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To Determine the Percentage of Isoniazid Monoresistant Tubercular Mutations Via Kat G V/S INH A Genes.

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ABSTRACT

Background: Some important challenges for TB control strategies include the increasing prevalence and rapid distribution of drug-resistant TB. Recently, this concern has been further intensified by reports of multi drug resistant (MDR) and extensively drug resistant-TB (XDR-TB). Although resistance to first and second line drugs poses the important risk to patients, resistance to isoniazid (INH) alone is also important. INH is the most potent anti-TB drug and is the main part of any first-line treatment regimen for TB. Our objective is to determine the percentage of isoniazid monoresistance mutations via Kat G v/s Inh A gene. **Methods:** We conducted a retrospective record review of 100 INH monoresistant TB patients without rifampicin resistance registered during Feb 2017 - March 2018. **Results:** Of the 100 INH monoresistant patients taken in a year, 82% were found to be resistant via Kat G gene and only 18% were found to be resistant for Inh A gene. **Conclusion:** In conclusion, our study showed increased prevalence of isoniazid resistance via Kat G gene mutation than with Inh A gene.

Keywords: Isoniazid, monoresistance, Kat G, Inh A gene.

INTRODUCTION

Isoniazid has been the backbone of tuberculosis chemotherapy for 6 decades. Resistance to isoniazid threatens the efficacy of treatment of tuberculosis disease and infection. The World Health Organization (WHO) estimated that 10 million incident cases of tuberculosis (TB) and 1.4 million deaths due to TB occurred globally as per Global TB report 2018.[1] The drug isoniazid is an essential element of all first-line treatment regimens for TB, with demonstrated high bactericidal activity and low risk of adverse events.[2,3]

Isoniazid is also highly effective in preventing disease in individuals infected quiescently with Mycobacterium tuberculosis.[4] Isoniazid resistance thus undermines the effectiveness of treatment of both TB disease and infection. M tuberculosis strains resistant to isoniazid have been observed in nearly 15% of TB cases globally.[1,5]

One of the key points of National Drug Resistance Survey showed INH resistance (16% in all with 11.6% in new and 25.09% in previously treated patients) being the driver for R resistance.[6] The World Health Organization (WHO) has proposed a wide-scale implementation of rapid molecular methods to screen patients at risk of MDR-TB. Rapid tests can provide results within days and thus enable rapid and appropriate treatment, decrease morbidity and mortality, and interrupt transmission.[4]

Among these, line probe assay (LPA) has been developed for the rapid detection of M. tuberculosis complex and its resistance to rifampicin (RIF) and isoniazid (INH). The assay detects mutations in the rpoB gene for RIF resistance, the katG gene for high-level INH resistance, and the inhA gene for low-level INH resistance from smear-positive or culture-positive sputum sample. However, 70–80% of INH resistance is associated with mutations in codon 315 of the katG gene.

Molecular line probe assay (LPA) technology for rapid detection of multi-drug resistant tuberculosis (MDR-TB) was endorsed by WHO in 2008. This assay requires only 1000- 10,000 cfu/ml for detection of mycobacteria. Results are obtained in 1 to 2 days. It has been validated for sputum positive samples only as yet, with a clear advantage of providing drug susceptibility status in as early as few hours. On smear positive sputum specimens, LPA holds a high sensitivity ≥ 97% and specificity ≥ 99

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% for the detection of RIF resistance alone or in combination with INH (sensitivity ≥ 90% specificity ≥ 99 %) on isolates of MTB.

INH resistance was classified as either low level or high-level when there was greater than 1% growth of M. tuberculosis complex in the presence of 0.2 µg/ml or 1 µg/ml of INH, respectively in one of the studies. The latest treatment regimen consists of Levofloxacin, Rifampicin, Ethambutol & Pyrazinamide to make a total of 4 effective drugs regimen given daily. The total duration of treatment will be 6 months. There is no Intensive or Continuation Phase as per the recent changes by RNTCP. Earlier, second line injectables were also included and the duration was of 9-12 months. All the patients from our study had the latter course as the newer changes were done after the study.

Although the prevalence of isoniazid (INH) resistance is much higher than that of RIF7,8, detection of INH-resistance has received lower priority, largely because the clinical impact of INH monoresistance is less pronounced. To address these issues, we conducted a retrospective evaluation of tuberculosis cases reported to the Chest and Tb Hospital, Amritsar. Our objectives were to identify and describe variation in treatment regimens for patients with INH mono-resistant tuberculosis, and to determine the prevalence of kat g and inh a gene mutations under program conditions.

MATERIALS AND METHODS

This study was conducted after approval from the institution's ethical committee. This was an observational retrospective study which was carried out in the Department of Chest and Tuberculosis, Government Medical College, Amritsar. The study included patients diagnosed with isoniazid monoresistant pulmonary tuberculosis, who came to outpatient department or were admitted in wards over a period of 1 year from Feb 2017 - March 2018. Participants who met the inclusion criteria were recruited after giving information regarding the study in their vernacular language and written informed consent was obtained.

Inclusion criteria:

1. Patients with isoniazid monoresistant pulmonary tuberculosis on LPA.
2. Age more than 10 yrs.
3. Patients with isoniazid monoresistant pulmonary TB via Inh A or Kat G gene mutation.

Exclusion criteria:

1. Patients with multidrug resistant (MDR) tuberculosis.
2. Patients detected with Rifampicin resistance on LPA and CBNAAT.
3. Patients with extrapulmonary MDR-TB.

Methodology:

Each patient was explained the purpose of the study and the need for complete co-operation was emphasized. Those who satisfied the inclusion and exclusion criteria were interviewed, examined and relevant investigations were performed on them to reach at a diagnosis. A pre-structured proforma was filled in all those cases which were included in the study.

History, general physical and respiratory examination, contact history, past history of ATT (anti-tubercular therapy) intake, history of any addiction and the following tests were undertaken:

a. Sputum for AFB (Acid Fast Bacilli): Morning samples will be collected.
b. Chest radiograph: Both PA and relevant side lateral view.
c. Line Probe Assay - LPA was not performed in specimens other than sputum.
d. Cartridge Based Nucleic Acid Amplification Test (CBNAAT)

With the help of these clinical, laboratory and radiological procedures and investigations a definitive diagnosis was reached to find out isoniazid monoresistant pulmonary tuberculosis via Kat G gene or Inh A gene.

RESULTS

The present study included 100 patients diagnosed with isoniazid monoresistant pulmonary tuberculosis under Revised National Tuberculosis Control Programme (RNTCP), coming to outpatient department or admitted in wards, to study prevalence and clinico-radiological features of isoniazid monoresistant pulmonary tuberculosis.

Table 1 - Type Of Gene Mutation

S. No.	Type of gene mutation	No. of patients	%age
1.	Kat G	82	82
2.	Inh A	18	18

Maximum patients in our study were resistant via Kat G gene.

DISCUSSION

H mono/poly resistance is known to be around thrice as prevalent as RR-TB.[6] Perhaps one of the most significant factors that impact on control is resistance to first and second line antimicrobials. A specific 6 months treatment regimen has been initiated to manage H mono-poly resistance with available first-line drugs strengthened with a fluoroquinolone.

Line Probe Assay (LPA) provides rapid diagnosis of R and H resistance as well as resistance to class FQ and class SLID. LPA can yield results in 72 hours. The resistance among all tuberculosis cases to any drug has ranged from 0% to 70.4%, to isoniazid from 0% to 60.3% and to rifampicin 0% to 44.4%. Isoniazid Mono-Resistance (IMR) is the most common form of mono resistance, and its world

prevalence is estimated to range between 0.0 to 9.5% globally (0.0 to 12.8% among new cases and 0.0 to 30.8% among retreated cases).[7]

Of the 100 INH monoresistant patients taken in a year, 82% were found to be resistant via Kat G gene and only 18% were found to be resistant for Inh A gene. Drug-resistant TB, like TB is a disease of poverty, expressed through microbial, clinical and programmatic channels. From a microbiological perspective, the resistance is caused by a genetic mutation that makes a drug ineffective against the mutant bacilli. In clinical settings, an inadequate or poorly administered treatment regimen allows drug-resistant mutants to become the dominant strain in a patient infected with TB. Clinical characteristics of patients have also been recognized where appropriately administered drugs have not achieved necessary drug levels to deal with all populations of mycobacteria. From a programmatic perspective, weak TB services lead to delay in detection and effective treatment of drug resistance and are unequipped to support patients to keep adherence to treatment and prevent ongoing transmission.[6]

Treatment outcomes and survival based on DST pattern in MDRTB showed that mono resistance either to FQ or AM, was independently associated with poor outcomes in patients with MDRTB and also suggested implementation of strategies to identify and cure these patients. For this reason, early DST for FQ and AM is mandatory before designing a regimen for MDR/RR/H mono-poly resistance TB patients.[8] This is confirmed by second line LPA that is sent after resistance to first line drugs is confirmed.

Ignorance of timely detection of resistance may compromise the effectiveness of global disease control. Further, with early availability of DST results, simplification and standardization of regimens would make treatment more practicable.8

A review published in 1986 of 12 British Medical Research Council (BMRC) clinical trials from sub-Saharan Africa, Hong Kong, and Singapore in the 1970's and 1980's described a low rate of treatment failure (2%) for INH resistant strains treated with an initial 4–5 drug regimen containing rifampin for at least six months.[9]

One of the studies done in San Francisco by Cattamenchi et al,[10] showed decreased all-cause mortality during tuberculosis treatment in INH mono-resistant compared to drug susceptible tuberculosis cases. Population-based studies have shown that M. tuberculosis strains harboring certain INH resistance mutations, including a serine to threonine substitution at amino acid position 315 of the katG gene, are less likely to generate secondary cases.[11,12] Similar studies are needed to establish whether these or other mutations impact M.tuberculosis virulence in addition to transmission. This study also identified prior treatment for latent or active tuberculosis as independent risk factors for subsequent INH mono-resistance.

CONCLUSION

In conclusion, our study showed increased prevalence of isoniazid resistance via Kat G gene mutation than with Inh A gene. The spread and transmission of INH- resistant bacilli are likely to pose a significant problem for Revised National TB Control Programme (RNTCP). Routine use of LPA can substantially reduce the time to diagnosis of RIF and/or INH-resistant TB and can hence potentially enable earlier commencement of appropriate drug therapy and thereby facilitate prevention of further transmission of drug resistant strains.

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