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Research Article

Nitrogen Base Sequence Analysis and Characterization of Mutations in Gene Coding Region That Can Lead to High Levels of Resistance in Tuberculosis Patients in Jayapura, Papua Province-Indonesia

Rosye H. R. Tanjung[1] and Yohanis Ngili[2*]

[1]Faculty of Mathematics and Natural Sciences, Department of Biology, University of Cenderawasih, Jayapura, Indonesia; [2]Faculty of Mathematics and Natural Sciences, Department of Chemistry, Biochemistry Research Group, University of Cenderawasih, Jayapura, Indonesia

ABSTRACT

The disease of tuberculosis in Papua Province, Indonesia, is very high based on data at the Papuan Provincial Health Department. The geographical and demographic conditions of the Papuan population cause the eradication of the disease more difficult. One cause of the development of this disease is the presence of anti-tuberculosis drug resistance. This study was conducted by analyzing samples from sputum of TB patients. The increasing number of HIV/AIDS sufferers has caused TB disease, WHO categorize as reemerging disease, especially in Papuan province of Indonesia, the number of people with HIV/AIDS is highest in Indonesia. The purpose of this research is to get information on MDR-TB relationship with coding genes, and review the results of several studies of *M. tuberculosis* genotype on isolates in Jayapura, Papua Province-Indonesia. Here, we reported that a change in nucleotide C1363A (Pro535His) in sensitive *M. tuberculosis* of several antituberculosis drugs demonstrated that only a few mutations in the rpob gene caused resistant properties. The results of this research open a new path paradigm focused on mutations in the area of gene promoter and noncoding region.

Keywords: Characterization; Rpob512; RNA polymerase β-subunit resistance; Tuberculosis; Papua-Indonesia Province

INTRODUCTION

The provincial government of Papua, Indonesia, has passed various methods and techniques to reduce the high number of tuberculosis diseases. Various programs are conducted to inhibit lanju transmission of this disease and even suppress the number of drug resistance in patients. TB disease is characterized by tissue death (necrosis) caused by slow type hypersensitivity, namely the phagocytic process and epitope presentation (identification of antigens) by macrophage cells on the surface of the cell resulting in a series of processes that trigger the reaction of T lymphocyte cells. continues to increase in TB treatment and control is a multidrug-resistant *M. tuberculosis* (MDR-TB) isolate, defined by the world health agency WHO as a RIF and INH-resistant *M. tuberculosis* isolate. Treatment of TB patients is usually done by administering three types of antituberculous drugs with the main options being rifampin (RIF) and isoniazid (INH), then accompanied by streptomycin or pyrazinamide [1].

As the number of HIV/AIDS sufferers increases, WHO categorizes TB disease as reemerging disease. As a result of rpoB mutations, especially in hotspots or RRDR (rifampin resistance-determining region), RIF cannot inhibit RNA polymerase because it cannot bind to the β-subunit. Meanwhile, INH requires an activation process by the catalase-peroxidase enzyme that produced by *M. tuberculosis* [2,3]. Data from the provincial health offices of Papua-Indonesia, showing more than 95% resistant of *M. tuberculosis*-caused by mutation of rpoB and approximately 65% of *M. tuberculosis* resistant to INH are caused by katG mutations. This study was conducted by analyzing samples from sputum TB patients in Papua Province-Indonesia [4].

Correspondence to: Yohanis Ngili, Biochemistry Research Group, Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Cenderawasih, Jayapura, Indonesia, E-mail: joe_ngili@yahoo.com

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MATERIALS AND METHODS

Isolation, characterization of phenotype and genotype characterization

Samples were obtained from Dok 2 Jayapura hospital and Abepura General Hospital in Jayapura-Papua-Indonesia Province. Suspension isolates are made by introducing *M. tuberculosis* colonies into sterile tubes containing physiological and glassbead solutions so as to achieve turbidity of McFarland. This process is done in Biochemistry Laboratory, Department of Chemistry, University of Cenderawasih, Jayapura-Papua, Indonesia. Genotypic characterization process was carried out to analyze 4 genes of *M. tuberculosis*, two genes that produced membrane proteins and the other two were rpoB gene and other secondary areas, such as katG which caused the resistance of *M. tuberculosis* to RIF and INH. The result of this primer pair amplification is called the non-variable band because it must always exist to mark the running of the multiplex PCR process [5-8].

Determining the sequence of other gene nucleotides is done to see how these genes relate to MDR-TB resistant traits. Research on the coding region of this gene performed on several MDR-TB isolates in Papua province represented high-grade resistance and low resistance levels. Determination of this nucleotide sequence has been done by using the services of Macrogen Inc., Seoul, South Korea. The entire sequence of nucleotide sequencing data was analyzed by DNAstar program using various applications in it as well as some applications used in Biochemistry Research Group, University of Cenderawasih.

RESULTS AND DISCUSSION

Analysis of rpoB genotype

The rpoB gene has a length of 4.8 kb while the rpoB gene nucleotide sequence analysis is performed successively on the nucleotide sequence 1.5-1.7 kb. It is possible that the amount of printed DNA added to the PCR mixture is too much so that the PCR reaction shows non-specific results. The use of three primers on multiplex PCR causes the temperature for primer adhesion to be more than one type. The presence of non-specific bands on PCR multiplex results did not affect genotype analysis for rpoB531, and rpoB526 because this technique was only used to study the existence of a variable DNA fragment band of 0.18 kb and 0.17 kb for the codon: rpoB526 and rpoB531. DNA samples used in multiplex PCRs are the result of cell lysis that is not measured by its DNA concentration, causing the DNA concentrations in each sample of multiplex PCR reactions to vary. Approximately more than 90% of RIF-resistant isolates are caused by the mutation of rpoB gene in the region of 81 pb. This area is called a hotspot area and known as RRDR [9-12]. The frequent mutations caused changes i.e., (TCG become TTG) and (CAC become GAC) or Ser531Leu and His526Asp [13].

The results of this study indicate that approximately 85 MDR-TB isolates are known to have multiplexed PCR mutations, while 19% of undetectable MDR-TB isolates undergo rpoB526 or rpoB531 mutations. Different results were obtained from P3 isolate samples and some isolates from several hospitals in Jayapura, i.e., no mutation with multiplex PCR rpoB526 but electroferogram result showed adenin mutation to guanine at position 1576, A1576G (rpoB526 codon).

A number of 12 amino acid residues in RNA polymerase β-subunit are involved in interaction with RIF. The substitution of 11 out of the 12 amino acid residues will give rise to RIF-resistant properties [14-16] mentioning that more than 90% of RIF resistance occurs due to a genetic change in the 81 bp RRDR fragment of rpoB gene encoding the β-subunit RNA polymerase. Meanwhile, several studies have mentioned mutations of rpoB gene outside the RRDR can also cause resistance.

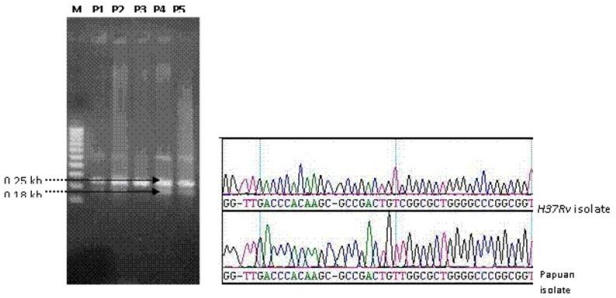

Figure 1: (left) The results of multiplex PCR gene rpob526 and rpob531. The results of the amplification of two bands 0.17 or 0.18 kb, and 0.25 kb indicated that the isolates were wild-type rpob526 or rpob531 as seen in isolates P3, P4, and other isolates while the results of one band amplification of 0.25 kb indicated mutant isolates rpob526 or rpob531 such as P2 isolates and other isolates; (M) 100 pb marker band; (right) Electroperogram of rpob gene on some isolates of MDR-TB. On graph shows the change in P1 nucleotide isolates compared to consensus isolates of *M. tuberculosis* H37Rv.

G1389C mutated mutations occur in isolates P5, P11, and P12 so it is suspected that this mutation is a form of rpoB *M. tuberculosis* gene polymorphism in addition to A1538T on isolate P1. Other bioinformatics researchers suggests that changes in rpoB gene residues at at RRDR 511, 512, 515, 521, and 529 positions do not significantly affect RIF's minimum inhibitory concentration MIC, but this study suggests that rpoB512 mutations can also cause high levels of resistance. Meanwhile, other research results suggest a change in 12 amino acids in the region (pocket) proteins resulted in the region being not in direct contact with the RIF.

The analysis of the determination of nucleotide sequence of isolate P4 was done in two directions, using primary and back primers and both showed the same result. Other studies were also conducted on the same genes [rpoB516]. The resistive nature of the RIF of the *M. tuberculosis* isolate acquired by Tracevska et al., is the probable outcome of [rpoB516], not [rpoB535] that the result of Tracevska et al., may be used to support the possibility [rpoB535] - which does not give rise to *M. tuberculosis* (Figure 1) [17-19].

Changes in rpoB526 and rpoB531 are mutations in the RRDR region which are widely known because they are most dominant

in RIF-resistant isolates. This study obtained 16 (38.1%) and 15 isolates (35.7%) for [rpoB526]- and [rpoB531]-, 3 isolates (7.1%) double mutants [rpoB526, rpo531]-, 3 isolates experienced mutations in other rpoB codons, and 5 isolates not detected experienced mutations in the rpoB segment analyzed, out of 42 MDR-TB isolates analyzed. The results of the Cummings et al., (2004) study of 103 RIF-resistant isolates showed 53, 31, and 20 isolates respectively for [rpoB531]- [rpoB526]- and other rpoB mutants. Meanwhile, only the katG315 mutation is known to cause resistance to INH, and this study only received 16 isolates [katG315]-.

The results of genotype characterization in the rpoB gene carried out in this study were lower than those reported by Pozzi et al., (1999) who conducted research in Italy, namely 56.7% of the rpoB531 mutation and 24.3% of the rpoB526 mutation occurred in isolates MDR-TB. Yue et al., (2003) who conducted research in China obtained 41% of MDR-TB isolates were [rpoB531]-, and the other 40% were [rpoB526]-. The research of Yue et al. is not different from the results of this study, namely the frequency [rpoB526]- and [rpoB531]- which occurs relatively balanced.

The primers we use in this study are as follows in (Table 1).

Table 1: Primer nucleotide sequences used in multiplex PCR.

Primer Name	Nucleotide Sequence	Temperature(° C)
Forward primer rpoB	5'-GTCGCCGCGATCAAGGA-3'	56
Reverse primer rpoB	5'-TGACCCGCGCGTACAC-3'	56
Inner primer rpoB531	5'-ACAAGCGCCGACTGTC-3'	48
Inner primer rpoB526	5'-GTCGGGGTTGACCCA-3'	50
Forward primer katG	5'-GCAGATGGGGCTGATCTACG-3'	64
Reverse primer katG	5'-AACGGGTCCGGGATGGTG-3'	60
Inner primer katG315	5'-ATACGACCTCGATGCCGC-3'	62

CONCLUSION

This study suggests that rpoB512 mutations can also cause high levels of resistance. Meanwhile, a change in some of the amino acids in the pocket area of the protein causes the area to be out of direct contact with the RIF. Most of the mutations occurring in the β-subunit RNA polymerase are found in region I (the position of amino acid residues 505 to 537) and region II (the position of amino acids 562 to 572). The results of the structural model analysis of RNA polymerase β-subunit proteins that bind RIF to T. aquaticus, show only a few amino acid residues that bind directly to RIF because they have the same polarity and can form bonds between nitrogen or oxygen, with RIF hydroxyl groups. Amino acid changes in this residue caused the greatest effect on the *M. tuberculosis* phenotype on the Mtb isolate in Papua Province of Indonesia.

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