DIFFERENCE OF PLASMA INTERLEUKIN-10 LEVEL IN PULMONARY TUBERCULOSIS PATIENTS AND HEALTHY NURSES WITH PULMONARY TUBERCULOSIS RISK

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ABSTRACT

Introduction: Tuberculosis is an emerging infectious disease. Indonesia is the third country with the most cases of tuberculosis in the world. Tuberculosis eradication program is only focused in case finding and treating tuberculosis patients. Health care workers are at risk of tuberculosis infection, but there is no examination yet for early detection of tuberculosis activity. To know the activity of tuberculosis, several examinations should be performed and one of them is IL-10. There is no IL-10 study yet in Indonesia, so a study about the difference of plasma IL-10 levels in lung tuberculosis patients and nurses at risk of lung tuberculosis is needed. Objective: to analyze the difference of plasma IL-10 of lung tuberculosis patients and nurses at risk of lung tuberculosis. Materials and methods : a cross sectional, observational analytical study of 10 nurses at risk of lung tuberculosis and 20 lung tuberculosis patients, has been conducted from March–August 2007, at the Dr. Soetomo General Hospital and Karang Tembok Hospital Surabaya. The lung tuberculosis patients included in this study were those with a positive Mycobacterium tuberculosis sputum culture and who had never been treated with anti-tuberculosis drugs. Nurses at risk of lung tuberculosis were those who had been working more than 2 years with negative Mycobacterium tuberculosis sputum culture, chest X-ray and TB-Dot. IL-10 examination (RayBio® Human IL-10 ELISA Kit; RayBiotech, Inc.) was performed using ELISA method. Differences of plasma IL-10 level in both groups were determined by two independent sample t-test. IL-10 cut-off value was determined by Receiver Operator Characteristics Curve and analyzed using Kappa agreement and McNemar test. Results : plasma IL-10 level in lung tuberculosis patients was 14.1 ± 5.3 pg/ml, while in nurses at risk of lung tuberculosis was 6.7 ± 3.3 pg/ml (p<0,001). IL-10 cut-off value was 8.6 pg/ml. Conclusion: plasma IL-10 level in lung tuberculosis patients was significantly higher than in nurses at risk of lung tuberculosis. IL-10 cut-off value was 8.6 pg/ml.

Keywords: plasma IL-10, lung tuberculosis patients, nurses at risk of lung tuberculosis.

INTRODUCTION

Tuberculosis is an infectious disease caused by Mycobacterium tuberculosis, the acid fast bacteria (AFB), which are obligate aerobic and facultative intracellular. Most of the bacteria Mycobacterium tuberculosis attacks the lungs in addition to other body organs (Goldberg, 2004). Mycobacterium tuberculosis infects one-third of world population with a mortality rate of 3 million people. Tuberculosis in Indonesia is the third cause of death in all age groups and number one for the group of infectious diseases (Anonymous, 2005). The source of transmission of tuberculosis is active tuberculosis patients with sputum containing Mycobacterium tuberculosis. The main transmission mode is via the respiratory tract (Anonymous, 2005). Individuals infected with Mycobacterium tuberculosis can occur following circumstances: (1) germs can directly destroyed by the body's immune response; (2) 10-15% will become active tuberculosis within 1-3 years; (3) most will have latent infections, that is, if there is a balance between immune response and germs. Latent tuberculosis do not show clinical symptoms but a positive tuberculin test (Flynn, 2001A). Ninety percent of adult cases of tuberculosis is reactivation of latent infection (Daley, 2004).

Host's protective immune response against Mycobacterium tuberculosis is dominated by cellular immunity humoral immunity with a little help (Nugraha, 2004). Th1 CD4 T lymphocytes (T helper 1) or Th2 (T helper 2) will be stimulated but the role of Th1 CD4 T lymphocytes are more dominant for the body's defense against the invasion of intracellular bacteria like Mycobacterium tuberculosis. Immunopathogenesis of pulmonary tuberculosis based on immunopathologic process due to the interaction of Mycobacterium tuberculosis with host immune responses and cytokines (Handojo, 2004a). The failure of the immune system
overcome infection by *Mycobacterium tuberculosis*. The Th1/Th2 paradigm is due to Th1-mediated immunity due to inadequate downregulation of Th1 immunity by Th2 responses (Abebe, 2005).

Th1 and Th2 immune response can be distinguished from the secreted cytokine (Zumla, 1998). Th1 cytokine pattern dominated by interferon gamma (IFN-gamma), which is the main mediators of protective immunity against *Mycobacterium tuberculosis* (Al-Attiyah, 2006). Th2 cytokine pattern is characterized by the secretion of Interleukin-4 (IL-4), IL-5 and IL-10 is associated with antiinflammatory effects (Crevel, 2002) and inhibits host innate response. The balance of Th1 and Th2 cytokines affect innate host response and is the key to the transition of cellular and humoral adaptive immunity (Strieter, 2002). Different patterns of cytokines are thought to help determine whether the cases of tuberculosis has been cured, be active or latent tuberculosis, so the research response to cytokines in tuberculosis patients and healthy individuals is useful to know better the pathogenesis of tuberculosis (Al-Attiyah, 2006). IL-10 mainly produced by Th2 lymphocytes and macrophages. IL-10 is antiinflammatory and related to reduced body defense and the development of chronic tuberculosis (Al-Attiyah, 2006). IL-10 shifts the immune response to a Th2 cytokine pattern, increased Th2 cell differentiation and suppresses Th1 cell activity (Strieter, 2002). IL-10 inhibits macrophage activity and negatively affect the mechanism microbicidal phagocyte cells, thus reducing the body's defense against *Mycobacterium tuberculosis* (Triencheri, 2001).

Cytokines are produced locally and are involved in a complex network of cytokine system with other cytokines on immunological processes (Olobo, 2001). Evaluation of cytokine responses in tuberculosis is best done from the place of infection, such as pleural fluid in tuberculosis pleuritis (Condos, 2001). Sampling is more difficult and invasive, so a lot of research done with the cytokine profile of peripheral blood mononuclear cell method (PBMC), ie analysis of peripheral blood mononuclear cells stimulated with antigen of *Mycobacterium tuberculosis* in vitro. This method has the disadvantage of a longer examination time for the incubation process, the working procedure is more complicated than checking a systemic cytokines in plasma (Barnes, 2005), and cytokine responses to stimulation in vitro may not reflect the actual situation in vivo (Dlugovitzky, 1997). Immunity in patients with tuberculosis can be reflected by some cytokine levels in plasma but until now there is still little to investigate the systemic cytokine levels in patients with tuberculosis despite plasma sampling is easier than checking procedures and requires no special equipment (Olobo, 2001). Local cytokine response to *Mycobacterium tuberculosis* is dominated by IFN-gamma and IL-10. Cytokine leakage from the tissue into systemic circulation, so that IFN-gamma and IL-10 can be detected in the plasma (Barnes, 2005). Research in Argentina, Ethiopia and South Korea showed that the levels IL-10 in circulation is higher in tuberculosis patients compared to healthy individuals (Dlugovitzky, 1997; Olobo, 2001; Jang, 2006), whereas other studies using PBMC showed a similar result is IL-10 levels in the supernatant fluid was higher in patients with tuberculosis (Barnes, 2000).

Risk of tuberculosis in health care workers who are exposed to *Mycobacterium tuberculosis* is an important issue in most countries, the incidence of infection related to Human Immunodeficiency Virus (HIV), which increased (Menzies, 1995). A health worker has a risk of 50-10% throughout their lives to experience the development of active tuberculosis (Schluger, 1998). Health workers in endemic countries serving patients with tuberculosis in large quantities, while the patient isolation facilities are still rare. Health workers are vulnerable to tuberculosis infection and there is no clear method to monitor the investigation of tuberculosis infection in health workers (Pai, 2005).

On the basis of such consideration and given the research profile of IL-10 in patients with pulmonary tuberculosis and tuberculosis-risk health nurses have not been studied in Indonesia, it is necessary to study differences in plasma levels of IL-10 in both groups to know better the pathogenesis of IL-10 against tuberculosis and can used to monitor incidence of tuberculosis in high-risk health workers. This study aimed to prove the difference in IL-10 plasma levels in patients with pulmonary tuberculosis and healthy nurses at risk of pulmonary tuberculosis.

**MATERIALS AND METHODS**

This study uses an observational analytic study using a cross-sectional study on 20 patients with pulmonary tuberculosis in BP4/RS Reef Wall and 10 healthy nurses at risk of tuberculosis in Pulmonary Disease Inpatient Unit Dr. Soetomo who meet the selection criteria sample, starting in March-June 2007.

Sample acceptance criteria were patients with pulmonary tuberculosis pulmonary tuberculosis adults (aged over 17 years) in BP4/RS Surabaya Coral Wall, a new case with positive sputum AFB smear, positive sputum culture of *Mycobacterium tuberculosis*, pulmonary tuberculosis support the radiological examination and were willing to participate in research. Sample rejection criteria were patients with pulmonary tuberculosis...
tuberculosis pulmonary tuberculosis with HIV infection, diabetes mellitus who are not regulated and has been receiving corticosteroid therapy or anti-tuberculosis drugs.

Sample acceptance criteria nurses at risk of pulmonary tuberculosis are healthy with no clinical symptoms of tuberculosis nurses who had served more than two years in the Inpatient Unit, Dr Pulmonary Disease. Soetomo with negative sputum smear examination, culture of sputum negative, TB-dot test is negative, negative HIV serological test, lung abnormalities are not visible on radiological examination and are willing to fill out and sign a consent form to participate in the research statement. Sample rejection criteria of healthy nurses at risk of tuberculosis is the nurse who had diagnosed as pulmonary tuberculosis and/or are suffering from respiratory tract infections or other infections.

Venous blood is inserted into the tube without anticoagulant for examination of TB-DOT and HIV serology and inserted into a tube containing heparin for inspection coagulant IL-10 plasma. Heparin plasma immediately separated by centrifuged 3000 rpm for 5 minutes at the sampling location. Plasma was divided in aliquot and immediately inserted into stereofoam box containing dry ice. Plasma samples immediately taken to the Central Health Laboratory Surabaya to be stored at a temperature of -70°C.

The method used for examination of IL-10 is the enzyme linked immunosorbent assay (ELISA). IL-10 are contained in the plasma will be bound by human anti-IL-10 monoclonal antibodies that have been coated on solid phase (pitting). Shaft, then washed and added to biotinylated anti-human IL-10 antibody. Pitting again washed to remove biotinylated antibody which is not bound. Conjugate streptavidin-horseradish peroxidase (HRP) is then added. Pitting again washed and substrate solution added to 3.3', 5,5'-tetramethylbenzidine (TMB), produces a blue color which is proportional to the level of IL-10 in plasma. Stop Solution changing the color blue to yellow, and color intensity was measured at a wavelength of 450 nm.

Reagents used are RayBio ® Human IL-10 ELISA Kit (RayBiotech, Inc.) For quantitatively measuring levels of human IL-10 in the serum, plasma, cell culture supernatants and urine. These reagents are used only for research, not for diagnostic or therapeutic purposes. Investigation performed at the Department/Clinical Pathology Faculty of Medicine Airlangga University/Dr. Soetomo. Strengthening the quality of the examination of IL-10 with precision control is looking for imprecision of duplicate samples examined. Control accuracy is not done because there is no accuracy control materials and reagents IL-10 is only for research only. Two independent samples t test to compare the level of IL-10 in both groups. Cut-off value to differentiate the two groups were determined by Receiver Operator Characteristics Curve (ROC) and tested with Kappa association test and McNemar test. P-value used in this study amounted to 0.05.

RESULTS

During the period March 2007 until August 2007 there were 20 patients who pass the selection as a research subject of pulmonary tuberculosis. A total of nine people (45%) of these patients twen th microscopic examination of sputum showed 2 + and 11 people (55%) with the results of a microscopic examination of sputum 3 + (according to the criteria according to the results of smear readings IUATLD). All (100%) patients with pulmonary tuberculosis under study showed positive results of sputum culture of Mycobacterium tuberculosis. Ten healthy people at risk of tuberculosis nurses who worked more than two years in the Inpatient Unit, Pulmonary Disease. Descriptive and meet the criteria for sample acceptance and rejection does not meet the criteria of the sample is included as samples. Range of years of service in Pulmonary Disease Inpatient Unit for 2-23 years (mean = 12.2 years, standard deviation = SD = 7.8 years). General characteristics of study subjects are described in Table 1.

Table 1. General characteristics of research subjects and patients with pulmonary tuberculosis health nurses at risk of pulmonary tuberculosis.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Tuberculosis patients</th>
<th>Healthy nurses</th>
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<tbody>
<tr>
<td>Age</td>
<td>Mean 35.6 yrs</td>
<td>Mean 40.9 yrs</td>
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<tr>
<td></td>
<td>SD 10.4 yrs</td>
<td>SD 10.0 yrs</td>
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<td></td>
<td>Range 21-56 yrs</td>
<td>Range 24-53 yrs</td>
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<tr>
<td>Sex</td>
<td>Male 10 (50%)</td>
<td>Male 2 (20%)</td>
</tr>
<tr>
<td></td>
<td>Female 10 (50%)</td>
<td>Female 8 (80%)</td>
</tr>
</tbody>
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Notes: a) p = 0.190; b) p = 0.235

Plasma levels of IL-10 pulmonary tuberculosis patients ranged from 7.4 pg/ml to 26.3 pg/ml with a mean of 14.1 pg/ml and SD 5.3 pg/ml. IL-10 plasma levels in healthy nurses at risk of tuberculosis ranged from 1.8 pg/ml to 12.4 pg/ml with a mean of 6.7 pg/ml and SD 3.3 pg/ml. Results of t test analysis showed that two independent samples of IL-10 plasma levels between patients with pulmonary tuberculosis and tuberculosis-risk health nurses have a significant difference (p <0.001). Plasma levels of IL-10 pulmonary tuberculosis patients was significantly higher (14.1 ± 5.3 pg/ml) than...
in healthy nurses at risk of pulmonary tuberculosis (6.7 ± 3.3 pg/ml).

Cut-off values to distinguish IL-10 group of patients with pulmonary tuberculosis and tuberculosis-risk health nurse determined using Receiver Operator Characteristics Curve (ROC) and tested with Kappa association test and McNemar test. Based on the ROC curve (figure 12) got the cut-off value of IL-10 at 8.6 pg/ml (Kappa association test p <0.001 and McNemar test p = 1.0). Imprecision calculated from the inspections conducted as many as 10 samples in duplicate. Difference of absorbance at each sample is calculated to get the SD. SD in this study amounted to 0.025 (which can be seen in appendix 7).

DISCUSSION

Risk of tuberculosis in health care workers who are exposed to Mycobacterium tuberculosis is an important issue in most countries, related to the incidence of HIV infection is increasing. A health worker has a risk of 50-10% throughout his life to experience the development of active tuberculosis. Health workers in endemic countries serving patients with tuberculosis in a large number of isolation facilities while patients are still rare. Health workers are vulnerable to tuberculosis infection and there is no clear method of examination for tuberculosis infection to monitor the health officer.

On the basis of such consideration and given the research profile of IL-10 in patients with pulmonary tuberculosis and tuberculosis-risk health nurses have not been studied in Indonesia, it is necessary to study differences in plasma levels of IL-10 in both groups to know better the pathogenesis of IL-10 against tuberculosis as well as expected examination of plasma IL-10 may help to monitor the incidence of tuberculosis in high-risk health workers.

This study has been carried out examination of IL-10 plasma from 20 patients with pulmonary tuberculosis and 10 healthy nurses at risk of pulmonary tuberculosis. Both research groups based on age and gender showed that the two groups were homogeneous with the statistical test (p = 0.190 age, gender p = 0.235). The results showed a significant difference in plasma levels of IL-10 among patients with pulmonary tuberculosis and healthy nurses at risk of pulmonary tuberculosis (p <0.001). Plasma levels of IL-10 pulmonary tuberculosis patients is higher than the plasma levels of IL-10 healthy nurses at risk of pulmonary tuberculosis. This is in accordance with the results of research Handzel (2007) stating that the level of IL-10 in the systemic circulation in patients with pulmonary tuberculosis were significantly higher than the control group in contact with pulmonary tuberculosis and tuberculosis risk (Handzel, 2007).

Individuals at risk of pulmonary tuberculosis with a high intensity of exposure to Mycobacterium tuberculosis occurs then there will be resistance of host immune response against these bacteria. Primary immune response in individuals who immunocompetent cellular immune response which is protective against Mycobacterium tuberculosis. Mycobacterium tuberculosis, macrophages will capture and produce IL-12. Produced IL-12 stimulates Th1 cells to secrete IFN-gamma that occurs in the protective mechanism of macrophage microbicidal for the host (Nugraha, 2004, Handojo, 2004a).

Balance of host immune responses and Mycobacterium tuberculosis disrupted in the group of pulmonary tuberculosis. Immune response shifted to Th2 cells by Mycobacterium tuberculosis has the ability to stimulate macrophages in the infected lung tissue to produce IL-10. Levels of IL-10 can inhibit mechanisms that increase microbicidal macrophages because IL-10 suppress Th1 cell proliferation and differentiation resulting in decreased secretion of IFN-gamma (Nugraha, 2004, Handojo, 2004a). Strong Th2 response in addition to systemic leakage of IL-10 cytokines from infected tissue into the systemic circulation causing an increase in IL-10 levels in plasma in patients with tuberculosis of the lung (Barnes, 2005).

IL-10 mainly produced by macrophages and Th2 lymphocytes and has the function of inhibiting the synthesis of several cytokines that are stimulated by macrophages, NK cells and T lymphocytes (IL-1, IL-2, IL-6, IL-8, IL-12 and GM-CSF) and also stimulates B cell proliferation and differentiation into antibody-producing cells (Conti, 2003). IL-10 inhibit the synthesis of several cytokines that stimulated macrophages after binding to 110-kd cellular receptors. IL-10 inhibits expression of cell surface molecules of major histocompatibility complex (MHC) class II (Opal, 2000). After binding to IL-10 receptor, the mechanism of IL-10 signals through JAK1 and STAT3 will be activated. STAT3 activates the genes to produce products that inhibit the inflammatory signal (Murray, 2006).

Evaluation of cytokine responses in patients with tuberculosis actually best done from the place of infection, such as pleural fluid in tuberculosis pleuritis (Condos, 2004). Sampling is more difficult and invasive, so a lot of research done with the cytokine profile of PBMC method. PBMC method has many weaknesses that a longer inspection time for the
incubation process, the working procedure is more complicated than checking a systemic cytokines in plasma (Barnes, 2005), and cytokine responses to stimulation in vitro may not reflect the actual situation in vivo (Dlugovitzky, 1997). Immunity in patients with tuberculosis can be reflected by the levels of cytokines in the plasma, in addition to sampling the plasma more easily than the examination procedure and does not require special equipment compared with PBMC (Olobo, 2001).

Immunoassay against systemic cytokine IL-10 can be performed from serum or plasma samples. Serum samples has many shortcomings which time a very short part of cytokines, leukocytes are activated during the clotting of blood can produce cytokines and the presence of protease in serum that can interfere with the examination. Plasma is said to be better than serum, but the plasma must be immediately processed and stored frozen (Handojo, 2003).

In this study, heparin plasma samples are processed quickly to address the weaknesses of plasma samples for examination of cytokines. Heparin blood samples were taken at the scene then immediately centrifuged to obtain plasma samples, and immediately inserted into the plasma stereofoam box containing dry ice. Plasma sample was immediately taken to BBLK to be stored at a temperature of -70 degrees C.

Cut-off value of IL-10 in this study is determined to be able to differentiate groups of patients with pulmonary tuberculosis and healthy nurses at risk of pulmonary tuberculosis. Cut-off value is determined using a Receiver Operator Characteristics Curve (ROC) and tested with Kappa association test and McNemar test. Based on the obtained ROC curve cut-off value of IL-10 at 8.6 pg/ml. There are two nurses who were healthy at risk of pulmonary tuberculosis with IL-10 plasma levels above the cut-off value. The first nurse was a healthy 25-year-old woman with years of service for four years. Plasma levels of IL-10 first nurse is 10.0 pg/ml. The second nurse was a healthy 43-year-old woman with years of service for 20 years. Plasma levels of IL-10 second nurse was 12.4 pg/ml.

IL-10 plasma levels exceeding the cut-off value in both the nurse is probably caused by the development of latent tuberculosis in these nurses into active pulmonary tuberculosis. For the diagnosis of active pulmonary tuberculosis, may be considered the gold standard examination of others, such as culture or polymerase chain reaction (PCR) from samples of broncho-alveolar lavage (BAL). Results IL-10 plasma high on both nurses are supported by research Novi (2007) showing levels of IFN-gamma (release assay) is high on the second nurse. This is because the immune response of the two nurses have not been successfully control *Mycobacterium tuberculosis* infection that occurred (Novi, 2007). There is a tuberculosis patient with plasma levels of IL-10 7.4 pg/ml (below the cut-off value). These patients were men aged 23 years and microscopic examination of sputum smear showed 2 +. Incompatibility results obtained in both study groups can also be influenced by the individual's immune response. Immune response is influenced by the degree of severity and long-suffering of tuberculosis as well as nutritional status is not examined in this study.

**CONCLUSIONS**

Plasma levels of IL-10 pulmonary tuberculosis patients is higher than health nurses at risk of tuberculosis, so the examination of plasma IL-10 can be used to help monitor the incidence of pulmonary tuberculosis in high-risk health workers. Cut-off value of IL-10 can differentiate between groups of patients with pulmonary tuberculosis and tuberculosis-risk health nurses amounted to 8.6 pg/ml. In the group of healthy nurses with higher levels of IL-10 high and the possibility of infection of other non-tuberculosis has been excluded, then the examination of culture or polymerase chain reaction (PCR) from samples cho-alveolar lavage (BAL) should be considered for the diagnosis of pulmonary tuberculosis.

**REFERENCES**


