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IFN-γ and IL-4 Secretion after Stimulation of EC610 Fusion Antigens (ESAT-6-CFP-10) in Patients With Active Pulmonary TB and Latent TB

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Abstract

Tuberculosis (TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis* (*M.tb*) and is still a major health problem in the world. Immunity against tuberculosis is very complex because it involves almost all components of the immune system. One of the cells that are responsible for cell-mediated immunity are lymphocytes, especially T helper lymphocytes are divided into Th1 (proinflammatory cytokines IFN-γ) and Th2 (anti-inflammatory cytokine IL-4). EC610 fusion antigens (ESAT-6-CFP-10) is a peptide containing a specific antigen *M.tb*. Order to determine the differences between the secretion of IFN-γ and IL-4 after stimulation EC610 fusion antigens (ESAT-6-CFP-10) in patients with active pulmonary TB and latent TB.Research with a quasi-experimental design in laboratory in vitro in patients with active pulmonary tuberculosis group of 21 samples and 28 samples of latent TB. Venous blood sampling in vitro anticoagulants heparin and PBMCs isolated using a Ficoll-Paque ™, then cultured with antigen fusion EC610 for 24-72 hours with a CO2 incubator at a temperature of 370 C. The levels of IFN-γ and IL-4 by ELISA method. Statistical analysis with alternative test non-parametric Mann Whitney. IFN-γ secretion in patients with active pulmonary TB group (6700 pg / ml) was higher than the latent (6000 pg / ml) after stimulation by antigen fusion EC610, but not significant (p = 0.769). While the secretion of IL-4 levels (180 and 60 pg / ml) there was a significant difference between the groups (p = 0.000).

Keywords : Tuberculosis, Interferon-γ, Interleukin-4, EC610 Fusion Antigens

Introduction

Tuberculosis (TB) is still a major health problem in the world. Indonesia is the country with the second largest number of new cases in the world after India. Mortality due to tuberculosis is estimated at 1.4 million deaths, although the number of deaths due to tuberculosis decreased 37% between 2000 and 2016, tuberculosis remains the 10 highest cause of death in the world in 2016.[1] In 2016 in Indonesia found the number of tuberculosis cases increased from year to year

previously that was as many as 351,893 cases. The number of new cases of positive smear pulmonary tuberculosis in South Sumatra province was found as many as 5,674 cases in 2016.[2] The discovery of new cases of pulmonary TB in Palembang in 2015 amounted to 1,305 cases.[3] Tuberculosis (TB) is an infectious infectious disease caused by the bacterium Mycobacterium tuberculosis (M .tb), ie aerobic germs that can live mainly in the lungs or in various other organs that have high oxygen partial pressure.[4] These bacteria are rod-shaped and are acid-resistant so they are known as Acid-Resistant Basil (BTA) .[5] Immunity against tuberculosis is very complex because it involves almost all components of the immune system both specific immunity and non-specific immunity, humoral and cellular immunity that arise naturally or acquired (acquired). One of the cells responsible for cellular immunity is lymphocytes especially T lymphocyte cells.[6] Based on the type of cytokines it produces, T helper lymphocytes are divided into Th1 which produces IFN-γ proinflammatory cytokines, TNF-α, IL-2, while Th2 that produces cytokines anti-inflammatory IL-4.[7] IFN- γ is the main cytokine for controlling M.tb infection. In tuberculosis, these cytokines are produced by CD4 + and CD8 + T cells, and Natural Killer (NK) cells. Although IFN-produksi production alone is not enough to control TB infection, these cytokines are needed for a protective response.[9] IL-4 has an inhibitory effect on proinflammatory cytokines through suppression of IL-1, TNF-α, IL-6, IL-8, and MIP-1α.[10] IL-4 prevents the activation of macrophages induced by IFN-γ, therefore IL-4 has the opposite effect to IFN-γ.[11] Immunological examination studies continue to develop to detect antibodies or immune responses to tuberculosis antigens. A combination of tests is needed to increase sensitivity and specificity.[12] Early secretory antigenic target protein 6-kDa (ESAT-6) and Culture filtrate protein 10-kDa (CFP-10) are complexes of various specific antigens secreted by M.Tb.[13] Antigens These antigens are encoded by the Regions of Difference (RD) gene locus 1. These antigens are secreted by M.tb when bacteria live. In latent TB, M.tb secretes specific, circulating and detectable antigens in the blood that act as a defense against the host immune response.[14] Research on tuberculosis continues to be carried out continuously in finding appropriate solutions to break the pathophysiological chain of biomolecular tuberculosis.[15] Other studies stated that there was a significant increase in IFN-pada in active TB. Another study also states that, the secretion of IFN-γ levels in Peripheral Blood Mononuclear Cell (PBMC) after stimulation of ESAT-6 antigens in patients with active TB is higher than latent TB and healthy people. This suggests that the Th1 response is more dominant against M.tb so it is more protective.[16,17] Other studies using T-SPOT to see IFN-γ secretion and IL-4 secretion in PBMC after stimulation of the ESAT-6 antigen in active TB patients have shown the concentration and production of IL-4 is lower compared to IFN-resi secretion when compared

with latent TB.[18,19] Research to look at IFN-γ and IL-4 secretion with PBMC after stimulation by EC610 fusion (ESAT-6-CFP-10) in both groups of active pulmonary TB patients and latent TB have never been done in Indonesia so researchers are interested in doing so. This research is expected to be used as an update in evaluating the pathogenesis of tuberculosis.

Methods

This study uses a quasi-experimental design (Quasi Experimental Design) in a laboratory in vitro with Posttest Only, Non-Equivalent Control Group Design in both groups, those with active pulmonary TB and latent TB who meet the criteria. Venous blood sampling in heparin anticoagulant tubes and PBMC was isolated using Ficoll-Paque ™, then cultured with EC610 fusion antigens for 24-72 hours with CO2 incubator at 370 C. Examination of IFN-γ and IL-4 levels by ELISA examination method. Statistical analysis with non-parametric Mann Whitney alternative test ($p < 0.05$). This research is ethical from the Health Research Ethics Commission of the Central General Hospital Mohammad Hoesin and the Faculty of Medicine, Sriwijaya University, Palembang.

Results

The number of samples that met the criteria were 21 samples of active pulmonary TB patients and 28 samples of latent TB. Characteristics of research subjects based on all research variables namely gender, age, education, BCG status, BMI, chest X-ray and QFT-Plus results can be seen in Table 1.

Tabel 1. Characteristics of Subject

Characteristics	Group	
	TB Paru Aktive n (%)	TB Latent n (%)
Subyek (n)	21 (100)	28 (100)
Sex		
Woman	13 (61,9)	21 (75,0)
Man	8 (38,1)	7 (25,0)
Ages (Years)	39,10±10,034	36,32±8,773
Educational		
Junior High School	3 (14,3)	
Senior High School	12 (57,1)	7 (25,0)
Diploma	2 (9,5)	12 (42,9)
Bachelor	4 (19,0)	9 (32,1)
BCG		
Yes	14 (66,7)	20 (71,4)
Not	1 (4,8)	
Unknown	6 (28,6)	8 (28,6)
Body Mass Index (BMI) (kg/m^2)		
Very Thin : <17	3 (14,3)	1 (3,6)
Thin : 17-18,5	6 (28,6)	1 (3,6)
Normal : 18,5-25,0	11 (52,4)	19 (67,9)
Fat : 25,0-27,0	1 (4,8)	4 (14,3)
Obesity : >27		3 (10,7)
Sputum BTA		
Negative	6 (28,6)	28 (100)
Positive	15 (71,4)	
BTA+1	13 (61,9)	
BTA+2	1 (4,8)	
BTA+3	1 (4,8)	
Toraks Rontgen		
Normal		28 (100)
Minimal Lesion	15 (71,4)	
Moderate Lesion	6 (28,6)	
QFT-Plus		
Negative		15 (53,6)
Positive	21 (100)	13 (46,4)
IFN-γ T.Spot		
Negative	4 (19,0)	22 (78,6)
Positive	17 (81,0)	6 (21,4)
IL-4 T.Spot		
Negative	6 (28,6)	22 (78,6)
Positive	15 (71,4)	6 (21,4)

IFN-gamma examination results after stimulation with EC610 fusion antigens in both groups of active pulmonary TB patients and latent TB each obtained a median with the following range of values, 6700 (9100 - 2000) pg / ml and 6000 (9000 - 2500) pg / ml. There was no significant

difference between IFN-kadar levels stimulated by EC610 fusion antigens in both groups of active pulmonary TB patients and latent TB (p = 0.769).

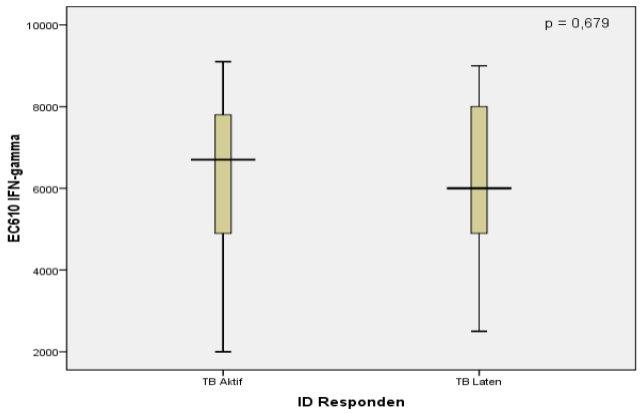

Figure 1. Interferon Gamma in Subject

The results of examination of IL-4 levels after stimulation with EC610 fusion antigens in both groups of active and latent pulmonary TB patients each obtained a median (middle value) with the following range of values, 180 (210 - 80) pg / ml and 60 (110 - 40) pg / ml. There is a significant difference between the levels of IL-4 stimulated by EC610 fusion antigens in both groups of patients with active pulmonary TB and latent TB (p = 0,000).

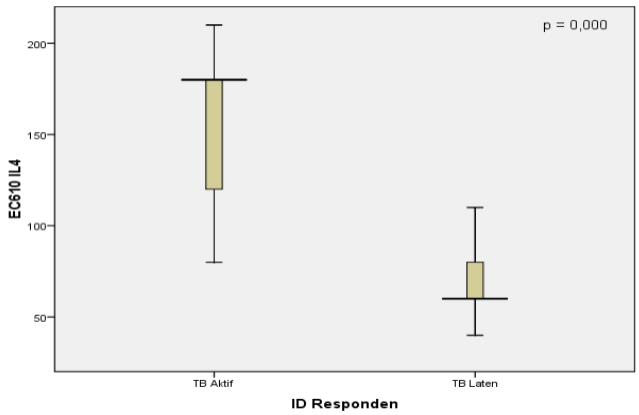

Figure 2. IL-4 Level in Subject of Study

Discussion

As the development of TB disease research, researchers discovered a new biomarker for M.TB infection, Interferon gamma (IFN-γ). IFN-γ appears as an immune reaction against M.TB bacteria in the body. This discovery led to the development of new immunological examinations by

measuring IFN-γ in the body qualitatively and quantitatively. This examination is called the Interferon Gamma Release Assay (IGRA). In this study the results of IFN-γ qualitatively as a gold standard with QuantiFERON-TB Gold Plus (QFT-Plus) examination showed positive results in the group of active pulmonary TB patients with 21 subjects (100%) and in the latent TB group of 13 subjects (46 , 4%). Other studies in patients with pulmonary TB who had HIV negative, sputum smear positive and GenXpert positive were 40 subjects with positive QFT-Plus results. Other studies also on subjects in contact with TB patients were screened for TST for latent TB, then a QFT-Plus examination obtained more positive results than negative results.[20-23] In some countries there have been many studies comparing the effectiveness of IGRA with TST. One of them is South Korea, a country with tuberculosis burden intermediates that has compared the results of TST, QFT and T-Spot. TB assays. By determining the cut off point for TST 10 mm, the sensitivity for T.SPOT (96.6%) was significantly higher than TST (66.7%) and QFT (70.1%). For specificity, QFT is higher than TST (91.6% versus 78.6%). The specificity of QFT is higher compared to T-SPOT TB (91.6% versus 84.7%). [24] Qualitative study results from IFN-sek secretion with PBMC after stimulation of EC610 fusion antigens and measured by Immunospot Analyzer (T.Spot-TB) in patients with active pulmonary TB as many as 21 subjects (100%) positive results, while in latent TB more negative results ie 22 subjects (78.6%) compared with positive results. Other studies obtained IFN-resi secretion with PBMC after ESAT-6 antigen stimulation showed positive results in 20 of 35 subjects (57.1%) in the active TB group and 35 out of 151 subjects (23.2%) in the latent TB group. 19 Meanwhile, the secretion of IL-4 levels with PBMC in the group of active pulmonary TB patients was 15 subjects (71.4%) positive results, and in latent TB more negative results were 22 subjects (78.6%) compared to the positive results of 6 subjects (21.4%). IFN-γ levels with PBMC settings the EC610 fusion antigen stimulation in the group of active pulmonary TB patients was higher than latent TB, but not significant ($p = 0.679$). This higher IFN-dapat level can be understood that there is a protective immune response against M.tb bacterial infection and proves that T lymphocytes are still functioning well. The absence of significant differences between the two groups was caused by the following things, first the group of active pulmonary TB patients were new cases of pulmonary TB patients who were diagnosed early and had not received therapy or less than one month of OAT consumption, where the patient's cellular immune response was quite good so producing high IFN-γ but not strong enough against M.tb bacteria so that the patient is infected with TB. In TB patients, IFN-kadar levels were previously high and will significantly increase after OAT treatment.[25] Second, nutritional status in both groups tends to be normal so that the fulfillment of protein intake can increase regeneration of damaged tissue also

accelerates the sterilization of M.tb bacteria with increasing the number of IFN-TN, TNF-α and Inducible Nitrit Oxide Synthase (iNOS) .[26] Comparison of the nutritional status of TB patients before treatment with during the advanced phase of treatment there is an increase in normal nutritional status, but there is no significant difference.[27] Third, this study did not assess genetic factors of each study subject so that it cannot prove how much influence the genetic factors on pulmonary TB. The influence of this genetic factor is IFN-γ + 874 T / A gene polymorphism, which is a gene that plays a role in producing IFN-γ more. This condition is considered to have a better protective effect against M.tb.[28] infection. The level of IL-4 secretion with PBMC after stimulation of EC610 fusion antigens in the group of active pulmonary TB patients is higher than latent TB. This means that IL-4 levels in the latent TB group were less responsive to EC610 fusion antigen administration when compared to groups of active pulmonary TB patients. Other studies of IL-4 secretion with PBMC after stimulation by ESAT-6 antigens in the median active TB group and their range of values were higher compared to latent TB. There is a comparison of cytokine secretion between IFN-γ and IL-4 which is lower. This is related to the immune response to certain antigens, namely ESAT-6 and may differ from other antigens. Research on PBMC culture stimulated by 38 kDa antigens obtained an average expression of IL-4 lymphocytes CD4 + + higher in the TB contact group than in healthy people, although not meaningful. The 38 kDa antigen has an effect on the exposed immune system so that it can induce CD4 + T lymphocytes and express these cytokines.[29] Increased IL-4 gene expression found in PBMC is associated with excessive coughing, night sweating, radiological diseases, and cavities. Other studies state Significantly increased IL-4 in TB patients. Increased CD8 + IL-4 T cells predicted progression from latent infection to active disease at 6/10 in healthcare workers. Flowcytometry analysis of CD8 + and CD4 + IL-4 cell production in blood from patients TB shows an increase in patients with cavities. mRNA IL-4 in PBMC is associated with pulmonary TB and subgroups with an increase in IL-4δ2 antagonists; the role of Th2 cytokines in TB requires further research. Several observations indicate that Th2 cytokines (IL-4 and IL-10) are associated with latent TB infection, reactivation and advanced TB. IL-4 is an anti-inflammatory cytokine produced by T cells and mast cells. IL-4 is a cytokine marker for Th2 cells, is the main stimulus for Th2 development from naive CD4 + cells.11 IL-4 can function as a T cell growth factor and induce T cells to express IL-2 receptors and produce IL-2. But it can also be an antagonist for IL-2 in several other cell types. IL-4 has an inhibitory effect on proinflammatory cytokines through suppression of IL-1, TNF-α, IL-6, IL-8, and MIP-1α.10 IL-4 prevents the activation of macrophages induced by IFN-γ, therefore IL-4 has the opposite effect to IFN-γ.11 These cytokines IL-4 can also inhibit the

development of Th1 and Th17 lymphocytes and participate in macrophage activation. Increased Th2 (IL-4) cytokine production by bronchoalveolar lavage cells (BAL) is a strong risk factor for TB transmission in South African patients. IL-4 has been involved in the conversion of latent TB infection to active TB. IL-4 has been postulated as a key in TB pathogenesis, especially with its ability to regulate nucleic nitric oxide synthase derivatives, Toll-like receptors 2, and activation of macrophages. Increased activity of the Th2 component can inhibit the role of IFN-γ in protective immunity against M.Tb infection.[30-36]

Conclusion

There is no difference level of Interferon gamma and IL-4 between patient active tuberculosis and latent tuberculosis after stimulation of antigen EC610 (ESAT-6-CFP-10).

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