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DIFFERENCES OF PLASMA INTERLEUKIN-6 AND TUMOR NECROSIS FACTOR-α LEVELS IN HEALTHY PEOPLE, RIFAMPICIN RESISTANT AND SENSITIVE PULMONARY TUBERCULOSIS PATIENTS

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ABSTRACT

Increased tuberculosis in the world is caused by increased HIV-infected and antituberculous drugs (rifampicin) resistant individuals. IL-6 and TNF-α play an essential role in explaining the different degrees of inflammation in Rifampicin Resistant (RR) and Rifampicin Sensitive (RS) pulmonary tuberculosis patients, and healthy people. The research aimed to analyze the differences in plasma IL-6 and TNF-α levels in healthy people, Rifampicin Resistant (RR), and Rifampicin Sensitive (RS) pulmonary tuberculosis patients. A cross-sectional study was conducted from July-September 2017. Thirty-nine subjects were classified into RR pulmonary tuberculosis (n=15), RS pulmonary tuberculosis (n=12) based on GeneXpert examination and treated by anti tuberculous drugs ≤ 1 month, and healthy people (n=12) based on AFB results, Thorax X-ray, and tuberculin tests. IL-6 and TNF-α were done in all subjects using ELISA U-CyTech®(Biosciences, Inc.). Anova analyzed differences of IL-6 and TNF-α levels between groups. The mean IL-6 levels (pg/mL) in RR and RS pulmonary tuberculosis patients, and healthy people were 54.56±59.13, 27.05±37.04, 4.42±2.83, respectively. The mean TNF-α levels (pg/mL) in RR and RS pulmonary tuberculosis patients, and healthy people were 263.54±327.58, 250.25±314.20, 9.04±5.89, respectively. The mean differences between IL-6 and TNF-α levels (pg/mL) between RR pulmonary tuberculosis patients and healthy people were 50.14±15.29 ($p<0.05$) and 254.59±8460 ($p<0.05$). Significant differences of mean IL-6 and TNF-α levels were found between RR pulmonary tuberculosis patients and healthy people.

Key words: Pulmonary tuberculosis, rifampicin resistant, rifampicin sensitive, healthy people, interleukin-6, tumor necrosis factor-α

INTRODUCTION

Tuberculosis is a disease that has long been known and is still a health problem of people in the world.[1,2] Mycobacterium tuberculosis as a bacterium that causes tuberculosis has been discovered by Robert Koch in 1882.[3] The spread of tuberculosis infection occurs through droplet nuclei with the airway as the port of entry of Mycobacterium tuberculosis (95%).[4] Increased tuberculosis infection is caused by increased HIV-infected and anti tuberculous drugs (rifampicin) resistant individuals.[5]

The incidence rate of pulmonary tuberculosis in the world in 2015 was 580.000 or 7.9 people per 100.000 population. New cases of RR tuberculosis in the world in 2015 were 3.9%, and cases of RR tuberculosis with previous anti tuberculous drugs in the world in 2015 were 21%. The incidence rate of pulmonary tuberculosis in Indonesia in 2015 was 32.000 or 12 people per 100.000 population. New cases of RR tuberculosis in Indonesia in 2015 were 2.8%, and cases of RR tuberculosis with previous anti tuberculous drugs in Indonesia in 2015 were 16%.[6] As many as 12.5% of RS pulmonary tuberculosis patients became RR pulmonary tuberculosis patients in TB DOTS/MDR Wards, Department of Pulmonology and Respiratory Medicine, Dr. Soetomo Hospital Surabaya in October 2016-July 2017. This is likely influenced by IL-6 and TNF-α. Besides tuberculosis, other infectious diseases, trauma, diseases which cause inflammation such as diabetes mellitus, liver disease, kidney disease, and others can influence the levels of IL-6, and TNF-α.

The role of IL-6 and TNF-α in the pathogenesis of Mycobacterium tuberculosis resistance to rifampicin remains unclear. IL-6 and TNF-α may be factors that increase the occurrence of pathological processes in RR pulmonary tuberculosis.[7,8] IL-6 dan TNF-α in RR and RS pulmonary tuberculosis have an essential role because it can explain the pattern of different inflammatory reactions.[7]

Ladel *et al.* suggested that IL-6 has a protective role against tuberculosis. In-vitro, CD4 T cells from M. tuberculosis-infected mice produce high levels of

IL-6 during the early immune response, and IL-6 induces mycobacterial growth inhibition in macrophages. Consistent with the functional effects of IL-6 in murine tuberculosis, analyses of human pulmonary tuberculosis and levels of IL-6 in human plasma point to a role of IL-6 in infection with M. tuberculosis. However, other studies showed that IL-6 was involved in the inability of the cellular immune response to eradicate tuberculosis.[2]

TNF-α was needed to control tuberculosis. TNF-α was also involved in the host pathological response to tuberculosis and often cited as a significant factor in lung tissue damage.[9] Increased TNF-α was positively correlated with clinical deterioration in tuberculosis patients.[10]

Ieremenchuk *et al*. and Correia *et al*. conducted researches on plasma IL-6 levels in RR and RS pulmonary tuberculosis and obtained increased IL-6 levels in RR pulmonary tuberculosis higher than RS pulmonary tuberculosis.[7,8] Eum *et al*. obtained TNF-α level in PBMC or whole blood of RS pulmonary tuberculosis higher than RR pulmonary tuberculosis.[11]

Research on plasma IL-6 and TNF-α levels in RR and RS pulmonary tuberculosis patients has not been done in Indonesia and also in the Dr.Soetomo Hospital Surabaya at this time. From this idea, this research should be done to know and compare plasma IL-6 and TNF-α levels in pulmonary tuberculosis patients with different sensitivity to rifampicin.

METHODS

The study was conducted in July-September 2017 using a cross-sectional design, and samples were taken consecutively. Subjects consisted of RR and RS pulmonary tuberculosis patients (anti tuberculous drugs ≤ one month) in the TB DOTS/MDR Wards, Department of Pulmonology and Respiratory Medicine, Dr. Soetomo Hospital Surabaya, and healthy people. The GeneXpert examination was performed in RR and RS pulmonary tuberculosis patients. Tuberculin tests were performed in healthy people. AFB results and thorax X-ray were performed in all subjects.

Rifampicin resistant pulmonary tuberculosis patients were determined based on the clinical examination and GeneXpert examination which showed positive DNA TB and resistant to rifampicin. Rifampicin sensitive pulmonary tuberculosis patients were identified based on the clinical study and GeneXpert examination which showed positive DNA TB and sensitive to rifampicin. Rifampicin resistant pulmonary tuberculosis patients and RS pulmonary tuberculosis patients infected with HIV, with autoimmune disease (lupus), trauma, sepsis, liver disease, kidney disease, hepatitis B, hepatitis C, diabetes mellitus, and was receiving immuno suppressant therapy or corticosteroids were excluded.

Healthy people were determined based on the normal results of thorax X-ray, negative AFB result, and tuberculin test<10 mm. Healthy people who had been diagnosed as pulmonary tuberculosis, with a history of liver disease, kidney disease, diabetes mellitus, HIV, autoimmune disease (lupus), were suffering from a respiratory infection or other infections, and was receiving immunosuppressant therapy or corticosteroids were excluded.

Laboratory examinations were performed in the Clinical Pathology Laboratory, Dr. Soetomo Hospital Surabaya. Plasma IL-6 and TNF-α levels were performed using Enzyme-Linked Immunosorbent Assay (ELISA) U-CyTech®(Biosciences, Inc.).

Statistical analysis was performed using SPSS ver 16.0. Differences of IL-6 and TNF-α levels between groups were analyzed by Anova if the data of each group was normally distributed or analyzed by Kruskal Wallis if the data of each group was not normally distributed. P-value <0.05 was considered as statistically significant, with a 95% confidence interval.

RESULTS AND DISCUSSION

The results of this study revealed that 27subjects were diagnosed as pulmonary tuberculosis. The GeneXpert examination which positive DNA TB and resistant to rifampicin were detected in 15 subjects, while GeneXpert examination which positive DNA TB and sensitive to rifampicin were detected in 12 subjects. Healthy people who were willing to participate in this research and meet the inclusion criteria obtained by 12 subjects.

Based on the median age of patients, RR and RS pulmonary tuberculosis patients in this research tend to occur in productive age. The majority of subjects in RS pulmonary tuberculosis in this research were males. The results of this research were similar to Correia's research in Brazil.[7] The ratio of male to female in RR pulmonary tuberculosis patients in this research was almost the same. The results of this research were similar to the study of the incidence of RR pulmonary tuberculosis at the Bahtera Mas Regional Hospital of Southeast Sulawesi Province in 2016 and Correia's research. Characteristics of subjects could be seen in Table 1.

Table 1. The characteristics of subjects

Variables	RR pulmonary tuberculosis (n=15)	RS pulmonary tuberculosis (n=12)	Healthy people (n=12)
Age (years)			
Median	43	39	30
Range	26-60	17-61	23-37
Sex:			
Male, n (%)	7 (47)	10 (83)	6 (50)
Female, n (%)	8 (53)	2 (17)	6 (50)

Table 2. Means (pg/mL), standard deviations, p-values of IL-6

Groups	Number of samples	Means	Standard deviations	p-values
RR pulmonary tuberculosis	15	54.56	59.13	
RS pulmonary tuberculosis	12	27.05	37.04	0.015
Healthy people	12	4.42	2.83	

The highest level of IL-6 in RR pulmonary tuberculosis patients was 145.53 pg/mL, while the lowest level of IL-6 in RR pulmonary tuberculosis patients was 0 pg/mL. The highest level of IL-6 in RS pulmonary tuberculosis patients was 102.01 pg/mL, while the lowest level of IL-6 in RS pulmonary tuberculosis patients was 0 pg/ml. Healthy people have IL-6 levels ranging from 0-7.67 pg/mL. The distribution of IL-6 in three groups could be seen in Figure 1.

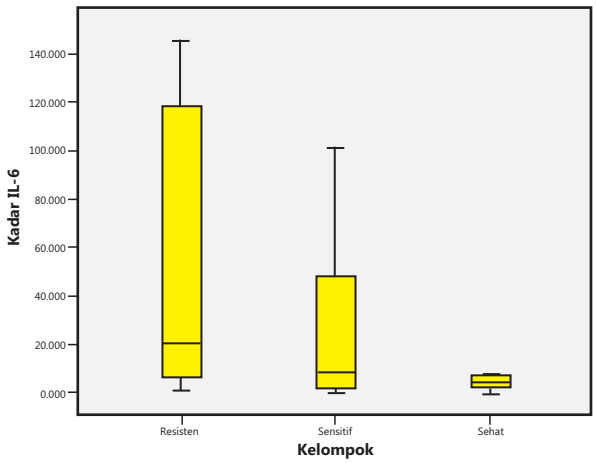

Figure 1.The distribution of IL-6 (pg/mL)

Means, standard deviations, and p-values of IL-6 in three groups can be seen in Table 2.

Mean IL-6 levels between RR pulmonary tuberculosis patients, RS pulmonary tuberculosis patients, and healthy people showed a significant difference ($p < 0.05$). Mean IL-6 of RR pulmonary tuberculosis patients was higher than mean IL-6 of RS pulmonary tuberculosis patients and healthy people. Mean IL-6 of RS pulmonary tuberculosis patients was higher than mean IL-6 of healthy people. This finding was due to the increased inflammatory response in RR pulmonary tuberculosis patients from RS pulmonary tuberculosis patients and healthy people, and increased inflammatory response in RS pulmonary tuberculosis patients from healthy people. The results of this research were consistent with Correia's research and Ieremenchuk's research.[7,8]

The process of phagocytosis rifampicin sensitive Mycobacterium tuberculosis by macrophages results in rifampicin sensitive Mycobacterium tuberculosis destroyed in the phagosome of macrophages. This process results in increased differentiation of M2 macrophages and decreased M1 macrophages. Rifampicin resistant Mycobacterium tuberculosis is phagocytosed by macrophages and is not destroyed in macrophage phagosomes resulting in increased differentiation of M1 macrophages and decreased M2 macrophages. Increased M1 macrophages produce higher levels of IL-6.[12]

Post Hoc analysis results showed that statistically significant differences were obtained in RR pulmonary tuberculosis patients with healthy people ($p < 0.05$), while RS pulmonary tuberculosis patients with healthy people and RR pulmonary tuberculosis patients with RS pulmonary tuberculosis patients showed no statistically significant differences. The mean differences of IL-6 between groups could be seen in Table 3.

Table 3. The mean differences of IL-6 (pg/mL) between RR pulmonary tuberculosis, RS pulmonary tuberculosis, and healthy people

Groups	Groups	Mean differences	Standard deviations	p-values
RR pulmonary tuberculosis	RS pulmonary tuberculosis	27.51	18.64	0.320
RR pulmonary tuberculosis	Healthy people	50.14	15.29	0.014
RS pulmonary tuberculosis	Healthy people	22.63	10.72	0.133

Table 4. Means (pg/mL), standard deviations, p-values of TNF-α

Groups	Number of samples	Means	Standard deviations	p-values
RR pulmonary tuberculosis	15	263.54	327.58	
RS pulmonary tuberculosis	12	250.25	314.20	0.038
Healthy people	12	9.04	5.89	

The mean differences of IL-6 that not significant between RS pulmonary tuberculosis patients and healthy people in this research were probably caused by *Mycobacterium tuberculosis* destroyed in RS pulmonary tuberculosis patients, so that the inflammatory response decreased. The mean differences of IL-6 that not significant between RR pulmonary tuberculosis patients and RS pulmonary tuberculosis patients were probably caused by data variations were too vast. Other intervening variables that could not be controlled or measured were probably as the cause of wide data variations.

The role of IL-6 in RR and RS pulmonary tuberculosis patients is crucial because it will glue information about the different degrees of inflammation. IL-6 is a proinflammatory cytokine group that initiates an inflammatory response.[7] Ieremenchuk stated that increased IL-6 showed a high inflammatory response activity and was shown maximally in RR pulmonary tuberculosis patients compared with RS pulmonary tuberculosis patients.[8] This inflammatory response was critical in detaining *Mycobacterium tuberculosis*.[7]

The highest level of TNF-α in RR pulmonary tuberculosis patients was 882.53 pg/mL, while the lowest level of TNF-α in RR pulmonary tuberculosis patients was 0 pg/mL. The highest level of TNF-α in RS pulmonary tuberculosis patients was 835.58 pg/mL, while the lowest level of TNF-α in RS pulmonary tuberculosis patients was 0 pg/mL. Healthy people have TNF-α levels ranging from 0-17.66 pg/mL. The distribution of TNF-αin three groups could be seen in Figure 2.

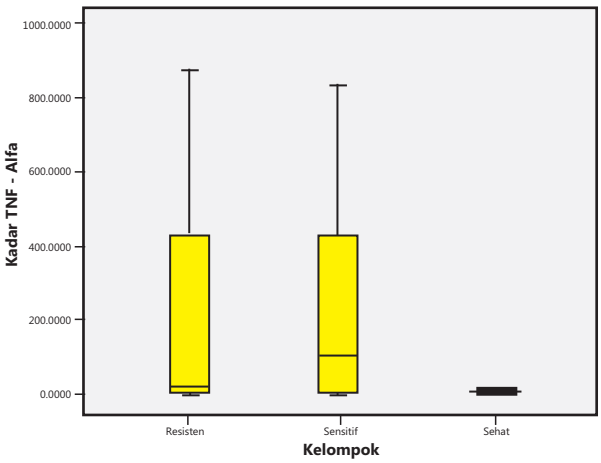

Figure 2. The distribution of TNF-α (pg/mL)

Means, standard deviations, and p-values of TNF-α in three groups could be seen in Table 4.

Mean TNF-α levels between RR pulmonary tuberculosis patients, RS pulmonary tuberculosis patients, and healthy people showed a significant difference ($p < 0.05$). Mean TNF-α of RR pulmonary tuberculosis patients was higher than mean TNF-α of RS pulmonary tuberculosis patients and healthy people. Mean TNF-α of RS pulmonary tuberculosis patients was higher than mean TNF-α of healthy people. This results was due to the increased inflammatory response in RR pulmonary tuberculosis patients from RS pulmonary tuberculosis patients and healthy people, and increased inflammatory response in RS pulmonary tuberculosis patients from healthy people.[10]

Eum *et al*. found that TNF-α levels in PBMC or

Table 5. The mean differences of IL-6 (pg/mL) between RR pulmonary tuberculosis, RS pulmonary tuberculosis, and healthy people

Groups	Groups	Mean differences	Standard deviations	p-values
RR pulmonary tuberculosis	RS pulmonary tuberculosis	13.28	124.02	0.994
RR pulmonary tuberculosis	Healthy people	254.49	84.60	0.024
RS pulmonary tuberculosis	Healthy people	241.21	90.72	0.054

whole blood of RS pulmonary tuberculosis patients were higher than those in RR pulmonary tuberculosis patients. This was due to decreased production of nitric oxide in RR pulmonary tuberculosis patients that correlated with TNF-α production from monocyte cells. The results of this research were different from the results conducted by Eum.[11] Junior stated that pulmonary tuberculosis patients with high levels of TNF-α showed a poor clinical condition.[13]

The phagocytosis process of rifampicin resistant *Mycobacterium tuberculosis* by macrophages results in rifampicin resistant *Mycobacterium tuberculosis* destroyed in the phagosome of macrophages. This process results in increased differentiation of M2 macrophages and decreased M1 macrophages. Rifampicin resistant *Mycobacterium tuberculosis* is phagocytosed by macrophages and is not destroyed in macrophage phagosomes resulting in increased differentiation of M1 macrophages and decreased M2 macrophages. Increased M1 macrophages result in higher levels of TNF-α.[12]

Post Hoc analysis results showed that statistically significant differences were obtained in RR pulmonary tuberculosis patients with healthy people ($p < 0.05$), while RS pulmonary tuberculosis patients with healthy people and RR pulmonary tuberculosis patients with RS pulmonary tuberculosis patients showed no statistically significant differences. The mean differences of TNF-α between groups can be seen in Table 5.

The statistically significant differences in TNF-α in this research was obtained only in patients with pulmonary tuberculosis RR with healthy people ($p < 0.05$), while the mean differences of TNF-α in RS pulmonary tuberculosis patients with healthy people and RR pulmonary tuberculosis patients with RS pulmonary tuberculosis patients showed no significant difference. The results of this research were similar to the results conducted by Eum.[11]

The mean differences of TNF-α that not significant between RS pulmonary tuberculosis patients and healthy people in this research were probably caused by *Mycobacterium tuberculosis* destroyed in RS pulmonary tuberculosis patients, so that the inflammatory response decreased. The mean differences of TNF-α that not significant between RR pulmonary tuberculosis patients and RS pulmonary tuberculosis patients were probably caused by data variations were too vast. Other intervening variables that could not be controlled or measured were probably as the cause of wide data variations.

This research has a limitation, namely intervening variables that could not be controlled or measured entirely.

CONCLUSION AND SUGGESTION

Differences of mean IL-6 and TNF-α levels were found between RR pulmonary tuberculosis, RS pulmonary tuberculosis, and healthy people, but statistically significant differences of mean IL-6 and TNF-α levels were found between RR pulmonary tuberculosis patients and healthy people. Further studies are needed using other better-differentiating markers, regarding the other intervening variables affecting IL-6 and TNF-α levels, as well as the effect of antituberculous drugs on IL-6, TNF-α, and other biomarkers in monitoring the course of tuberculosis cure.

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