Changes in ARB Sputum Positivity, BMI and TNF-α Levels of Pulmonary TB Patients with ARB Positive During One-month Intensive Treatment

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Abstract

Backgoround: TNF-α plays a role in the occurrence of anorexia and weight loss in pulmonary tubercolusis (TB) patients. Evaluation of treatment response can be done with clinical, microbiological and radiological evaluation. In patients without cough, Body Mass Index (BMI) and TNF-α levels can be used to evaluate clinical treatment response.

Objectives: The aim of the study is to measure the Acid Resistant Bacteria (ARB) sputum positivity decrease, TNF-α levels and increased BMI after 2 weeks and 4 weeks intensive treatment of pulmonary TB patients with new case of ARB positive.

Methods: The sample size was 14 people with ARB positive pulmonary TB cases, consisting of 9 men and 5 women. It was done sputup smear, BMI and TNF-α examination before treatment, at week 2 and week 4 of intensive phase treatment

Result: At the end of the second week, TNF-α levels decreased significantly compared to baseline TNF-α levels with $p = 0.015$. There was a non significant increase of BMI at the end of the second and fourth week with $p = 0.309$ and $p = 0.270$ respectively. The decrease in ARB sputum smear positivity at the end of the second week compared to the fourth week was not statistically significant with $p = 0.157$. There was a strong correlation between TNF-α and IMT levels before treatment with negative correlation, $r = -0.702$ and $p = 0.005$.

Conclusion: Levels of TNF-α at the end of the second week decreased significantly compared to baseline TNF-α levels. There was a strong correlation between TNF-α levels and BMI prior to treatment.

Keywords: *Acid Resistant Bacteria Sputum, Body Mass Index, TNF-α levels, pulmonary Tuberculosis, new cases, intensive phase*

Introduction

Tuberculosis (TB) is an important public health problem worldwide. It is estimated that about one-third of the world's population has been infected by the Mycobacterium tuberculosis. The WHO data 2012 stated that Indonesia takes the fourth place in the world with the number of 0.40-0.50 million people with tuberculosis after India (2.0-2.5 million sufferers), China (0.9-1.1 million sufferers) and South Africa (0.40-0.60 million sufferers) [1].

Body weight loss due to TB is caused by pro-inflammatory cytokines TNF-α produced by dendritic cells and macrophages. TNF-α is known as cachectin that works in the hypothalamus by suppressing the eating desirE center causing anorexia and cachexia. At the onset of OAT treatment, there was an increase in TNF-α levels, whereas longitudinal studies concluded that TNF-α levels fluctuated during OAT treatment. Other studies reported an increase in TNF-α levels in TB patients although not significant [2].

Body Mass Index (BMI) is the result of weight (kg) divided by height (m) squared. BMI is a simple method to monitor adult nutritional status. BMI is very sensitive to sudden changes in the body for example due to

infectious diseases or decreased appetite. Evaluation of weight gain can be used to assess the clinical treatment progress of TB patients [3].

The number of ARB germs declines rapidly after OAT reatment begins, 80-85% of pulmonary TB patients become non-infectious after about 2 weeks of treatment. It is estimated that more than 50% of TB sufferers remain infectious after 2 weeks of treatment [4]. The best assessment of the OAT therapy response is the TB bacilli eradication of sputum. Some researchers have found ARB decreased after receiving OAT therapy for approximately 2 weeks [5].

Surgery, OBG, ENT Eye Disease Sciences are not rare getting a case with surgical indications in a patient who simultaneously suffers from ARB positive pulmonary TB. The Pulmonology and Respiratory Medicine is often consulted in such cases. Should the operation be delayed and wait until the intensive phase is complete (sputum conversion has occurred)? And the answers given are often different. From the description above, it is necessary to examine the decrease of ARB sputum positivity, TNF-α level and BMI increase after 2 weeks and 4 weeks of intensive treatment to determine the relationship between the sputum positivity level of BTA and BMI, as well as the relationship between decrease in ARB sputum positivity and TNF-α after 2 weeks and 4 weeks of intensive phases.

Method

The subjects were pulmonary TB patients who met the inclusion and exclusion criteria. Inclusion criteria were male and female, aged 15-60 years, positive new case oof pulmonary TB, and willing to seek treatment at DOTS Unit of Pulmonology Department, Dr. Soetomo General Hospital Surabaya, Indonesia. Exclusion criteria were DM, malignant and AIDS / HIV disease, patients could not excrete sputum spontaneously, did not get standard OAT 4 FDC, there are major side effects of OAT [6].

This was analitical observational study. The study procedure included interview, physical examination, TNF-α level examination, and ARB smear sputum examination. Interviews included anamnesis about Diabetes Mellitus (DM) symptoms, malignancy and HIV/AIDS risk factors. Physical examination included general examination, weight, height, tension, pulse, breath frequency. Physical examination was also intended to look for signs of malignancy and HIV/AIDS more specifically [7].

Examination of TNF-α levels was performed by taking venous blood with a syringe of 5 cc, then centrifuged for several minutes. The serum was taken, then put into the tube and stored in the refrigerator with a temperature of -70^{o}C. Measurements of TNF-α levels were performed by ELISA. The steps of TNF-α examination were as follows (1). Reagents preparation, samples and standard solutions as controls (2). Add 100 µl standard solution or sample, incubate for 2.5 hours at room temperature (3). Add 100 µl biotin antibody, incubate for 1 hour at room temperature (4). Add 100 µl of Streptavidin solution, incubate for 45 minutes at room temperature (5). Add 100 µl of One-Step Substrate TMB reagent, incubated for 30 minutes at room temperature (6). Add 50 µl Stop Solution and read immediately on 450 nm wave.

Sputum smear of ARB examination was done by taking spontaneous sputum of patients into 2 sputum pots. Sputum taken for examination was spontaneous sputum and morning sputum. Spontaneous sputum was labeled with name, age, register number, date of specimen taking then sent to Microbiology Unit for sputum painting examination by Ziehl Nielsen method. Ziehl Nielsen painting results were read on an IUALTD scale [8].

The data were recorded and collected, then analyzed using the SPSS computer statistical program (SPSS, Inc., Chicago, IL). Sputum positivity rate of ARB, BMI and TNF-α levels were presented in tables. The correlation between sputum positivity level of ARB and BMI, between sputum positivity of ARB and TNF-α level was tested using Pearson correlation test with $p < 0.05$[9].

Result

Respondents Characteristics

Smear sputum results of ARB according to IUALTD score at the time of pulmonary TB diagnosis were 2 ARB scanty sputum (14.28%), 6 ARB sputum 1+ (42.86%), 4 ARB sputum 2+ (28.57%) and 2 ARB sputum 3+ (14.28%). At the end of second week of treatment, 9 patients (64.29%) converted to negative, 4 patients (28.57%) with ARB smear sputum 1+ and 1 patient (7.14%) with ARB smear sputum 2+. At the end of the fourth week of treatment, there were 11 patients (78.57%) converted to negative, 2 patients (14.28%) with ARB smear sputum 1+ and 1 patient (7.14%) with

ARB smear sputum 2+ (Table 1).

Analysis Results

Paired sample t test was used to determine the significant difference of BMI in the fourth week and preliminary condition, second week and preliminary condition, fourth week and second week of treatment. The result of paired sample t test of BMI was p = 0.309, 0.107 and 0.270. TNF-α in the fourth week compared to preliminary TNF-α was p = 0.015 while second week compared to preliminary TNF-α was p = 0.026. The TNF-α in fourth week compared to second week was p = 0.990 (Table 2). The Wilcoxon statistical test was used to compare the decrease in the ARB sputum positivity in the fourth week and second week. The result was p = 0.157 (Table 3).

Before conducting a correlation test, there should be a significance test among decreased ARB sputum positivity, BMI and TNF-α levels at the end of the second and fourth week (table 4). The significant test of TNF-α and initial BMI obtained p = 0.005, thus it could be concluded that there was a significant difference. Pearson correlation was performed to determine the correlation between TNF-α and initial BMI (table 5). The correlation between TNF-α and initial BMI obtained r = -0.702 (table 6).

Table 1. ARB sputum examination

ARB sputum (IUATLD)	Preliminary		Second week		Forth week	
	Total	%	Total	%	Total	%
Negative	0	0.00	9	64.29	11	78.57
Scanty	2	14.28	0	0.00	0	0.00
1+	6	42.86	4	28.57	2	14.28
2+	4	28.57	1	28.57	1	7.14
3+	2	14.28	0	0.00	0	0.00
Total	n=14	100	n=14	100	n=14	100

Table 2. Paired sampel t test on BMI and TNF-α

Variable		p
BMI	4th week and preliminary 2nd week and preliminary 4th weeek and 2nd week	0.309 0.107 0.270
TNF-α	4th week and preliminary 2nd week and preliminary 4th weeek and 2nd week	0.015 0.026 0.990

Table 3. Wilcoxon test between decreased ARB sputum positivity at 4th and 2nd week

Variable	p
ARB sputum	0.157

Table 4. Significant test between decreased ARB positivity with BMI and TNF-α at 2nd and 4th week

Week	Variable	p
2nd	Decrease ARB sputum positivity and BMI	0.446
	Decrease ARB sputum positivity and TNF-α	0.855
4th	Decrease ARB sputum positivity and IMT	0.975
	Decrease ARB sputum positivity and TNF-α	0.386

Table 5. Significant test between TNF-α and BMI at 2nd and 4th week

Week	p
Preliminary 2nd 4th	0.005 0.184 0.095

Table 6. Pearson correlation test between TNF-α and BMI

Week	Pearson correlation
Preliminary	-0.702

Discussion

Nutritional status is one of the factors that determine the body system function including the immune system. The immune system is needed by humans to protect the body against infection. When the immune system is weak, *M. tuberculosis* germs easily enter the lungs, multiply and spread throughout the body through blood vessels or lymph nodes [10]. People infected by *M. tuberculosis* germs does not always suffer from tuberculosis, depending on the immune system. When the immune system is strong then the germ remains asleep in the body (dormant) and will not develop into a disease. When the immune system is weak, germs will develop and cause tuberculosis [11].

Body Mass Index (BMI) can be used to assess the patient's nutritional status. BMI is a simple and sensitive way to determine adult nutritional status. BMI is defined as body weight (kg) divided by height (m) squared. There are several levels of nutritional status based on BMI that is very thin (<17 kg/m^2), thin (17.0-18.4 kg/m), normal (18.5-24.9 kg/m^2), overweight (25.0-26.9 kg/m^2), fat (27-28.9 kg/m^2) and obese/very fat (≥29 kg/m^2). A person is said to be malnourished if BMI <18.5 kg/m^2.

In this study, 9 (64.29%) of 14 patients had been converted at the end of second week of OAT treatment.

This was due to many died M. tuberculosis germs because of OAT. The conversion rate in the second week was 50%. Tuberculosis is an infectious disease that is often accompanied by nutritional and metabolic disorders. Response to infection is associated with increased energy expenditures and tissue damage levels [12].

Energy expenditures needed to fight through M. tuberculosis infection. Characteristics of patients characterized by loss of appetite and weight loss. Complex changes occur in macronutrient metabolism such as proteins, carbohydrates and fats. Increased protein breakdown leads to reduced muscle mass in patients with tuberculosis. Tuberculosis patients also experience loss of protein (nitrogen), which is the result of impaired absorption of diarrhea, fluid loss, electrolytes and other nutrient reserves [13].

Sputum of ARB is an important thing that should be evaluated in pulmonary tuberculosis patients. According to the Health Department, ARB sputum evaluation is done at the end of the second month, fifth month and sixth month of treatment. This study reported decreased ARB sputum quantitatively and qualitatively at the end of the second week and the fourth week of treatment. Quantitatively, 4 (28.57%) patients still had ARB positive at the end of second week and 3 (21.43%) at the end of the fourth week. At the end of the fourth week, there was a conversion of ARB sputum as much as 11 (78.58%) from 14 patients. ARB declined rapidly after starting OAT treatment, 80-85% of pulmonary tuberculosis patients became non-infectious after 2 weeks. The sputum conversion after four weeks was 52%. This study obtained the conversion rate at the end of second week by 64.29% and the fourth week of 78.58% [14].

In the intensive phase, mycobacterium was killed quickly within 2 weeks thus the infectious patients became non-infectious and had clinical improvement. The result of patient's sputum is influenced by various things such as the ability of the sufferer for adequate cough, small sputum volume (ideally 5-10 ml), sputum consistency (mucoid or purulent). The ARB sputum conversion is strongly influenced by appropriate drug, appropriate dosage, regular drug taking, nutritional status and immune status [15].

Weight loss due to tuberculosis caused by TNF-α produced by dendritic cells, T lymphocytes and macrophages. TNF-α is known as cachectin that works in the hypothalamus by suppressing the appetite center causing anorexia. Increased TNF-α can be detected in the MN-cell supernatant of peripheral blood of pulmonary tuberculosis patients [16]. Immune response pulmonary tuberculosis patients is active before and after treatment, and rreporting an increase of TNF-α levels during treatment although not significant. Recent studies reported significant increases in TNF-α levels after recognition of the muramyl dipeptide bond (MDP). Alveolar macrophage activity was significantly associated with TNF-α [17].

At the onset of OAT treatment there was an increase of TNF-α. Longitudinal studies reported that TNF-α levels fluctuated during treatment. The TNF-α in tuberculosis patients before treatment were significantly higher than in healthy controls (23.19 ± 12.78 vs 14.22 ± 7.17 pg/mL, with $p < 0.05$). After standard OAT treatment for 6 months, TNF-α levels in both groups were similar. It could be concluded that there was correlation between TNF-α and weight.

Conclusion

It could be concluded that TNF-α levels at the end of the second week decreased significantly compared to baseline TNF-α levels. There was an increase BMI by the end of the second week and the fourth week. There was no significant different of ARB sputum positivity at the end of the second week compared to the fourth week. There was a strong correlation between TNF-α levels and BMI prior to treatment with negative direction.

Ethical Clearance: The study has been approved by ethical comittee in Dr.Soetomo General Hospital Surabaya, Indonesia.

Conflict of Interest: The author reports no conflict of interest of this work.

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