INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 80
2021 Nov; 28(1): 1-
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

Analysis of MTb Rapid Molecular Test Performance Towards Microscopical Acid Fast Bacilli Examination at Labuang Baji General Hospital

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ABSTRACT

Tuberculosis (TB) is a global health problem, which is the third leading cause of death of all infectious diseases around the world, included Indonesia. Acid Fast Bacilli (AFB) smear and rapid molecular assay for *Mycobacterium tuberculosis* (MTB) are the old and new examinations required for MTB laboratory diagnosis. This study aimed to compare the performance of MTB rapid molecular assay and AFB smear in diagnosis and screening for TB patients. This observational retrospective study used a cross-sectional approach, with a purposive sampling technique of 559 patients with suspected TB in Labuang Baji Hospital, Makassar. This study was conducted from March 2019 to June 2019 by taking data from medical records from January 2018 to December 2018 at Labuang Baji Hospital, Makassar. Three hundred and forty-nine subjects were males (62.4%), and 210 subjects were females (37.6%). This study revealed sensitivity and specificity of 98.57% and 84.96%, respectively for MTB rapid molecular assay, and 68.65% and 99.44%, respectively for AFB smear, this shows that MTB rapid molecular assay was superior to AFB smear in diagnosing TB patients.

Keywords: Tuberculosis, AFB smear, MTB rapid molecular assay

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTb) that can affect the lungs and other organs. *Mycobacterium tuberculosis* was first introduced in 1882 by Robert Koch. Robert Koch's presentation upon announcing *Mycobacterium tuberculosis* for the first time earned him a Nobel award in the health category. *Mycobacterium tuberculosis* is also known as Koch Tuberculosis is an important worldwide community health problem. World Health Organization (WHO) has stated TB to be a Global Emergency since 1992. Estimate reports of TB patients globally reached a million people in 2018 with two-thirds of these patients domiciling in eight main countries, where Indonesia was in third place following India and China.[1]

Riskesdas' reports in 2018 stated there were 1,017,290 cases in Indonesia, and South Sulawesi alone was predicted to have 33,693 TB cases. According to the global burden of disease study, TB is the second cause of death worldwide. The number of TB in Indonesia according to microscopical examination reached 759 out of every 100,000 people at the age of 15 above, with more male patients than females, and more urban patients compared to rural patients.[2,3]

Mycobacterium tuberculosis is usually basil shaped with the length of 1–10 μm, width 0.2–0.6 μm, dormant and acid fast with Ziehl Neelsen staining, the bacteria appear red under microscopic examination. The source of infection is acid-fast bacilli of TB-positive patients through droplet nuclei that are emitted during coughing and sneezing. The infection will happen if healthy subjects inhale air containing droplet nuclei through the mouth or nose, traveling to the upper respiratory tract from the bronchus reaching the alveoli.[3]

Tuberculosis lung examinations in this study are sputum laboratory microscopical smear (acid-fast bacilli sputum), Xpert MTB/RIF/Rapid Molecular Test (RMT). Microscopical sputum examination is one of the most effective ways to diagnose TB. Technically the AFB sputum examination is inexpensive, easy to be done, and very specific for high prevalence areas, but its' sensitivity is 60% compared to sputum culture. Microscopic smear examination has a 5000–10,000 per mL linearity in detecting bacteria in sputum. Drug resistance is diagnosed by using standardized *Mycobacterium tuberculosis* drug resistance tests available in Indonesia, such as the rapid test and conventional method.[4]

An automatic molecular test using a cartridge

that fulfills the Nucleic Acid Amplification Test (NAAT) to detect TB bacteria and rifampicin resistance rapidly and simultaneously is potentially revolutionary and can change TB treatment and control. Rapid molecular testing of MTB-RIF is more accurate than conventional testing because it directly decomposes bacterial DNA while identifying the mutation on the rpoB gene that is related to rifampicin resistance. Rapid molecular tests give accurate results in less than two hours, with minimum biosafety and conditional training, and can be placed in a non-conventional laboratory. Rapid molecular testing has a sensitivity of 98% and linearity of 50–150 per mL. Technology supported by WHO is used in 88 countries.[5]

This study was held to compare the performance of RMT and AFB smear in diagnosing TB.

METHODS

This study was observational retrospective using a cross-sectional approach using purposive sampling technique, with 559 samples from Labuang Baji General Hospital, Makassar. This study was held from March to June 2019 with medical record sampling from January–December 2018 at Labuang Baji General Hospital Makassar.

Inclusion criteria were patients suspected with TB with an age range of 18–70 years old and had complete medical record information and both AFB and RMT results. Microscopical sputum AFB is a sputum smear stained with ZN to detect acid-fast bacteria under the microscope. Microscopical acid-fast bacilli results are classified according to the International Union Against Tuberculosis and Lung Disease (IUATLD), reported as: Negative: no AFB 0/100; Scanty: 1-9/per 100 fields; 1+: 10–99/per 100 fields, 2+: 1-10/field; and 3+: > 10 AFB in 1 field, examining at least 20 fields. Rapid Molecular Test (RMT) using the Polymerase Chain reaction (PCR) method using the GeneXpert by the measurement for fluorescence and algorithm in the system with results of Rif resistance detected; Rif resistance intermediate, Rif resistance not detected, MTB detected.[5,6]

Authorization of this study was given by the Ethical Study Committee of the Medical Faculty of Hasanuddin University/Labuang Baji Hospital, Makassar with article no 298/UN4.6.5.31/PP36/2019.

RESULTS AND DISCUSSION

Five hundred fifty-nine (559) samples were fulfilling the inclusion and exclusion criteria with 349 respondents were male and 210 were female with the highest population in the 45–65 years old range (Table 1).

The male population with TB was 26.1%, which was higher than the female population of 23.3%. Patients were mostly in the 45–65 years old age group.

Both gender and age groups had normal data distribution whilst AFB and RMT groups were not normally distributed.

Table 2. Suitability analysis of AFB and RMT MTB results

RMT	Microscopical AFB		
	Positive (+)	Negative (-)	Total
Positive	138	63	201
Negative	2	356	358
Total	140	419	559

AFB: Acid-Fast Bacilli, RMT: Rapid Molecular Test, *Fisher test, $p < 0.05$

Table 2 was analyzed using the Fisher test that showed a significant correlation from the results using microscopical AFB and RMT ($p < 0.05$).

Table 1. TB patients' characteristics

Parameter	Positive TB N (%)	Negative TB N (%)	P-value*
Gender			
Male	91 (26.1%)	258 (73.9%)	
Female	49 (23.3%)	161 (76.7%)	
Age			
< 26 years old	20 (25.6%)	58 (74.4%)	
26-45 years old	50 (22.9%)	168 (77.1%)	
45-65 years old	58 (29.6%)	138 (70.4%)	
> 65 years old	12 (17.9%)	55 (82.1 %)	
AFB	140 (25.04%)	419 (74.96%)	
RMT	201 (35.96%)	358 (64.04%)	0.000

AFB: Acid Fast Bacilli, RMT: Rapid Molecular Test, *Kolmogrov-Smirnov test, $p < 0.05$

Table 3. Sensitivity and specificity of RMT testing towards microscopical AFB

Variable	Value (%)
RMT examination towards AFB	
Sensitivity	98.57
Specificity	84.96
PPV	68.65
NPV	99.44
AFB examination towards RMT	
Sensitivity	68.65
Specificity	99.44
PPV	98.56
NPV	84.96

PPV: Positive Productive Value, NPV: Negative Predictive Value

Table 4. Results of diagnostic examination with microscopical AFB compared to RMT method

RMT	Microscopical AFB			
	Negative	Positive		
		+1	+2	+3
Negative	356	2	-	-
Very low	18	8	2	1
Low	22	17	8	4
Medium	18	23	30	16
High	5	5	10	14
Total	419	55	50	35

AFB: Acid Fast Bacilli; RMT: Rapid Molecular Testing

Table 4 shows that there is a difference between TB microscopical examination and RMT, where 63 patients were stated positive from RMT and negative from microscopical AFB.

Table 5. Diagnostic results of RMT compared to culture

RMT		Culture		Total
		Positive	Negative	
Resistance	Very low	3	1	4
	Low	4	0	4
	Medium	8	0	8
	High	5	0	5

RMT: Rapid Molecular Test; DST: Drug Susceptibility Test

Table 5 shows a concordance from the RMT results with the culture results, excluding one sample that tested low resistance for RMT, which had a negative culture result.

Patients detected resistance towards OAT with the RMT method were 21 samples that were followed-up with MTB culture and DST. According to Table 5, there was a concurrence between RMT and culture results, only one patient tested false positive with "very low" results. This might be due to the low count of bacteria causing it hard to grow on the MTB culture.[2]

The results of this study (Table 1) were in line with Fernandes *et al*. where the youngest patient was 18 and the oldest 81 years old, with more males than females. Campos *et al*. study in 129 patients with lung TB but negative AFB in Barcelona also stated that the mean age for patients with TB was 46.6 years old with 74 male (57.4) and 55 female (42.6%) patients. The high rate of TB in the male productive age is thought to be caused by high productivity that causes the infection of TB to easily happen from both positive or negative AFB patients with TB.[7,8]

According to the Fisher test, there was suitability between the negative microscopical TB and negative RMT in 356 samples even though there were 2 false-negative samples. This study had similar results to the study by Chaisson and Duong *et al*. in which 233 patients (77%) from 301 patients were detected positive by RMT and 8 were negative. This could be due to mutation in the bacteria causing the RMT to not be able to detect the bacteria, or due to < 50 bacteria/mL. Bacteria found in microscopical AFB examination were acid-fast bacilli of other species or *Mycobacterium* other than tuberculosis (MOTT). Acid-fast bacilli sputum examination cannot differentiate *M.tuberculosis* from other acid-fast bacilli (MOTT), where Xpert MTB/RIF can only detect TB bacteria. RMT sensitivity towards microscopical AFB examination was 98.7%, specificity 84.96%. This was in concurrence with Sharma *et al*. where RMT had a higher sensitivity and specificity of 99.3% and 95.7%.[9,10]

There were 63 positive samples in RMT that were negative in microscopical AFB, this could be due to RMT linearity of 50–150 per mL. This study had similar results to a study by Claessens *et al*. that found false-positive cases from the Xpert MTB/RIF examination. This could be due to contamination of other bacteria, even though RMT technology has a very small chance for contamination due to its closed system, and the surface where the sample is processed is always sterilized thoroughly to prevent contamination of other bacterial DNA. Another reason that can cause false positivity in RMT is *M.tuberculosis* DNA stays in the patients even after therapy. The rapid molecular test cannot differentiate active from inactive TB, while a clinical diagnosis of TB diagnoses active cases. Incorrect

sputum sampling can also cause a lack of AFB bacteria that can cause an error in microscopical AFB examination. A microscopical AFB examination is a widely used diagnostic procedure, but has limitations in detecting bacteria less than 5000–10.000 CFU/mL, inadequate AFB staining, unstable reagent storing, inadequate skills in microscope reading, and unable to detect resistant bacteria. High sensitivity and specificity of RMT can be used to detect TN bacteria.[11,12]

Sputum AFB examination is used worldwide to diagnose TB, but culture is still the gold standard in diagnosing TB. Patients with a negative sputum AFB test are less infectious than patients with positive results, but can still be a source of infection. Microscopical examination can detect *mycobacterium* with a minimal concentration of 5000 bacteria/mL sputum, whereas less is needed to be able to infect other people. Due to that, humans that are in contact with AFB negative lung TB patients are still at risk to be infected by *M.tuberculosis* and becoming actively infected patients. Tuberculosis patients with negative AFB but positive cultures have a 26% possibility to transmit TB, whereas patients with negative cultures but positive Chest X-rays have a 17.5% risk of infecting others.[3] Mnyambwa *et al.* in the Netherlands found that patients with AFB negative but positive culture have an 18% chance of infecting other people.[13]

Patients detected of resistance towards OAT by RMT were 21 samples, that were followed with MTB culture and DST examination. There was a concordance between RMT results and culture and only one patient was falsely positive with very low results. This might be due to the small number of bacteria so it was hard to grow in MTB culture.[2]

CONCLUSIONS AND SUGGESTIONS

Rapid molecular testing is superior compared to microscopical AFB for TB patient screening.

Further study is needed to see the suitability of the TB examination method to increase the sensitivity and specificity in detecting TB bacteria; Further study of whether a patient's obedience to control during therapy will affect TB bacterial results using different methods is needed.

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