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Diagnostic Value of Encode TB IgG and IgM Rapid Test to Support Pulmonary Tuberculosis Diagnosis

Notrisia Rachmayanti[1], Aryati[1], Tutik Kusmiati[2]

[1] Department of Clinical Pathology,Faculty of Medicine, Airlangga University/Dr.Soetomo Hospital, Surabaya, Indonesia. E-mail: dr_aryati@yahoo.com
[2] Department of Pulmonology and Respiration Medicine, Faculty of Medicine, Airlangga University/Dr. Soetomo Hospital, Surabaya, Indonesia

ABSTRACT

Diagnosis of tuberculosis can be established through the detection of antigens by Acid Fast Bacilli (AFB), microscopy, culture, and Polymerase Chain Reaction (PCR). The World Health Organization (WHO) 2012 issued a recommendation not to use antibody detection in the diagnosis of tuberculosis. However, there is high demand from clinicians to detect anti-tuberculosis antibody in patients who are challenging to do a bacteriological examination. The purpose of this research was to determine the diagnostic value of anti-*M.tuberculosis* IgG and IgM Encode TB to support lung tuberculosis diagnosis. This study was a cross-sectional by using consecutively sampling, which was performed in the Dr. Soetomo Hospital, Surabaya, Indonesia, from November 2017 until May 2018. A total of 52 patients were included and evaluated for clinical or bacteriological examination using AFB microscopy or PCR (Gene Xpert) as the gold standard and tested the anti-*M.tuberculosis* IgG and IgM with immunochromatography. Encode Tuberculosis (TB) IgG was positive in 12 patients from the tuberculosis group and one false-positive in the non-tuberculosis group. The diagnostic sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of Encode TB IgG dan IgM were 35%, 94%, 92%, 43% and 55.7%, respectively. The specificity was high that the positive result was considered as TB; the sensitivity was low that the negative results were not excluded from TB. Encode TB IgG/IgM rapid test was not recommended to use as a single diagnostic test and must be combined with other diagnostic tests to increase the sensitivity.

Keywords: Tuberculosis, immunochromatography, Encode TBIgG, IgM

INTRODUCTION

Tuberculosis is a significant health problem in the world that affects around ten million people each year and is one of the top ten leading causes of death worldwide. According to the WHO report in 2015, tuberculosis cases in Indonesia were estimated to be around one million cases per year, with 100,000 deaths each year. Indonesia succeeded in reducing tuberculosis mortality and morbidity, from more than 900 per 100,000 population in 1990 to 647 per 100,000 people in 2015. The current indicator of Millennium Development Goals (MDGs) for tuberculosis in Indonesia has only succeeded in a target to reduce the incidence rate. For this reason, more considerable and more integrated efforts are needed for Indonesia to achieve the goal of Sustainable Development Goals (SDGs) with 90% reduction in deaths caused by tuberculosis and an estimation of 80% reduction in tuberculosis incidence by 2030 compared to 2015.[1,2]

Natural infections stimulate antibodies production against *Mycobacterium tuberculosis*. Lyaschenko *et al.*, in 1998 in New York, performed a study using ten panels of *M.tuberculosis* protein. This study showed that the increase in serum immunoglobulin titers against *M.tuberculosis* antigens during the clinical presentation was found in 90% of patients. Antibodies formed as humoral immune responses will bind to extracellular *M.tuberculosis* and cause an opsonization process, which then leads tomacrophage activation. Activated macrophages via Fcγ receptors will facilitate the process of phagocytosis. The phagocytosis process is continued by phagolysosome fusion to kill *M.tuberculosis* and present its peptides through class II MHC. Antigen presentation through class II MHC will activate Th1 and result in increased secretion of IL-2, TNFα, and increased formation of tuberculosis granuloma. In conditions of active tuberculosis, macrophages are unable to play a role in eliminating tuberculosis, resulting in the stimulation of Th2 cells. Th2 stimulation causes the release of IL-4. High levels of

IL-4 in sputum play a role in increasing high antibodies in the serum of patients with active pulmonary tuberculosis.[3]

Diagnosis of tuberculosis can be established through the detection of antigens and antibodies. Antigen was detected by performing AFB, culture, and PCR. Acid-fast bacilli test uses Ziehl Nielsen staining of the patients sputum. This method is easy, fast, and inexpensive despite low sensitivity (<50%) and requires around 104 AFB/mL to obtain a positive result. Culture as the gold standard for the diagnosis of tuberculosis is more effective than microscopic examination because it has a sensitivity of around 80-85% and requires fewer bacilli around 10 AFB/mL. However, culture needs a longer time to be performed. Polymerationchain reactionis performed with expensive molecular rapid testing which is currently recommended by WHO.[4,5]

Detection of anti-*M.tuberculosis* antibodies are currently being developed more for diagnosis than for therapeutic follow-up.[3] The WHO in 2012 issued a recommendation not to use antibody detection as a diagnosis of tuberculosis because of its varying sensitivity and specificity. However, thereare high demands from clinicians to detect anti-tuberculosis antibody in patients with difficulty in doing the bacteriological examination. Therefore, anti *M.tuberculosis* antibody testing is used in the laboratory.

At present, a rapid diagnostic instruments with the basis of serological tests has been developed to detect anti-tuberculous antibodies using the Enzyme-Linked Immunosorbent Assay (ELISA) and Immunochromatography (ICT) methods. Immunochromatography methods are available as cassette formats to detect total Immunoglobulin (Ig), a combination of IgG and IgM, and combination of IgG, IgM, and IgA.

This study aimed to determine the diagnostic value of anti-tuberculosis IgG and IgM using the rapid TB Encode test with the ICT method to support the diagnosis of *pulmonary tuberculosis.*

METHODS

This research was a cross-sectional study performed at Dr. Soetomo Hospital Surabaya, Indonesia, as a referral hospital. Subjects who met the inclusion criteria were consecutively involvedd. The subjects were outpatient patients of the Pulmonary Department and hospitalized in Pulmonary Inpatient Rooms from November 2017 to May 2018.

The study was performed on 52 patients who were evaluated based on clinical and/or bacteriological findings of AFB microscopy or PCR and detected the antibody examination Encode TB IgG and IgM using a rapid test. Patients were divided into 34 TB patients and 18 non-TB patients. Patients in the tuberculosis group aged over 18 years who were suspected of having pulmonary tuberculosis infection and were positive in the rapid molecular test using PCR and AFB microscopic examination and were willing to be the subject of research by signing informed consent. Patients in the non-TB group were patients with non-TB pulmonary disease based on clinical examination and radiological findings and were negative in the bacteriological AFB microscopy or PCR rapid molecular test. Icteric, lipemic, hemolysis sample, and patients who have received tuberculosis treatment were excluded from the study.

This research has received approval from the Health Research Ethics Committee of Dr. Soetomo Hospital Surabaya with number 643/Panke.KKE/XI/2017. Approximately 3 mL of blood was taken from each subject. Serum was obtained by centrifugation at 3,000 g for 15 minutes. Serum was placed in a liquots and stored in the refrigerator at -80ºC until analysis of IgG and IgM was performed.

Anti-tuberculosis IgG and IgM were detected using the Encode TB IgG and IgM rapid test from China with lot number 20170433. The method used was lateral flow chromatographic immunoassay with the principle of a double antigen sandwich. The was carried out according to standard procedures. The results were obtained as positive qualitative data indicated by the formation of burgundy color in the test pad due to the presence of antibodies to the patient serum against *M.tuberculosis*.[6]

Data on patient characteristics, including age, and sex, were statistically analyzed using SPSS ver. 17.0 and Minitab 16.p <0.05 indicated a significant difference. The diagnostic sensitivity, diagnostic specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio, and diagnostic accuracy were analyzed.

RESULT AND DISCUSSION

The study subjects were 52 people divided intoTB group,which consisted of 34 people consisting of 20 males and 14 females. The number of non-TB patients were 18 people with 14 males and 4 females.

Table 1. Characteristics of patients on gender and age

Characteristics	TB Patients (n=34)	Non-TB Patients (n=18)	P- value
Male, n (%)	20 (59%)	14 (77.7%)	0.143
Female, n (%)	14 (41%)	4 (22.3%)	
Mean of age	46.5 ±31.4	58.2 ±21.2	0.07
Range of age	22-83 years	45-87 years	

Table 2. Result of Encode TB IgG and IgM on TB and non-TB groups

Test	TB Group (n=34)	Non-TB Group (n=18)
Positive Encode TB IgG	12 (35%)	1 (5.6%)
Negative Encode TB IgG	22 (65%)	17 (94.4%)
Positive Encode TB IgM	0 (0%)	0 (0%)
Negative Encode TB IgM	34 (100%)	18 (100%)
Positive Encode TB IgG and IgM	12 (35%)	1 (5.6%)
Negative Encode TB IgG and IgM	22(65%)	17 (94.4%)

Table 3. Diagnostic value of Encode TB IgG and IgM

Diagnostic Test	Sensitivity (%)	Specificity (%)	PPV %	NPV %	PLR	NLR
Encode TB IgG	35%	94%	92%	43%	5.8	1.4
Encode TB IgM	0%	100%	0%	100%	-	-
Encode TB IgG and IgM	35%	94%	92%	43%	5.8	1.4

PPV: Positive Predictive Value
NPV: Negative Predictive Value
PLR: Positive Likelihood Ratio
NLR: Negative Likelihood Ratio

IgG and IgM anti-*M.tuberculosis* Encodes TB rapid test was performed on all patients. Three different people read the results at the same time, and the results were not significantly different. This finding showed that the Encode TB test was quite objective.

The positive results of anti-*M.tuberculosis* IgG was found in the serum of 12 people in the TB group, and one false-positive result was found in the serum of one person in the non-TB group. The false-positive result was obtained in patients with tongue cancer with pulmonary metastases with negative AFB and PCR results. Negative results of the IgM test were obtained in all subjects in the TB and non-TB groups. Result of the Encode TB IgG and IgM rapid test are shown in Table 2, and the diagnostic value of Encode TB IgG and IgM rapid test were shown in Table 3.

The humoral immune response plays a role in active tuberculosis infection, which can be measured by the detection of anti-*M.tuberculosis* antibodies. Welch *et al.* in 2008-2010 in Utah United States examined immunoglobulin G, which reacted to the *M.tuberculosis* antigen Mtb81, Mtb8, Mtb48, DPEP (MPT32) and 38kDa using the In Bios IgG TB assay. Previous studies showed that Mtb81 and MPT32 are specific markers for active tuberculosis; however, the sensitivity decreased when used together. The 38 kDa protein was found in the immunodominant *M.tuberculosis* culture filtrate with high specificity, but it showed a low sensitivity if it was used singly. In Bios combined several antigens to maximize sensitivity and specificity.[7]

Lyaschenko *et al.* in 1998 in New York used 10 *M.tuberculosis* protein filtrates, such aESAT-6, 14kDa, MPT63, 19kDa, MPT64, MPT51, MTC28, Ag58B, 38kDa, and KatG antigens. Increased serum anti-tuberculosis immunoglobulin levels were found in 90% of patients; however, none of the antigens was universally recognized by all patients with active tuberculosis.[3] Low sensitivity in anti-*M.tuberculosis* Encode TB IgG test was likely due to limited antigen used in the Encode rapid test which used CFP10 and ESAT 6.

Velayudhana *et al.* investigated the serum binding of active TB patients to TB proteins using

1,200 copies of the *M.tuberculosis* proteins. The results showed that the recognition of *M.tuberculosis* protein by individuals with active tuberculosis was only limited to 0.5%. It was following the study by Deng *et al*. using more protein expression, which showed the same results. The range of antigens that could be recognized was very narrow when compared with *M.tuberculosis* proteins obtained were the same. This fact may be related to a decrease in antibody affinity of patients with active tuberculosis to directly bind to live bacteria.[3] The anti-tuberculosis IgG sensitivity of Encode TB test was low (35%), probably related to a decrease in the affinity of antibodies to the *M.tuberculosis* antigen.

Encode TB is a laboratory test with the ICT method and qualitative results. When the levels of antibodies are below the detection limit, the device will give a negative outcome.[6] False-negative results found in this study were possibly due to the low level of patient antibodies below the detection limit and also probably due to inadequate packaging techniques.

Tuberculosis infection in humans stimulates the formation of IgG and IgA against *M.tuberculosis* antigens in Bronchioalveolar Lavage (LAB). IgG is mostly found in the lower respiratory tract, in contrast IgA is mostly found in the upper respiratory tract.[4] When infection occurs, IgG and IgA tuberculosis can enter the circulation enabling the detection in the serum. IgM is an antibody in the acute phase, while tuberculosis infection is a chronic infection. This finding might be the reason that IgM titers did not increase and negative results were found in this study.

False-positive results were obtained in one patient in the non-TB group. The patient was diagnosed with tongue cancer with pulmonary metastases. The result of false-positive in the Encode TB test can occur due to an infection other than *M.tuberculosis*,which has a similar epitope, such as *Mycobacterium* other than tuberculosis (MOTT). *Mycobacterium* other than tuberculosis such as *Mycobacterium africanum* or *Mycobacterium bovis* can befound in the oral cavity with poor hygiene.

CONCLUSION AND SUGGESTION

The Encode TB test is a fast examination to detect antibodies to the serum, which is easy to apply and does not require equipment or special abilities of the technicians. In this study, the specificity was high that the positive results were still considered as TB, but the sensitivity was low that the negative result could not exclude TB. The sensitivity of anti-tuberculosis IgG and IgM was low that the TB IgG/IgM rapid test was not recommended to be used as a single diagnostic test for the diagnosis of pulmonary tuberculosis but should be combined with clinical and advanced laboratory examination.

Based on the results of this study, it was recommended to increase the sensitivity of the Encode TB test by selecting more specific antigens with the proper concentration. It was also necessary to develop further research to combine the Encode TB test with other diagnostic tests to improve diagnostic sensitivity.

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