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VALİDİTY OF EXAMİNATİON OF COMPLEX SPECİFİC ANTİGEN MYCOBACTERİUM TUBERCULOSİS RAPİD IMMUNOCHROMATOGRAPHY METHOD İN PATİENTS WİTH PULMONARY TUBERCULOSİS

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ABSTRACT

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosi*s which can be transmitted from one person to another by inhaling droplets coughed or sneezed by an infected person. One of the most recent technologies today is the rapid immunochromatography (ICT) method for testing TB antigens. This test detects antigens secreted by Mycobacterium tuberculosis, namely the early secretory antigenic target 6 kDa protein (ESAT6), culture filtrate protein (CFP 10), and Mycobacterium tuberculosis protein (MPT64) encoded by region of difference genes (RDI, RD2, and RD3). The purpose of this study was to determine the validity of TB ICT antigens in diagnosing pulmonary tuberculosis. The research method used is a diagnostic test with a cross-sectional study design. The population of this study was all pulmonary TB patients who were examined at the Prof. Hospital Laboratory. W.Z. John. The samples used in this study amounted to 81 samples which were preceded by smear examination and then 47 samples of M. tuberculosis culture on Lowenstein Jensen media which was used as the gold standard, the results of the examination were 41 samples growing and 6 samples not growing. The sensitivity and specificity of the Rapid ICT TB antigen test were 75.61% and 100%, respectively. The conclusion of the rapid ICT TB antigen test has high validity, so it can be used as an alternative laboratory test for the diagnosis of pulmonary TB.

Keywords: *Pulmonary Tb, Complex Specific Antigen, Microscopic TB*

INTRODUCTION

Tuberculosis (TB) is currently still a public health problem both in Indonesia and internationally so it becomes one of the goals of sustainable health development (SDGs) (Kementerian Kesehatan RI, 2021). Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis which can be transmitted from one person to another by inhaling small droplets (droplets) from coughs or sneezes from an infected person (Sharma & Sarkar, 2018). TB can attack other parts of the body such as the glands, bones, and nervous system, especially the lungs (Raja et al., 2008). The main symptoms are a cough for two weeks or more, a cough accompanied by additional symptoms, namely sputum mixed with blood, shortness of breath, body feeling weak, decreased appetite, decreased body weight, malaise, sweating at night without physical activity, and fever for more than one month (Kemenkes, 2020).

Globally, it is estimated that 10 million people will suffer from TB in 2019. Despite the decrease in new TB cases, it is not fast enough to achieve the target of the 2020 END TB Strategy, which is to reduce TB cases by 20% between 2015 – 2020. From 2015 – To 2019, the cumulative decrease in TB cases was only 9% (World Health Organization 2020, 2020).

Research conducted by the Indonesian Ministry of Health found the number of TB cases of all types in East Nusa Tenggara Province totaling 5,350 cases with details of cases in men as many as 3053 (57.07%) cases and women as many as 2297 (42.93%). In each province throughout Indonesia, more cases occur in men than women. Three Regencies/Cities in East

Nusa Tenggara Province with the highest number of sufferers in the last <1 year, namely West Sumba (1.2%), East Sumba (0.7%) and Central Sumba (0.7%) and within >1 year the last were Nagekeo (2.3%), Central Sumba (2.0%) and Kupang Regency (1.9%) while the Districts/Cities with the highest treatment were West Sumba (62.9%), East Sumba (52.7 %) and North Central Timor (50.5%) (Kemenkes, 2020).

Laboratory tests for diagnosing pulmonary TB currently have limitations, for example, the microscopic sensitivity of AFB is low, the culture method takes a very long time, or molecular methods require special expertise (Siddiqi et al., 2003). Serological tests to detect M. tuberculosis antibodies using the rapid immunochromatography (ICT) method have been widely used (Bekmurzayeva et al., 2013). In 2011, WHO issued a policy statement recommending not using commercial antibody detection reagents because of their low sensitivity, which varies between 1% to 60%, and specificity of 53–99%. Diagnostic methods that are easy, fast and accurate are needed to improve diagnosis and increase the efficiency of TB control in overcoming these limitations (Gustiani et al., 2014).

Currently, an ICT examination method has been developed that is fast, easy, and practical and does not require special expertise to detect TB antigens (Drancourt et al., 2016). The material for detecting TB antigens on this tool uses a combination of three monoclonal antibodies against specific antigens ESAT6, CFP10, and also MPT64 which are secreted by M. tuberculosis during their active period. The rapid ICT TB antigen test uses a sputum specimen of a suspected pulmonary TB patient (Lawn et al., 2012). The purpose of this study was to determine the validity of examining complex specific antigen RD1-RD3 (ESAT6, CFP10, and MPT64) in the sputum of pulmonary TB patients using the rapid ICT method as a diagnostic marker for pulmonary TB (Gustiani et al., 2014).

METHOD

This study used a diagnostic test with a cross-sectional design (Knottnerus & Muris, 2003). The research was conducted after taking care of Research Ethics with No. LB.02.03/1/0029/2022 from the Kupang Ministry of Health Polytechnic Ethics Commission. Sputum collection for all patients who come to do pulmonary TB examination in the laboratory of Prof. Hospital. W.Z Johannes Kupang. The samples examined were 81 samples. The sample was then subjected to the microscopic examination of AFB with ziehl Nielsen staining which is also known as an acid-fast bacterial stain (Derese et al., 2012). The AFB-positive samples were then planted in the Lowenstein Jensen Medium Base medium. Furthermore, the media that had been planted was incubated for 30 days at 370C in the incubator. Bacterial colonies growing on seed media were taken in 1-3 eye loops mixed homogeneously with 200 micron SD TB Ag MPT64 Rapid extraction buffer solution. Pipette 100 microns of the homogeneous culture and then drop it into the sample well of the Rapid test SD TB Ag MPT64 Rapid. Then read the results in the 15th minute whether positive or negative. Positive if 2 lines appear on the Control (C) and Test (T) lines. Negative if only one line appears in area C. The data obtained were analyzed using descriptive statistics which included patient characteristics such as age, sex, and history of TB treatment. The sensitivity, specificity, positive predictive value (NPP), negative predictive value (NPN), and accuracy of the ICT method test were calculated compared to the AFB test.

RESULTS AND DISCUSSION

The study was conducted on pulmonary TB patients who carried out examinations in the laboratory during the study period as many as 81 samples were recorded with the results of the examination presented in table 1:

Table 1 Characteristics of patients with suspected pulmonary TB

Variable	Frekuensi	%
Gender		
Male	50	61,73
Female	31	38,27
	81	**100,00**
Ages		
12-21 Teenager	7	8,64
22-35 Young Adult	30	37,04
36-45 Adult	9	11,11
46-55 Middle Aged	18	22,22
56-65 Early Ages	9	11,11
>65 Elderly	8	9,88
	81	**100,00**
History of Therapy		
New	70	86,42
Follow Up	11	13,58
	81	**100,00**
Microscopist Test		
Positive	47	58,02
Negative	34	41,98
	81	**100,00**

Microscopic examination of pulmonary tuberculosis carried out during the study period can be seen in table 2:

Table 2. Results of Microscopic Examination of TB

Variabel	F	Sputum BTA	
Gender		BTA (+)	BTA (-)
Male	50	29 (35,80%)	21 (25,93%)
Female	31	18 (22,22%)	13 (16,05%)
	81	47 (58,02%)	34 (41,98%)
Umur			
12-21 Teenager	7	5	2
22-35 Young Adult	30	19	11
36-45 Adult	9	6	3
46-55 Middle Aged	18	9	9
56-65 Early Ages	9	2	7

>65	**Elderly**	8	3	5
		81	47 (58,02%)	34(41,98%)

The World Health Organization stated that according to several studies, the incidence of pulmonary TB in women is lower than in men because fewer women with pulmonary TB seek treatment at healthcare facilities. Another study conducted by Kristina (2020) concerning the potential for transmission of pulmonary TB to families of sufferers found that positive TB cases in males were greater than in females, namely 52.9% (Kristini & Hamidah, 2020).

Examination of Complex Specific Antigen

The test material for detecting Complex Specific Antigens, in this case, the MPT64 protein, is all positive sputum samples in the AFB test. Rotate the loop five turns clockwise then pull the loop from the cryo tubes. A total of 100 µl was taken using a pipette and then dripped into the MPT64 cassette (in area S). The interpretation of the results was carried out after 15 minutes. Positive results (+) will form a red line on the test and control. Negative results (-) will form a red line only on the control.

Table 3. Inoculation Examination of Lowenstein Culture and TB Ag MPT64.

	Sum	
The Tes	N	%
Lowenstein Culture		
Growing	41	87,23
Ungrowing	6	12,77
Total	47	100%
Rapid TB Ag MPT64		
Positive	31	65,96
Negative	16	34,04
Total	47	100%

Table 4. Examination Validity

Type of Validation	**Value (%)**
Sensitivities	75,61
Spesifisitas	100
Positive Predictive Value	100
Negative Predictive Value	78,72

Testing the validity of testing TB rapid ICT antigen on M. tuberculosis culture media on Lowenstein Jensen media obtained a sensitivity value of 75.61%, a specificity value of 100%, a positive predictive value of 100%, and a negative predictive value of 78.72%. The TB antigen test using the rapid immunochromatography method for isolating examination material has validity in the form of moderate sensitivity and high specificity to the gold standard of the M. tuberculosis culture method on Lowenstein Jensen's media. Antigen detection using the TB Ag

rapid test can be used as an alternative test for the rapid diagnosis of pulmonary TB, in addition to other tests (bacterial smear culture and microscopy).

MPT64 is a protein fluid used for rapid identification tests conducted by researchers in Africa with high sensitivity and specificity results. The sensitivity and specificity of the results of the current study are different from studies conducted by other studies, Sari research (2011) stated that the sensitivity value of the TB Ag rapid test conducted in Surabaya was 72.6% and a specificity of 90.9%. That research concluded that the TB Ag rapid test could be considered as a new diagnostic tool for the diagnosis of pulmonary tuberculosis, especially in healthcare settings that do not have laboratory technicians who are experienced in examining the staining results of acid-resistant bacilli (Sari & Aryati, 2016). According to research conducted by Shen et al (2011), it was stated that to identify ESAT6/CFP10 by rapid ICT, a minimum concentration of M. tuberculosis in culture isolates is required of 3x104 to 3x105 CFU/ml. While the culture method is a method for growing and multiplying bacteria, then with 500 AFB it can produce positive cultures. False negatives can also be caused by damage to the epitope and recombinant antibody which becomes the epitope for capturing TB antigens on the device, resulting in a negative test. This false negative value will affect the sensitivity value.

CONCLUSION

The results of the research conducted on Pulmonary Tuberculosis patients, it can be concluded that: The total sample of suspected pulmonary TB patients was 81 samples, then AFB examination obtained positive results in as many as 47 samples and negative as many as 34 samples. The sensitivity value of the Mtb identification test using rapid Ag TB MPT64 was 75.61% and Specificity was 100%. The positive predictive value (NPP) of the Mtb identification test using rapid Ag TB MPT64 was 100% and the Negative Predictive Value (NPN) was 78.72%.

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