Adenosine Deaminase (ADA) Levels in Suspected Tuberculosis Patients with Results of Interferon-Gamma Results Release Assay (IGRA) Positive and Negative

by Adenosine Deaminase (ada) Levels In Suspected Tube With Results Of Interferon-gamma Results Release A

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ORIGINAL ARTICLE

Adenosine Deaminase (ADA) Levels in Suspected Tuberculosis Patients with Results of Interferon-Gamma Results Release Assay (IGRA) Positive and Negative

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ABSTRACT

Introduction: Tuberculosis is one of the major public health problems and remains a global health issues including Indonesia, which is caused by the bacterium Mycobacterium tuberculosis (MTb). Adenosine deaminase (ADA) is an enzyme in purin catabolism process which catalyze ademosine into inosine and deoksiadenosine into deoksiinosine and plays an important role in lymphoid cell differentiation. ADA is elevated in TB pleural. Aim of this study was to determine ADA levels in suspected TB patients. **Materials and Methods:** This study was a cross sectional analytic observational. The subjects were 60 samples, 30 samples with IGRA positive and 30 samples with IGRA negative. The participants's age range from 18 to 90 years old. The examination of IGRA by ELISA method, ADA by enzymatic colorimetry method. This study was conducted from January to April 2020 at Gleneagles Diagnostic Centre Laboratory in Surabaya. **Results:** There was a significant difference of ADA levels in IGRA positive and negative ($p< 0.05$), which means the ADA value of TB patients are higher than non TB patients. **Conclusion:** As there is still needed further research to determine the benefits of ADA relate to other examinations, to interprete ADA levels and IGRA in TB cases should be compared to clinical symptoms and radiology reports.

Keywords: Adenosine deaminase, Interferon gamma release assay, Tuberculosis

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INTRODUCTION

Tuberculosis (Tb) remains a major public health concern, according to the World Health Organization (WHO) about one-third of the world's population has been infected with tuberculosis, Indonesia ranks third highest prevalence of tuberculosis after India and China. These three countries account for nearly 50% of all Tb cases in the world. Based on the latest Global Tuberculosis Report, in 2018 the number of people infected with TB in the world was 9,011,100 million (1).

In Indonesia, the total prevalence rate of Tb incidence is 316 per 100,000 population or total about 845,000 cases (2). Deaths from Tb without HIV amounted to 35 per 100,000 population (1). This indicates that tuberculosis is a problem in Indonesia. East Java province ranks third highest after West Java and Central Java with a total of 56,445 cases (2). In Surabaya, the total prevalence of Tb is 242.83 per 100,000 population or about 7,007 cases (3).

Based on data at the Gleneagles Diagnostic Centre Surabaya, the report of Tb suspected patients from January to November 2019 was 592 registers. The number of suspected TB patients is a referral from some pulmonologists (4).

In the tuberculosis eradication program, conventional smear microscopy with the Ziehl-Neelsen (ZN) stain is a rapid and practical method for detecting acid-fast bacilli (AFB) (5). The gold standard examination is bacterial culture, but it takes time while Tb treatment is better

to given soon (5). There are currently several screening methods to help diagnose suspicions of tuberculosis infection. Various biomarkers are developed in detecting Tb suspect so that they can establish diagnosis more quickly, accurately and noninvasively (6). Biomarkers often used in the diagnosis of suspected TB patients today are Adenosine Deaminase (ADA) and Interferon Gamma (IFN-γ) Release Assay (6). ADA is an enzyme that catalyzes adenosine and deoxyadenosin into inosin and deoxy-inosin, playing an important role in the proliferation and differentiation of lymphocytes, especially T lymphocytes (7).

ADA levels will increase in various diseases because of stimulated cellular immunity, so ADA is an indicator of active cellular immunity (8). Conditions that trigger the immune system such as Mycobacterium tuberculosis infection can increase the amount of ADA production in the area of infection (8). ADA levels increase in tuberculosis due to stimulation of T lymphocytes by microbial antigens (9). In two different studies, ADA examinations showed high specificity of 91-96% and 92.1-98.6% for tb diagnosis (10).

In addition to ADA, the IGRA test is a specific and sensitive current test for tuberculosis screening, working by measuring the cellular or T cell immune response to TB infection (11). T cells in TB-infected individuals will be activated in response to antigen sensitization in the form of specific peptides Mycobacterium tuberculosis. The T cells will produce interferon gamma (IFN-γ) which is measured in the examination (11).

Clinical diagnosis of pleural TB is still a major challenge, a single diagnostic test such as ADA or IFN-γ measurements often show low sensitivity. However, the performance of the IFN-γ test in pleural fluid and peripheral blood combined with ADA measurements has not been well studied (10,11). The combination of ADA and IFN-γ in the pleural fluid showed greater performance compared to the combination of ADA measurements in the pleural fluid and IFN-γ in the serum (12). More research is needed to improve diagnostic usability using this method (12). Based on the description above, researchers are interested in conducting research on differences of ADA levels in suspected TB patients with positive and negative IGRA results.

MATERIALS AND METHODS

Samples

This study is an analytical observational study with a cross sectional approach to 60 samples of suspected TB patients consisting of 30 samples with positive IGRA results and 30 samples with negative IGRA during January 2020 to April 2020. In this study, independent variables are suspected TB patients with positive and negative IGRA results, while dependent variable is ADA examinations. The population is suspected TB patients based on referrals from pulmonologist. The study sample was part of a population that met the inclusion criteria of patients ≥ 18 years old. Sampling is done by purposive sampling. All human procedures were approved by the Ethics Committee for Human Experimentation of the Trisensa Diagnostic Centre with number: 018/ I/ RM/ 2019.

Analysis of IGRA level

The IGRA examination uses the ELISA method while the ADA examination uses the enzymatic method of colorimetry.

Statistical analysis

Followed by an independent t-test sample. The effectiveness of the statistic test is determined if the significant value < 0.05.

RESULTS

The subjects in this study were ADA levels in tuberculosis suspected patients with Positive and Negative IGRA results obtained from pulmonologist in the Clinical Laboratory Gleneagles Diagnostic Centre Surabaya in January-April 2020. The total number of samples was 60 with details of 30 samples group for the positive IGRA and 30 samples of the Negative IGRA group. The character of the research subjects of the two groups can be seen in Table I.

The results of the ADA level examination in the positive IGRA sample obtained an abnormal result of 36.7% (11 samples), while the ADA level in the negative IGRA sample obtained an abnormal result of 13.3% (4 samples). There was a 63.6% difference in the number of abnormalities (7 samples) between positive and negative IGRA samples, indicating that the abnormal percentage of ADA levels was higher in the positive IGRA sample (Table II).

Table III shows the results of the distribution of samples based on age in positive IGRA samples with abnormal ADA levels of 36.7% namely patients aged 18-40 years as much as 54.5%, ages 41-60 years as much as 27.3% and ages 61-80 years as much as 18.1%. This shows that patients with the most abnormal ADA levels in the productive age range are between 18-40 years. Based on the sex in a positive IGRA sample with abnormal ADA levels consisting of 45.4% of men and 54.5% of women, this indicated that there was no significant percentage difference between men and women.

The results of kolmogorov Smirnov's normality test analysis showed that all normal distribution data then continued the independent t-test test obtained = 0.020 ($p < 0.05$), which means that there is a difference in ADA levels in tuberculosis suspect patients with Positive and Negative IGRA results.

Table I: Basic characteristics of research subjects

Characteristic	Group		Total
	IGRA Positive	IGRA Negative	
1. Number of Sample	30	30	60
2. Age Group (Years) :			
- 18 - 40	15 (50%)	7 (23.3%)	Age range of Subjects : 18-90 years
- 41 - 60	7 (23.3%)	9 (30%)	
- 61 - 80	8 (26.7%)	12 (40%)	
- 81 - 100	0 (0%)	2 (6.7%)	
3. Gender :			
- Man	15 (50%)	11 (50%)	
- Woman	15 (50%)	19 (50%)	

Table II: Comparison of abnormal ADA levels in IGRA positive and negative

	IGRA Positive	IGRA Negative
Percentage of Abnormal ADA Levels (≥ 15 IU/L)	36.7% (11 Samples)	13.3% (4 Samples)

Table III: Abnormal ADA levels in IGRA positive

Characteristic	IGRA Positive		Total
	Total sample of Abnormal ADA	Percentage	
Based on Age Group (Year):			
18-40	6	54.5%	Samples
41-60	3	27.3%	
61-80	2	18.1%	
Gender			
Man	5	45.4%	
Woman	6	54.5%	

DISCUSSION

This study showed that levels ADA and IGRA in samples has good combination, the test together significanly improve diagnostic performance on Tuberculosis infection. Based on our experience, the combining test is a practical test that could be applied clinically. Despite the progress in the diagnosis and treatment of TB, TB is still a major health problem worldwide, particularly in developing countries (12).

The IGRA test is one of the examinations of the diagnosis of tuberculosis infection in the blood serology by detecting an immune response to specific antigens M. tuberculosis (MTb) namely ESAT-6 (Early Secreted Antigenic Target 6) and CFP-10 (Culture Filtrate Protein 10) (11). The mechanism is as follows, after being inhaled into the pulmonary alveoli, MTb will soon be phagocytized by alveolar macrophages. Naturally the manifestations arising from the interaction between MTb and macrophages are divided into 2 stages, non-specific and specific immune responses. In the early stages before the activation of T lymphocytes, the immune response formed is nonspecific, where the interaction between germs and macrophages leads to several possibilities. MTb may be destroyed by alveoli macrophages or, MTb destroys macrophages or even multiplies them. The interaction of MTb germs in these macrophages produces ESAT-6 and CFP-10 specific antigens. MTb germs can also enter blood vessels or lymph vessels and spread throughout the body (13).

In the next stage there will be activation of T lymphocytes to form a specific immune response. MTb germs that have been phagocytized by macrophages can be destroyed in several ways. Germ antigens can be presented through MHC type I to CD8+ cells that are cytotoxic so that they can lysis macrophages containing germs. Germ antigens that have been processed can also be presented via MHC type II to CD4+ T cells. TCD4+ cells themselves consist of two subpopulations, namely Th1 and Th2 cells, each of which can produce cytokines that play a role in the regulation of the immune system. Th1 cells produce IL2 and IFN-ɣ which activate macrophages to lysis germs that have been phagocytes (13).

IFN-ɣ is the main cytokine involved in the immune response to MTb, plays a role in macrophage activation and has a very important function in Cell Mediated Immunity against intracellular microbes. In inactivated T cells, IFN-ɣ is not expressed so that the protein cannot be detected. IGRA testing uses 2 specific antigens, ESAT-6 and CFP-10, which are only obtained in MTb. The principle of IGRA examination is that lymphocytes in blood samples will be exposed to ESAT-6 and CFP-10 antigens in QFT tubes during incubation 16-24 hours, lymphocytes in individuals infected with MTb germs are able to hit those antigens and will secrete IFN-ɣ and IL-10. IFN-ɣ formed is checked by elisa sandwich method to provide positive IGRA results (14).

ADA is an enzyme involved in the process of purine catabolism that catalyzes the change of adenosine to inosin and deoxyadenosin to deoxyinosin and plays an important role in lymphoid cell differentiation. Its activity is high in conditions that stimulate cellular immunity. ADA is mainly produced by avtivated macrophages (7). There are two ADA isomers, ADA1 and ADA2. ADA1 is found in all cells. In people with TB pleurisy more dominant ADA2. Conditions that trigger the immune system such as MTb infection can increase ADA levels in the body. This is in accordance with the researchers' expectations that positive IGRA results followed by increased ADA levels are indicating of MTb infection.

The age range of the study subjects was 18-90 years, because TB affects almost all age groups. In positive IGRA with abnormal ADA levels, the highest percentage in the age range of 18-40 years was 54.5% and the lowest in the age range of 61-80 years was 18.1%. These results are similar to several other studies, including a study by Tay & Tee (15) in Singapore that examined retrospectively from 2007-2011 on 160 samples of TB pleural fluid estimated the average age of patients 44 years as well as research by Amalia & Pradjoko (7) obtained an average age at 39 years. This is in accordance with research Werdhani, RA (16) which states that 75% of TB sufferers are found at the most productive age, this is likely due to high mobility so that the risk of exposure to MTb bacteria is also higher. In the younger age group, most are caused by primary infection while in old age other than by primary infection can also occur due to tb reactivation (5, 7, 15).

In negative IGRA with abnormal ADA results were obtained in the age range of 65-82 years as much as 13.3%. Although proven not to be infected with MTb, indicated by negative IGRA results but clinicians suspect tb suspect based on the symptoms of patients. This can happen because in that age range the ability of immune and physiological were decreases. While the increase in ADA levels can be caused by other bacterial infections. It is appropriate that ADA levels may increase when there is stimulation of cellular immunity.

The study data showed that the ratio of men and women was almost the same or there was no significant difference, men by 45.4% and women by 54.5%. This is not in accordance with the statistical data sourced from the Surabaya Health Office (3), the Indonesian Ministry of Health (2) and WHO (17) which states that the number of TB sufferers in men is more than women. The cause of the difference in this study may be due to differences in the sample population. This causes the number of incidents cannot reflect in accordance with existing statistical data (2, 3, 17).

Another study with almost the same research results, obtained by Dogra et al (18), in India reported a balanced proportion of men and women with a ratio of 1: 1 and research conducted by Amalia & Pradjoko (7) also got comparable results between men and women. There is no meaningful difference between men and women because MTB germs can affect anyone (6, 7, 18).

The sample group with positive IGRA obtained the results of normal ADA levels examination as much as 63.3% and abnormal as much as 36.7%. While in the sample group with negative IGRA obtained the results of examination of normal ADA levels as much as 86.7% and abnormal as much as 13.3%. There was a 63.6% difference in the number of abnormality between positive and negative IGRA samples. This is in accordance with various studies that show a significant difference in ADA values between groups of TB and non-TB where the value of TB patients is higher (10).

Finding rapid, accurate, and affordable MTb diagnostic tests ia an essential part of Tb control. Special challenges was emerging in immunosuppressed patients, such as Tb with HIV, extrapulmonary Tb and infection Tb in lower concentration of bacteria has challenging in diagnostic. The combination tests of ADA and IGRA might be valuable in these high risk population. Future studies are needed to validate our combination test in more spesific case. In summary, combining Adenosine Deaminase and Interferon-Gamma Release Assay (IGRA) performs better than individual test in diagnosis of Tb. The combination diagnosis has very high diagnostic rate, can be eaasily and safely carried out.

CONCLUSION

The study found a significant difference between Adenosine Deaminase (ADA) levels in tuberculosis suspect patients between positive and negative results of Interferon Gamma Release Assay (IGRA).

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GRADEMARK REPORT

FINAL GRADE

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GENERAL COMMENTS

Instructor

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