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Research article

Differences of Interleukin-18 and Interleukin-10 Levels in Pulmonary Rifampicin Resistant dan Rifampicin Sensitive Tuberculosis Patients in Dr. Soetomo Hospital Surabaya

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ABSTRACT

Rifampicin is an anti-tuberculosis drug used in short-term treatment regimen for tuberculosis (TB) patients. Resistance to rifampicin causes the prolonged duration of tuberculosis treatment. Interleukin-18 (IL-18) is a pro-inflammatory cytokine which acts in controlling the growth of *M. tuberculosis*, while Interleukin-10 (IL-10) is an anti-inflammatory cytokine which acts in limiting tissue damage and maintain tissue homeostasis. IL-18 and IL-10 is important in explaining the different degrees of inflammation (mild, moderate and severe) in rifampicin-resistant (RR) and rifampicin-sensitive (RS) pulmonary TB patients. The purpose of this study is to determine different levels of IL-18 and IL-10 in new TB patients with RR and RS. A retrospective cohort study with a cross-sectional design. 50 subjects were examined and grouped into two groups, namely pulmonary TB with RR ($n = 25$) and pulmonary TB with RS ($n = 25$). IL-18 and IL-10 were measured using the ELISA Method. Differences in IL-18 and IL-10 levels between groups were analyzed using the Mann-Whitney test. The mean level of IL-18 (pg/ml) in RR and RS pulmonary TB patients were 1273.53 ± 749.86 and 787.96 ± 589.28 respectively. The mean level of IL-10 (pg/ml) in RR and RS pulmonary TB patients were 125.25 ± 118.32 and 128.81 ± 135.77 respectively. The mean level of IL-18 in RR and RS pulmonary TB patients were found to have a significant difference, while the mean level of IL-10 did not have a significant difference. This circulating level of IL-18 and IL-10 can be used as a marker of inflammation degrees in pulmonary RR-TB and RS-TB patient.

Keywords: Interleukin-18, Interleukin-10, Tuberculosis, Rifampicin Resistant, Rifampicin Sensitive

ABSTRAK

Rifampisin adalah rejimen dasar pengobatan jangka pendek untuk penderita tuberculosis (TB). Resistensi terhadap rifampisin menyebabkan durasi pengobatan tuberculosis menjadi lebih lama. Interleukin-18 (IL-18) adalah sitokin Proinflamasi yang berperan dalam mengontrol pertumbuhan *M. tuberculosis*, sedangkan Interleukin 10 (IL-10) adalah sitokin anti-inflamasi yang berperan membatasi kerusakan jaringan dan mempertahankan homeostatis jaringan. IL-18 dan IL-10 berperan penting untuk menjelaskan derajat inflamasi (ringan, sedang dan berat) yang berbeda pada penderita TB paru dengan rifampicin resistant (RR-TB) dan rifampicin sensitive (RS-TB). Tujuan penelitian ini adalah mengetahui perbedaan kadar IL-18 dan IL-10 pada penderita RR-TB dan RS-TB. Penelitian ini merupakan penelitian cohort retrospektif dengan rancangan cross-sectional. Sebanyak 50 subjek penelitian diperiksa dan dikelompokkan menjadi dua kelompok yaitu kelompok RR-TB ($n=25$) dan kelompok RS-TB ($n=25$). Pemeriksaan IL-18 dan IL-10 dilakukan dengan Metode ELISA. Perbedaan kadar IL-18 dan IL-10 antara kelompok dianalisis menggunakan Mann-whitney. Rerata kadar IL-18 (pg/ml) pada penderita RR-TB dan RS-TB adalah 1273.53 ± 749.86 dan 787.96 ± 589.28 . Rerata kadar IL-10 (pg/ml) pada penderita RR-TB dan RS-TB adalah 125.25 ± 118.32 dan 128.81 ± 135.77 . Rerata kadar IL-18 pada penderita RR-TB dan RS-TB ditemukan memiliki perbedaan signifikan, sedangkan rerata kadar IL-10 pada penderita RR-TB dan RS-TB tidak

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memiliki perbedaan yang signifikan. Nilai kadar IL-18 dan IL-10 ini dapat digunakan sebagai penanda derajat inflamasi pada penderita RR-TB dan RS-TB.

Kata Kunci: Interleukin-18, Interleukin-10, Tuberculosis, Rifampicin Resistant, Rifampicin Sensitive

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INTRODUCTION

In 2018, The World Health Organization (WHO) was stated that Tuberculosis (TB) is one of the top ten causes of death worldwide. About 10.4 million people suffer from TB and 1.7 million people die from this disease. More than 95% of deaths from TB occur in low and middle-income countries. India, Indonesia, China, the Philippines, Pakistan, Nigeria, and South Africa are countries that accounted the most cases of TB.¹ According to the Basic Health Research of Indonesia the prevalence of patients diagnosed with TB in 2013 was 0.4% with the five highest provinces which are West Java, Papua, DKI Jakarta, Gorontalo, Banten and West Papua. Of the entire population diagnosed with TB, only 44.4% were treated with a program medicines.²

Rifampicin Resistant is defined as a TB case that is declared resistant to rifampicin. TB strains resistant to rifampicin may be either sensitive or also resistant to isoniazid, which for the latter is considered as Multidrug Resistant-Tuberculosis (MDR-TB) based on the GeneXpert test results. This is due to the lower mutation rate of isoniazid (2.56×10^8 CFU / ml *M. tuberculosis* colonies) compared to the mutation rate of rifampicin (6×10^{10} CFU / ml *M. tuberculosis* colonies), so that it can be said that TB patients that are resistant to the rifampicin drug are also resistant to isoniazid, but this comparison varies greatly between countries and patient groups.^{3,4} Rifampicin is an antibiotic that has efficient antimicrobial action which combined with isoniazid which considered to be the basis of a short-term treatment regimen for TB. Rifampicin in *M. tuberculosis* targets the RNA polymerase β -subunits by binding and inhibiting the extension of RNA messenger. The role of

rifampicin is to inhibit active growth and slow metabolism (slow-growing) of bacilli.³

Interleukin-18 (IL-18) was first described and used in rat serum which was intraperitoneally inoculated with endotoxin and was referred to as “Interferon-gamma (IFN- γ) inducing factor”.⁵ Inside the human body, IL-18 is constitutively expressed by several cells, namely macrophages, kupffer cells, keratinocytes, osteoblasts, adrenal cortex cells, intestinal epithelial cells, microglial cells, and synovial fibroblasts.⁶ IL-18 is a pro-inflammatory cytokine that works synergistically with Interleukin-12 (IL-12) to induce IFN- γ production. The expression of IL-18 is regulated in chronic inflammatory diseases mediated by Th1. IL-18 can also contribute to the protection against mycobacteria. It is found that rats with IL-18 deficiency also have a decrease in IFN- γ levels.⁷

Interleukin-10 (IL-10) is an anti-inflammatory cytokine which has a crucial role in preventing inflammatory, pathological autoimmune⁸ and allergies.⁹ Deficiency or decreased expression of IL-10 can increase the inflammatory response to microbes but on the other hands, it can also cause the development of infectious diseases such as TB and several of autoimmune diseases.⁸ IL-10 can also increase the continuity of *M. tuberculosis* and its growth in macrophages by suppressing the partial maturation of phagosomes which depend on the activity of the signal transducer and activator of transcription 3 (STAT3)¹⁰. Currently, IL-10 increases survival and intracellular growth Mycobacteria by suppressing innate and adaptive immune responses.¹¹

This study will describe how different levels of IL-18 and IL-10 in pulmonary TB patients with rifampicin resistant and rifampicin sensitive,

where IL-18 and IL-10 can play an important role in explaining the different degrees of inflammation between these two groups.

MATERIALS AND METHODS

Study Population

This study was conducted in the Department of Clinical Pathology, Dr. Soetomo Hospital from August to November 2018. This study included 50 patients who were selected from the TB-DOTS/MDR Clinic of Dr. Soetomo Hospital. The study protocol has been approved by the Ethical Review Committee of Dr. Soetomo Hospital (0488/KEPK/VIII/2018). The data of all patients were collected after taking informed consent from patients. The age of patients ranged from 17 to 75 years old. The patients were assigned into two groups. The first group consisted of 25 patients with rifampicin-resistant pulmonary TB and the second group also consisted of 25 patients with rifampicin-sensitive pulmonary TB. Patients with HIV-AIDS, hepatitis, autoimmune diseases, diabetes mellitus, liver and kidney disease were excluded from this study. Also, patients treated with corticosteroid or immunosuppressive drugs were excluded, along with patients who had received anti-tuberculosis for more than one month because it can cause bias in the results of the examination

Sample Preparation

Four milliliters of blood were drawn aseptically from the basilic vein of each patient. Blood specimens were collected by using vacutainer venipuncture then stored in the serum separator tube. The tube contains a separation gel in the base of the tube which separates the serum from the whole blood. The blood sample was collected then was centrifuged at 3000 rpm for 10 minutes, the serums were then stored and freeze at -80°C for further use.

Enzyme-linked Immunosorbent Assay (ELISA) Analysis

The frozen serums were thawed at room temperature and cytokine IL-18 and IL-10

levels were then measured using the Human Sandwich-ELISA kit from *Elabscience*® done as the manufactures instructions. The cytokine concentrations in samples were calculated using the standard curve generated from recombinant cytokines, and the results are expressed in picograms per milliliter (pg/ml).

Statistical Methods

The result is presented as the mean \pm s.d. Statistical significance was calculated by the *Mann-Whitney test* to see differences between IL-18 and IL-10 in patients with pulmonary RR-TB and pulmonary RS-TB. The *p values* < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Clinical Characteristics of Subjects

The clinical characteristics of the 25 patients with pulmonary RR-TB and 25 patients with pulmonary RS-TB are summarized in Table 1. The clinical type of all TB patients were all pulmonary TB.

IL-18 Level

The highest level of IL-18 found in pulmonary RR-TB patients was 2486 pg/ml, and the lowest 58.39 pg/ml, while the highest level of IL-18 in pulmonary RS-TB patients was 1990 pg/ml and the lowest was 106.06 pg/ml. The mean, standard deviation, and *p-values* of IL-18 levels in these two groups are shown in Table 2. The mean level of IL-18 between pulmonary RR-TB and RS-TB patients were showed significant differences ($p < 0.05$). The differences of IL-18 in pulmonary RR-TB and pulmonary RS-TB patients are shown in the boxplot in Figure 1.

Table 1. Clinical Characteristics of the Population Studied.

	Pulmonary RR-TB	Pulmonary RS-TB
Gender, male/female	18/7	11/14
Median age (range)	37.00 (23-67)	43.00 (18-63)

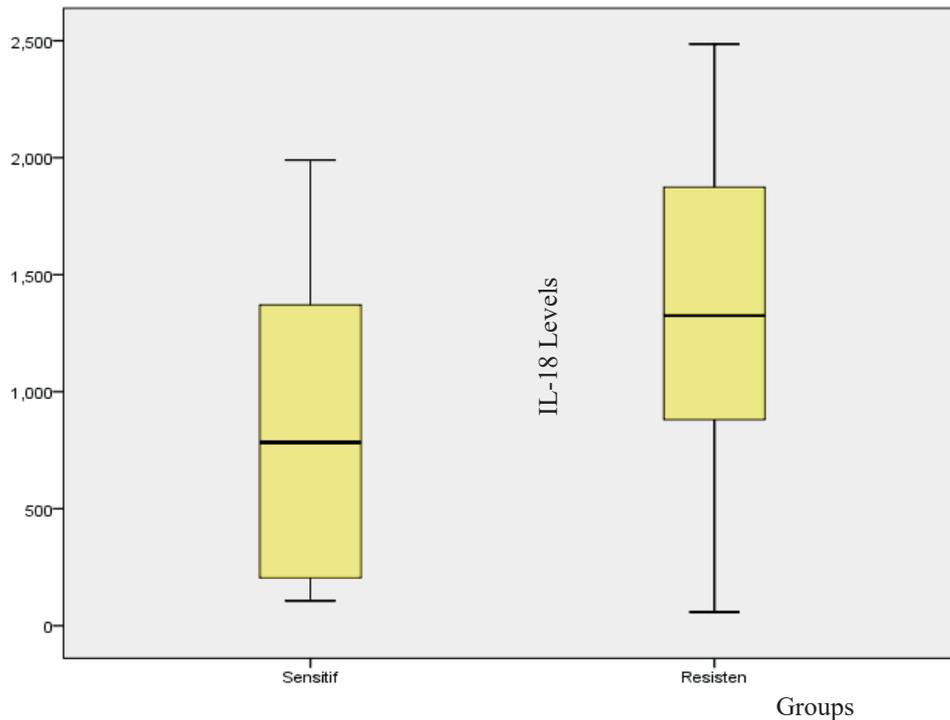


Figure 1. IL-18 Levels in Pulmonary RR-TB and Pulmonary RS-TB. This result shows that an increase in IL-18 levels in the blood was found to be significantly higher in pulmonary RR-TB patients compared to pulmonary RS-TB, meaning a higher increase in the inflammatory process for pulmonary RR-TB patients compared to pulmonary RS-TB patients. This results is also accordance with the result of Wang et al¹² study.

Table 2. The Mean and Standard Deviation of IL-18 in Pulmonary RR-TB and Pulmonary RS-TB

Group	N	Mean	Standard deviation	p-value
Pulmonary RR-TB	25	1273.53	749.86	0.017
Pulmonary RS-TB	25	787.96	589.28	

n = number of samples
p < 0,05 = significant

The IL-18 level between pulmonary RR-TB and RS-TB patients found in this study has a mean of 1273.53 ± 749.86 pg/ml and 787.96 ± 589.28 pg/ml respectively. This shows that the increasing level of IL-18 in the blood was found to be significantly higher in pulmonary RR-TB than in pulmonary RS-TB. This results in this study also in accordance with the result of Wang et al¹² study. Wang et al¹² also stated that the IL-18 serum was found to be higher in patients with pulmonary RR-TB (131.03 ± 94.92) compared to drug sensitive TB (94.28

± 57.10) and healthy controls (61.66 ± 24.78). The resistance to rifampicin in TB is caused by mutations in the bacterial chromosome (rpo β gene). Mutations in this gene will cause changes in the structure and activity of drug targets that results in generating bacterium *M. tuberculosis* that cannot be eliminated using rifampicin which has an impact on increasing the number of said bacteria in the host body.¹³ This increase in the number of bacteria causes macrophages as a first-line defense against the invasion of these bacteria and mediates the innate immune response through the introduction of pathogens and an increase in inflammatory reactions. Increased macrophage activation in RR pulmonary TB infection will increase the production of proinflammatory cytokines that play a role for the mechanism of killing *M. tuberculosis*.¹⁴

Rifampicin plays an important role in TB treatment because of its bactericidal effect that can eliminate *M. tuberculosis*.¹⁵ When pulmonary TB patients are resistant to rifampicin, the

growth of *M. tuberculosis* will increase and cannot be controlled. Macrophages as the first-line defense will fight the bacterial invasion and mediate innate immune responses through the introduction of pathogens and the activation of inflammatory reactions. Macrophages will polarize to various functional conditions such as M1 which is classically activated and M2 which is alternatively activated. Macrophage polarization into M1 is important for the elimination of intracellular *M. tuberculosis*. Activation of M1 macrophages through the TLR2 signal pathway can be beneficial for the host to inhibit growth and the survival of *M. tuberculosis*.^{16,17} Increased activation of M1 macrophages in newly infected RR pulmonary TB will produce pro-inflammatory cytokines which play a role in the mechanism of eliminating *M. tuberculosis*. This causes the level of pro-inflammatory cytokines to be higher in RR pulmonary TB serum compared to RS pulmonary TB. The level of pro-inflammatory cytokines in both RR and RS pulmonary TB is found to be higher compared to the level of anti-inflammatory cytokines to suppress growth and the survival of *M. tuberculosis*.¹²

Increased level of IL-18 in the patients' serum is also suspected to indicate that there has been a leak of cytokines from the tissues to the circulation. This is supported by various studies which stated that a high concentration of IL-18 are found in TB patients with advanced disease, high fever, and extensive radiographic infiltrates.^{7, 18} Increased levels of IL-18 as a pro-inflammatory cytokine in RR pulmonary TB patients are associated with various pathological conditions in the patients themselves. Patients with pulmonary RR-TB with high levels of IL-18 were also found to have higher ESR and CRP levels compared to pulmonary RS-TB patients and healthy people. ESR and CRP have been used as markers for the diagnosis of pulmonary TB that reflect pathological processes in the patient's body. Increased CRP and ESR indicate that an acute inflammatory process has occurred in pulmonary TB patients.¹² Higher IL-18 levels found in pulmonary RR-TB patients compared to pulmonary RS-TB patients in this study confirmed various previous studies which stated

that IL-18 levels were significantly increased in patients with severe pulmonary TB.

IL-10 Level

The highest level of IL-10 in pulmonary RR-TB patients was 465.77 pg/ml, and the lowest was 1.57 pg/ml, while the highest level of IL-10 in pulmonary RS-TB patients was 552.11 pg/ml and the lowest level was 1.36 pg/ml. The mean, standard deviation, and *p-values* of IL-10 level in these two groups are shown in Table 3. The mean of IL-10 level between patients showed no significant differences ($p > 0.05$). The differences of IL-10 in pulmonary RR-TB and pulmonary RS-TB patients are shown in the boxplot in Figure 2.

IL-10 is an anti-inflammatory cytokine that works by inhibiting the ability of myeloid cells such as macrophages and dendritic cells to activate Th1 cells. Initially, IL-10 is known to be secreted by antigen-stimulated Th2, but it is now known that IL-10 is not only secreted by Th2, but also secreted by a subset of CD4 + T cells, including Th1 and Th17, B cells, neutrophil cells, and macrophages.¹⁷ IL-10 is generally thought to modulate the ability of the immune response and allow bacterial elimination without damaging the host tissue, but in some cases the absence of IL-10 makes the immune response more effective in eliminating pathogens, but resulting in more damage to the tissue and affects the survival of the host.^{20, 21}

The mean level of IL-10 in pulmonary TB patients with RS and RR in this study were 128.81 ± 135.77 pg/ml and 125.15 ± 118.32 pg/ml respectively. This shows that IL-10 levels in RS were found to be higher than in RR pulmonary

Table 3. The Mean and Standard Deviation of IL-10 in Pulmonary RR-TB and Pulmonary RS-TB

Group	N	Mean	Standard deviation	p-value
Pulmonary RR-TB	25	125.15	118.32	0.961
Pulmonary RS-TB	25	128.81	135.77	

n = number of samples
 $p > 0,05$ = not significant

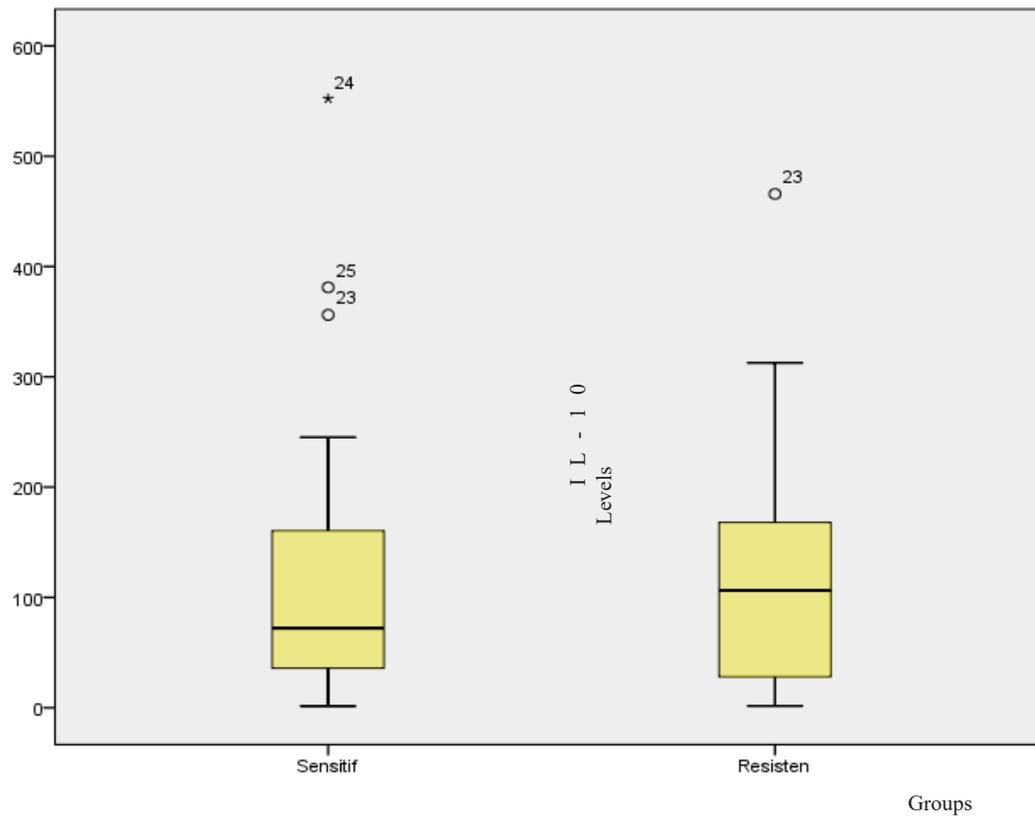


Figure 2. IL-10 Levels in Pulmonary RR-TB and Pulmonary RS-TB Patients. The results shows showed no significant differences between these two groups.

TB, although statistically did not have a significant difference ($p > 0.05$). The results of this study are following a study conducted by Butov et al²² which stated that the mean level of IL-10 in MDR-TB patients' serum before and after 2 months of treatment were found to be lower when compared to non-MDR TB patients and healthy people. This result is in accordance with the result of Lihawa²³ and Peñaloza²⁴ study. Lihawa and Yudhawati²³ in Dr. Soetomo Hospital showed that descriptively IL-10 levels in MDR-TB patients were found to be lower than non-MDR TB, but statistically no significant differences were found. Peñaloza²⁴ was stated that during non-MDR *M. tuberculosis* infection, IL-10 production is important for host survival, but the role of IL-10 in the immune response of patients with MDR pulmonary TB molecularly has not been found with certainty. This insignificant difference in IL-10 may indicate a tendency of static state occurring during the acute phase of TB levels IL-10 due to the role of macrophages which secrete

more proinflammatory cytokines to protect the host from *M. tuberculosis*. It is evidenced in this study by the discovery of IL-18 levels that were higher than the IL-10 levels in each group. High levels of IL-10 can only be found in chronic TB infections.²⁵

CONCLUSIONS

The level of IL-18 is higher in patients with pulmonary RR-TB compared to pulmonary RS-TB. This circulating level of IL-18 and IL-10 can be used as a marker of inflammation degrees in pulmonary RR-TB and RS-TB patients.

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CONFLICT OF INTEREST

There is no conflict of interest that has to be declared in this study.

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