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INTERLEUKIN LEVEL 17 AS A BIOMARKER IN IMPROVING TREATMENT OF LUNG TB BEFORE TREATMENT AND AFTER 2 MONTHS OF OAT TREATMENT

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Abstract

Background

Tuberculosis is an important public health problem in the world. Indonesia ranks third in the world after China and India. The cause of TB is Mikrobakterium tuberculosis. Interleukin 17 (IL-17) is a cytokine that plays an important role in the pathogenesis of TB. Therefore, this study examined serum IL-17 levels before treatment and after 2 months of OAT treatment to determine its relationship with sputum smear conversion and therapeutic success.

Aim

Analyzing changes in IL-17 levels in smear positive pulmonary TB patients before treatment and after 2 months of receiving anti-tuberculosis drug treatment (OAT).

Method

Observational research with a cohort - prospective data collection method. Thirty subjects with positive smear pulmonary TB were taken blood samples, before treatment and after 2 months of OAT treatment to assess changes in IL-17 levels

Results

Of 30 patients with positive smear pulmonary TB disease, the IL value of 17 before receiving OAT treatment was 75.2 (11.3 - 281.0) and after 2 months of treatment decreased to 35.1 (4.7 - 141.2) with $P < 0.05$. By using the Spearman correlation obtained a significant correlation between IL-17 levels before and after treatment ($p < 0.05$) and $r = 0.72$,

Conclusion

From the results of the study concluded that there were significant differences in IL-17 levels before and after two months of intensive phase treatment.

Introduction

Background:

Tuberculosis (TB) is an infectious disease caused by the bacteria *Mycobacterium tuberculosis*. The immune system has an important role in the pathophysiology of TB. The protective response to TB involves the secretion of proinflammatory cytokines (such as TNF- α , IL-12, IL-1 β and IFN- γ , IL-17) and to prevent excessive damage due to these proinflammatory factors, the body secretes anti-inflammatory cytokines (such as TGF- β and IL-10) which plays an important role in the immune system. (Dheda K, 2010)

Interleukin 17 (IL-17) is a cytokine that plays an important role in the pathogenesis of TB. These cytokines are able to regulate antibacterial activity and inflammatory processes to fight further infections from germs. (WHO Global Report 2016).

The success of TB control is highly dependent on the diagnosis and appropriate treatment. In addition, it is also influenced by efforts to monitor and evaluate treatment. Therefore, in this study an examination of plasma IL-17 levels was carried out at the beginning of treatment and up to the sixth month to determine its relationship with



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sputum conversion and therapeutic success. It is hoped that knowing the changes in IL-17 can be a clue to predict the success of TB therapy. (WHO Global Report 2016).

Method:

Observational research with a cohort - prospective data collection method. Thirty subjects with positive smear pulmonary TB were blood samples taken, before treatment and after 2 months of OAT treatment to assess changes in IL-17 levels.

Result:

This study was conducted on as many as 30 people with new cases of tuberculosis with the aim to determine the comparison of Interleukin-17 levels in the blood of patients with pulmonary tuberculosis before and after 2 (two) months of intensive phase treatment. As for the characteristics of the subjects in this study can be seen in table 1 below:

Table 1. Characteristics of Research Subjects

Characteristics		N	%
Gender	Man	21	70.0
	Women	9	30.0
Age (Year)	< 40	10	33.3
	40 - 49	6	20.0
	50 - 59	6	20.0
	≥ 60	8	26.7
AFB smear	Scanty	3	10.0
	Positif 1	8	26.7
	Positif 2	8	26.7
	Positif 3	11	36.7
		30	100.0

As many as 70% of sufferers are male and only 30% are female. The most common age range in patients is under 40 years which reaches one third of cases (33.3%). The sufferers who are in the age range 40-49 years and 50-59 years each amounted to 6 people (20%).

All subjects in this study were new cases of pulmonary TB patients confirmed by sputum smear examination (direct smear sputum). Sputum smear examination results are grouped into scanty, +1, +2, and +3 according to the criteria established by the IUATLD (International Union Against Tuberculosis and Lung Disease).

Table 2. Relationship between TB bacilli density with IL-17 levels

	IL - 17 (pg/ml)		p value
	mean ± SD	median (min - max)	
Scanty	70.1 ± 34.1	77.3 (33.1 - 100.1)	0.78
Positif 1	94.6 ± 85.6	61.7 (23.2 - 281.0)	
Positif 2	78.7 ± 32.7	87.4 (13.4 - 121.6)	
Positif 3	69.0 ± 52.5	72.3 (11.3 - 185.9)	

Kruskal Wallis Test

Table 2 shows that there is no significant relationship between the density of TB bacilli with IL-17 levels in the blood of pulmonary TB patients. The changes in IL-17 levels can be seen in the following table:



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Table 3. Comparison of IL-17 levels before and after Treatment

	IL - 17 (pg/ml)		p value
	mean \pm SD	median (min - max)	
before treatment	78.5 \pm 56.3	75.2 (11.3 - 281.0)	<0.001*
after treatment	39.2 \pm 29.0	35.1 (4.7 - 141.2)	

*) significant with the Wilcoxon Test

Based on the normality test using the Shapiro-Wilk test, the levels of Interleukin in 17 TB patients both before and after treatment were not normally distributed. Therefore, to see the average difference between the two, a non-parametric test was performed using the Wilcoxon test.

Table 3 shows that there were significant changes in IL-17 levels in the blood of pulmonary TB patients before and after undergoing intensive phase of treatment for 2 months ($p < 0.001$).

The next objective of this study is to determine whether there is a relationship between changes in IL-17 levels before and after treatment with sputum conversion at the end of the intensive phase (2 months of treatment), as can be seen in table 4 below:

Table 4. Relationship between Changes in IL-17 levels with Sputum Conversion

	N	Δ IL-17	p value
Conversion	26	- 47.6 \pm 28.8	0.002*
No Conversion	4	+ 14.7 \pm 14.05	

*) significant with the Mann Whitney test

Table 4 shows that of the 30 subjects of the study, there were 4 TB patients who did not experience conversion at the end of the intensive phase of treatment. There were significant differences in changes in IL-17 levels during treatment between the groups undergoing conversion and those who did not undergo sputum conversion.

The group of patients who experienced sputum conversion showed a decrease in IL-17 levels by 47.6 pg / ml during treatment for 2 months. In contrast, the group of patients who did not experience sputum conversion actually showed an increase in IL-17 levels of 14.7 pg / ml during 2 months of treatment, and this difference was statistically significant ($p < 0.05$). This phenomenon increasingly emphasizes the important role of IL-17 levels in tuberculosis immunopathogenesis.

The next objective of this study was to determine the correlation between IL-17 levels before and after treatment. The correlation can be seen through the following table 5:

Table 5. Correlation of IL-17 before and after treatment

	IL-17
p value	<0.001*
koefisien (r)	0.72

*) significant with the Spearman Correlation Test

Based on table 5, there is a significant correlation between IL-17 levels before and after treatment ($p < 0.05$), with the correlation strength being strong / high ($r = 0.72$). To clarify these correlations, IL-17 levels are depicted in the scatter diagram as follows:

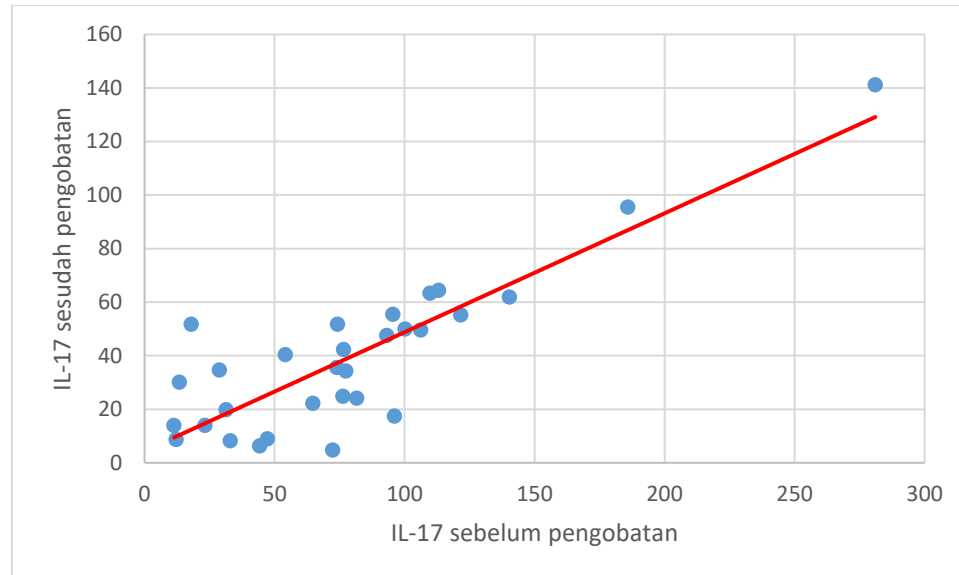


Figure 1 IL-17 stocking diagram before and after treatment

Figure 1 shows a scatter plot diagram that shows a diagonal line that leads to the right, which means the coefficient (r) is positive. This means that if IL-17 levels of TB sufferers before treatment are high, then IL-17 levels after treatment will also remain higher than TB sufferers whose IL-17 levels before treatment are lower.

Discussion

A study has been conducted which aims to determine the comparison of Interleukin-17 levels in the blood of pulmonary tuberculosis patients before treatment and after 2 (two) months of intensive phase treatment. A total of 30 people with tuberculosis were included as subjects in this study. All subjects in this study were new cases of pulmonary TB patients confirmed by sputum smear examination (direct smear sputum). Of all research subjects, 70% of sufferers were male. Patients with pulmonary tuberculosis who were the subjects in this study were more in the age range under 40 years (33.3%). (Ziljstra & Hecken, 2012)

This study found that there was a significant decrease in IL-17 levels in the blood of pulmonary TB patients after undergoing intensive phase of treatment for 2 months. IL-17 levels before treatment by 78.5 pg / ml decreased dramatically by almost half to 39.2 pg / ml after undergoing treatment for 2 months.

The results of this study are in line with the results of research obtained by Emizola et al who conducted an identical study in Malang. He also found that there was a decrease in IL-17 levels, as treatment and clinical improvement of the patient. IL-17 levels at the beginning before treatment were 225 pg / ml, the levels then dropped to 200 pg / ml in the second month of treatment, and the levels decreased even after finishing treatment at 6 months to 122 pg / ml. The study also found a correlation coefficient that was not much different from this study that is equal to 0.517 (moderate).

This decrease in IL-17 levels reflects a reduction in Th1's immune response to TB infection, because IL-17 is an important cytokine in inducing an optimal Th1 response, maintaining the integrity of granulomas by limiting neutrophil deaths, and protective immunity against TB infection. (Chen YC, 2013).

The results of this study are in accordance with the study by Chen et al., Which obtained a significant decrease in IL-17 levels after administration of OAT. The work of IL-17 itself, besides being a proinflammatory cytokine, can also induce several other proinflammatory cytokines such as IL-1, IL-6, IL-8, and TNF α .



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Studies conducted by Singh and George in India in 2018 have proven the important role of IL-17 in TB immunopathogenesis. Singh and George conducted experiments on mice by weakening Th-17 cells, and keeping Th1 cells active, then injecting tuberculosis bacilli antigens. These findings indicate that Th17, through the IL-17 compounds it produces, has a very important role in determining the immune response to tuberculosis (Singh S, George MG, et al, 2018)

The study of Emizola et al found that the decrease in IL-17 levels was in line with the success of OAT therapy which was marked by improvement in clinical symptoms, improvement in photorax features, and conversion of putums to negative smear. This shows that the success of OAT therapy is marked by a significant decrease in the level of IL-17. Then the decrease in plasma IL-17 levels during OAT therapy can be considered as one of the biomarkers of successful therapy in pulmonary TB patients. In addition, this biomarker can be further evaluated in patients with extrapulmonary TB and pediatric TB who cannot be evaluated for therapeutic results through sputum examination.

Conclusion

Based on the results of research previously stated, there are significant differences in IL-17 levels before and after two months of intensive phase treatment, where IL-17 levels have decreased from 78.5 pg / dl to 39.2 pg / dl ($p < 0.001$). There is no relationship between the level of sputum smear density with IL-17 levels in the blood of patients with new cases of pulmonary TB. There was a significant difference in IL-17 levels between pulmonary TB patients who experienced sputum conversion and those who did not experience sputum conversion after two months of treatment ($p > 0.002$). There was a positive correlation between IL-17 levels before and after treatment ($p < 0.001$; $r = 0.72$). IL-17 levels can be used as a biomarker of successful tuberculosis treatment.

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