MDR TB–The Lethal Sound of Silent Mutations

Saeeda Baig

Department of Biochemistry, Ziauddin University, Karachi, Pakistan

The emergence of the multidrug-resistant tuberculosis (MDR-TB) strains, poses a serious health challenge to developing countries, including Pakistan, which is the 4th highly vulnerable MDR-TB country in the world. The consistent increase in annual TB rates is due to the emergence of resistance of MDR-TB strains to isoniazid (INH), rifampicin (RIF), streptomycin, pyrazinamide, and ethambutol, the first-line drugs for TB control. In Pakistan, approximately 570,000 TB cases were reported in 2019 with an incidence rate of 263/100,000. It is attributed mostly to a high burden of MDR-TB, RR-TB (rifampicin resistant), as well as isoniazid resistance. Prevalence of pre-XDR-TB (pre-extensive drug resistance) is also reported, including MDR-TB, fluoroquinolone and amikacin, kanamycin, and capreomycin, the second-line injectales.

Research around the world has identified that tuberculosis drug resistance is not only caused by inadequate or failed treatment but also due to the molecular basis of drug resistance which includes data on silent mutations concerning first-line TB drugs, leading to transmission of resistant strains. Silent or synonymous mutations, such as those on codon 514 of *rpoB,* warns the emergence of *Mycobacterium tuberculosis* rifampin-susceptible strains.[1] Studies from Pakistan have also reported silent mutations at various codons. Sequence analysis of MDR-TB isolates resistant to isoniazid and rifampicin, at codon 528 (CGC to CGT) in the *rpoB* gene, a silent mutation detected in the RRDR region.[2] Another study from Pakistan reported 531 and 513 codon mutations on *rpoB* gene. A double mutation in the *rpoB* gene was observed in 12% of the cases. Mutations at codon 315 and 299 *of katG* gene were observed. The control group had 28.6% MDR positive cases, whereas, the treated group were 100% positive.[3] In 2022, the tuberculosis isolated data from Pakistan, from 2003 to 2020 was assessed by using the whole genome sequencing. *Microbacterium tuberculosis* genetic diversity showed the most significant association with the *nusG* gene, the potential transmissible phenotype ($p = 5.8 \times 10^{-10}$) and the cause of circulating drug resistance mutations in Pakistan.[4]

Correspondence to: Prof. Saeeda Baig, Department of Biochemistry, Ziauddin University, Karachi, Pakistan
E-mail: saeeda.baig@zu.edu.pk

Received: June 07, 2024; Revised: July 19, 2024; Accepted: July 24, 2024
DOI: https://doi.org/10.29271/jcpsp.2024.08.867

Studies from Iran reported by Norouzi *et al*. showed silent mutations on other codons.[5] They detected in one of the MDR isolates, in the *inhA* gene at nucleotide 649 showing L649L a silent synonymous substitution. Rifampin susceptible studies conducted in Mexico, using the mycobacteria growth indicator tube (MGIT) system, showed rifampin-resistance in 13 isolates tested through Xpert® MTB/RIF assay. A discrepancy in results was observed during the DNA sequencing analysis. A silent mutation, P514P was detected in 7 (53.8%) isolates, whereas, three different types of missense mutations (S531L, D516Y, and L511P) were observed in three isolates and three were without any mutation. The researchers concluded that DNA sequencing through specialised centres should be necessitated for all Xpert® MTB/RIF-diagnosed rifampin-resistant cases for early detection of the emergence of disputed, silent mutations or hetro-resistance.[6]

The emergence of resistant strains is attributed to the mutations in the genome of *Mycobacterium tuberculosis*, altering some genes in the targeted anti-tuberculosis drugs, including *rpoB* gene and *katG* gene. Researchers submitted resistant clinical isolates to DNA sequencing to determine the frequency of disputed and silent mutations. The resistance mechanism which develops in Mycobacterium tuberculosis is generally different from other bacteria. It has been found to be associated with the structural changes in the genome of *Mycobacterium tuberculosis*.

Silent or synonymous mutations are considered benign or neutral ever since the 1960s, when the genetic code was resolved that protein sequence is not altered because of their presence. They are especially ignored in the studies where investigations are being done on disease-causing mutations. Some recent researchers claimed that the synonymous mutations are not benign. To check this hypothesis, Shen *et al.* conducted an experiment in 2022 by introducing mutations in yeast cells.[7] Using CRISPR/Cas9 genome editing, they targeted 21 genes for nonsense, non-synonymous or synonymous mutation, and constructed 8,000 mutant strains in yeast cells. They were surprised to find out that in contrast to synonymous mutations, 22.8% were neutral, 75.9% of them were significantly harmful, whereas 1.3% were significantly beneficial.

Researchers have discovered that synonymous mutations can influence and alter the genetic process of translation, starting from initiation by binding of transcription factor (TF), altering transcriptional process, from splicing of pre-mRNA to its folding and stability, hindering initiation, and efficiency of translation totally modifying the protein sequence with loss of function.[8,9]

These findings imply that identifying synonymous mutation is equally important since they, like non-synonymous mutations, are also virtually involved in causing some diseases. Worldwide studies show a region-specific continuous evolution of MDR-TB. A comprehensive research evaluation is required for the detection of mutations and the development of effective strategies for TB control. Since MDR-TB is linked to genomic variants that alter the basic mode of action causing TB resistance, it is, therefore, possible by understanding the basic changes in drug targets genotypic resistance to various drugs could be predicted and used for treatment. Identification of lineages of virulent strain types and drug resistance will help in phylogenetic studies of transmission clusters to assist in targeting the source.[4]

REFERENCES

1. Alonso M, Palacios JJ, Herranz M, Penedo A, Menendez A, Bouza E, *et al*. Isolation of mycobacterium tuberculosis strains with a silent mutation in rpoB leading to potential misassignment of resistance category. *J Clin Microbiol* 2011; **49(7)**:2688-90. doi: 10.1128/JCM.00659-11.
2. Qazi O, Rahman H, Tahir Z, Qasim M, Khan S, Ahmad Anjum A, *et al*. Mutation pattern in rifampicin resistance determining region of rpoB gene in multidrug-resistant mycobacterium tuberculosis isolates from Pakistan. *Int J Mycobacteriol* 2014; **3(3)**:173-7. doi: 10.1016/j.ijmyco.2014.06.004.
3. Aftab A, Afzal S, Qamar Z, Idrees M. Early detection of MDR Mycobacterium tuberculosis mutations in Pakistan. *Sci Rep* 2021; **11(1)**:16736. doi: 10.1038/s41598-021-96116-x.
4. Napier G, Khan AS, Jabbar A, Khan MT, Ali S, Qasim M, *et al*. Characterisation of drug-resistant mycobacterium tuberculosis mutations and transmission in Pakistan. *Sci Rep* 2022; **12(1)**:7703. doi: 10.1038/s41598-022-11795-4.
5. Norouzi F, Moghim S, Farzaneh S, Fazeli H, Salehi M, Nasr Esfahani B. Significance of the coexistence of non-codon 315 katG, inhA, and oxyR-ahpC intergenic gene mutations among isoniazid-resistant and multidrug-resistant isolates of Mycobacterium tuberculosis: A report of novel mutations. *Pathog Glob Health* 2022; **116(1)**:22-9. doi: 10.1080/20477724.2021.1928870.
6. Rafael LL, Raquel MS, Rogelio FA, Miroslava FP, Isabel JGA, Paola RTS. Discordant results between genotypic and phenotypic assays (Xpert MTB/RIF *vs.* BACTEC MGIT 960 system) for detection of RIF-resistant *Mycobacterium tuberculosis* isolates in a high burden region. *Infect Genet Evol* 2021; **96**. doi:10.1016/j.meegid.2021.105142.
7. Shen X, Song S, Li C, Zhang J. Synonymous mutations in representative yeast genes are mostly strongly non-neutral. *Nature* 2022; **606(7915)**:725-31. doi: 10.1038/s41586-022-04823-w.
8. Chamary JV, Parmley JL, Hurst LD. Hearing silence: Non-neutral evolution at synonymous sites in mammals. *Nat Rev Genet* 2006; **7(2)**:98-108. doi: 10.1038/nrg1770.
9. Walsh IM, Bowman MA, Soto Santarriaga IF, Rodriguez A, Clark PL. Synonymous codon substitutions perturb cotranslational protein folding *in vivo* and impair cell fitness. *Proc Natl Acad Sci U S A* 2020; **117(7)**:3528-34. doi: 10.1073/pnas.1907126117.