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The Effect of KatG S315t Mycobacterium Tuberculosis Gene Mutation on the Conversion Rate of MDR-TB Patients with Shorter-Course Treatment

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ABSTRACT

Introduction. Resistance to the isoniazid (INH) drug is most often caused by a mutation of the KatG S315T M.Tb gene. MDR TB treatment with short-term WHO mixes using high-dose INH drugs is considered less effective in this mutation condition because it causes high resistance to INH. The effectiveness of MDR TB treatment can be seen from the sputum smear conversion rate. This study was aimed to determine the effect of the S315T katG gene mutation on the treatment response of patients with MDR TB who received WHO short-term alloys at Dr Moh Hoesin general hospital, Palembang, Indonesia. **Methods.** This study uses observational analytic with a prospective cohort approach. The study subjects were MDR TB patients at Dr Moh Hoesin general hospital, Palembang, Indonesia, and a PCR-RFLP examination were performed to see the katG gene, followed by sputum smear evaluation at the end of the first and second months of treatment to assess the speed of conversion. Data analysis using SPSS 25 with the chi-square statistical test. **Results.** The frequency of katG S315T M.Tb gene mutations was 51.85%. The majority of MDR TB sufferers experience rapid conversion (92.59%). 64.29% of the katG S315T gene mutation group experienced sputum smear conversion after one month, 28.57% after two months, and 7.14% after three months of treatment. There was no significant difference in conversion speed in the two groups (p = 0.741). **Conclusion.** There was no effect of the S315T M.Tb katG gene mutation on the speed of sputum smear conversion of MDR TB patients who received short-term alloy treatment.

1. Introduction

Multi-Drug Resistance Tuberculosis (MDR TB) is a global health problem, including Indonesia. Based on the WHO Global Tuberculosis Report 2019 data, Indonesia ranks fifth highest. MDR TB is TB with the least resistance to isoniazid (INH) and rifampicin. The diagnosis of MDR TB in Indonesia is made quickly by examining the TCM GeneXpert MTB / RIF so that when positive test results are referred to as rifampicin-resistant tuberculosis. Even so, Tadesse M et al. and Stagg HR et al. reported about 90% of cases of resistance to rifampicin accompanied by INH resistance.[1 -5]

Isoniazid is a very important first-line OAT because it is used to treat TB and MDR TB. INH enters the M.Tb cell as a prodrug by passively diffusing, INH is then activated by the enzyme catalase-peroxidase (katG) expressed by the M.Tb katG gene to become its active form. The active INH will then inhibit the biosynthesis of the M.Tb cell wall mycolic acid. A mutation of the katG gene causes about 50% to 80% of INH resistance, and the majority of the modifications are changes in serine to threonine in codon 315 (S315T). This mutation causes a high level of resistance to INH with MIC

ranging from 1-10 µg / ml. Dalla costa ER et al. reported that 83% M.Tb with an S315T katG mutation caused a high degree of resistance with MIC with two µg / ml. Chesov et al. reported the incidence of katG S315T gene mutations in patients with MDR TB so high in Moldova that it was inappropriate to be given INH because high-dose INH was only useful for INH resistance due to inhA gene mutation.[6-12]

The World Health Organization in 2016 introduced a short-term MDR-TB treatment that uses high-dose INH drugs (10-15 mg/kg) in addition to 6 other OAT. The success of treatment with this alloy is reported to be up to 80%. In the katG gene mutation state, INH's use becomes ambiguous because M.Tb has been declared a high level of resistance to INH and high dose INH increases the risk of side effects.[13]

The effectiveness of MDR TB treatment was assessed by sputum smear conversion and M.Tb culture. Lv et al. and Shibabaw et al. reported that sputum smear conversion and M.Tb culture were critical early indicators of treatment efficacy and determined the treatment length for TB MDR patients. To assess the effect of the S3GT katG gene mutation on the short-term alloy treatment, this study needs to be done, so that it can be linked to the choice of OAT in compiling MDR treatment regimens.[14,15]

2. Methods

This study uses observational analytics with a prospective cohort approach. The study subjects were MDR TB patients, and 27 samples were obtained. PCR-RFLP examination to see 315 KatG codon alleles was carried out in the microbiology laboratory. BTA sputum evaluation is performed at the end of the first and second months of treatment to assess conversion speed. In this study, the patient experienced conversion as an independent variable, G S315T gene mutation as a dependent variable and mutations other than G S315T kat, malnutrition, comorbidities as confounding variables. Data analysis using SPSS 25 with the chi-square statistical test.

3. Results

This research was conducted at Dr Moh Hoesin general hospital, Palembang, which took place from February 2019 to May 2020. Samples were divided into two groups: 13 (48.15%) katG gene wild type katG genes and mutant katG S315T M.Tb gene as many as 14 (51, 85%) people.

Table 1. General characteristics, laboratory, radiology, and duration of conversion of research subjects

Characteristics	Groups	
	Wild type gene katG M.Tb n=13 (%)	Mutant gene katG M.Tb n=14 (%)
Gender		
- Male	6 (46,15)	8 (57,15)
- Female	7 (53,85)	6 (42,85)
Age (Year)		
- 18 – 23	1 (7,69)	0 (0)
- 24 – 34	5 (38,46)	4 (28,58)
- 35 – 44	3 (23,07)	5 (35,71)
- > 44	4 (30,77)	5 (35,71)
Body mass index (kg/m^2)		
- Underweight	7 (53,85)	10 (71,42)
- Normoweight	6 (46,15)	4 (28,58)
Sputum Acid Resistan Basili		
- Positive 1	2 (15,385)	5 (35,715)
- Positive 2	9 (69,23)	4 (28,58)
- Positive 3	2 (15,385)	5 (35,715)
Molecular rapid test		
- *M.Tb detected low*	2 (15,385)	3 (21,42)
- *M.Tb detected medium*	9 (69,23)	4 (28,58)
- *M.Tb detected high*	2 (15,385)	7 (50)
Chest Thorax X-ray		
- Minimal lesion	3 (23,08)	1 (7,14)
- Moderate lesion	5 (38,46)	5 (35,71)
Conversion		
- One month	9 (69,235)	9 (64,29)
- Two months	3 (23,075)	4 (28,57)
- ≥three months	1 (7,69)	1 (7,14)

Table 2. Effects of the S315T M.Tb katG gene mutation on the sputum smear conversion rate

Conversion	Groups		*p*	OR (95% CI)
	Wild type gene katG M.Tb n=13 (%)	Mutant gene katG M.Tb n=14 (%)		
Quick	12 (92,31)	13 (92,86)		
Slow	1 (7,69)	1 (7,14)	0,741	1,04 (0,247 – 4,373)

4. Discussions

In this study, 51.85% of katG S315T M.Tb gene mutations were obtained. Seifert M et al. reported 300 mutation points in the katG gene and 64% in the codon allele 315. About 95% of the katG codon 315 gene mutation due to the serine amino acid substitution into threonine. The incidence of katG S315T M.Tb gene mutations varies in each country. Allagapan C et

al. reported katG S315T M.Tb gene mutations' rate ranged from 50% -95% worldwide, 71% in India. Chekov D et al. reported 88.1% of these mutations in Moldova. Bollela VR et al. found a difference in the incidence of variations in the KatG S315T M.Tb gene that is 84.2% of the M.Tb isolates with MDR TB in Mozambique and 54.5% in Brazil.[11,16-18]

In this study, the majority (66.67%) of MDR TB sufferers had sputum smear conversion after one month, 25.92% after two months, and only 7.4% after three months of treatment. BTA sputum conversion that occurs within two months of treatment is stated as a fast conversion. It was found that 92.31% of the wild type katG gene group 315MTb and 92.86% of the group mutation katG S315T M.Tb gene experienced rapid conversion. There was no effect of the S3GT15T M.Tb katG gene mutation on the sputum smear conversion rate of MDR TB patients who received short-term alloys with high doses of INH in Palembang RSMH (p = 0.741). This study shows the success of MDR TB treatment, where 92.59% of MDR TB sufferers experienced conversion in the first two months after receiving short-term alloy treatment. It can be concluded that MDR TB treatment at Dr Moh Hoesin general hospital, Palembang has been going very well by implementing a short regimen who use high doses of INH.

In the treatment of MDR TB with this short-term blend, ethionamide drugs are also used. Vilcheze et al. wrote about the effectiveness of ethionamide to kill M.Tb that had high INH resistance levels due to the mutation of the katG gene. It seems that the occurrence of M.Tb katG gene mutations is rarely accompanied by M.Tb resistance to ethionamide drugs. In contrast to variations in the M.Tb inhA gene that cause low INH resistance levels, cross-resistance to ethionamide is often found.[19]

One possible cause of the influence of the katG S315T M.Tb gene's mutation to the speed of sputum smear conversion in this study is that some of the enzyme catalase-peroxidase (katG) is thought to be still active and able to activate INH so that MDR TB treatment is always useful. Dalla costa ER et al. reported that about 30-40% of the katG enzyme was still active in the S315T katG mutation condition. This active katG enzyme will activate the INH pro drug so that it will eventually inhibit the synthesis of the M.Tb wall. In his writings, approximately 83% of M.Tb strains undergoing the S315T katG mutation cause a high level of resistance with MIC ≥ 2 μg / ml, but still show successful treatment.[10]

The possible cause of the absence of the influence of the katG S315T M.Tb gene mutation to the conversion rate of sputum smear can also be caused by the full range of minimal inhibitory concentration (MIC) INH in the mutation state of the kat.G S315T M.Tb gene. Researchers have not found publication data about MIC INH in the state of the Gene P.15T M.Tb gene mutation in Indonesia. Seifert et al. (2015) reported that the S315T katG mutation was related to the MIC 2 range to more than ten μg / ml.[16]

Lempens P et al. researched M.Tb isolates in patients with MDR TB in Belgium showed the majority of resistance to INH caused by the mutation of the katG gene. In this study, a correlation between genotype and phenotype was performed. The katG and inhA gene mutations condition, both single and both, is associated with MIC INH levels in M.Tb isolates. This mutation causes a wide MIC range. This study used two groups of samples, namely, isolate samples from the WHO data banks and MDR TB patients undergoing short-term treatment regimens in Bangladesh. There was a slight difference in the level of INH resistance due to the two groups. In isolated samples from the WHO data banks,

resistance due to mutation of the S315T katG resulted in 82.7% moderate level mutations (MIC 3.2-12.8 µg / ml) without high-level resistance. Whereas most sputum samples of MDR TB patients in Bangladesh showed an average level of resistance, 9.1% experienced a high level of resistance (MIC,219.2 µg / ml). In conclusion, Lempen P et al. writes that there is still an effective treatment with high-dose INH drugs (15-20 mg/kg) in resistant TB INH due to the katG S315T gene. In general, the S315T katG mutation causes a moderate level of resistance of 6.4 µg / ml. Rieder et al. and Otto-Knapp et al. have compared the INH drug sensitivity test's genotype and phenotype results. They reported that a single katG mutation had a wide MIC range, and the majority of strains with low to moderate resistance were still effective with usual or high dose INH administration. The majority of S315T katG mutations show MIC of less than 15 mg / L, so WHO recommends giving INH at a 15-20 mg/kg dose for effective treatment of this mutation.[20-22]

If the S315T katG gene mutation and the -15C / T promoter mutation inhA M.Tb gene occur together, there will be very high resistance to total resistance to INH drugs with MIC 19.2 µg / ml. Previously Pasaribu R, reported his research with the results found no mutation promoter -15C / T inhA gene in patients with MDR TB in Palembang RSMH. In this study, it is also possible that no mutation of the KatG S315T M.Tb gene together with the -15C / T promoter mutation of the M.Tb inhA gene in MDR TB patients in Palembang RSMH so that resistance to INH is not total and high-dose INH still has good effectiveness treatment.[23]

In addition to M.Tb genetic mutation factors and treatment regimens, several other factors influence the speed of sputum conversion in the management of MDR TB, including age, smoking, nutrition, sputum smear examination results at the beginning of treatment more than positive 1, extensive radiological lesions, the number of resistant drugs, diabetes mellitus and medication adherence.[24-27]

5. Conclusion

There was no effect of the Ser315Thr M.Tb katG gene mutation with the speed of sputum smear conversion in patients with MDR TB who received high-dose INH adjuvant therapy in short-term alloy treatment.

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