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Research Article

The Correlation of Profiles Resistance Rifampicin and Isoniazid with Acid Fast Bacilli Gradations in Multidrug Resistance TB Patients

Abdul Rahman Bahmid[1]*, Soedarsono[2], Ni Made Mertaniasih[3] and Isnu Pradjoko[2]

[1]Resident of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo Hospital Surabaya, Indonesia

[2]Lecturer of and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo Hospital Surabaya, Indonesia

[3]Department of Clinical Microbiology, Faculty of Medicine, Universitas Airlangga Surabaya, Indonesia

***Corresponding Author:** Abdul Rahman Bahmid, Resident of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo Hospital Surabaya, Indonesia.

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Abstract

Background: Multi-Drug Resistance Tuberculosis (MDR-TB) is a global health problem. Rifampicin resistance (RR) is caused by mutations in the rpoB gene, resistance of INH is caused by mutations in katG or inhA. Fitness is defined as the ability of pathogens to survive, replication and transmission. The fitness of drug resistance strain is one of the determinants MDR-TB/RR spreading. Estimation of strain MDR-TB fitness were assessed by the gradation of AFB smear in new case and previous treatment of MDR-TB Pulmonary TB patients

Method: The study was conducted at RSUD Dr. Soetomo Surabaya from November 2021 to March 2022. To detect genetic mutations conducted by Line Probe Assay (Genoscholar™NTM+MDR TB II, NIPRO Japan), and AFB examination using Ziehl Neelsen staining microscopy. Analyze correlation of resistance gene with AFB gradation using chi square test and Fischer's exact

Results: There were 33 subjects recruited, 16 MDR/RR-TB patients new cases and 17 MDR-TB/RR patients with previously treatment. The new cases patients had more rpoB mutations, while patients with previously treatment had more double mutations (rpoB+katG/inhA). Higher AFB smear grades were significant associated with MDR/RR-TB previously treatment, otherwise no significant correlation in all TB cases of resistance in RR and HR with AFB gradation. Among the lower AFB grades revealed rpoB and rpoB+katG/inhA mutations were 57,14% and 42,86%. In the Higher AFB grades revealed rpoB and rpoB+katG/inhA mutations were 47,37% and 52,63% respectively.

Conclusion: There is no correlation between profile resistance of rifampicin and INH with AFB gradation.

Keywords: *TB-MDR/RR; rpoB; inhA; katG; AFB*

Background

MDR TB caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) that is resistance to first line anti tuberculosis drugs, including rifampicin (RIF) and isoniazid (INH) with or without being accompanied by resistance to other first-line anti-TB drugs. The global burden of MDR-TB is increase. In 2019 overall there were an estimated 465,000 cases of MDR-TB with a mortality rate of around 182,000. Indonesia is one of the 30 countries with the highest MDR-TB burden in the world, with an estimated 2.4% of MDR-TB new cases and 13% of MDR-TB previous treatment [1,2].

The prevalence of MDR-TB in patients with previous treatment is much higher than in patients without history of treatment/new cases. However, recently new cases of MDR-TB patients are increasing, as a consequence all TB patients are required to undergo drug sensitivity test, especially in regions with a high burden of MDR-TB. WHO has issued a policy statement to improve the diagnosis of drug resistant TB through molecular methods such as Line Probe Assay (LPA). LPA detects RIF resistance by identifying mutations in *rpoB* gene, and INH resistance by detecting mutations in *katG* and *inhA* genes [3,4].

Acid fast Bacilli (AFB) microscope examination is a simple tool and is generally used for diagnosis and monitoring of treatment. Patients with higher AFB grade indicates higher bacillary load and may increasing the risk of transmission drug resistance. Research by Soedarsono., *et al.* at RSUD dr. Seotomo Surabaya, which involved 433 sputum samples of MDR-TB patients with AFB positive, stated that the resistance pattern of first-line anti-TB drugs is not significant relationship with AFB grading [5].

AFB grade is a significant to estimate the risk of transmission, patients with high AFB grading are more likely to transmit the disease than patients with low AFB grade. MDR-TB patients with high AFB grade is a risk factor for transmission of drug-resistant strain of *M. tuberculosis* in the community. To estimate the fitness of drug-resistant strain we tried to assess the relationship of AFB gradations with RIF and INH profiles resistance in new cases and previous treatment MDR-TB patients.

Methods

This study is an observational analytical study with a Cross Sectional study design. Samples were collected from MDR-TB patients who are treated from November 2021 to March 2022 in Dr. Soetomo hospital. Subject collected based on the GeneXpert sputum examination, by excluding TB-HIV patients and non-tuberculous *Mycobacteria* (NTM) co-infection. LPA test with Genoscholar NTM+MDRTB II was performed to assess RIF and INH profiles resistance. AFB smear examination was performed when the patient is diagnosed with MDR-TB. Sample sputum were taken from new and previously treated MDR-TB patients.

Results

There were 33 MDR-TB patients, consisting of 16 new cases and 17 patients previous treatment. the study subjects were dominated by the female (69.7%), Based on the age profile shows the youngest age range is 18 years old and the oldest is 70 years old. The most age range 43 - 50 years old (24.2%). In the group of MDR/RR TB previous treatment had more of a history of DM (58.8%) than patients without comorbidities (41.2%). Most subjects have normal nutritional status (51.5%). The most of subjects in this study did not have habit of smoking (81.8%).

Frequency distribution based on suspected MDR-TB

Based on the criteria for suspected MDR-TB patient, the relapses after category 1 had a total of 8 patients (47.1%), followed by failed category 1 as many as 4 patients (23.5%). The rpoB+inhA/katG mutation was more commonly found in the criteria for relapse cases after category 1, which was 7 patients (Table 1).

Characteristic	TB-MDR new cases (n = 16)	TB-MDR previous treatment (n = 17)	Total N = 33
Gender			
Men	4 (25%)	6 (35,3%)	10 (30,3%)
Woman	12(75%)	11 (64,7)	23 (69,7%)
Age			
19 - 26	4 (25%)	3 (17,6%)	7 (21,2%)
27 - 34	1 (6,2%)	2 (11,8%)	3 (9,1%)

35 - 42	3 (18,8%)	2 (11,8%)	5 (15,2%)
43 - 50	5 (31,3%)	3 (17,6%)	8 (24,2%)
51 - 58	2 (12,5%)	2 (11,8%)	4 (12,1%)
59 - 66	1 (6,2%)	3 (17,6%)	4 (12,1%)
67 - 70	0	2 (11,8%)	2 (6,1%)
Comorbid DM			
Yes	6 (37,5%)	10 (58,8%)	16 (48,5%)
No	10 (62,5%)	7 (41,2%)	17 (51,5%)
Nutritional Status (BMI)			
Underweight	5 (31,25%)	4 (23,5%)	9 (27,3%)
Normal	6 (37,5%)	11 (64,7%)	17 (51,5%)
Overweight	5 (31,25%)	1 (5,9%)	6 (18,2%)
Obesity class 1	0	1 (5,9%)	1 (3,0%)
History of smoking			
Yes	3 (18,7%)	3 (17,6%)	6 (18,2%)
No	13 (81,3%)	14 (82,4%)	27 (81,8%)

Table 1: *Characteristics of the subject of study.*

Criteria for suspected TB-MDR	rpoB (resist RIF)	rpoB+inhA/katG (resist RIF+INH)	Total
Patients failed treatment of category 1	2	2	4 (23,53%)
TB patients who are not converted after Category 1		2	2 (11,76%)
Relapse cases after category 1	1	7	8 (47,06%)
TB Patients drop out treatment	2	1	3 (17,65%)
Total	4	12	17

Table 2: *Frequency distribution based on the criteria of suspected TB-MDR and its resistance profile.*

RIF and RIF+INH resistance profiles in new case and previous treatment MDR/RR TB patients

In the new cases of TB-MDR/RR patient the most mutations were obtained in the rpoB gene (12 patients), but in previous treatment was more of a double mutation (rpoB +inhA/ katG). Therefore significant differences resistance pattern between new cases and previous treatment MDR/RR-TB patients (Table 3).

Mutation Genes	TB-MDR/RR		P-value
	New cases	Previous Treatment	
rpoB (resistance RIF)	12	5	
rpoB + katG/inhA (resistance RIF+INH)	4	12	0,032
Total	16	17	33

Table 3: *Distribution frequency of rpoB, katG and inhA mutations in new cases and previous treatment TB-MDR/RR-TB patients.*

Difference of AFB gradation in new cases and previous treatment MDR/RR-TB patients

To determine difference of AFB gradations in new case and previous treatment of MDR/RR-TB patients, we grouped the AFB gradations into 2 groups, namely low (negative, scanty, 1+), and high (2+, 3+). We found MDR/RR-TB patients previously treatment was significant associated with a higher AFB gradation (Table 4).

BTA gradations	TB-MDR/RR		p
	New cases	Previous Treatment	
High	6	13	0,024
Low	10	4	
Total	16	17	33

Table 4: *Differences of AFB gradation in new cases and previously treatment MDR/RR-TB patients.*

The relationship of *M. tuberculosis* mutation resistant to RIF and RIF+INH with AFB gradation

To analyze the relationship between *M. tuberculosis* mutations and AFB gradations, resistance genes were grouped into rpoB and rpoB+katG/inhA with low and high AFB. Statistical tests with $p > 0.05$. There was no significant association between *M. tuberculosis* resistant RIF and INH with AFB gradations (Table 5).

Mutation genes	BTA gradations		Total	p
	Low	High		
rpoB	8	9	17	0,579
rpoB + (katG/inhA)	6	10	16	
Total	14	19	33	

Table 5: *Analysis of the relationship between genetic mutations of M. tuberculosis and AFB gradations in MDR/RR TB patients.*

Discussion

Most of the subjects in this study were female, which was 69.7% compared to male (30.3%). There is not accordance with WHO reported of TB incidence in 2019, which states that TB cases globally are dominated by men around 56% [1]. This may be due to the low position of women in family to decision-making, so that women's were limited to access and control in the management of resources for health [6]. A study of Muslih., *et al.* reported the risk factors for TB in women include a history of contact, exposure to kitchen smoke pollution, and poor ventilation which not accordance with health requirements [7]. Based on the distribution of age groups in MDR-TB patients, the most age range were 43 - 50 years old and 19 - 26 years old. A meta-analysis reported that MDR-TB was 2.5 times more common in younger less than 65 years old. Epidemiological studies show that young age is the risk factors for TB transmission [8].

In this study the group of previously treatment MDR-TB patients had more comorbid DM as much as 58.8%. Saktiawati's study in Yogyakarta (2018) reported that DM has a significant correlation with incidence of MDR-TB, and has a 6.8 times risk of developing MDR-TB [9]. Patients with DM are associated with poor TB treatment responses. It has been reported that patients with DM had lower concentrations of anti-TB drug, decreased plasma concentrations of anti-TB drug is associated with failure treatment and the emergence of drug resistance. A study of Nijland., *et al.* reported that TB-DM patients in Indonesia had 53% lower rifampicin concentrations than TB without DM [10].

Four criteria suspected MDR-TB were obtained in this study, namely patients who failed treatment category 1, TB patients who did not convert after category 1 treatment, TB patients who relapsed after category 1 and patients returned after drop out. Relapse cases were dominant with 47.06%. This is in accordance with study of Soedarsono., *et al.* (2020) reported that of the 426 TB-MDR patients with treatment history, 160 patients (36.9%) were TB relapse [5]. *M. tuberculosis* mutation pattern in relapses cases were found to had more rpoB +inhA/katG mutations. KatG mutations are commonly found in MDR-TB or INH monoresistent patients. A study reported that katG mutations have a significant relationship with poor therapeutic outcomes [11]. The most frequent causes of relapses are irregular medication, non-standard drug regimens therapy, poor medication adherence, and the presence of unknown drug resistance before. Suboptimal drug concentrations will reduce effectiveness therapy and emergence of drug resistance [12].

The mutation pattern *of M. tuberculosis* showed significant differences between new cases and previously treatment MDR/RR-TB patients, thus causes differences of drug resistance patterns. The new cases MDR/RR patients were found more single mutations in the rpoB gene (R resistance), while in patients with previously treatment there were more double mutations in rpoB and katG or rpoB and inhA (R+H resistance). This is in accordance with study of Ahmed., *et al.* were found patients with previous treatment had more R+H resistant patterns [13]. A study of Alvarez's reported a new case patients had more RIF resistant than INH-resistant, suggests that RIF-resistant strains (rpoB mutations) tend to be more likely a high transmission ability in the community than strains with rpoB+katG mutations [14]. Gagneux's., *et al.* found that *M. tuberculosis* rpoB mutations especially in codon 531 had not decrease fitness in both laboratory and clinical strains [15]. This may causes a high transmission of RIF-resistant strain in new cases MDR/RR TB patients.

In this study was conclude a higher AFB grade was significant with previously treatment MDR/RR-TB patients. This is in accordance with study of Kassa., *et al.* (2021) in Ethiopia reported that in MDR-TB patients was found a higher AFB smear 3+ (34.4%) and significantly related to the previous TB treatment [16]. Patients with higher AFB smear grade can be a source of transmission and spread of MDR-TB strains in the community.

There was no significant relationship between mutations of *M. tuberculosis* resistant RIF and INH with AFB gradations. Gene mutations have a negative impact on physiological function resulting in reduced of bacterial fitness. The rpoB, katG, and inhA genes are have a function in the replication process and bacillus survival. Although drug-resistant strain often suffer an initial reduction in fitness, they continue to evolve by acquiring one or more secondary-site mutations that can improve or even restore the fitness of these strain over time, these process is known as compensatory evolution. This is part of the evolution of drug resistance which is related to the fact that mutations can have an impact on changes in bacterial fitness [17]. Song., *et al* reported that the rpoB mutation strain had different mutations in the *rpoC* gene. This study reported that the decrease in fitness due to the rpoB mutation will be compensated by the rpoC mutation which will restore the transcription activity of RNA polymerase [18]. Some laboratory studies state that *M. tuberculosis* undergoing katG mutation can maintain catalase-peroxidase activity due to the presence of mutations in the ahpc promoter gene (*alkyl hydroperoxide reductase*). Ahpc mutation causes ahpc overexpression as compensation for loss of katG activity that can protect bacillus from organic peroxide damage [7,20].

This may be a causes insignificant differences in AFB grade in each of the resistance genes. Mutations that initially decrease bacillus fitness including the replication rate, over time there will be compensation in each gene that will increase fitness of these strain. To find out this requires examination of whole genome sequencing. In addition, genetic background may also influence this. The Beijing strain is often associated with MDR strain, the Beijing strain might be better adapted to compensate for the loss fitness due to mutations.

Limitations of the Study

This study used spontaneous sputum that had limitations, including poor sputum samples that can effect accuracy LPA test. In addition, we did not observe other mutations causing other first-line or second-line anti-TB resistant that may have an effect on the virulence of *M. tuberculosis*.

Conclusion

There is no relationship between the RIF and RIF+INH resistance profiles and the AFB gradation. A high AFB grade were significantly associated with the patient's TB treatment history. This indicates the high bacillary load in MDR/RR-TB patients which is a risk factor for transmission in the community.

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