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Clinical Characteristics and Genetic Patterns of Isoniazid Mono Resistance in Pulmonary and Extra- Pulmonary Tuberculosis

Dr.G.Rajyalakshmi, Prof. Dr. K.V.V.Vijaya Kumar MD, Dr.Ch.R.N. Bhushan Rao MD, Dr. A. Ayyappa MD, Dr. B. M. S. Patrudu MD, Dr. P. V. Siva Ram MD, Dr. K. Phanindra Vasu, Dr .C. L. N. Yamini Priyanka
Andhra Medical College, Visakhapatnam, India

Corresponding Author: Dr. G. Rajyalakshmi**,** Andhra Medical College, Visakhapatnam, India

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Abstract

Background: Isoniazid is an important first line drug for the control of TB that we cannot afford to lose. Resistance to isoniazid is the most common form of drug-resistance in tuberculosis and its incidence is on the rise. Mutations associated with Isoniazid resistance are more complex and occur in multiple genes (inhA & katG). The aim of this study was to evaluate the clinical characteristics and genetic patterns of resistance to Isoniazid via inhA and katG genes in INH-monoresistant TB patients.

Methods: One hundred and nineteen patients with INH-monoresistant TB confirmed by 1st Line LPA and liquid culture DST during March 2018 to March 2019 were retrospectively enrolled. The patients' clinical characteristics, mutations of INH resistance were analyzed.

Results: Of the 119 INH monoresistant patients included, male predominance was seen in the current study 74(62.2%) vs 45(37.8%). The majority of patients 107 (89.9%) had no prior history of TB treatment. The most common comorbidity was diabetes mellitus (n = 20; 16.8%), followed by the use of immunosuppressive medication (n = 13; 10.9%). Eight cases (6.72%) had extra-pulmonary TB. Of the 111 cases (93.2%) with pulmonary involvement, 50 cases (45%) had at least one cavitary lesions on chest x-ray. 86 cases(72.2%) were found to be resistant via katG gene mutation, 27 cases (22.6%) had inhA gene mutation & 6 cases(5.04%) had both. katG mutation was present in 10/12 patients with a history of previous INH usage-for the treatment of active TB(8 cases) & who received IPT(4 PLHA cases).

Conclusion: Accurate drug sensitivity testing for all patients is critical for global TB control. A history of latent or active tuberculosis treatment was associated with subsequent INH mono-resistance. Routine use of LPA can substantially reduce the delay in diagnosis & enable earlier initiation of appropriate drug regimen for INH monoresistant tuberculosis.

Keywords: Isoniazid monoresistance, mutation, inhA & katG.

Introduction

Tuberculosis remains one of the most challenging global health problems as resistance to first-line antimycobacterial drugs continues to rise in many countries worldwide. Resistance to isoniazid (INH) is the most common form of mono resistance in drug resistant tuberculosis. The global averages of isoniazid resistance without concurrent rifampicin resistance were 7.2% (95% CI: 6.2–8.2%) in new TB cases and 11.6% (95% CI: 9.9–13.3%) in previously treated TB cases [1].

INH is one of the cornerstones of anti-tuberculosis treatment, as it exhibits mycobactericidal activity by inhibiting mycolic acid biosynthesis [2]. INH resistance commonly occurs due to mutations in the katG gene or the inhA regulatory regions. kat G encodes catalase-peroxidase, an enzyme that converts INH to its biologically active form. As mutations in katG , particularly at codon 315, confer high-level INH resistance, INH is ineffective for the treatment of Mycobacterium tuberculosis with this mutation profile [3]. The inhA regulatory region encodes nicotin-amide adenine dinucleotide-dependent enoyl-acyl carrier protein reductase, the primary target of active INH, as well as ethionamide (ETH) and prothionamide (PTH). inhA mutations cause low-level resistance to the drug, which means that high doses of INH may be effective against M.tuberculosis.

katG mutations tend to be more frequent (42–95% of isolates), while inhA mutations occur in 6–43% of isolates; around 10% of M.tuberculosis isolates have both mutations [4]. The RNTCP of India introduced the programmatic management of drug resistant TB services (PMDT) in 2007 and is now scaling up services across the country. As per PMDT guidelines [5], Rapid identification of DR TB is achieved by using a combination of NAAT (CBNAAT/TruNAAT), first- and second-line LPA and Liquid culture DST for specific drugs.

Recognition of INH resistance patterns and the frequency of katG and inhA mutations in different geographic areas may help to guide decision making about standardization of treatment regimens or individualized treatment, for INH monoresistant TB and also in the case of MDR- or XDR-TB, as in these settings the number of effective available drugs is limited [6].

The aim of this study was to evaluate the clinical characteristics and to determine the frequency of inhA and katG mutations for those patients with INH-monoresistant TB.

Materials & Methods

This retrospective study was carried out on patients of the Department of Pulmonary Medicine at Govt. Hospital for Chest and Communicable Diseases, Andhra Medical College, Visakhapatnam, Andhra Pradesh from March 2018 to March 2019.

Study Design: A Retrospective observational study.

Study Location: This was tertiary care teaching hospital-based study done in the Department of Pulmonary Medicine at Govt. Hospital for Chest and Communicable Diseases, Andhra Medical College, Visakhapatnam, Andhra Pradesh.

Study Duration: March 2018 to March 2019.

Sample size: 119 patients.

Subjects & selection method: The study population was drawn from consecutive INH monoresistant Tuberculosis patients who had drug susceptibility tests (DST) performed and were treated at the Govt. Hospital For Chest & Communicable Diseases, Visakhapatnam from March 2018 through March 2019.

Isoniazid mono-resistance was defined as tuberculosis episodes with isoniazid resistance confirmed with 1st line Line Probe Assay and wherever required testing with Liquid culture - drug susceptibility testing (DST) was also done. Patients with MDR Tuberculosis were excluded.

Procedure methodology: Information on the following variables – Age, Sex, Pulmonary or Extra-pulmonary TB, type of previously treated TB case, Chest X-ray, HIV status and associated comorbidities - into a structured data collection format by reviewing the patient records. The information on patients' samples that were tested with LPA and the results of the tests were extracted from culture and drug susceptibility (DST) registers. Data were double entered, validated and analyzed using SPSS software (Version 21.0).

Results

A total of 1357 tuberculosis patients were identified in our hospital from March 2018 to March 2019, of whom 945 cases (69.63%) were tested with LPA and 119 cases (12.5%) were detected with INH monoresistance and were included in this study. The baseline demographics and clinical characteristics of these patients are listed in Table 1. The mean age of the study population was 44.28 years with majority of the cases (n=39; 32.8%) in the 21-40 years age group followed by 41-60 years age group (n=37; 31.1%). There is a male predominance (n=74; 62.2%) and majority of patients (n=107; 89.9%) patients had no prior history of TB treatment. The most common comorbidity was diabetes mellitus (n=20; 16.8%), followed by the use of immunosuppressive medication (n=13; 10.9%). Four patients (3.36%) were identified as HIV positive. Of the 111 cases (93.2%) with pulmonary involvement, 50 cases (45%) had at least one cavitary lesions present on chest x-ray.

Mutations conferring INH resistance were reported for all 119 patients. Eighty-six patients (72.2%) had katG mutation, twenty-seven patients (22.6%) had inhA mutation alone while 6 patients (5.04%) had both katG & inhA mutations. MUT 1 of katG gene probe was positive in 73 cases (84.88%) and MUT 2 of katG in 8 cases (9.30%) and both were positive in 5 cases (5.81%). MUT 1 of inhA gene probe was positive in 25 cases (92.5%) where in two cases both MUT 1 and MUT 2 was positive (7.40%). In six cases with coexistence of inhA & katG mutations, MUT 1 of katG probe and MUT 1 of inhA probe were positive.

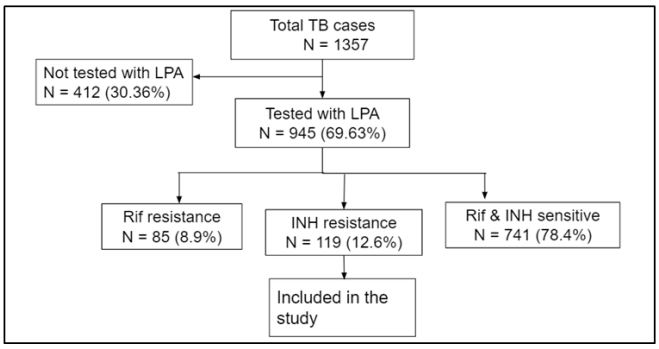

Figure 1: Line Probe Assay (LPA) testing and results among tuberculosis patients attending Govt Hospital for Chest & Communicable Diseases, Visakhapatnam, Andhra Pradesh, India from March 2018 to March 2019.

Of the total 119 INH-resistant M. tuberculosis cases that were detected by LPA almost every case was also detected by phenotypic DST. However, there were fifteen cases where phenotypic DST was not performed due to unknown reasons. katG mutation was present in ten out of twelve (83.33%) patients with a history of previous INH usage either for the treatment of active TB (5 out of 8 cases) & who received IPT (3 out of 4 PLHA cases) listed in table 2. katG mutation was detected in 71.02% of patients without previous treatment with INH (76/107). However, the difference between the two groups was not statistically significant

(p = 0.38). katG mutation was detected in five out of eight cases (62.5%) that had extra pulmonary tuberculosis. In this study, the most common site for extra pulmonary INH monoresistant TB was Lymph node (n= 4; 50%), followed by skeletal/joint involvement (n=2; 25%), pleural fluid/pus in one case (n=1; 12.5%) and a rare scenario of recurrent breast abscess in one case (n=1; 12.5%).

Table 1: Demographic and Clinical characteristics

Characteristic	Total patients (n = 119)
Sex	
Male	74 (62.2%)
Female	45 (37.8%)
Mean Age	44.28 years
Comorbidities	
Diabetes mellitus	20 (16.8%)
Immunosuppressive medication usage	13 (10.9%)
HIV positive	4 (3.36%)
Malignancy	3 (2.52%)
Chronic kidney disease	2 (1.68%)
Prior history of TB treatment	
No prior TB treatment	107 (89.9%)
Prior TB treatment	8 (6.72%)
INH preventive therapy	4 (3.36%)
Disease site	
Pulmonary	111 (93.2%)
Extrapulmonary	8 (6.72%)
Extent of pulmonary disease	
Cavitary lesions in chest radiography	50 (45%)
Isoniazid resistance	
Both katG & inhA mutations	6 (5.04%)
Only inhA mutation	27 (22.6%)
Only katG mutation	86 (72.2%)

Table 2: Previous use of INH to treat latent tuberculous infection or active TB and rate of detection of katG mutations

INH-resistance by kat G mutation	Previous use of INH	
	Yes	No
Yes	10 (83.33%)	76(71.02%)
No	2 (16.67%)	31 (28.98%)
Total	12	107

Discussion

Commercial liquid culture systems and molecular LPAs have been endorsed by the World Health Organization (WHO) as the gold standard for the rapid detection of drug resistance to 1st and 2nd line anti tuberculosis drugs. However, due to their technical complexity, high cost and the need for sophisticated laboratory

infrastructure, the use of these techniques has been limited in many resource constrained settings [7]. New LPAs are easier to perform and more accessible than commercial liquid culture. They also provide important information that can help clinicians initiate suitable treatment for drug-resistant TB more rapidly. LPAs also provide information about the level of INH resistance, depending on the mutation detected as well as providing an accurate diagnosis for MDR-TB [8].

The frequency of detected katG mutations was higher in the current study (n=86; 72.2%). According to various studies, the frequency of katG mutations in INH-resistant M.tuberculosis may vary between 31.8% and 96.9% [9–11]. Reports from different regions of the world show high variations in the proportion of katG and inhA mutations. Despite the high variability, katG mutations are consistently more frequent than inh A mutations [12].

In this study, less than one fourth (n=27; 22.6%) of the isolates showed low-level INH resistance (inhA mutation) and for these patients, high doses of INH would be a possible option in the management of INH monoresistant TB; while Thionamides may be a good option in settings with katG mutations, provided information on drug resistance is available to the clinicians. However, it is important to be aware of the possibility of cross resistance between low-level INH resistance and ETH, as reported by Schaaf et al. in South Africa [13] and Vadwai et al. [14] in India.

In the present study, high-level INH resistance (katG mutations) were more frequent in isolates obtained from patients with a history of previous INH for the treatment of latent tuberculosis infection (LTBI) or active TB than in patients without a history of INH (83.33% vs. 71.02%); however, this difference was not statistically significant (p= 0.38), probably because of the small number of INH-resistant patients.

katG gene mutation at MUT 1 region (S315T) in 73 cases (84.88%) and inhA gene mutation at MUT 1 region (C15T) in 25 cases (92.5%), were the most frequent mutations in this study and this is supported by various studies [15]. In six cases with coexistence of inhA & katG mutations, MUT 1 (S315T) of katG probe and MUT 1 (C15T) of inhA probe were positive. A study by Campbell et al., using 212 INH resistant isolates from both WHO and CDC laboratory archives estimated the global frequency of the katG315 mutation to be 85%, inhA-15 to be 17%, and their cumulative frequency 91%; however, isolates used for this study were selected to provide a diverse set of mutation patterns, and therefore may not accurately represent true global frequencies [16].

A more recent study conducted by Rodwell et al., using 348 INH resistant isolates from four geographically diverse countries estimated the global frequencies of katG315 and inhA-15 to be significantly higher at 86% and 34% respectively. The cumulative frequency of both mutations with the addition of inhA-17 was 96% [17]. Prevalence of drug resistant TB varies significantly by region, making it reasonable to expect regional variation in the frequencies of specific mutations that drive INH resistance [18].

The clinical presentation of INH monoresistant tuberculosis in this study is similar to drug susceptible tuberculosis as most of the patients presented with cough, fever, night sweats, weight loss, and hemoptysis. (data not shown).

It is important to recognise the limitations of this study. First, this study is limited by its retrospective design and the operational nature of the study relying on accuracy of hospital records that were routinely

maintained. Hitherto, this was a small case study, and further prospective studies are encouraged. Second, patients with INH monoresistance could have had additional resistance to pyrazinamide and/or ethambutol and this could have been missed as liquid culture DST and/or testing for individual drug susceptibility was not done for all cases.

Conclusion

In conclusion, katG315 mutation (high level resistance) is the most common mutation conferring Isoniazid monoresistance. Newer rapid molecular tests are helpful to understand the INH drug resistance mutation profile and also to guide treatment decisions. Prior treatment for latent or active tuberculosis is associated with subsequent INH mono-resistance. This concerning association with Isoniazid preventive therapy deserves further evaluation.

The performance of molecular-based diagnostic tests for drug resistant TB is intrinsically linked with not only the regional frequencies of mutations being detected, but also the diversity of resistance-conferring mutations being detected by diagnostic tests. Both of these factors influence the maximum sensitivity which rapid molecular tests can be expected to achieve. Further research is needed to determine if unknown genes or specific mutations account for the unexplained phenotypic INH resistance, or if a more inclusive combination of mutations in previously identified genes. This would allow for tailoring of molecular tests to specific regions, better interpretation of the molecular tests being used, and improved therapy recommendations.

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