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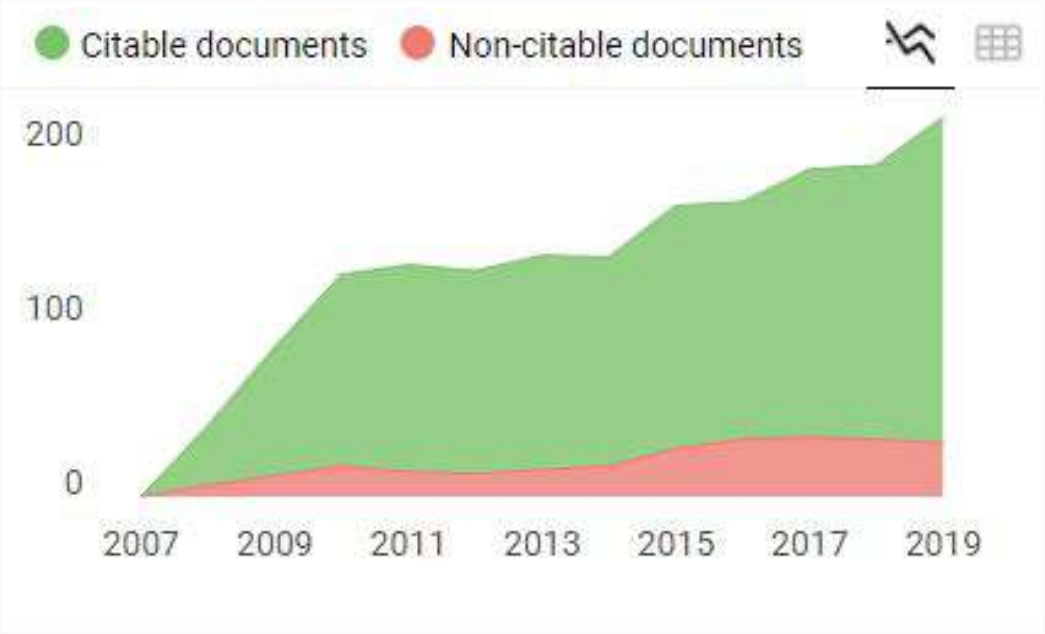

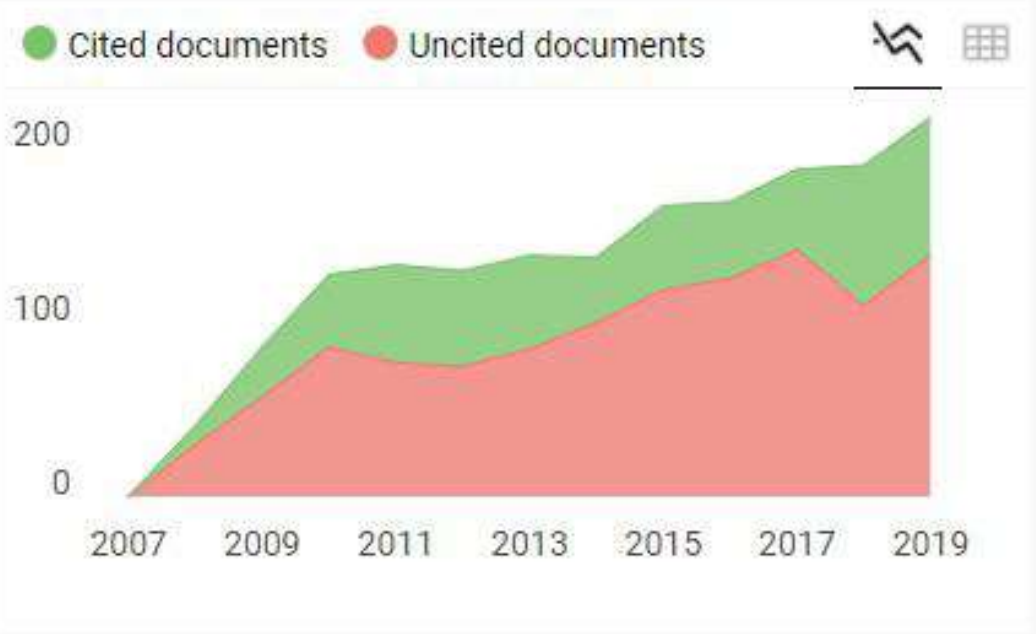

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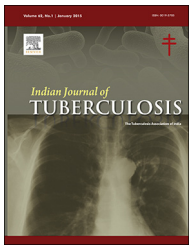

Original article

The comparison of pleural fluid TNF-α levels in tuberculous and nontuberculous pleural effusion

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ABSTRACT

Background: Tuberculous pleural effusion is the manifestation of Mycobacterium tuberculosis infection in pleura. With existing means, it is difficult to establish the diagnosis of tuberculosis (TB) and non-TB pleural effusions; thus, establishing the diagnosis of TB pleural effusion and non-TB pleural effusion is still a clinical problem. Tumour necrosis factor alpha (TNFα) is a potent inflammatory cytokine that plays an important role in immunity to Mycobacterium tuberculosis infections, this level of cytokine increases in pleural effusion due to tuberculosis.
Objective: To compare the TNF-α level of pleural fluid in TB and non-TB pleural effusion.
Methods: The samples in this study that fulfilled the inclusion criteria were patients with non-TB pleural tuberculosis effusion in the inpatient ward in Pulmonology Unit Dr. Soetomo General Hospital Surabaya, male and female, aged between 15 and 60 years. The data is divided into two: primary data and secondary data of patients who fulfilled inclusion and exclusion criteria. The data with normal distribution was analyzed using independent *t*2 test and if the data distribution is abnormal, it was analyzed using Fisher's exact test.
Results: There were 22 subjects divided into 2 groups that were 11 patients with TB pleural effusion and 11 patients with non-TB pleural effusion. The TNF-α level of pleural fluid in TB pleural effusion was 25.43 ± 13.55 pg/mL. The TNF-α level of pleural fluid in non-TB was 5.98 ± 1.89 pg/mL. The serum TNF-α level in TB pleural effusion was 83.22 ± 88.15 pg/mL. The serum TNF-α level in non-TB was 68.54 ± 57.88 pg/mL. There was higher level of TNF-α pleural fluid in TB pleural effusion than in non-TB pleural effusion (25.43 ± 13.55 pg/mL vs 5.98 ± 1.89 pg/mL, *p* value <0.05). The serum TNF-α level in patients with TB pleural effusion was higher than TNF-α serum level of non-TB pleural effusion. There was no significant difference between TNF-α level of pleural fluid and serum TNF-α levels in the TB pleural effusion group (*p* value >0.05).
Conclusion: The TNF-α level of pleural fluid in TB pleural effusion was higher than non-TB pleural effusions and there was no significant difference between serum TNF-α levels in the TB pleural effusion group and in the non-TB pleural effusion group.

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1. Introduction

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis and is a major cause of morbidity and mortality in developing countries. Indonesia is on the fourth rank after India, Africa and China. Pleural effusion in TB is a manifestation of Mycobacterium tuberculosis infection in the pleura with an incidence rate of approximately ±31%.[1,2] Medical record data in Pulmonary Ward of dr. Soetomo General Hospital Surabaya in 2012 found 37 patients with TB pleural effusion per year while there were 39 patients in 2013. The golden standard of TB pleural effusion is a conventional test in discovering Mycobacterium tuberculosis. Conventional methods such as acid fast bacilli smear and Mycobacterium tuberculosis culture from pleural fluid are often found to be negative due to the small number of germs. On the other hand, the right and effective diagnosis is important in controlling the disease. Given the process of hypersensitivity reactions in TB, there are some biomarkers for diagnostic testing of TB pleural effusion, such as examining IFN gamma levels in pleural fluid.[3]

Tumor necrosis factor alpha (TNFα) is a cytokine derived from Th1 cells that play an important role in immunity against Mycobacterium tuberculosis infection. This cytokine contribute to granuloma formation, which controls the disease progression. The mycobacteria antigen in the pleura interacts with T cells which is previously sensitized by mycobacteria. This will trigger a delayed type hypersensitivity and cause a caseous necrosis of granuloma which will subsequently affect the pleural capillary permeability towards the protein resulting in pleural effusion.[4]

TB pleura effusion is an exudative pleural effusion mostly caused by Mycobacterium tuberculosis. With existing means, it is difficult to establish the diagnosis of TB and non-TB pleural effusions; thus, establishing the diagnosis of TB pleural effusion and non-TB pleural effusion is still a clinical problem. Previous study on differential diagnostic markers for both TB and non-TB pleural effusions had significantly higher levels of TNF-α pleural fluid in the TB pleural effusion group compared with the non-TB pleural effusion group.[5]

In Indonesia, research on the level of TNF-α pleural fluid in TB pleural effusions has not been done; therefore, the researchers are intended in conducting the comparison the TNF-α levels of pleural fluid in TB and non-TB pleural effusion.

2. Methods

This research was cross sectional analytic observational study. The samples in this study that fulfilled the inclusion criteria were patients with TB and non-TB pleural tuberculosis effusion in the inpatient ward in Pulmonology Unit Dr. Soetomo General Hospital Surabaya, male and female, aged between 15 and 60 years, and signed the informed consent.

The variables were divided into independent and dependent variable in which the independent variable was TNF-α level of pleural fluid while the dependent variable was the pleural effusion of TB and non-TB. The TNF-α level of pleural fluid and TNF-α were examined with human TNF alpha ELISA kit.

The samples were undergone pleural fluid aspiration and collected their venous blood. It was examined the TNF-α of pleural fluid and peripheral blood serum. The examination began with collecting 3 cc of pleural fluid began, then put in a tube and stored in a refrigerator with a temperature of −70 °C. The measurement of TNF-α level was performed using ELISA kit by centrifuging the pleural fluid sample for 20 min. The supernatant obtained was added to TNF-α reagent. In examining the serum, venous blood was taken as much as 3 cc; then, it was centrifuged for several minutes. It was put in a tube and stored in a refrigerator with a temperature of −70 °C. Afterwards, the examination of TNF-α level was measured by ELISA kit by adding TNF-α reagent in patients' serum.

The data is divided into two: primary data and secondary data of patients who fulfilled inclusion and exclusion criteria. The data with normal distribution was analyzed using independent t2 test and if the data distribution is abnormal, it was analyzed using Fisher's exact test.[6,7]

3. Results

In the TB pleural effusion group, there were 7 (63.6%) male and 4 (36.4%) female patients. In the non-TB pleural effusion group, there were 5 (45.5%) male and 6 (54.5%) female patients. The mean age in the TB pleural treatment group was 27 years old, with the youngest age of 16 and the oldest age of 40 years old. The most age group was 16–25 years old that were 5 (45.5%) patients. The mean age in the non-TB pleural treatment group was 61.3 years old, with the youngest age of 47 years old and the oldest age of 78 years old. The most age group was 46–55 years old that were 4 (36.4%) patients (Figs. 1 and 2). The independent t-test results showed that there was a significant difference between the age group in TB pleural effusion and the age group in non-TB pleural effusion with p value <0.05. The TB pleural effusion group had a younger age than the non-TB pleural effusion group.

The normality included the age data, TNF-α pleural fluid level and serum TNF-α level were examined by using Kolmogorov Smirnov test. It was obtained that the age data, TNF-α level of pleural fluid and serum TNF-α level were normally distributed with $p > 0.05$. The result of Chi-square test between gender and TNF-α level of pleural fluid in the TB and non-TB pleural effusion group concluded that there was no significant association between gender and TNF-α level of pleural fluid with $p > 0.05$. The result of Pearson correlation test between age and TNF-α level of pleural fluid in TB and non-TB pleural effusion group showed that there was no significant association between age and TNF-α fluid level with $p > 0.05$.

The mean TNF-α level of pleural fluid in the TB pleural group was 25.43 pg/mL, with the lowest levels of 11.17 pg/mL and the highest level of 55.12 pg/mL. On the other hand, the mean TNF-α level of pleural fluid in the non-TB pleural effusion group was 5.98 pg/mL, with the lowest level of 3.35 pg/mL and the highest level of 10.20 pg/mL. The independent t test results showed that there was a significant difference between TNF-α level of pleural fluid in TB and non-TB pleural effusion group with p value <0.05 (Fig. 3). The mean TNF-α serum level in TB pleural effusion group was 83.22 pg/mL, with the lowest level of 12.62 pg/mL and the highest level of

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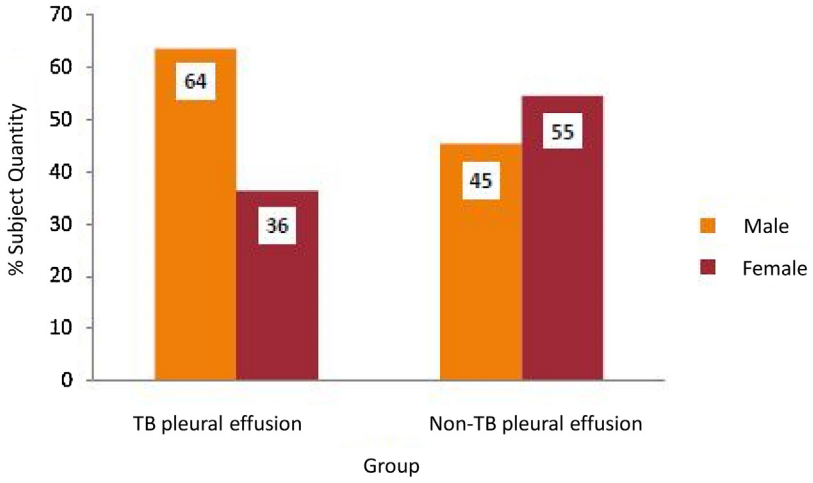

Fig. 1 – The characteristics of research subjects based on gender.

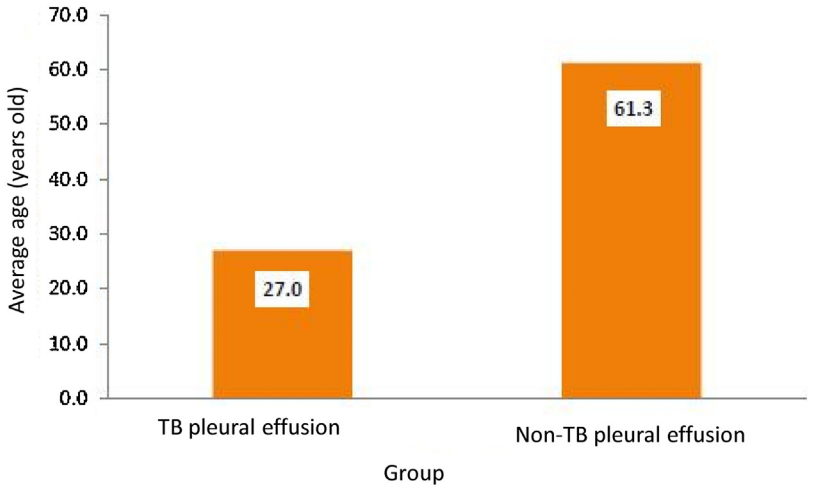

Fig. 2 – The characteristics of research subjects based on age.

259.69 pg/mL. On the other hand, the mean TNF-α serum level in the non-TB pleural effusion group was 68.54 pg/mL, with the lowest level of 13.08 pg/mL and the highest level of 203.80 pg/mL. The independent *t* test results showed that there was no significant difference between TNF-α serum level in TB and non-TB pleural effusion group with p value >0.05 (Fig. 4). The result of paired t test showed that there was no significant difference between TNF-α level of pleural fluid and TNF-α serum level in the TB pleural effusion group with p value >0.05 (Fig. 5).

In the non-TB pleural effusion group, the average TNF-α pleural fluid level was 5.98 pg/mL, with the lowest level of 3.35 pg/mL and the highest level of 10.20 pg/mL. On the other hand, the mean TNF-α serum level was 68.54 pg/mL, with the lowest level of 13.08 pg/mL and the highest level of 203.80 pg/mL. The result of paired *t* test showed that there was a significant difference between TNF-α level of pleural fluid and TNF-α serum level in the non-TB pleural effusion group with p value <0.05 (Fig. 6).

4. Discussion

There were 22 subjects divided into 2 groups that were 11 patients with TB pleural effusion and 11 patients with non-TB pleural effusion. In the TB pleural effusion group, there were 7 (63.6%) male and 4 (36.4%) female patients. In the non-TB pleural effusion group, there were 5 (45.5%) male and 6 (54.5%) female patients. This is in accordance with WHO Global Tuberculosis Report in 2013 which is male to female ratio of 1.5:1.[1] The Chi-square test results concluded that there was no significant association between gender and TNF-α level of pleural fluid with $p > 0.05$. This means gender is not associated with high levels of TNF-α pleural fluid.

The age characteristic in the TB pleural effusion group was 27.0 years old, with the youngest age of 16 years old and the oldest age of 40 years old. The most age group is 16–25 years old that is 5 (45.5%) patients. The mean age in the non-TB pleural treatment group was 61.3 years old, with the youngest age of

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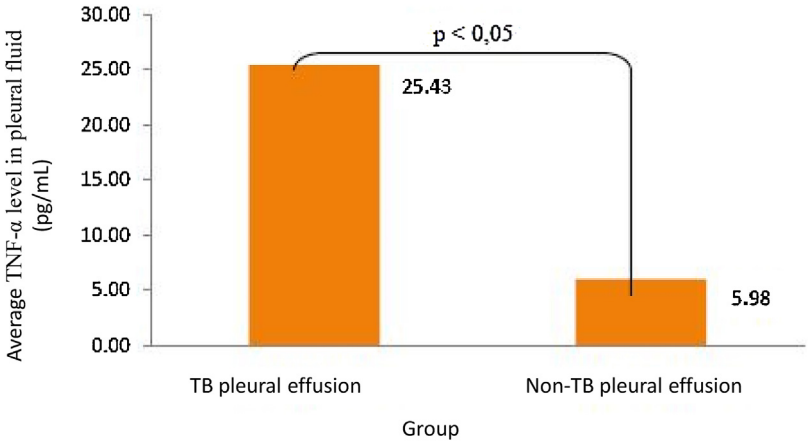

Fig. 3 – The comparison of TNF-α pleural fluid level between TB and non-TB pleural effusion group.

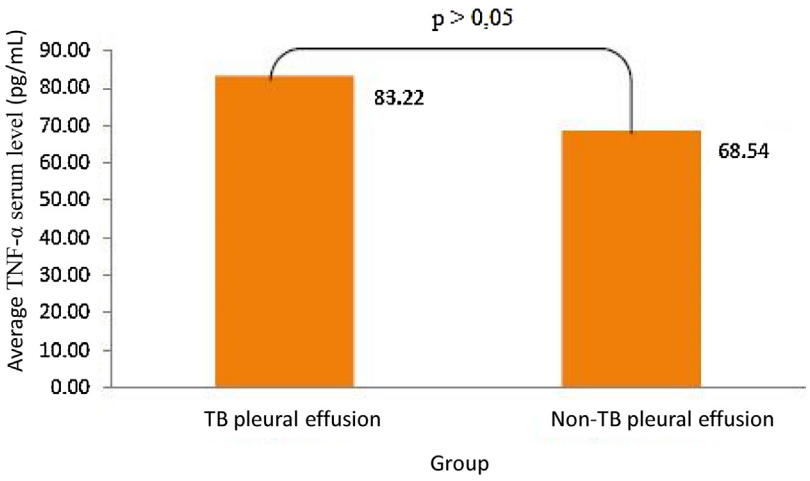

Fig. 4 – The comparison of TNF-α serum level between TB and non-TB pleural effusion group.

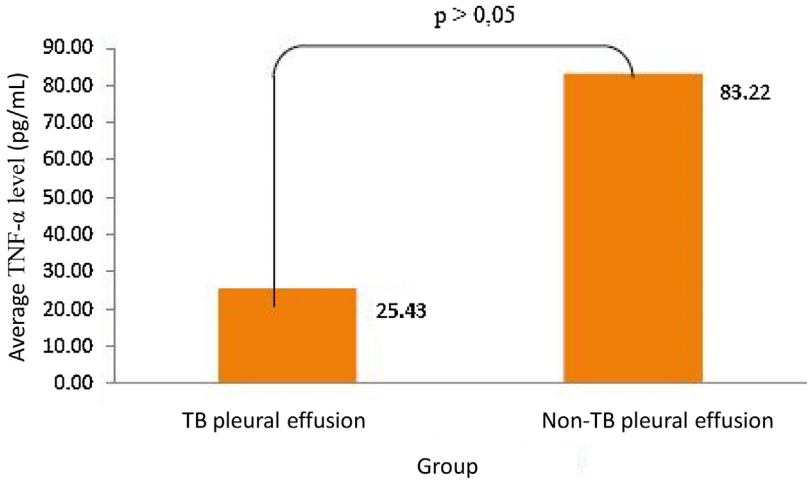

Fig. 5 – The comparison of TNF-α pleural fluid level and TNF-α serum level between TB pleural effusion group.

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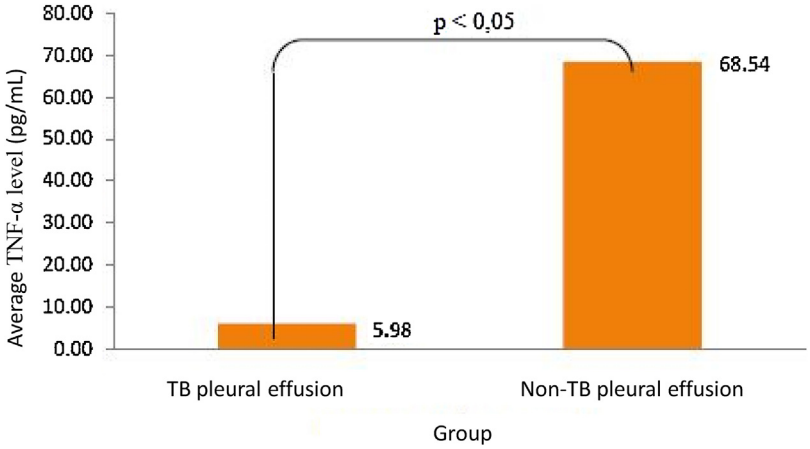

Fig. 6 – The comparison of TNF-α pleural fluid level and TNF-α serum level between non-TB pleural effusion group.

47 years old and the oldest age of 78 years old. The most age group was 46–55 years old that were 4 (36.4%) patients. The independent t test results showed that there was a significant difference between the age group in TB pleural effusion and the age group in non-TB pleural effusion with p value <0.05. Pearson correlation results concluded that there was no significant association between age and TNF-α level of pleural fluid with $p > 0.05$. It can be concluded that age is not associated with high levels of TNF-α fluid pleural. The TB pleural effusion group had a younger age than the non-TB pleural effusion group, according to a study conducted in Korea in 2012 that reported TB disease occurred in the productive age group between 15 and 49 years old.[8] It indicates that the susceptible age group to TB is the productive age group and males are more susceptible than females.[1,8] The result of the normality test by using Kolmogorov Smirnov test showed that the data was normally distributed.

In this study, the mean TNF-α fluid effusion in the TB pleural effusion group of 25.43 pg/mL, with the lowest levels of 11.17 pg/mL and the highest levels of 55.12 pg/mL. Yamada et al. obtained TNF-α levels in pleural fluid of 37.8 ± 11.7 pg/mL. Tahhan et al. obtained TNF-α level of pleural fluid at 65.4 ± 136.9 pg/mL whereas Ambade et al., on TB and non-TB pleural effusion obtained TNF-α level of 195.5 ± 292.1 pg/mL.[5,9,10] The mean TNF-α pleural fluid in the non-TB pleural effusion group was 5.98 pg/mL, with the lowest level of 3.35 pg/mL and the highest level of 10.20 pg/mL. It shows a major protective immune response mediated by local CMI by macrophages that work with Th1 lymphocytes. The Th1 lymphocyte complex with specific antigen of Mycobacterium tuberculosis presented in the pleural cavity through IL-12 will trigger the secretion of cytokines from Th1 cells including TNF-α.[11] The assessment of TNF-α level of TB pleural fluid effusion indicates its role in the body's defense mechanisms especially the process of granuloma formation, elimination of intramacrophage bacillary antigens, and the formation of fibrosis that inhibits disease progression.[12,13] The study examined experimental rats, in which rats with deficiency of this receptor would be more susceptible to the occurrence of Mycobacterium tuberculosis infection. This is due to the inability of macrophages to produce TNF-α.[14]

The mean TNF-α serum level in the TB pleural effusion group was 83.22 pg/mL, with the lowest level of 12.62 pg/mL and the highest level of 259.69 pg/mL. On the other hand, the mean TNF-α serum level in the non-TB pleural effusion group was 68.54 pg/mL, with the lowest level of 13.08 pg/mL and the highest level of 203.80 pg/mL. The independent t test results showed that there was no significant difference between TNF-α serum level in TB and non-TB pleural effusion group with p value >0.05.

Tahhan et al. obtained TNF-α serum level of 2.55 ± 5.23 pg/mL which is lower than the result of this study.[10] However, it is similar to the study conducted by Andrate et al. that obtained higher TNF-α serum level than the level of TNF-α pleural fluid. TNF-α serum levels were 9055.6 pg/mL, 1519.9 pg/mL and 2848.0 pg/mL affected by the clinical severity of Mycobacterium tuberculosis.[15]

The mean TNF-α level of pleural fluid in the TB pleural group was 25.43 pg/mL, with the lowest levels of 11.17 pg/mL and the highest level of 55.12 pg/mL. On the other hand, the mean TNF-α level of pleural fluid in the non-TB pleural effusion group was 5.98 pg/mL, with the lowest levels of 3.35 pg/mL and the highest levels of 10.20 pg/mL. The independent t test results showed that there was a significant difference between TNF-α level of pleural fluid in TB and non-TB pleural effusion group with p value <0.05 (25.43 ± 13.55 pg/mL vs 5.98 ± 1.89 pg/mL). It is in accordance with a research conducted by Tahhan et al. that higher TNF-α level of pleural fluid than the serum level (65.4 ± 136.9 pg/mL vs 54.5 ± 144.2 pg/mL; $p < 0.001$) while Ambade et al., obtained higher TNF-α level in TB group than non-TB group (195.5 ± 292.1 pg/mL vs 59.7 ± 128.9 pg/mL; $p < 0.01$).[5,10] It can be concluded that TNF-α level of pleural fluid is higher in TB pleural effusion than in non-TB pleural effusion group.

In this study, four patients with pleural effusion in pneumonia had TNF-α level of 6.74 pg/mL, 4 pg/mL, 5.87 pg/mL and 5.45 pg/mL. It is considered lower compared to TNF-α level of pleural effusion in TB pleural effusion. The previous study conducted by Yamada et al. found that the level of TNF-α in TB pleural effusion was higher than TNF-α in pleural effusion caused by inflammation (9.2 ± 2.3 pg/mL vs 37.8 ± 11.7 pg/mL).[9] They also attained lower TNF-α level in

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malignant pleural effusion of 6.3 ± 0.7 pg/mL than in TB pleural effusion (37.8 ± 11.7 pg/mL).[9] Among 11 patients with non-TB pleural effusion in this study, seventeen of whom were patients with malignant pleural effusion. TNF-α level of pleural fluid was also found to be lower than the mean level of TNF-α pleural fluid in TB. Lie et al. obtained higher TNF-α level of pleural fluid (45.55 ± 15.58 pg/m/L) than TNF-α level in malignant pleural effusion (17.18 ± 4.84 pg/mL).[16] Ambade et al. also obtained the mean TNF-α level in TB pleural effusion of 195.5 pg/mL, TNF-α level of pleural effusion because by pneumonia of 55 pg/mL and TNF-α level in malignant pleural effusion of 61 pg/mL.[5]

In the TB pleural effusion group, the mean TNF-α pleural fluid level was 25.43 pg/mL, with the lowest levels of 11.17 pg/mL and the highest level of 55.12 pg/mL. On the other hand, the mean TNF-α serum level was 83.22 pg/mL, with the lowest level of 12.62 pg/mL and the highest level of 259.69 pg/mL. The result of paired *t* test showed that there was no significant difference between TNF-α level of pleural fluid and TNF-α serum level in the TB pleural effusion group with *p* value >0.05 (25.4 ± 13.55 pg/mL vs 83.22 ± 88.15 pg/mL).

In the non-TB pleural effusion group, the average TNF-α pleural fluid level of 5.98 pg/mL, with the lowest level of 3.35 pg/mL and the highest level of 10.20 pg/mL. On the other hand, the mean TNF-α serum level was 68.54 pg/mL, with the lowest level of 13.08 pg/mL and the highest level of 203.80 pg/mL. The result of paired *t* test showed that there was a significant difference between TNF-α level of pleural fluid and TNF-α serum level in the non-TB pleural effusion group with *p* value <0.05 (5.98 ± 1.89 pg/mL vs 68.54 ± 57.88 pg/mL). The TNF-α serum level was higher than TNF-α level of pleural fluid.

The high concentration of cytokines in pleural fluid reflects local immune stimulation. It occurs because of the migration of T cells from the periphery to the site of the disease. Thus, TNF-α cytokines are secreted at the site of the disease to increase the level of TNF-α cytokine in pleural effusion than TNF-α level in plasma.[17,18] This study obtained different results with Prabha et al.'s research on 46 patients with TB pleural effusion encountered increased level of TNF-α significantly than the level in plasma.[19] The role of TNF-α as proinflammatory cytokines that have immunoprotective role to control the growth of Mycobacterium tuberculosis as well as the detrimental role in immunopathology of TB. In patients with weight loss, TNF-α serum levels are elevated; thus, this mediator is presumed to play an important role in cortex.

Andrate et al. in his study regarding the association between TNF-α level and clinical TB severity was found that

Table 2 – The normality data of age, TNF-α level of pleural fluid and TNF-α serum level.

Data	*p* value	
	TB pleural effusion	Non-TB pleural effusion
Age (years old)	0.995	0.996
TNF-α level of pleural fluid (pg/mL)	0.884	0.932
TNF-α serum level (pg/mL)	0.601	0.912

Table 3 – The association of gender and age with TNF-α level of pleural fluid.

TNF-α level of pleural fluid (pg/mL)		TB pleural effusion	Non-TB pleural effusion
Chi-square test	Gender	0.545	1.000
Pearson correlation	Age	0.967	0.393

Table 4 – The association of gender and age with TNF-α serum level.

TNF-α serum level (pg/mL)		TB pleural effusion	Non-TB pleural effusion
Chi-square test	Gender	0.576	1.000
Pearson correlation	Age	0.910	0.243

Table 5 – The Comparison of TNF-α level of pleural fluid between TB and non-TB pleural effusion group.

Group	TNF-α level of pleural fluid (pg/mL)	
	mean ± SD	*p* value
TB pleural effusion	25.43 ± 13.55	0.001
Non-TB pleural effusion	5.98 ± 1.89	

Table 1 – The characteristics of research subjects.

Characteristics	Group		*p*
	TB pleural effusion	Non-TB pleural effusion	
Gender			
Male	7 (63.6%)	5 (45.5%)	0.392
Female	4 (36.4%)	6 (54.5%)	
Age (mean ± SD)	27.0 ± 7.6	61.3 ± 9.7	0.000
16–25 years old	5 (45.5%)	0 (0.0%)	
26–35 years old	4 (36.4%)	0 (0.0%)	
36–45 years old	2 (18.2%)	0 (0.0%)	
46–55 years old	0 (0.0%)	4 (36.4%)	
56–65 years old	0 (0.0%)	3 (27.3%)	
66–75 years old	0 (0.0%)	3 (27.3%)	
76–85 years old	0 (0.0%)	1 (9.1%)	

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Table 6 – The comparison of TNF-α serum level between TB and non-TB pleural effusion group.

Group	TNF-α serum level (pg/mL)	
	mean ± SD	*p* value
TB pleural effusion	83.22 ± 88.15	0.649
Non-TB pleural effusion	68.54 ± 57.88	

Table 7 – The comparison of TNF-α level of pleural fluid and TNF-α serum level in TB pleural effusion group.

Variable	TB pleural effusion group	
	mean ± SD	*p* value
TNF-α level of pleural fluid	25.43 ± 13.55	0.073
TNF-α serum level	83.22 ± 88.15	

Table 8 – The comparison of TNF-α level of pleural fluid and TNF-α serum level in non-TB pleural effusion group.

Variable	Non-TB pleural effusion group	
	mean ± SD	*p* value
TNF-α level of pleural fluid	5.98 ± 1.89	0.005
TNF-α serum level	68.54 ± 57.88	

patients with lower body weight had higher serum TNF-α level (15,468.54 ± 4580.54 pg/mL) than patients without weight loss (2904.98 ± 1367.89 pg/mL) with *p* value <0.05. This suggests that, besides being caused by virulence levels, it is also caused by the effect of TB pathogenesis that is affected by clinical severity marked by weight loss conditions.[2,4] Andrate et al.'s research obtained the association between BB with high TNF-α serum level. Patients with a low weight obtained higher TNF-α serum level than patients without decreased weight (Tables 1–8).

5. Conclusion

The level of TNF-α pleural fluid in TB pleural effusions were higher than in non-TB pleural effusions and there was no significant difference between TNF-α serum levels in TB and non-TB pleural effusion group.

Conflicts of interest

The authors have none to declare.

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