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Increasing of IL-17 and IL-23 levels in Acid-Fast Bacilli Conversion of Drug Resistant Tuberculosis Patients after One Month Treatment

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ABSTRACT

Background: In Drug-Resistant Tuberculosis (DR-TB), high IL17 level was corelated with severe tissue damage while low level was associated with mortality. High levels of IL-17 and IL - 23 were found in patients with a high bacterial load. TB treatment is carried out by combination of anti-tuberculosis drugs for a period of 9 to 24 months. This study aimed to analyze the differences in IL-17 and IL-23 levels before and after treatment compared to healthy controls, and their relationship with conversion AFB smear.

Methods: A prospective cohort study was conducted with total 38 subjects diagnosed as DR-TB using a molecular rapid test and 34 normal subjects as case and control groups. Examination of sputum AFB, IL-17 and IL-23 levels were carried out at the start of treatment and 30 days after treatment. Treatment was given in short term and individul drugs resistant regiments.

Results: The average levels of IL-17 and IL 23 before treatment compared to those after treatment showed no significant difference (p=0.153; p= 0.328). When compare to healthy controls group, the mean levels of IL-17 and IL 23 before treatment was not significantly different (p= 0.883; p= 0.928), whereas after 1 month treatment were significantly different (p= 0.047; p=0.042). An increase of IL-17 levels followed by an increase in IL-23 levels (p=0,000 r=.0.872). TB treatment affected the conversion rate of 97.4% with linear association.

Conclusion: The levels of IL-17 and IL-23 after treatment were increasing and significantly different with healthy group. These related with the high AFB conversion rate.

Keywords: Drug-Resistant Tuberculosis, Drug Resistant Tuberculosis Treatment, Acid-Fast Bacilli, Interleukin-17, Interleukin-23

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INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by the *Mycobacterium tuberculosis* (*Mtb*) and is the tenth causes of death due to infectious diseases. If TB not treated properly, it could cause complications and death [1]. Globally, tuberculosis cases in 2019 are estimated at 10 million cases (range, 8.9 - 11.0 million) with 1.2 million cases of death, including 208,000 of them HIV positive people. Two thirds of TB cases in the world were found in 8 countries, there are India (28%), Indonesia (8.5%), China (8.4%) Philippines (6%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%) and South Africa (3.6%). Based on the WHO 2020 report, Indonesia's position is in the second highest contributor to TB cases after India, the number has increased from 331,703 cases in 2015 to 562,049, in 2019, and for drug-resistant TB incidence, Indonesia is in the 5th rank [2].

TB disease can be cured with properly treatment for a long period of time, called TB patient control program or known as the DOTS (Directly Observed Treatment Short course) program that has been carried out, the program has succeeded in increasing recovery by up to 87%, but there have been many reports of recurrence cases (relapse) as well as treatment failure, as well as the incidence of resistance TB, both primary and secondary, are the problems that hinder tuberculosis control programs. Programmatic Management of Drug Resistant TB requires patient adherence in undergoing DR TB treatment as in Indonesian National TB Program Short-Term Regiment guideline injectable takes 9-11 months and long-term (individual) therapy guidance for 18-24 months. [1]

Drug-resistant tuberculosis is a condition in which *Mycobacterium tuberculosis* is resistant to AAT (Anti Tuberculosis Treatment), namely (i) Rifampicin-Resistant Tuberculosis (RR-TB: resistant to RIF), (ii) Multidrug-resistant Tuberculosis (MDR-TB: resistant to INH and RIF) and (iii) Extensively-resistant Tuberculosis (XDR) strain of DR that is resistant to fluoroquinolone (FQ), which is the first-line drug for curing TB disease, and one of the second-line injectable drugs, namely amikacin, kanamycin, or capreomycin), and (iv) pre XDR if strain of DR that is resistant to fluoroquinolone (FQ), or resistant to one of the second-line injectable drugs [3]. Globally in 2019 there were 78% DR-TB cases and 61% RR/DR TB cases with a total of 206,030 cases, an increase of 10% from 186,883 cases in 2018 and MDR TB cases in Indonesia were ranked the 5th highest in the world with as many as 24,000 cases of RO TB (WHO, Global Tuberculosis Report, 2020). The situation of pulmonary tuberculosis in the world is getting worse with increasing cases causing a decrease in the success rate of TB treatment from 85.7% in 2017 to 84.6% in 2018.[1]

The success treatment is influenced by several factors

including the body's immunity, especially the cellular immune response. Previous studies have suggested that IL-17 secreting Th17 cells may play an important role in the development of tuberculosis infection. Low IL-17 and IFN-γ levels and high IL-4 levels were found in patients with active tuberculosis. IL-17 and IFNγ levels tended to increase during conversion in active tuberculosis disease, whereas IL-4 levels tended to decrease during conversion. [4]. Th17 cells are a subset of T cells that respond strongly to IL-23. The IL-23 / IL-17 axis appears to act as an important modulator of the immune response associated with all phases of *Mtb* infection, with a protective role reported in a mouse TB model [5].

Management of MDR TB requires a long and more complex period and requires greater costs which can hinder the success of treatment. Conversion of sputum smear also determines the infectious potential and disease transmission of drug-resistant TB patients and leads to worse treatment outcomes [6,7]. In a study conducted by Kim et al, at 4 weeks of treatment the sputum conversion rate was 52.7% in the drug-sensitive TB group and 45.7% in the MDR and XDR TB groups. Based on research conducted in 2019 at dr Saiful Anwar Malang Hospital, it was found that 35.3% AFB sputum conversion time was obtained in the first month [8].

Based on the description above, the high incidence of tuberculosis, especially in Indonesia and the large number of TB-resistant cases, is an obstacle to successful therapy, this study will determine whether there are differences in IL-17 and IL-23 levels before and 30 days after DR TB treatment as well as the relationship between IL-17 and IL-23 levels and conversion of smears after one month of treatment.

MATERIAL AND METHODS

Research design

The study design was a Cohort Prospective Study. The research was conducted at the dr. Saiful Anwar General Hospital (Rumah Sakit Saiful Anwar/RSSA) Malang. Samples were all new patients with drug-resistant pulmonary tuberculosis (DR TB) who attended pulmonary clinic or were hospitalized at RSSA Malang from August to October 2020. The healthy control group was taken from a healthy population. Determination of Rifampicin Resistance is carried out with the Molecular Rapid Test or Xpert/*Mtb* Rif while for second line drugs susceptibility it was carried out through Line Probe assay at the Center for Health Laboratory (BBLK/Balai Besar Laboratorium Kesehatan) Surabaya. The levels of IL-17 and IL-23 in serum were measured by Enzyme Linked Immunosorbent Assay (ELISA) technique at the Central Laboratory of RSSA Malang. The Acid-Fast Bacteria (AFB) examination was carried out at the Microbiology Laboratory of RSSA Malang. The results of the sputum smear were examined before and after treatment. It is categorized as a conversion if the sputum AFB is negative within 30-day interval post DR TB treatment. This research has received approval from the Ethics Commission with an ethical Approval number 400/187 / K.3 / 302/2020.

Data collection technique

Sampling was obtained by consecutive sampling method that fulfills the inclusion and exclusion criteria in MDR TB Lung Clinic at dr Saiful Anwar Hospital Malang. The inclusion criteria were: (i) patients who had been diagnosed with drug-resistant pulmonary tuberculosis (RO TB) through the Xpert/*Mtb* Rif examination with the results of "*Mtb detected, Rif resistant detected*" *very low, low, medium* or *high* and had never received TB drug combination therapy or had received TB drug combination therapy for less than 1 week (<7 doses), (ii) aged 18 - 65 years, (iii) willing to take part in the study and signed an informed consent form while the exclusion criteria were patients who had received DR TB for ≥ 1 months, patients with HIV positive, and pregnant patients.

Drug regiments

Twenty subjects of case group were administrated with injectable short-term regimen with kanamycin/capreomycin, moxifloxacin, high-dose isoniazid, pyrazinamide, ethambutol, ethionamide, clofazimine and B6 vitamin and two subjects treated with short term regimen non injectable. Another sixteen subjects were treated with Individual Treatment Regimen, on the basis of 5 types of TB drugs that are still effective, consist of 4 types of second line TB drugs plus pyrazinamide.

Sputum AFB examination

Microscopic examination of AFB with Zielh Nielsen staining used a scale from the International Union Against Tuberculosis and Lung Disease (IUALTD), namely Negative, Scanty, 1+, 2+, 3+.

Examination of IL-17 and IL-23 levels:

The levels of IL-17 and IL-23 in the serum of DR-TB patients were measured by Enzyme Linked Immunosorbent Assay (ELISA) technique at dr Saiful Anwar Hospital Central Laboratory. Blood samples that have been taken and given the initials name and date of collection are taken to the laboratory to be centrifuged for serum, which is then stored in a refrigerator (-80°C). After all serum samples have been collected, then analyzed by ELISA kit (Elabscience). Standard working solution was added of different concentrations to the first two columns: Each concentration of the solution is added into two wells side by side (100 uL for each well). Samples were added to other wells (100 uL for each well). The plate was covered with sealer provided in the kit and incubated for 90 min at 37°C. The liquid should be removed of each well, and immediately 100 µL of Biotinylated Detection Ab/Ag working solution was added to each well. After covered with plate sealer, the Plate was gently mixed up and incubated for 1 hour at 37°C. The solution was aspirated from each well, and 350 uL of wash buffer was added to each well. The plate was soaked for 1~2 min and the solution were aspirated from each well and pat it dry against clean absorbent paper. This wash step repeated for 3 times, a microplate washer can be used in this step and other wash steps. Complete removal of liquid at each step is essential. After the last washing, the remained wash buffer was removed by aspirating or decanting. Invert the plate and pat it against thick, clean absorbent paper. 100 µL of HRP (Avidin Horseradish Peroxidase) Conjugate working solution was added to each well and the plate incubated for 30 min at 37°C. The solution was aspirated from each well, and the wash process wash repeated for five times as conducted in step of washing, 90 µL of substrate reagent was added to each well and the plate was incubated for about 15 min at 37°C. Protect the plate from light. 50 µL of Stop Solution was added to each well. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm. The absorbance obtained was converted to the equation in the standard curve so that the IL-17 and IL-23 levels were obtained and expressed in units of pg / ml.

Data processing and analysis techniques

Data processing and analysis was carried out using IBM

SPSS version 18.0 software. Analysis of differences in levels of IL-17 and IL-23 at day 0 and day 30 based on the conversion status of sputum smear smears using the Wilcoxon test (for pre- and post-comparisons), as well as the Mann Whitney test (for pre-control and post-control comparisons). The relationship between IL-17 and IL-23 levels (before and after treatment) and the results of smear examination were analyzed using the Spearman correlation test with a confidence degree of 95%, or α = 0.05

RESULTS AND DISCUSSION

Data Description Characteristics of Research Subjects

A total of 38 samples of new patients with drug-resistant pulmonary tuberculosis (DR-TB) and 34 healthy control samples were included in this study. Demographic data for both the case group and the healthy group are in **table 1**.

Table 1. Distribution based on demographic factors of initial AFB results.

	Case Group		Control Group	
Demography	**AFB Negative**	**AFB Positive**		**P value**
Sex				
Male	6 (8.3%)	13 (18.1%)	17(23.6%)	0.943 [C]
Female	7(9.7%)	12 (16.7%)	17(23.6%)	
Age (years old) (mean±SD)	40.85±14.53	47.4±11.59		0.138 [t]
< 20	1 (1.4%)	0 (0.0%)	0(0%)	0.016 [C]
20-59	11(15.3%)	20 (27.8%)	34(47.2%)	
>59	1 (1.4%)	5 (6.9%)	0(0%)	
Residence				
Malang	5 (6.9%)	10 (13.9%)	34(47.2%)	0.000 [C]
Outside Malang	8 (11.1%)	15 (20.8%)		
BMI				
Underweight (<18.5)	7 (9.7%)	12 (16.7%)	0(0%)	0.000 [C]
Normal (18.5-24.9)	6 (8.3%)	13 (18.1%)	28(38.9%)	
Overweight (>=25)	0 (0%)	0 (0%)	6(8.3%)	
Comorbid				
None	8 (11.1%)	17 (23.6%)	34(47.2%)	0.005 [C]
DM	3 (4.2%)	7 (9.7%)	0 (0%)	
DM, HT	1 (1.4%)	1 (1.4%)	0 (0%)	
DM, CAD	1 (1.4%)	0 (0%)	0 (0%)	
Xpert/*Mtb*Rif				
None	0 (0%)	0 (0%)	34 (47.2%)	
detected very low	3 (4.2%)	1 (1.4%)	0 (0%)	0.000 [C]
detected low	1 (1.4%)	3 (4.2%)	0 (0%)	
detected medium	5 (6.9%)	16 (22.2%)	0 (0%)	
detected high	4 (5.6%)	5 (6.9%)	0 (0%)	

[A]=Uji ANOVA; [t]= t test; [C]= Chi square test

In Table 1, the comparison of the number of samples of men and women between groups of AFB negative cases, AFB positive cases and controls is not significantly different. There was a significant difference when compared the number of samples of productive and non-productive age among groups of negative cases, positive cases and control there was a significant difference, where more samples were aged <= 59 years than those aged> 59 years. The results of the comparison of the residence of the respondents between the negative case groups, positive cases and controls showed a significant difference, because the number of patients in the positive and negative cases group came more from outside Malang, but for the control group more who lived in Malang. The comparison results of BMI among negative cases, positive cases and controls showed a significant difference, because BMI in normal controls were higher than the case groups. The results of the comparison of Comorbid between the negative cases, positive cases and controls showed a significant difference, because the group with a negative initial smear result and the group with a positive initial smear result had a significantly different number of samples of patients with comorbidities. Management of DRTB requires a long and complex period and requires greater costs which can hinder the success of treatment and increasing probability of drop out from treatment. [9]. Success treatment is influenced by several factors including age, most often found a young or productive age, 15-49 years, gender also affects men more than women because men have smoking and alcoholism which lowers the body's immunity so it is easy to be at risk of exposure to tuberculosis, then the factor of residence is very influential because a bad and slum house or place of residence results in the transmission of TB disease, where the *Mycobacterium tuberculosis* can survive in a dark place that is less exposed to sunlight, as well as educational

factors, where someone with a higher education will have broader knowledge and easily understand TB treatment compared to someone with low education. Apart from demographic factors, the impact of comorbidities such as HIV (Human Immunodeficiency Virus), DM (Diabetes mellitus) is a factor that can affect worse treatment outcomes, and the role of less or no interaction between health workers and patients and families so that the process of DR TB treatment is poorly implemented [10].

Acid-Fast Bacteria (AFB) smear before and after treatment

The results of DR TB treatment based on AFB sputum (initial measurement results up to 1 month) can be seen at the time of the start of treatment, there were 10 samples with negative results, 2 samples with AFB scanty results, 11 samples with AFB +1 results, 5 samples with AFB + results. 2, and 10 samples with AFB +3 results. Then after TB treatment for 1 month, the results obtained were 37 samples with AFB negative, 1 person with AFB +1 (**table 2**)

Table 2. Distribution of DR TB treatment results based on AFB (baseline measurement results up to 1 month)

	AFB (Acid Fast Bacilli) sputum before and after treatment					P value
	Negative	Scanty	+1	+2	+3	
Before	10 (13.2%)	2 (2.6%)	11 (14.5%)	5 (6.6%)	10 (13.2%)	0.000
After	37 (48.7%)	0 (0%)	1 (1.3%)	0 (0%)	0 (0%)	

The test results showed a significance value of p= 0.000, which means that the group with AFB (initial measurement results up to 1 month) was negative, +1, +2, and +3 had a number of samples of patients with significantly different AFB results, because the number of patients in the two groups who had different AFB results were abundant.

Table 3. Distribution of DR TB treatment results based on AFB (before and after treatment)

	AFB Negative (-)	**AFB Positive (+)**	**P value**
TB Treatment			
Before	10 (13.2%)	28 (36.8%)	0.000
After	37 (48.7%)	1 (1.3%)	

The results of TB treatment based on AFB (before and after treatment) can be seen at **table 3**. At the start of treatment, there are 10 samples with negative results, and 28 people with positive smear results. Then after 1 month of TB treatment, 37 samples were smear negative (97.4%), and 1 person was smear positive. The test results showed a significance value of 0.000 (p <0.05).

Based on the national TB guidelines in Indonesia, the results of Xpert *Mtb Rif* or positive smear sputum are called bacteriologically diagnosed TB so although subjects with negative smear results but the results of Xpert *Mtb Rif* are *Mtb* detected and Rifampicin resistant detected, are still included as RR TB or DR TB

Interleukin-17 and interleukin 23 levels before and after treatment

Based on data normality testing using the Shapiro-Wilk test, numerical data for IL-17 before treatment, IL-17 after treatment, IL-23 before treatment, IL-23 after treatment, IL-23 healthy control and IL-17 healthy control all have significance values less than 0.05 (p <0.05), it can be concluded that all numerical data have an abnormal distribution, further testing uses analysis in non-parametric statistics.

Table 4. The results of IL-17 and IL-23 measurements among case group (before and after DR-TB treatment) and in normal control group.

Variable	**Before DR-TB treatment**	**After DR-TB treatment**	**Control**
	(mean±SD**)**	**(mean**±SD**)**	**(mean**±SD**)**
IL-17 (pg/ml)	789.75±1041.62	1140.28±1170.24	501.39±407.48
IL-23 (pg/ml)	1320.36±2291.05	1705.24±2355.82	578.35±519.19

The comparative test results for the levels of IL-17 and IL-23 between before and after 1 month of TB treatment and compared with healthy controls, are as follows: Comparative test result for IL-17 levels before treatment (averaged 789.75 pg /ml), and those after treatment (average 1140.28 pg / ml), was not significantly different (p: 0.153). Comparison between normal control group (average 501.39 pg/ml), also was not significantly different, (p: 0.883). In contrast IL 17 levels of the group after 1 month of treatment was significantly different (p= 0.153) (**Figure.1**)

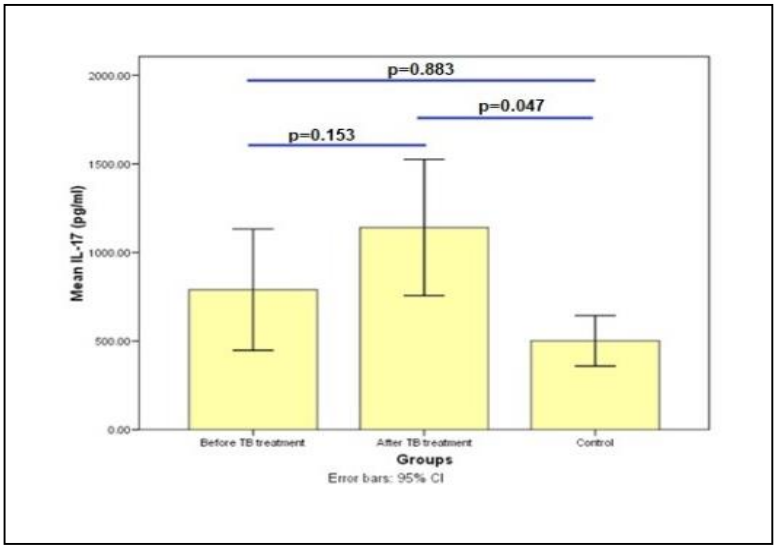

Figure 1. The comparison of IL-17 levels among groups.

There were no significant differences of IL-17 levels between before and after TB treatment of case group, as well as between the control and both before treatment case groups. Interestingly there were significant differences of IL-17 levels between the control and both after treatment case groups

The level of IL-17 before and after treatment is not significantly different maybe caused by many factors. IL-17 has been implicated in the pathogenesis of many autoimmune and inflammatory diseases, such as rheumatoid arthritis, psoriasis, multiple sclerosis, asthma, inflammatory bowel disease, and periodontal disease and recent evidence also suggests that IL-17 plays an important role in cardiovascular disease including coronary atherosclerosis [11]. In this study these factors were not examined. The standard deviation difference was wide because there are still quite a lot of data that are classified as outliers; this is what makes the variance so wide. During the incidence of *Mtb*, Th1 and Th17 cross-link with each other and this may be important for the immunopathological events that occur not only with *Mtb* infection but also in another *Mycobacterium*. During the process of tuberculosis infection, IFN-N and IL-17 are induced due to the interaction of *Mtb* pathogens with the immune system. These two potent proinflammatory cytokines can induce the expression of other chemokines that promote the recruitment of immune cells and the formation of granulomas. In the chronic phase, a balance between Th1 and Th17 cytokine responses needs to occur to be able to control *Mtb* growth while limiting immunopathology. This is because excessive IL-17 release can result in prolonged neutrophil recruitment and tissue damage [12].

The averaged levels of IL-23 in before TB treatment group was 1320.36 pg /ml, compared to those in after treatment group (average level of 1705.24 pg /ml), was not significantly different. Similary an average IL 23 level before treatment group compared to those of healthy control (578.35 pg/ml) was not significant different (p= 0.928), while those of the group after TB treatment compared to the healthy control group was significantly different (p= 0.042). (**Figure 2)**

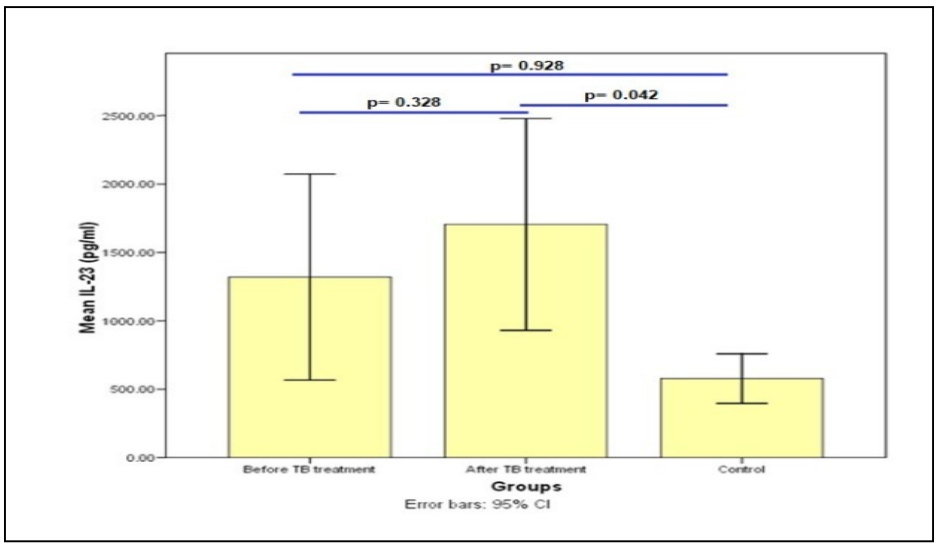

Figure 2. The comparison of IL-23 levels among groups.

There were no significant differences of IL-23 levels between before and after TB treatment of case group, as well as between the control and both before treatment case groups. Interestingly there were significant

differences of IL-23 levels between the control and both after treatment case groups.

The level of IL-17 and IL-23 before and after treatment is not significantly different. It maybe caused by different regiments (short term regiment injectable, short term regiment non injectable and individual regiment) that administrated for DR-TB patient. This study used the therapy that was only for one month.

Short term regimen injectable consist of 4 until 6 months initial phase of Kanamycin or Capreomycin injection, Moxifloxacin, Etionamid, high dose Isoniazid, Clofazimin, Ethambutol and Pyrazinamid and the continuous phase for five month Moxifloxacin, Clofazimin, Ethambutol, Pyrazinamid and short term regimen non injectable consist of initial phase for 4 until 6 months Levofloxacin, Etionamid, Clofazimin, Ethambutol, Pyrazinamide and high dose Isoniazid and Bedaquilin for 6 month Bedaquilin and the continuous phase for 5 months Levofloxacin, Clofazimin, Pyrazinamide and Ethambutol, for the individual regimen treatment consist of at least five effective drugs chosen from four second-line core drugs (such as Levofloxacin or Moxifloxacin Kanamycin Capreomycin Etionamid, Linezolid, Clofazimin, Cycloserin, Bedaquilin, Delamanid) plus additional Pyrazinamide. [13,14,15]

In this study the evaluation of the IL-17 and IL-23 levels was carried out for only one month of treatment, because we want to evaluate the changes in IL-17 and IL-23 levels after treatment have associated with sputum AFB conversion. The conversion is expected to reduce the transmission of *Mycobacterium tuberculosis*, so it is necessary to evaluate IL-17 and IL- 23 levels at least after the intensive phase of DR TB treatment is completed, which is 4- 8 months.

However the IL-17 and IL-23 levels were increased anf signifantly different with the healthy control group. Tuberculosis is a chronic disease that requires constant expression of cellular immunity to limit bacterial growth. The constant expression of immunity also results in chronic inflammation, which requires regulation. Meanwhile, Th1 CD4 + cells produce IFN-γ which is needed to control bacterial growth and maintain a mononuclear inflammatory response. Another group of T cell induced by *Mtb* infection is Th17 which is capable of producing IL-17 which is a strong inflammatory cytokine capable of inducing chemokine expression and cell recruitment to parenchyma tissue. Both IL-17 and Th17 responses to *Mtb* are largely dependent on IL-23. Although both Th17 and Th1 cells were induced after primary infection with *Mtb*, the protective response was significantly dependent on the absence of Th1 cells but not in the absence of Th17. In contrast, in vaccinated animals, the absence of Th17 cell memory resulted in loss of Th1 response and memory [12].

Correlation of AFB measurement results and Interleukin-17 and interleukin 23

To determine the relationship between the baseline AFB measurement results and after 1 month of treatment with IL-17 and IL-23 levels, it can be seen from the test results with the Spearman correlation **(Table 5).**

Table 5. Measurement results for baseline AFB and after 1 month of treatment with IL-17 and IL-23 levels

Correlation test results between	Coefisient correlation	p value
DR TB treatment with AFB (before and after treatment)	-0.716	0.000
IL-17 and IL 23 pg / ml) before and after treatment	0.872	0.000
IL-17 (pg / ml) before and after treatment	0.174	0.133
IL-23 (pg / ml) before and after treatment	0.168	0.147

Based on the results of the correlation test above between DR TB treatment and AFB (initial measurement results up to 1 month), the correlation coefficient value was -0.716 with a significance value of p= 0.000. So, it can be concluded that there is a significant relationship between TB treatment and AFB (initial measurement results up to 1 month), with a negative correlation direction, which means that TB treatment will reduce the results of patients who were initially positive (+1, +2 or +3), becomes negative or the BTA results decrease and vice versa.

There was a significant effect between TB treatment and AFB (initial measurement results up to 1 month), based on the results of the study can be shown in the form of a linearity graph as follows**: (Figure 3)**

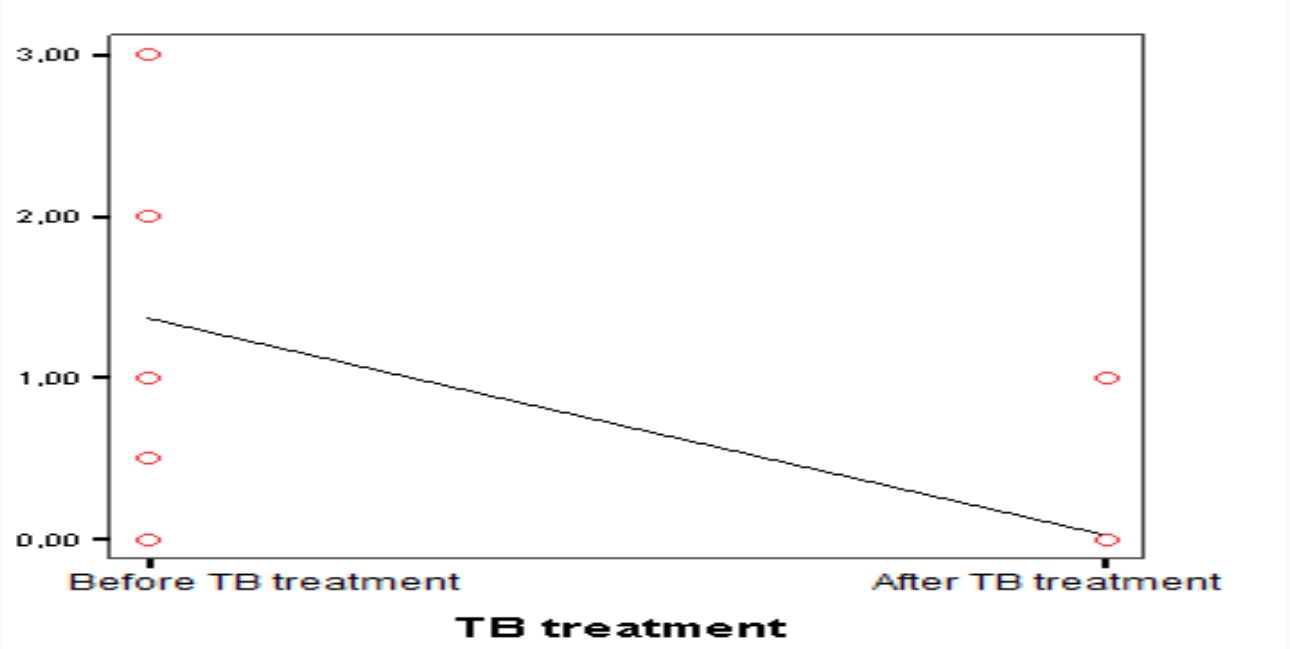

Figure 3. Graph of linearity between TB treatment and AFB (before and after treatment).

This means that the presence of TB treatment will reduce the patient's BTA results, which were initially positive (+1, +2 or +3), become negative or the results of the BTA decrease and vice versa.

Based on the linearity graph above, it can be seen that the regression line between TB treatment and AFB (before and after treatment) points to the lower right. This proves the linearity between TB treatment and AFB (before and after treatment).

Based on the results of the correlation test between IL-17 levels (before and after treatment) and IL-23 levels (before and after treatment), the correlation coefficient value was 0.872 with a significance value of 0.000. So, it can be concluded that there was a significant relationship between IL-17 levels (before and after treatment) and IL-23 levels (before and after treatment), with a positive correlation, which means an increase IL-17 levels from the baseline until 1 month after TB treatment, will be followed by an increase in IL-23 levels from the baseline reading until 1 month after TB treatment and vice versa. based on the results of the study can be shown in the linearity graph as follows:

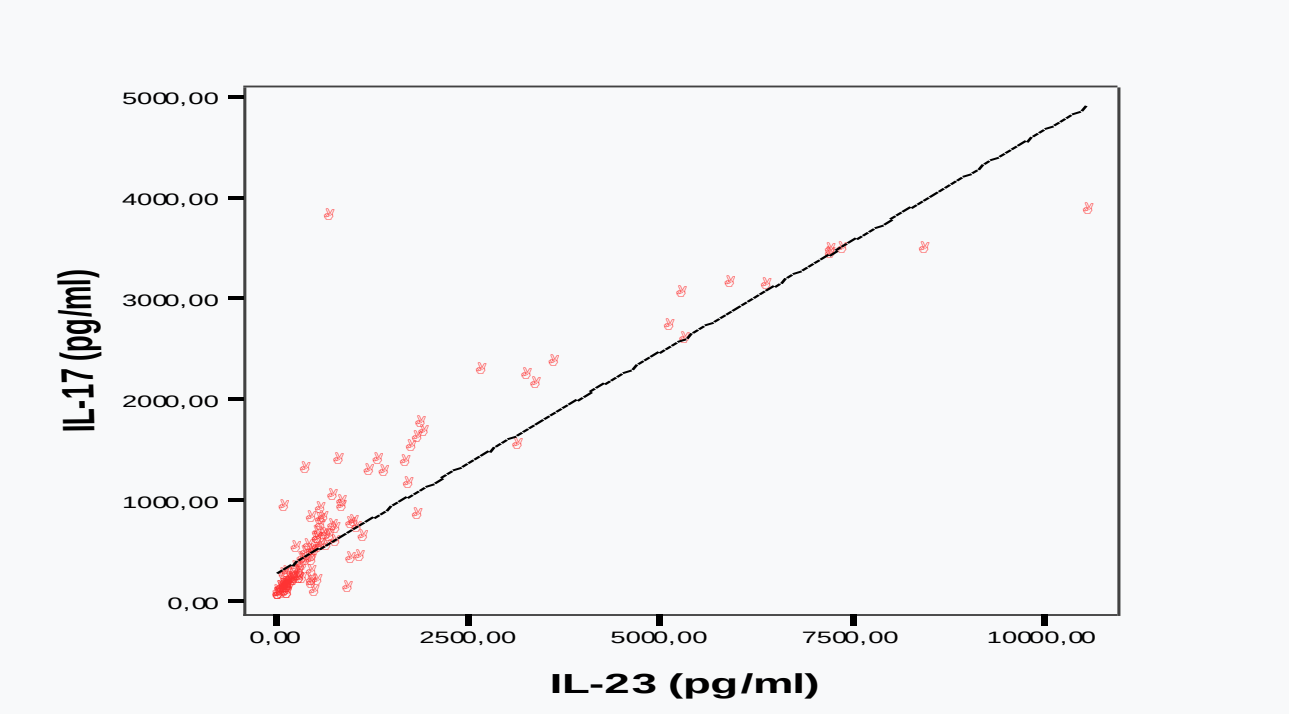

Figure 4. Graph of the linearity between IL-17 levels (before and after treatment) and IL-23 levels (before and 1 month after TB treatment).

There was a significant correlation between IL-17 levels (before and after treatment) and IL-23 levels (before and after treatment),

Based on the linearity graph above, it can be seen that the regression line between IL-17 levels (before and after treatment) and IL-23 levels (before and after treatment) points to the upper right. This proves that there is a linearity between IL-17 levels (before and after treatment) and IL-23 levels (before and after treatment). This means that an increase in IL-17 levels from the initial measurement results to 1 month after TB treatment, will be followed by an increase in IL-23 levels from the initial measurement results until 1 month after TB treatment, and vice versa.

T cell receptors can be one of the main sources of IL-17 which responds to IL-23 stimulation. Recent studies have shown that IL-23 and other cytokines associated with Th17 can induce the proliferation and expansion of Vγ2Vδ2 T cells in phosphoantigen (E) -4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP), and cells that have a multi-effector function to produce cytokines from IL-17, IL-22, IL-2 and IFN-γ. Autocrine production of IFN-γ and IL-2, furthermore, increases expansion as IL-23 / HMBPP withdrawal is stimulated by Vγ2Vδ2 T cells. The STAT3 dependent signaling pathway is involved in IL-23 expansion of Vγ2Vδ2 T cells. Data from studies using PBMC in TB patients demonstrated that the IL-23 / IL-17 axis is likely to be impaired by overexposure to stimulated *Mtb* antigen in chronic infection [5]. Th17 cells produce the cytokine IL17A, here in after referred to as IL-17, IL-17F, IL-22 and IL-26 in humans. CD 8 T cells, γδ T cells, and invariant natural killer T cells also produce IL-17. Th17 cells are positively regulated by TGFβ, IL-1β, IL-6, IL-21 and IL-23 and negatively regulated by Th1 and Th2 cytokines. IL-17 is thought to be an immunopathological intermediate in experimental animal models for autoimmune and infectious diseases. IL-17 has an influence on TB pathology [12].

The increase in Th17 cells due to the absence of IL-27 will cause premature death of the experimental animal in addition to increasing protection from *Mtb* infection. Patients with TB drug-resistant show high IL-17 expression, with severe tissue damage, showing IL- 17 produced by T cells can play a role in the immune state of pathology. Multivariate analysis showed that low serum IL-17 was associated with patient mortality during two months of anti-TB treatment. [4]. Interleukin-17 in pleural or pericardial fluid is not much, IL-17 expression against mycobacteria is not detected in healthy people, or in people infected with *Mtb* or in patients with TB pericarditis, these data indicate that IL-17 does not play a role in TB disease in specific location. [16]. A decreased Th17 response is related to the clinical presentation of patients with TB infection. Suppression of the Th17 response through down regulation of IL6R expression is probably an important mechanism for the development of active TB. [17, 19, 20]. Interaction of IL-17 and IL-23 plays a role in the immunopathological process of tuberculosis. IL-23 deficiency will decrease the Th 17 cell response. At the beginning of infection, IL-17 produced by innate immune cells will recruit neutrophils to the site of infection and form mononuclear granulomas as the initial response of the body. The IL-17 response occurred more rapidly in vaccinated mice compared to those without vaccination. Cellular immune response by Th1 cells secretes IL-12 and IFNy, help to limit the infection. Th1 cell response is reduced in the absence of IL-17. Apart from enhancing the Th1 cell response, interleukin 17 also attracts neutrophils and monocytes into the granuloma. In chronic infection, interleukin 17 maintains granuloma integrity and reduces neutrophil death. The absence of a Th17 response results in the fragility of the granuloma and the spread of *Mtb*.[17].

Therefore, the balance of Th1 and Th17 cellular immune responses is important to maintain the integrity of granulomas [18,19]. Interleukin (IL) -23 is a heterodimeric cytokine consisting of the IL-12B (IL-12p40) and IL-23A (IL-23p19) subunits, and its functional receptors include IL-12Rβ1 and IL-23R. IL-23 is mainly produced by antigen-presenting cells. In vitro, *Mtb* can induce human dendritic cells and alveolar macrophages to produce IL-23. In vivo, IL-23α expression was increased in PBMC in nonhuman primates in the early stages of mycobacterial infection; then, his expression decreased to a normal level. In addition, levels of IL-12p40, a subunit of IL-23, were higher in the serum of TB patients than healthy individuals and decreased after administration of anti-TB therapy. Interleukin-23 can mediate its effects on the natural and adaptive immune systems expressing IL-23R. Th17 cells are a subset of T cells that respond strongly to IL-23.

The IL-23 / IL-17 axis appears to act as an important modulator of the immune response associated with all phases of *Mtb* infection, with a protective role reported in mouse TB models. Receptor T cells can be one of the main sources of IL-17 which responds to IL-23 stimulation. Our study recently demonstrated that IL-23 and other cytokines associated with Th17 can induce the proliferation and expansion of Vδ2Vδ2 T cells in HMBPP ((E) -4-hydroxy-3-methyl-but-2-enyl pyrophosphate). Vaccination / infection of mycobacteria in macaques increases the ability of IL-23 to function as a cation multi-effector to produce cytokines from IL-17, IL-22, IL-2 and IFN-γ. Autocrine production of IFN-γ and IL-2, in turn, increases expansion such as IL-23 / HMBPP withdrawal via STAT3-dependent signaling pathways (Signal Transducers and Activators of Transcription 3). Data from studies using PBMC from TB patients suggest that the IL-23 / IL-17 axis is likely to be dysregulated or damaged by overexposure to stimulated *Mtb* antigen in chronic infection, findings suggesting that cytokines associated with Th17 and Th17 / Th22 cells can is devoted to the immune response to *Mtb* infection and may be involved in protective immunity against primary *Mtb* infection. There are few experimental data involving inconsistent immune responses to Th17-linked cytokines in TB patients. In the primary phase of *Mtb* infection, the STAT3 pathway is stimulated in phagocytes (monocytes / macrophages), but its protective effect is suppressed by immunosuppressive cytokines from host cells stimulated by *Mtb*. The STAT3 pathway and the Th17-associated cytokines appear to be affected by *Mtb* infection in the following way, after infection, virulent *Mtb* organisms survive in phagocytes, and *Mtb* antigen directly simulates the STAT3 signaling pathway to regulate host immunity. The initial antigenic target secreted 6 kDa (ESAT-6) is an important virulence factor. *Mtb* infection induces the production of immunosuppressive cytokines including IL-10 to influence STAT3 activation. Interleukin (IL) -10 and expression levels of STAT3 and pSTAT3 increased significantly in the first week after TB infection. IL-10 production was highly correlated with STAT3 and pSTAT3 protein expression. In vitro, IL-10 can regulate protective phenotypes in *Mtb*-infected phagocytes including

macrophages. IL-10 has an immunosuppressive effect on the initial response of *Mtb*-infected macrophages which are partly dependent on STAT3. Interaction of *Mtb* with rapidly differentiating monocytes activates the STAT pathway, which likely participates in IL-10 gene expression. STAT3 activation leads to inhibition of the cytokines IL-6, IFN-γ, TNF-α and MIP-1β (inflammatory protein macrophages-1β). [5,20,21].

Based on the results of the correlation test between the TB treatment group (before and after TB treatment) with IL-17 levels (pg/ml), the correlation coefficient value was 0.174 with a significance value of 0.133. So, it can be concluded that there is no significant relationship between the TB treatment group (before and after TB treatment) with IL-17 levels (pg / ml), in other words, the level of IL-17 is not related to the TB treatment group (before and after TB treatment). Likewise, the results of the correlation test between the TB treatment group (before and after TB treatment) with IL-23 levels (pg /ml) obtained a correlation coefficient value of 0.168 with a significance value of 0.14 So it can be concluded that there is no significant relationship between the TB treatment group (before and after TB treatment) with IL-23 levels (pg / ml), in other words, the level of IL-23 is not related to the TB treatment group (before and after TB treatment).

CONCLUSION

One month of DR TB treatment increased the sputum AFB conversion.The levels of IL-17 and IL-23 after DR-TB treatment were increasing and significantly different compared to healthy group. These related with high AFB conversion rate. We suggest to evaluate the level of IL-17 and IL-23 before and after the initial and continuous phase of DR-TB treatment and further large sample studies are needed.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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