RESEARCH ARTICLE

Molecular Docking Analysis of the T450A Mutation of the Gene rpoB from Leprosy Patients in Papua, West Papua and North Maluku, Indonesia

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ABSTRACT:
Leprosy persists to be a health problem in Indonesia, especially in the provinces of North Maluku, West Papua and Papua. Early diagnosis and complete treatment with multidrug therapy (MDT) remain the key strategy for reducing the disease burden. One of the major components of MDT is rifampicin which in certain cases in several countries, M. leprae resistance to this drug issue has been reported albeit only a few. This research aimed to detect and analyze polymorphism in M. leprae rpoB gene that was isolated from leprosy patients in three provinces: North Maluku Province, West Papua Province and Papua Province, Indonesia. The identification of mutations in the M. leprae rpoB gene was carried out by aligning the results of DNA sequencing with the reference strain. The 3D structure of rpoB was derived using the Swiss Model. The T450A, S456L, and H451Y variants of RNA Polymerase B subunits were constructed using FoldX based on the wild-type structure. The structures were repaired, and protein stability was evaluated using foldX under the Yasara viewer. The QC of the rpoB M. leprae homology models was conducted with Ramachandran Plot modeling using PROCHECK. The difference in binding affinity between native protein and T450A, S456L, and H451Y variants were analyzed using molecular docking. rpoB gene of M. leprae contains a mutation found in nucleotide of 1348 bp. The mutation triggered the conversion of the amino acid Threonine to Alanine in the amino acid to 450 rpoB subunit B. The structure of 3D RNA Polymerase Subunit B was constructed using rpoB Mycobacterium tuberculosis with PDB code 5UH5 as template. According to Ramachandran Plot, the percentage of residues in the most favored regions are 91.9%, and there was no significant number of residues in the disallowed regions. The results of molecular docking showed that the T450A variant had the same binding affinity with the native protein which was -8.9 kcal. Binding affinity on the S456L and H451Y variants increased by -7.3 kcal and -8.2 kcal, respectively. According to Molecular Docking analysis, T450A variant did not affect the energy binding between RNA polymerase and rifampicin.

KEYWORDS: Mycobacterium leprae, rpoB, rifampicin.
Papua (11,48), North Maluku (4.54), and Papua (4.06)2-3. Moreover, the prevalence of leprosy in other countries such as India is high as well9.

Early diagnosis and complete treatment with multidrug therapy (MDT) remain the key strategy for reducing the disease burden of leprosy1. One of the major components of leprosy MDT is rifampicin5, which in certain case in several countries, M. leprae resistance toward this drug issue has been reported albeit only a few1. Rifampicin has been standardized and formulized for leprosy, thus could be combined with another treatments6-9. Rifampicin also utilized in tuberculosis and hepatic damage patients as well, so this is considered semi-broad spectrum antibiotic10-11. The number of leprosy patients tested for resistance globally is too small to allow for accurate estimates of drug resistance. However, several high-burden countries have reported cases of drug resistance among new and previously treated patients1-12. Rifampicin resistance case astonishingly exists even at the low level, not only for relapsed case but also novel case13. Despite of the current low rate of resistance, this circumstance provides crucial reason for AMR 

The most important problem in drug-resistant detection of leprosy is M. leprae remains unable to be cultured by conventional method16,17. The availability of genomic sequences from M. leprae and increased understanding of the genetic basis of drug resistance in mycobacteria led to the development of molecular methods for the detection of mutations associated with dapsone, rifampicin, and fluoroquinolone. Some missense mutations (mutations conferring an amino acid change) confer resistance to dapsone (mutation in the folP1 gene), rifampicin (mutation in the rpoB gene) and the quinolones (mutation in the gyrA gene). As these mutations are clustered within each respective gene, regions determining drug resistance were described as dapsone resistance-determining region (DRDR), rifampicin resistance-determining region (RRDR) and quinolone resistance-determining region (QRDR)14. The sequencing result was aligned with M. leprae TN (NC_002677.1 GenBank) to determine the presence of drug resistance mutations. The DRDR area for rifampicin is at about 1225 bp to 1503 bp in the rpoB gene17.

rpoB gene codes the synthesis of RNA polymerase which is the main target of rifampicin. The binding site for rifampicin has been located at the β subunit encoded by the rpoB gene18. Substitution of key amino acids would thus result in conformational changes and defective binding of the drug19. During Nisha’s team research, they have used the computational approach to investigate the molecular and structural properties of the RMP binding to both native and mutant S425L rpoB20. Furthermore, a lot of of study analyzed the presence of SNP within rpoB gene through Bioinformatics approach19-21. More specifically, the molecular docking protocol will be devised to observe whether the conformation and binding between the protein and the drug will be altered accordingly24,25. Thus, this research aimed to detect and analyze polymorphism in M. leprae rpoB gene that was isolated from leprosy patient in three provinces; North Maluku Province, West Papua Province and Papua Province, Indonesia.

MATERIAL AND METHODS:

This study was a cross section and has received approval from the Ethics Committee of Research and Development of Health Ministry of Indonesia. The study sample was the extracted DNA from ear incision taken from leprosy patients then examined molecularly. Molecular examination begins with DNA extraction process using Qiagen Kit, followed by Polymerase Chain Reaction (PCR) process. The PCR reagent mix composition consisted of Go Taq Green Master Mix 2X, set primers (forward and reverse) 10 pmol, DNA template 0.5μg and nuclease free water. The primers used to amplify the rpoB gene in this study were R35'-CAATATCCGTTCCGGTGTC-3' as a forward primer and R4 5'-GTATTCGATCTCGTGCAGTA-3' as reverse primers. PCR was adjusted with pre-denaturation temperature at 95°C for 5 minutes, denaturation at 94°C for 1 minute, annealing at 60°C for 30 seconds, extension at 72°C for 1 minute and final extension at 72°C for 10 minutes. The sequencing process uses R5 5'-ACGCTGATCAATATCCGTCCGGTGGTC-3' forward sequencing primers and R6 5'-CGACAA TGAACCGATCAGAC-3' reverse sequencing primer15. The PCR conditions were arranged in sequence, pre-denaturation at 94°C for 5 minutes, denaturation at 95°C for 1 minute. Annealing at 60°C for 30 seconds, Extension at 72°C for 1 minute, and final extension at 74°C for 10 minutes. The cycle was repeated for 40 cycles. ExoSap IT purified the PCR result with a ratio of 2:5. A sequencing process followed the purified PCR product. The sequencing cycle uses BigDye Terminator v3.1 5X, BigDye Terminator buffer 5X, 100 ng molded DNA, and primer 0.8 pmol. DNA pGEM-3Zf was used as a positive control and Primary control -21 M13 as a positive control primer. The reaction of the sequencing cycle was carried out under conditions: 96°C 1 min, 96°C 10 sec, 50°C 5 sec, 60°C 4 min. The cycle was repeated 25 times later the result of the sequencing cycle is purified by XTerminator Solution and SAM solution 5:22,5. The sample volume
used is 5μL. The tube contained of premix got the vortexed sample for 30 minutes then in the centrifuge for 1 minute. The supernatant was inserted into a 20μl wellbore slab and read by using 3500 Genetic Analyzer. The sequencing results were then processed in the gene bank to identify the presence of mutations in the rpoB gene.

**Homology modeling of rpoB M. leprae:**
The rpoB amino acid sequence (accession number NP_301284.1) was downloaded from Mycobrowser server ([https://mycobrowser.epfl.ch/](https://mycobrowser.epfl.ch/)) in FASTA format. The 3D structure of rpoB was derived using Swiss Model ([https://swissmodel.expasy.org/](https://swissmodel.expasy.org/)). The T450A, S456L, and H451Y variants of RNA Polymerase Subunit B were constructed using FoldX26, based on the wild-type structure. The structures were repaired, and protein stability was evaluated using foldX under the Yasara viewer.

**Quality Control (QC) of the Homology models:**
The QC of the rpoB M. leprae homology models was conducted with Ramachandran Plot modeling using PROCHECK Server in [https://servicesn.mbi.ucla.edu/PROCHECK/](https://servicesn.mbi.ucla.edu/PROCHECK/) based on the established protocol27.

**T450 Mutation Effect Prediction:**
Interatomic interaction prediction between native protein and mutant was accomplished using Dynamut9 server ([http://biosig.unimelb.edu.au/dynamut/](http://biosig.unimelb.edu.au/dynamut/)).

**Molecular docking:**
Docking analysis was performed by Auto Dock Vina28, which is integrated with a PyRx application ([https://sourceforge.net/projects/pyrx/](https://sourceforge.net/projects/pyrx/)). Docking results were visualized using PyMol 1.8.6 and Biovia Discovery Studio 2016 Client. The structure of rifampicin was obtained by separating the rifampicin’s molecules that bounded within the crystal of M. tuberculosis RNA-polymerase (PDB: 5UHB).

**RESULT AND DISCUSSION:**
Detection of mutations in the rpoB M. leprae gene associated with rifampicin resistance necessarily needs to be performed. It is useful to comprehend the effectiveness of rifampicin as one of the components of MDT in the fight against leprosy. Papua is one of the provinces with high leprosy burden so that any information about mutations, especially rpoB gene that can be used to accelerate leprosy elimination in the Papua Province. Leprosy treatment in Indonesia is in accordance with the treatment program by WHO, actually with Multi Drug Therapy (MDT). MDT consists of rifampicin, dapsone and clofazimine (lamperne)29. Resistance to anti-leprosy drugs, such as dapsone, rifampicin and fluoroquinolones, has been described since 1967 using in vivo models30. However, this method requires a long time and expensive cost31. WHO recommends drug resistance detection for MDT by PCR and direct DNA sequencing. A comprehensive understanding of the molecular consequences of these mutations can provide valuable insight into how resistance develops and the pre-emptive identification of likely resistance mutations32.

Detection of rpoB mutations was performed on leprosy patients who had been approved or who had not been approved by MDT. Of the total 200 patients found, 1 polymorphism was found in the M. leprae rpoB gene. The obtained polymorphism was the conversion of Alanine to Guanin in the nucleotide of 1348bp (Fig. 1). Eventually the existence of polymorphism found within Jayapura-originated M. leprae rpoB gene revealed after alignment with reference sequence (M. leprae TN (NC_002677.1). These changes in nucleotides caused alteration in the amino acid Threonine to Alanine (T450A) (Fig. 3). Mutations were revealed in a mixed state between wild type and mutant types. Mixing sequences in the rpoB and gyrA genes from leprosy patients had also been approved in previous studies33,34.

**Fig. 1:** Strains Alignment comparison between Papuan M. leprae with M. leprae TN which presents two different point mutation in a single sequence. The mutations were observed in nucleotide 1348 bp and nucleotide 1467 bp.
Fig. 2: Alignment between *M. leprae* strains from Papua and *M. leprae* TN revealed two mutation points within a sequence. This mutation was provided by an electropherogram that exhibited in a mix sequence (bottom) when compared to wildtype (top). A mix consisting of Guanine (mutant) and Adenine (wildtype); B. mix sequences consist of Guanine (wild type) and Adenine (mutant).

The revealed mutations were in mixed sequences where in certain position found two peaks of electropherogram. Thus, indicated that there were two different strains within a single patient, mutant type and wild type (Fig. 2).

The quality control of the homology modeling result was analyzed with the Ramachandran plot using PROCHECK software package. The percentage residues in the most favored regions are 91.9%, and no significant number of residues in the disallowed regions (Fig. 4).

Fig. 3: *M. lepra* wild type and mutant type amino acid sequence comparison. The amino acid alteration was Threonine to be alanine. The amino acid alteration was triggered by the mutation in the first point whereas the second mutation point triggered no mutation (nonsense mutation).

Fig. 4: The plot already shown that the protein model is indeed stable enough, as it did not fare beyond 10% residues in the unfavorable region. In this regard, the protein model is usable and could leverage the further molecular simulation protocol.
The picture below shows prediction of interatomic interaction between native protein and mutant T450A *M. leprae*. The structure difference of native Threonine to Alanine compared to mutant type exhibited slightly different atomic interaction (Fig. 5). It could be seen that based on the Fig. 5, the native is observed to have at least 8 hydrogen bonds, while the mutant T450A is having at least 7 hydrogen bonds. Although the sum amount of bonding is similar, there are slight variations.

![Native and mutant residues are colored in light-green and are also represented as sticks alongside with the surrounding residues which are involved on any type of interactions](image)

The effect of the T450A mutation was analyzed by the molecular docking approach. As a comparison, mutants that have been confirmed to be resistant to rifampicin are H451Y and S456L. The results of molecular docking were displayed in the form of interactions between the residues that built up *M. leprae* rpoB and rifampicin. Types of interactions included hydrogen bonds, conventional hydrogen bonds, carbon hydrogen bonds, unfavorable donor, Alkyl, Pi-Alkyl (Fig. 4). Comparison of binding energy between native protein, T450A mutants, H451Y and S456L with rifampicin shows that native protein and T450A mutants possessed the similar energy while H451Y and S456L mutants had increased energy (Table 1).

![Rifampicin norma](image)

Rifampicin normally binds to RNA polymerase, which inhibits the transcription process in bacteria. A change in the order of nucleotides (mutations) in the gene causes rifampicin not to bind to RNA polymerase so that the transcription process in bacteria persists leaves the effects of rifampicin in leprosy patients to be ineffective. The effect of the T450A mutant has not been confirmed in vivo, so that our team analyzed the effect of polymorphism by using the in-silico approach. Homology modeling of *M. leprae* rpoB using Swiss server model was ensured if their modeling result is in accordance to the Ramachandran plot quality control protocol. The template used was rpoB *Mycobacterium tuberculosis* with PDB code 5UH5. Prediction of interatomic interaction between native and mutant T450A on the Dynamut server exhibited less ionic interaction on T450A mutants compared to native protein (Fig. 4).

![Figure Description:](image)

| S. No | variant          | Stability kcal/mol | Binding affinity |
|------|------------------|--------------------|------------------|
| 1    | Native           | 505.43             | -8.9             |
| 2    | S425L (S456L)    | 501.86             | -7.3             |
| 3    | H420Y (H451Y)    | 504.25             | -8.2             |
| 4    | T419A (T450A)    | 504.23             | -8.9             |
In the Fig. 5, it is shown that the variant that did not obtain unfavorable Donor-Donor interaction, and having pi-Cation (S456L), is the one that having the most positive binding affinity. It means that those residues are interfering the affinity between the protein and the ligand.

Crystallographic data of \textit{M. tuberculosis} rpoB interacts with rifampicin through hydrogen bonds in residues Q435, Q438, F439, H451, R454 and S45638. According to the results of docking, the interaction between rifampicin and RNA polymerase in native protein and T450A variants was less significant. Hydrogen bonds formed in native protein occur in ARG173, GLN438, PHE439, ARG454 and SER 456 residues. In the T450A variant, hydrogen bonds were slightly reduced. However, hydrogen bonds still occurred in ARG173, PHE439, and SER 456. In the S456L variant, no hydrogen bonds are formed. It formed at LEU456 residue, but it becomes another interaction, the Pi-Alkyl. The H451Y variant has three hydrogen bonds, namely ARG173, PHE439, and SER 456 (Fig. 5). In the binding energy calculation, the T450A and native variants had the similar binding energy which is -8.9 kcal/mol. This indicated that the T450A mutant did not affect the binding energy between RNA polymerase and rifampicin. H451Y and S456L mutants have increased their binding energy respectively to -7.3 kcal/mol and -8.2 kcal/mol compared to native protein (Table 1). Both of these variants have been confirmed to be resistant to rifampicin19-21. In another study also found the difference of binding energies observed in the docking study, especially that RMP is less effective in the treatment of M. tuberculosis strain compared with rifampicin. H451Y and S456L mutants have increased their binding energy respectively to -7.3 kcal/mol and -8.2 kcal/mol compared to native protein (Table 1).

**CONCLUSION:**

We found a T450A mutation in the rpoB \textit{M. leprae} gene from Papua. Based on in silico analysis, these mutations did not affect sensitivity to rifampicin. However, this vivo test is still needed to confirm the effect of the T450A mutation.

**ACKNOWLEDGEMENT:**

Thanks to the National Institute of Health Research and Development for funding this research. Thanks also to the Head of Papua Institute of Research and Development Center for Biomedicine that allows researchers to conduct research in its laboratory. Then, the heartfelt support of the Institute of Research and Community Empowerment (LPPM) of the Indonesia International Institute for Life Sciences (I3L) should be highly appreciated. The last but not least we would like to thank Sister Vera Yoku and Olief Robaha for assisting in research’s sampling.

**CONFLICT OF INTEREST:**

The authors declare no conflict of interest.

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