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Original Article

Phenotypic Low-level Isoniazid Resistance as a Marker to Predict Ethionamide Resistance in *Mycobacterium tuberculosis*

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Abstract

Background: Tuberculosis is one of the most prevalent diseases in Pakistan. Pakistan has the highest burden of MDR-TB in the Eastern Mediterranean region. Ethionamide is an anti-tuberculous drug frequently used to treat MDR-TB. Its drug susceptibility testing is not easily available in resource limited settings. Since it acts on the same target protein as isoniazid (inhA protein encoded by inhA gene), we sought to find out if phenotypic isoniazid resistance can be a marker of ethionamide resistance. **Materials and Methods:** This was a retrospective observational study conducted at the Aga Khan University hospital section of microbiology. Data was retrieved between 2011 to 2014 for all culture positive MTB strains. All culture positive MTB isolates with susceptibilities to isoniazid and ethionamide recorded were included in the study. Isoniazid and ethionamide susceptibilities were performed using agar proportion method on Middlebrook 7H10 agar. Rate of Ethionamide resistance between low-level isoniazid resistant, high level isoniazid resistant and isoniazid sensitive MTB was compared. **Results:** A total of 11,274 isolates were included in the study. A statistically significant association ($P < 0.001$) was found between Ethionamide resistance and low-level isoniazid resistance (26.6%) as compared to high-level isoniazid resistance (8.85%) and isoniazid sensitivity (0.71%) in MTB strains. However this association was not seen in XDR-TB strains. **Conclusion:** Low level isoniazid resistance may be used as marker for phenotypic ethionamide resistance and hence guide clinicians' choice of antituberculous agent for MDR-TB in Pakistan. Further studies involving detection of genotypic association of isoniazid and ethionamide susceptibilities are needed before a final conclusion can be derived.

Keywords: Ethionamide, isoniazid, *Mycobacterium tuberculosis*

Introduction

Antituberculosis (TB) drug resistance is a major public health problem. Ethionamide is a Group 4 anti-TB antibiotic. It is a structural analog of isoniazid and acts on the same target as isoniazid, i.e., *inhA* protein. The association of mutations in the promoter region *inhA* gene with isoniazid resistance is well established.[1] Cross-resistance between isoniazid and ethionamide has been documented in other regions.[2,3]

In this study, we aimed to seek if low-level isoniazid resistance could be used as a surrogate marker to predict ethionamide resistance and whether it can guide clinicians' choice of selecting antituberculosis therapies to manage multidrug-resistant TB (MDR-TB) patients.

Material and Methods

This was a retrospective study conducted in the Microbiology Section of Aga Khan University Hospital clinical laboratories.

The AKUH mycobacteriology laboratory is a WHO Supranational Reference Laboratory for TB and receives samples from across the country. Laboratory data of samples received from 2011 to 2014 were retrieved, and all culture-positive *Mycobacterium tuberculosis* (MTB) isolates with susceptibility testing performed for isoniazid and ethionamide were included in the study. Drug susceptibilities were performed using agar proportion method on Middlebrook agar 7H10 using isoniazid in concentration of 0.2 μg/ml and 1 μg/ml and ethionamide 5 μg/ml. Previous studies have shown that although testing two different concentrations of isoniazid does not alter the overall susceptibility results; it can help to predict cross-resistance between isoniazid and its structural analogs.[4] Sensitivity, specificity, negative predictive value (NPV), and

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positive predictive value (PPV) of isoniazid resistance for determining ethionamide resistance were calculated. Subgroup analysis to determine the rate of ethionamide resistance in low- and high-level isoniazid-resistant MTB strains was also calculated. Exemption to ethical approval was granted by the Ethical Review Committee, Aga Khan University.

Results

During the study period, records of 17,184 strains were recovered from the laboratory database. Among these, isoniazid and ethionamide susceptibility data were available for 11,274 MTB strains which were included in the analysis, whereas 5910 strains had incomplete information and were excluded from the study. Strains from all across Pakistan were included; 3080 isolates from Karachi, 1437 from Lahore, 1187 from Peshawar, 145 from Islamabad, and 505 from Quetta.

Out of 11,274 strains, 5939 were isoniazid resistant (including both low level and high level) and 5335 were isoniazid sensitive. Ethionamide resistance was seen in 629 (5.57%) strains [Table 1].

Sensitivity, specificity, NPV, and PPV of isoniazid resistance as a marker of ethionamide resistance were 93.80%, 49.76%, 99.27%, and 9.93% with a significant $P < 0.001$. As *inhA* mutation is associated with low-level isoniazid resistance, we further divided the isoniazid-resistant MTB strains into low- and high-level isoniazid-resistant groups and compared the rate of ethionamide resistance. Our findings showed ethionamide resistance to be 26.6% in low-level isoniazid-resistant MTB strains as compared to 8.85% in high-level isoniazid-resistant MTB strains [Figure 1].

A subgroup analysis was performed by further dividing low- and high-level isoniazid-resistant MTB strains into non-MDR, MDR, preextensively drug-resistant (XDR), and XDR groups. Among strains with low-level isoniazid-resistant (non-MDR, MDR, and pre-XDR) rate of ethionamide resistance was 26%–28% [Table 2]. Moreover, low-level isoniazid resistance was significantly associated with ethionamide resistance ($P < 0.001$) among all groups except XDR-TB strains.

Discussion

TB is a global health concern. According to the WHO report 2015, the prevalence of TB in Pakistan is about 361 cases/100,000.[5] It currently ranks fifth among the high TB burden countries, and although studies have shown nonuniformity in drug resistance rates among different regions in a country,[6] Pakistan still has the fourth highest prevalence of MDR-TB globally.[7] MDR-TB is caused by MTB resistant to at least isoniazid and rifampicin (RIF). Agents used to treat MDR-TB include second-line drugs; amikacin, kanamycin,

Table 1: Frequency of ethionamide resistance in low- and high-level isoniazid-resistant and isoniazid-sensitive *Mycobacterium tuberculosis* strains

	Ethionamide resistance	Ethionamide sensitive	Total
Low-level isoniazid resistant	96	264	360
High-level isoniazid resistant	494	5085	5579
Isoniazid sensitive	39	5297	5335

Table 2: Comparison of ethionamide resistance between different classes of isoniazid-resistant *Mycobacterium tuberculosis*-C

Classes of INH-resistant MTB-C	Frequency	Ethionamide resistance (%)	χ^2 (*P*)
Non-MDR			
High level	596	24 (4.02)	<0.001
Low level	210	55 (26.19)	
MDR			
High level	2143	176 (8.2)	<0.001
Low level	80	21 (26.26)	
Pre-XDR			
High level	2586	227 (8.77)	<0.001
Low level	64	18 (28.12)	
XDR			
High level	254	67 (25.3)	0.7029
Low level	6	2 (33.3)	

MTB: *Mycobacterium tuberculosis*, MDR: Multidrug-resistant, XDR: Extensively drug-resistant, INH: Isoniazid

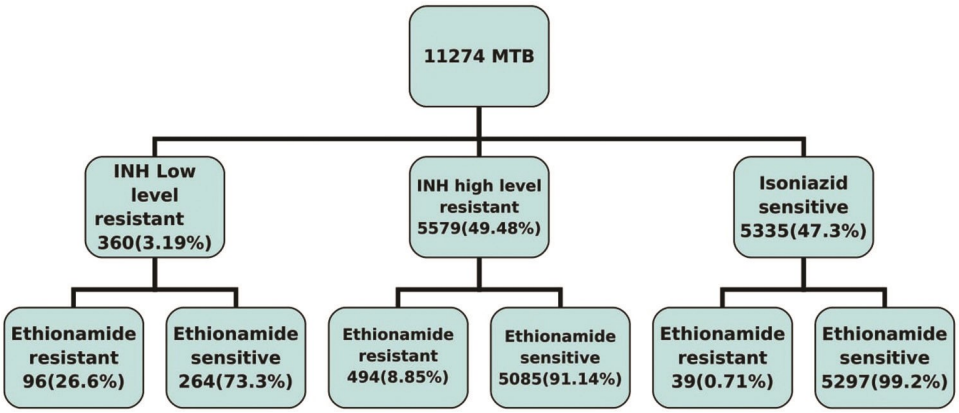

Figure 1: Flow diagram representing total number of MTB strains included in the study and their devision into different sub groups.

capreomycin, fluoroquinolones, cycloserine, and thioamides: ethionamide and prothionamide.[8]

Ethionamide is readily available in Pakistan and considering its low cost and oral route of administration is one of the frequently used agents to treat MDR-TB. It is available as a pro-drug which undergoes activation by *ethA* enzyme to convert into its active form. It functions by disrupting the mycobacterial cell wall. Ethionamide is structurally related to isoniazid and both drugs act on the same target site, i.e., *inhA* protein. *inhA* protein, encoded by *inhA* gene, is a nicotinamine adenine dineucleotide-dependent enoyl-acyl carrier protein, responsible for fatty acid chain elongation and mycolic acid synthesis. Association of ethionamide resistance with *inhA* promoter and gene mutations has been already been explored previously.[9,10] Our findings are consistent with previous studies which have shown a higher rate of ethionamide resistance among MTB strains with low-level isoniazid resistance and *inhA* mutation.[11,12] High incidence of ethionamide resistance in MTB strains showing mutations in *inhA* promoter and *inhA* gene has also been reported from a study in India.[13]

Genetic diversity among MTB exists, and the distribution of resistance gene may alter in different geographic areas.[14] Molecular tests are recommended by the WHO which can be used to rapidly detect mutations associated with resistance in MTB. These include Xpert MTB/RIF and line probe assays (LPAs).[15] Currently approved Xpert MTB/RIF only detects mutation in the *rpoB*; however, LPAs can detect mutations in *rpoB* along with *katG* and *inhA* newer version of LPA (MTBDRsl) for second-line drugs has also been approved by the WHO which can detect resistance in fluoroquinolones and injectable anti-TB drugs by detecting mutations in gyrA/gyrB and rrs/eis, respectively, but does not detect mutations associated with ethionamide resistance. Previous studies have employed LPAs as a rapid means of detecting *inhA* mutation and associated phenotypic ethionamide resistance.[16] Knowledge regarding the presence or absence of *inhA* mutation using rapid molecular test such as LPA can be useful in enabling clinicians to decide in a timely manner whether to prescribe ethionamide for the treatment MDR-TB. Since LPAs are increasingly being used as a rapid test for the detection of MDR-TB, our results suggest that in this population, detection of *inhA* mutations on LPA increases the possibility of ethionamide resistance and highlights the need for drug sensitivity testing. Ethionamide resistance is not only attributed due to mutations in inhA gene but also due to *ethA* and *ethR* gene mutations:[17,18] the *ethA* protein is itself regulated by ethR protein encoded by the *ethR* gene. There is thus also a need to explore the presence or absence of these mutations in MTB strains from our region, and to study the extent to which ethionamide resistance in our population is attributed to *inhA* or *ethA*/*ethR*.

Our study is limited by its retrospective nature and a single-center setting. However, the study was conducted on a large sample size and included strains from across the country. Mutations associated with isoniazid and ethionamide resistance were not explored, and studies evaluating the prevalence of *inhA*, *ethA,* and *eth*R mutations in addition to *inhA* are needed to better understand the association between ethionamide resistance and low-level isoniazid resistance in this population.

Conclusion

In our population, low-level isoniazid resistance may predict phenotypic ethionamide resistance and hence guide clinicians. However, molecular studies detecting *inhA* and other mutations are further needed before definitive conclusions can be drawn.

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Conflicts of interest

There are no conflicts of interest.

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