on gender, age, smoking history, Brinkman Index, level of education, occupational, and fibrosis lesson

Variable		N (%)
Gender		
Male		31 (60.80)
Female		20 (39.20)
Total		51 (100)
Age		
15-30 Years		16 (31.4)
31-45 Years		12 (23.5)
46-60 Years		22 (43.1)
61-75 Years		1 (2.0)
Total		51 (100)
Average Age (Year)	40.75 ± 14.3	
Smoking History		
Yes		26 (51.0)
No		25 (49.0)
Total		51 (100)
Brinkman Index		
Mild		10 (38.4)
Moderate		10 (38.4)
Severe		6 (23.2)
Total		26 (100)
Level of Education		
< Senior High School		24 (47.1)
Senior High School		16 (31.4)
Diploma		4 (7.8)
Bachelor		7 (13.7)
Total		51 (100)
Occupational		
Indoor		29 (56.9)
Outdoor		22 (43.1)
Total		51 (100)
Fibrosis Lesion		
Yes		42 (82.4)
No		9 (17.6)
Total		51 (100)

From the table, we showed the majority of participants were men consisted of 31 people (60.80%), while the female 20 (39.20%). Based on age, the highest age group was the age group of 46-60 years, which consisted of 22 people (43.1%), with an average age of 40.75 years. Study subjects who had a smoking history were 26 people (51.0%), with mild and moderate Brinkman Index in 10 people

(38.4%), respectively and severe Brinkman Index in 6 people (23.2%) and subjects who had no smoking history were 25 people (49.0%). The characteristics of the study subjects based on the presence of fibrosis lesions, there were 42 subjects (82.4%) with fibrosis lesions on the chest X-ray and 9 subjects without fibrosis lesions in the chest X-ray (17.6%).

Table 2: TGF-β level of study subject

Variable	Min - Max	Mean	SD	Median
TGF-β (pg/ml)	692 – 19100	6690.45	4913.46	5190.00
Smoking History (+)	692 – 16700	6621.50	4856.77	5370.00
Smoking History (-)	1590 - 19100	6762.16	5070.95	4780.00

Table 2 shows that TGF-β level in the study subjects obtained an average level of 6690.45 pg/ml ± 4913.46 pg/ml, median 5190 pg/ml, with a minimum value of 692 pg/ml and maximum value 19100 pg/ml. The study subjects with smoking history status obtained an average level of TGF-β were 6621.50 pg/ml ± 4856.77 pg/ml SD, median 5370.00 pg/ml, and a minimum value of 692 pg/ml and a maximum of 16700 pg/ml. In the study subjects without smoking history status, the average level of TGF-β was 6762.16 pg/ml ± 5070.95 pg/ml SD, median 4780.00 pg/ml, and a minimum value of 1590 pg/ml and a maximum of 19100 pg/ml.

Table 3: Correlation between TGF-β level and pulmonary fibrosis

Fibrosis Lesion	TGF-β Level				
	Mean	SD	Median	Min-Maks	p-value
(+)	7628.02	4928.38	7185.00	692-19100	0.001*
(-)	2315.11	505.83	2177.00	1620- 3430	

*Significant with the independent t-test correlation test.

The correlation between TGF-β level and the presence or absence of pulmonary fibrosis in a chest X-ray can be seen in Table 4. Based on the independent t-test, the p-value was 0.001. This result indicated that there was a significant correlation between TGF-β and the presence of pulmonary fibrosis in a chest X-ray as indicated by the presence or absence of lesions in post-TB patients ($p < 0.05$). In other words, the value of TGF-β was significantly related to the presence of fibrosis lesions.

Discussion

In this study, the majority of the subject was male (n = 31, 60.80%). This is in line with a study conducted by Adhanta SR et al., (2009), Chung et al., (2011) and Manji et al., (2016) which found that male gender is more common in TB cases [11], [12], [13]. In this study, the majority of participants was in the age group 46-60. According to the Indonesian Ministry of Health for the Prevention of Tuberculosis 2016, the most vulnerable groups of TB infections were young and productive age group [14]. On the other hand, based on 2013 Foundational Health Research in Indonesia, the age group > 45 years old has a higher prevalence among other groups [15].

This study found that 42 subjects (82.4%) had fibrosis lesions on chest X-ray. The fibrosis lesions also presented in the chest X-Ray of patients that had been recovered from TB. The presence of sequelae lesions is varied. Menon et al., (2015) found 40.36% of patients had residual lesions, including: fibrosis (38.7%), cavity (21.4%), bronchiectasis (4.3%) and calcification (3%) [16]. The presence of these fibrosis lesions correlates with TGF-β levels. Based on the Independent T-Test, the p-value was 0.001. This result indicated that there was a significant correlation between TGF-β level and the presence of pulmonary fibrosis in chest X-ray ($p < 0.05$).

TGF-β plays a role in growth regulators and cell differentiation. TGF-β is produced as an inactive form in part of a larger molecule that requires an acid or enzymatic process to activate. TGF-β is present in various tissues and interacts with specific cell membrane receptors [17], [18].

The importance of TGF-β in causing and maintaining fibrosis was seen in high levels of TGF-β in various fibrosis diseases as shown by few studies [19], [20]. This positive feedback process could explain the important role of TGF-β in promoting, strengthening and perpetuating fibrosis. Therefore, a potential therapeutic target for fibrosis might involve disconnecting one of the links with TGF-β induction found in myofibroblasts [19], [20].

TGF-β contributes to the differentiation of myofibroblasts by stimulating the expression of α-sma. TGF-β also activates myofibroblasts and then produce greater amounts of Extracellular Matrix protein, stimulating chemotaxis migration of fibroblasts to the inflammation site, and increasing myofibroblast ability to contract. In addition to increasing α-sma expression, TGF-β induces stress fibre formation and adhesion complex maturation. TGF-β also induces the biochemical release of contraction of stress fibres. In the other study, TGF-β also acts as the main mediator of EMT, providing a further source for myofibroblasts. This explains the TGF-β involvement in the pathogenesis of fibrosis found in various organs and tissues. TGF-β is considered a "major switch" in fibrotic processes, plays a role in parenchymal and interstitial fibrosis, as well as vascular remodelling. Thus the therapeutic target in diseases with fibrogenesis should target the TGF-β pathway. Many attempts have been made to block TGF-β. Pirfenidone, recently approved for the treatment of IPF, weakens the production and work of TGF-β, albeit with an unclear mechanism [21], [22], [23], [24].

In conclusion, there is a correlation between TGF-β level with the presence of pulmonary fibrosis in chest X-ray in post-pulmonary tuberculosis patients.

References

1. Kementerian Kesehatan Republik Indonesia Direktorat Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan, Pedoman Nasional Pengendalian Tuberkulosis: Indonesia Bebas Tuberkulosis. Jakarta, 2014

2. WHO, Global Tuberculosis Report. Geneva, 2016

3. Tarigan AP, Pandia P, Eyanoer P, Tina D, Pratama R, Fresia A. Obstructive lung disease as a complication in post pulmonary TB. InIOP Conference Series: Earth and Environmental Science. 2018; 125(1):012154). https://doi.org/10.1088/1755-1315/125/1/012154

4. Tambunan DT, Tarigan AP, Sinaga B, Pandia P, Eyanoer PC. Correlaton of serum tumor necrosis factor-alpha level with pulmonary function in post pulmonary tuberculosis patients. Respirology. 2017; 22:218-218. https://doi.org/10.1111/resp.13207_342

5. Harada S, Harada Y, Kitara Y, Takamoto M, Ishibashi T, Sininoda A. Tuberculosis sequelae: clinical aspects. 1990; 831-838.

6. Kim HY, Song KS, Goo JM, Lee JS, Lee KS, Lim TH. Thoracic sequelae and complication of tuberculosis. 2001; 839-860. https://doi.org/10.1148/radiographics.21.4.g01jl06839 PMid:11452057

7. Branton, M & Kopp, JB. TGF-β and fibrosis. Editions scientifiques et medicales Elsevier SAS. 1999; 1349-1365. https://doi.org/10.1016/S1286-4579(99)00250-6

8. Leask A & Abraham. TGF-β signaling and the fibrotic response. The FASEB Journal. 2004; 18(7):816-827. https://doi.org/10.1096/fj.03-1273rev PMid:15117886

9. McCartney-Francis N, Mizel D, Wong H, Wahl L, Wahl S. TGF-β regulates production of growth factors and TGF-β by human peripheral blood monocytes. Growth Factors. 1990; 4(1):27-35. https://doi.org/10.3109/08977199009011007 PMid:1707635

10. Deveci F, Akbulut HH, Turgut T, Muz MH. Changes in serum cytokine levels in active tuberculosis with treatment. Mediators of inflammation. 2005; 2005(5):256-62. https://doi.org/10.1155/MI.2005.256 PMid:16258192 PMCid:PMC1533887

11. Achanta S, Tekumalla RR, Jaju J, Purad C, Chepuri R, Samyukta R, Malhotra S, Nagaraja SB, Kumar AM, Harries AD. Screening tuberculosis patients for diabetes in a tribal area in South India. Public Health Action. 2013; 3(1):43-7. https://doi.org/10.5588/pha.13.0033 PMid:26393069 PMCid:PMC4463145

12. Chung Kuei-Pin, Chen Jung-Yueh, Lee Chih-Hsin, Wu Huey-Dong, Wang Jann-Yuan, Lee Li-Na, et al. Trends and predictors Changes in Pulmonary Function after treatment for Pulmonry Tuberculosis. Clinical Science. 2011; 66(4):549-555. https://doi.org/10.1590/S1807-59322011000400005 PMid:21655745 PMCid:PMC3095809

13. Mohammed M, Grace S, Simon M, Rose M, Ahmad J, Ferdinand M. Lung function among patients with pulmonary Tuberculosis in Dar es Salaam- a cross-sectional study. BMC Pulmonary Medicine. 2016; 16:58. https://doi.org/10.1186/s12890-016-0213-5 PMid:27107713 PMCid:PMC4842294

14. Peraturan Menteri Kesehatan Republik Indonesia Nomor 67 Tahun 2016 Tentang Penanggulangan Tuberkulosis. Jakarta, 2016.

15. Riskesdas. Laporan Riset Kesehatan Dasar Tahun 2013. Kementerian Kesehatan. Jakarta, 2013.

16. Menon B, Nima G, Dogra V, Jha S. Evaluation of the radiological sequelae after treatment completion in new cases of pulmonary, pleural, and mediastinal tuberculosis. Lung India: Official Organ of Indian Chest Society. 2015; 32(3):241-245. https://doi.org/10.4103/0970-2113.156233 PMid:25983409 PMCid:PMC4429385

17. Assoian RK, Fleurdelys BE, Stevenson HC, Miller PJ, Madtes DK, Raines EW, Ross R, Sporn MB. Expression and secretion of type beta transforming growth factor by activated human macrophages. Proceedings of the National Academy of Sciences. 1987; 84(17):6020-4. https://doi.org/10.1073/pnas.84.17.6020 PMid:2888109 PMCid:PMC298999

18. Blakytny R, et al. Latent TGF-β1 activation by platelets. Journal of Cell Physiology. 2004; 67-76. https://doi.org/10.1002/jcp.10454 PMid:14978736

19. Fernandez IE, Eickelberg O. The impact of TGF-β on lung fibrosis: from targeting to biomarkers. Proceedings of the American Thoracic Society. 2012; 9(3):111-6. https://doi.org/10.1513/pats.201203-023AW PMid:22802283

20. Gerarduzzi C, Di Battista JA. Myofibroblast repair mechanisms post-inflammatory response: a fibrotic perspective. Inflammation Research. 2017; 66(6):451-65. https://doi.org/10.1007/s00011-016-1019-x PMid:28040859

21. Martin GE, Ask K, Gilpin SE, Kolb M, Gauldie J. The transforming growth factor-beta (TGF-β) family and pulmonary fibrosis. Drug Discovery Today: Disease Mechanisms. 2006; 3(1):99-103. https://doi.org/10.1016/j.ddmec.2006.03.006

22. Massague' J. Receptors for the TGF-β family. 1992; 1067-1070. https://doi.org/10.1016/0092-8674(92)90627-O

23. Laurent GJ, McAnulty RJ, Hill M, Chambers R. Escape from the matrix: multiple mechanisms for fibroblast activation in pulmonary fibrosis. Proceedings of the American Thoracic Society. 2008; 5(3):311-5. https://doi.org/10.1513/pats.200710-159DR PMid:18403325 PMCid:PMC2643217

24. Shi M, Zhu J, Wang R, Chen X, Mi L, Walz T, Springer TA. Latent TGF-β structure and activation. Nature. 2011 Jun;474(7351):343-351. https://doi.org/10.1038/nature10152 PMid:21677751 PMCid:PMC4717672