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Relationship of Mutation of Codon S 315 T KatG Mycobacterium Tuberculosis Gene with The Incidence of Multidrug Resistance Tuberculosis in South Sumatra

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Abstract

MDR TB is the condition of M. tuberculosis (Mtb) that is resistant to at least isoniazid (INH) and rifampisin. This condition caused by certain genomic mutation in some Mtb spesific gene. Some gene that had role in INH mutation are katG, inhA, ahpC, ndh and kasA gene. The most common gene that mutates is katG gene, around 50-80 %, and the most mutation(64%) found in codon S 315T. Aim to know the correlation between Mtb codon S 315 T katG gene mutation and prevalence of multidrug resistance tuberculosis in South Sumatera This observational study with cross sectional approach was conducted in RSMH Palembang collaborated with Clinical Microbiology Laboratory Faculty of Medicine Universiats Sriwijaya from January to July 2019. The MDR TB group was 50 subjects the drug sensitive TB group was 21 subjects. Mtb katG codon S 315 T gene was isolated from each subjects using the PCR-RFLP method with the Mspl enzyme. KatG codon S 315 T gene in this study was visualized in 40 subjects only 26 subjects of MDR TB and 14 subjects of drug sensitive TB group. Result the frequency distribution genetic of MDR TB group was 65.38% wildtype and 34.62% mutant; in drug sensitive TB group was 92.86% wildtype and 7.14% mutant. The codon S 315 T katG gene mutation in this study was not correlated with the incidence of MDR TB (p = 0.07, OR = 6.8 (0.77 - 61.40, 95% CI)).Conclusion the codon S 315 T katG Mtb gene mutation was found in 34,62% subjects, but not significantly related to the incidence of MDR TB in South Sumatra.

Keywords: mutations, codon 315 katG genes, MDR TB

Introduction

Tuberculosis (TB) is still a global health problem, especially in Indonesia, because Indonesia is a country with a TB burden and a high prevalence of multi-drug resistance tuberculosis (MDR TB). Based on data from the Global Tuberculosis Report 2018 issued by the World Health Organization (WHO), Indonesia ranks third for sensitive TB with 842,000 estimated new cases, while for MDR TB it ranks seventh with an estimated new case of 23,000 cases.[1,2] MDR TB data in South Sumatra, since starting treatment for MDR TB in 2014, 532 cases of MDR TB have been found and 229 patients have been treated. TB control currently faces challenges posed by the global spread of M. tuberculosis (Mtb) strains that are resistant to standard anti-tuberculosis (OAT) drugs.[3,4] MDR TB is resistance to at least isoniazid and rifampicin which are the two most effective anti-tuberculosis drugs.[5,6,7,8] Mtb resistance to OAT occurs because of mutations. Mtb mutations occur naturally, where if the germ develops more than 1 x 10^6 there will be a single germ that is mutated against isoniazid. Mutations can be induced by inadequate therapeutic levels of the drug, mainly due to non-compliance during drug consumption. Therefore improper and irregular use of OAT can affect mutations in genes that encode OAT targets.[9,10,11,12] It has been found that Mtb resistance to OAT is due to certain genomic mutations in some specific Mtb genes. OAT resistance in Mtb is caused by the presence of additional strands in the mutation of the target gene. Some gene mutations that are known to be associated with first-line OAT resistance include isoniazid (INH). The mechanism of biomolecular Mtb resistance to INH is influenced by mutations in several genes, including katG gene, inhA gene, ahpC gene, ndh gene and kasA gene. Of the several genes that play a role in the INH mutation, the gene that most often occurs is the katG gene around 50-80% .[8,9,12]

All this time, the diagnosis of MDR TB is quickly carried out by rapid molecular test (TCM) examination which only checks the rpOB gene (ripamficin gene) so that if the positive TCM results are referred to as rifampicin resistant tuberculosis (RR RR). Whereas to get results for isoniazid resistance must wait for culture and resistance that requires a long time. So far there has not been a quick test for the examination of the INH gene, although the literature states that most patients who have experienced resistance to ripamficin (around 90%) also have resistance to INH. INH is an important first-line anti-tuberculosis. Mtb is very sensitive to INH. INH enters the Mtb cell as a passive diffuse prodrug, INH is then activated by the enzyme catalase peroxidase expressed by the katG Mtb gene to become its active form. The active INH

will then inhibit the biosynthesis of the Mtb cell wall mycolic acid.[12,13] The katG gene mutation causes a loss of enzyme catalase peroxidase activity. In several studies stated that the degree of resistance of Mtb in each region varies depending on the location of the gene mutation. Up to now, our area does not yet have data on the location of the MtB germ gene mutation. Some studies suggest the location of the katG gene mutation is different. Marva et al (USA, 2015) in their systematic review concluded that there were mutations in several katG gene codons, and the most mutations (64%) were found in the S 315T codon. Mutations in the S 315 T codon found in the katG gene are more common in MDR TB germs compared to TB germs that are only resistant to INH (monoresistant).[14] Research Jainagul Isakova et al (Kyrgyz Republic, 2018), Bostanabad et al (India) , 2008) and Marahatta et al (Nepal, 2011) found that the most katG gene mutations were located at codon 315 Ser (AGC) → Thr (ACC) around 88.6%, [15,16,17] while the Nurul-Ain et al study (Malaysia, 2015), Rosye and Yohanis research in Papua (2016) found no mutations in codon 315 but found mutations in the 316 Gly codon (GGC) → Cys (TGC) / base 946 G → T and codon 290 Ala (ACC) → Val (GTA) / base 896C → T and codon 238 with changes in the amino acid Leu → Arg.[18,19] Then this research is needed to determine the location of gene mutations in germ populations circulating in South Sumatra and where the location of the most mutations found in genes katG, so that it can be linked to events and man numbers MDR TB clinical assessment in South Sumatra.

Methods

The study design was observational with a cross sectional study approach, conducted at Palembang RSMH in collaboration with the Clinical Microbiology Laboratory of FK UNSRI Palembang from January - July 2019. The sample of the case group (MDR TB) was 50 people while the control group (TB SO) was 21 people. Each group was tested for S315T genotype katG M. tuberculosis gene using the PCR-RFLP method with the Mspl enzyme. Visualization of the katG gene in codon 315 in this study was only found in 40 samples, 26 samples in the case group (MDR TB) and 14 samples in the control group (TB SO).

Results

In this research, the katG gene codon 315 mutation was identified using the polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method using the Mspl enzyme. Because of the limitations of the existing tools in the Microbiology Laboratory Faculty of Medicine Universitas Sriwijaya there are only PCR-RFLPs and are not able to carry out gene sequencing, so this study only uses PCR-RFLP. the katG didn't appear. This could be due to the limitations of the tool and the sensitivity of the PCR-RFLP tool for the katG gene to only 85%. Visualization of the katG gene in codon 315 in this study was only found in 40 samples, 26 samples in the case group and 14 samples in the control group. Visualization of the katG gene in codon 315 consists of G allele (wildtype / normal) if there is a band of 153 bp, and allele C (mutant) if there is only one band of 132 bp, as shown in the figure below:

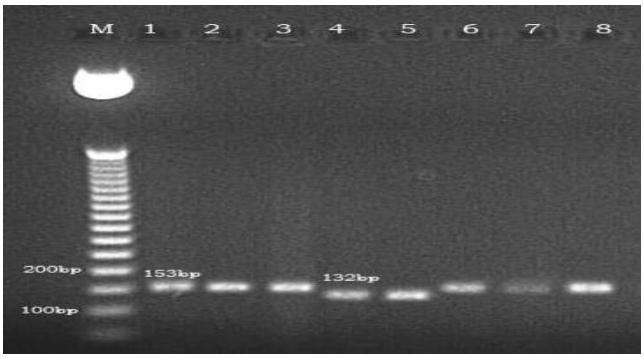

Figure 1. Electrophoresis of codon 315 katG genes after restriction using the Mspl enzyme. M = Marker, marker DNA; G allele on the path 1,2,3,6,7,8 C allele on the 4,5 path

The frequency distribution of 315 codons of katG genes in the case group was 65.38% wildtype and 34, 62% mutant, whereas in the control group was 92.86% wildtype and 7.14% mutant. Mutant genes were more likely to be found in the case group, compared to the control group. In this study, mutant genes and normal / wildtype genes were more numerous in the case group than in the control group. However, from the statistical calculation, it was obtained

Kodon 315	Group		*P*	OR (95% CI)
	Case n = 26	Control n = 14		
Mutant	9	1		
Wildtype	17	13	0,07	6,8 (0,77 – 61,40)

Fisher exact test, $p < 0,05$; *OR = Odds ratio, CI = Confidence interval*

that the mutation of codon 315 gene katG had no effect on the incidence of MDR TB. This can be seen from the p = 0.07 odd ratio of 6.8 (0.77 - 61.40, 95% CI).

Discussion

Of the 71 research subjects who met the acceptance criteria after genetic visualization in codon 315, only 40 subjects were found, 26 in the case group and 14 in the control group. This study only uses PCR-RFLP and gene sequencing was not carried out due to the limitations of the tools available at the Microbiology Laboratory Faculty of Medicine Universitas Sriwijaya which only had PCR-RFLP. The genetic frequency distribution of codon 315 in the case group was 65.38% wildtype and 34.62% mutant, whereas in the control group it was 92.86% wildtype and 7.14% mutant. The frequency of mutant genes in the case group is 34.62%, which represents the frequency of the 315 codon mutation in the katG gene of MDR TB patients in this study. This result is not in accordance with the study of Marva, et al (USA, 2015) which showed that the mutation of the katG gene in codon 315 occurred about 64%. This difference can occur because in this study we only examined point mutations in the 315 codon katG gene. It could be mutations that occur in our population not only at the codon, or also occur at other codon points that we did not do the examination in this study. Some studies that show mutations in codons include Nurul-Ain, et al (Malaysia, 2015), finding the katG gene mutation in codon 238.[18] Rosye & Yohanis research (Papua, 2016) found mutations in codon 316 and codon 290.[19] Mutant genes in codon 238.[18] the control group was found 7.14%. This illustrates that among the sample of sensitive TB patients there were 7.14% there were monoresistent cases of INH. The case of monoresistant INH was not detected before because the one examined for the diagnosis of MDR TB currently is TCM which only checks resistance to rifampicin drug (rpOb gene). The results of this study indicate that in sensitive TB patients,

there are around 7.14% that there has been a monoresistence of INH that cannot be diagnosed if it only relies on TCM examination. The codon 315 MTG gene mutation in this study had no effect on the incidence of MDR TB, with a p = 0.07 odd ratio of 6.8 (0.77 - 61.40, 95% CI). Although statistically the 315 codon MtG gene mutation mutation was not significantly different, but the 315 codon katG mutation had a 6.8 times risk of the risk of developing MDR TB (0.77 - 61.40, 95% CI).

Conclusion

In this study a 315 gene tuberculosis katG codon mutation was obtained, 34.62% but not significantly related to the incidence of MDR TB in South Sumatra.

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