Journal of Infectious Diseases & Research
JIDR, 3(1): 99-106
www.scitcentral.com
ISSN: 2688-6537
Original Research Article: Open Access

Study of Gene Patterns in First Line-Line Probe Assay (FLLPA) Leading to Isoniazid Monoresistance

Sumalata C*, Anil Kumar B and Rajesham A

*State TB Training and Demonstration Centre, Telangana, India.

Received May 28th, 2019; Revised June 06th, 2019; Accepted June 08th, 2019

ABSTRACT

Introduction: First Line-Line Probe Assay (FLLPA), a molecular test helps in diagnosing resistance of two First line ATT drugs-Isoniazid and Rifampicin. The genes causing Isoniazid resistance are enoyl acyl carrier protein (*acp*), reductase (*Inh A*), catalase–peroxidase (*Kat G*), alkyl hydroperoxide reductase (*ahpC*), oxidative stress regulator (*oxyR*), β-ketocyl acyl carrier protein synthase (*KasA*). FLLPA will be identifying only Inh A and Kat G genes for Isoniazid.

Aims and Objectives: The study endeavor to understand the gene patterns determining Isoniazid resistance due to deletion of *Kat G* and *Inh A*.

Methods: The study was an observational study, which took place in Intermediate Reference laboratory at Hyderabad for duration of three months i.e. May 2018 to July 2018. All the samples processed in First line -Line Probe Assay (FLLPA) found to be resistance due to either *Kat G* or *Inh A* gene were recorded, and the gene patterns were studied by applying appropriate statistics.

Results: The total tests performed which detected Isoniazid resistance are 185 of which 123 (66.5%) were resistance due to *Kat G* gene and 55 (29.7%) due to *Inh A*. Seven samples (3.8%) showed Resistance to both. There were 3 cases in which the resistance due to *Kat G*, all loci i.e. *Kat G* Locus Control, Wild type I and Kat G Mut 1 & 2 were absent. In one case of Isoniazid resistance due to Inh A there were loci, wild type 2 and Mut2 were present while rest of all loci were absent. There were few rare interpretations which implied Isoniazid Resistance.

Conclusion: The study showed most of the Isoniazid resistance was due to deletion of *Kat G* gene indicating high level resistance.

Keywords: Isoniazid, Monoresistance, FL-LPA, *Kat G*, *Inh A*

INTRODUCTION

Tuberculosis, caused by Mycobacterium tuberculosis, poses many challenges to community in terms of treatment, adherence and adverse drug reactions. Drug resistance is a serious problem itself posing many difficulties for physicians to tailor the regimen. There is immense need for study of wild types of genes to the growing needs of management of drug resistant TB. There were many changes in programmatic management of drug resistant TB.

Line Probe Assay and CBNAAT (Cartridge based Nucleic Acid Amplification Test) are two molecular technologies which diagnose TB and also detect resistance. CBNAAT detects resistance for Rifampicin whereas Line Probe Assay helps in diagnosing resistance for two drugs i.e. Isoniazidand Rifampicin. These two molecular tests have revolutionalized the diagnosis of drug resistant TB.

Isoniazid monoresistance accounts 16 % of all notified TB cases. There was no particular treatment in the previous guidelines. The genes causing Isoniazid resistance are enoyl acyl carrier Protein(*acp*), reductase (*Inh A*), catalase–peroxidase (*Kat G*), alkyl hydroperoxide reductase (*ahpC*), oxidative stress regulator (oxy R), β-Ketocylacyl carrier protein synthase (Kas A). Of all the above-mentioned genes FLLPA will be identifying only for *Inh A* and *Kat G* genes for Isoniazid.

Corresponding author: Sumalata C, Epidemiologist, State TB Training and Demonstration Center, Irramnuma, Hyderabad, Telangana state, India 500038, Tel: 09866469294; E-mail: drsumachittiboyina@gmail.com

Citation: Sumalata C, Kumar AB & Rajesham A. (2020) Study of Gene Patterns in First Line-Line Probe Assay (FLLPA) Leading to Isoniazid Monoresistance. J Infect Dis Res, 3(1): 99-106.

First Line-Line Probe Assay which is a molecular technology which detects resistance of Isoniazid and Rifampicin. The use of this genotypic test has hastened to know the status of resistance of Isoniazid and Rifampicin, helping the treating physician to tailor the treatment regimen.

Isoniazid monoresistance posed another challenge in management of DRTB. There were many regimens followed in different countries. There was a clear mention of management in Isoniazid Monoresistance in Technical Operation Guidelines in 2016 under Revised National Tuberculosis Control Program(RNTCP) in India

The most common pattern will guide the management of Isoniazid Monoresistance Drug management, especially determining the use of other first Line drugs.

OBJECTIVE OF STUDY

To study the gene patterns in determining Isoniazid resistance by FLLPA.

METHODS

The study was an observational study which took place in Intermediate Reference Laboratory (IRL), Hyderabad, Telangana, India for period of 3 months (May 2018 to July 2018).

All the consecutive samples which were processed in FL-LPA and found to be resistance to Isoniazid only due to either *Kat G* or *Inh A* gene were noted, and the gene patterns were studied. All the data was recorded in Microsoft Excel and appropriate statistics applied.

Inclusion criteria

1) All the consecutive Pulmonary samples processed with FL-LPA and found resistance to Isoniazid.
2) All the samples collected were irrespective of HIV status and age of the patients.

Exclusion criteria

1) All extra pulmonary samples were not included in study.
2) All samples which are smear negative concentration and which are processed in Liquid culture and then subjected to FLLPA.

RESULTS

The total tests performed which detected Isoniazid resistance were 185 during the period May 2018 to July 2018. Of which 123 (66.5%) were resistance due to *Kat G* gene and 55 (29.7%) due to *Inh A*. Seven Tests (3.8%) showed Resistance to both **(Table 1)**.

Table 1. Gene patterns for resistance due to *Kat G*.

	Present	Absent
Locus control	119	4
Wild type	12	111
Mut 1	113	10
Mut 2	2	121

There were four cases in which the resistance was due to Kat G, all loci i.e. *Kat G* locus control, Wild type I and *Kat G Mut 1 & 2* were absent which prompted resistance to Isoniazid.

There were 119 cases were mutation was expressed in *Mut 1 and two in Mut* 2 which indicated that high dose Isoniazid cannot be used. Regimen has to be designed in such a way that Isoniazid shall not be included.

In 106 cases of Isoniazid resistance to Kat G, the expression was - locus control was present, Wild Types were absent, *Mut 1* present and *Mut 2* present. This clearly indicated that Isoniazid cannot be used even at higher dose and regimen has to be tailored accordingly.

There was one case where in resistance to Isoniazid inferred as absence in locus control and mutation but presence of wild type1 was expressed.

Expression of presence of locus control and all other (wild type, *Mut1 & Mut2*) were absent in five cases.

There was expression of both *Mut 1 and Mut 2* in one case.

All the above instances discouraged the use of Isoniazid even at higher doses. There were 55 cases of Isoniazid resistance due to *Inh A* **(Table 2)**.

Table 2. Gene patterns for resistance due to *Inh A*.

Inh A	Present	Absent
Locus Control	55	0
Wild Type 1	15	40
Wild Type 2	52	3
Mut 1	50	5
Mut 2	1	54
Mut 3a	1	54
Mut 3b	0	55

Locus control was present in all cases. Wild type I was absent in 40 cases and in 2 cases wild Type II were not seen. *Mut* were expressed in 50 cases, *Mut II* and *Mut 3a* in only one case. Expression of *Mut 3b* was not seen in all cases.

In one case, expression of locus control, wild type I, wild type II, *Mut* I were seen, and rest were not seen.

In one case, *Inh A*, wild type II, and *Mut* 2 are seen and rest were absent. In all above cases, where resistance due to Isoniazid was due to Inh A gene, high dose of Isoniazid could help.

There were 7 cases where there was resistance expression both due *Inh A* and *Kat G* gene. The expressed gene patterns were as follows **(Table 3 and Figure 1)**.

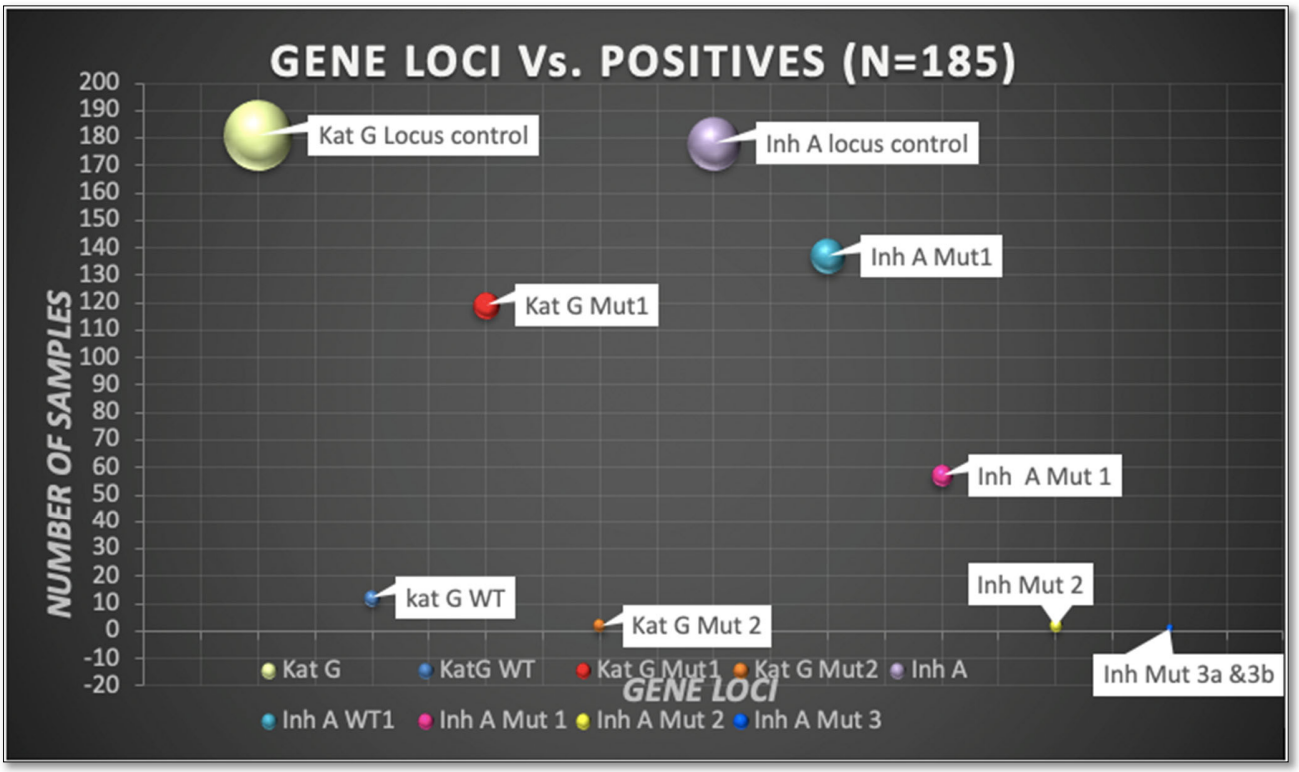

Figure 1. Graph showing Gene patterns in Isoniazid Resistant sample.

Table 3. Gene patterns observed for resistance to both genes *Inh A* and *Kat G*.

Gene/loci	Present/Absent
Kat G locus control	Present in all 7
Wild type I	Absent in 5 cases
Mut 1	Absent in only case
Mut 2	Absent in all cases
Inh A Locus Control	Present in all 7
Wild Type I	Absent in 5 cases
Wild Type II	Present in all 7
Inh Mut I	Absent in 2 cases
Inh Mut 2	Absent in 6 cases
Inh Mut 3a	Absent in all cases
Inh Mut 3b	Absent in all cases

DISCUSSION

Isoniazid was first synthesized in Prague in 1912 and is critically important first line drug. The prodrug isoniazid inhibits mycolic acid synthesis and mycobacterial cell wall formation [1]. Isoniazid monoresistance is estimated to be about 16-22% in National Drug Resistance survey 2016. Resistance is most frequently caused by mutations in genes coding for a bacterial catalase-peroxidase enzyme or a enoyl-acyl carrier protein reductase, although multiple other genes are implicated in isoniazid resistance [2].

First Line LPA is molecular tests which gives information on resistance of both Isoniazid and Rifampicin. Genes that cause Isoniazid resistance in FLLPA are *Kat G* and *Inh A*. *Kat G* resistance indicates high level resistance and *Inh A* indicates low level resistance to Isoniazid. Inh A gene also gives information whether to use Ethionamaide (Eto), a second line drug for TB or not. The samples collected were processed for decontamination and subjected to FLLPA after smear reading showed positive. LPA includes the step and reading is done expression of bands.

Reasons for false-negative LPA may include the following: reagents not equilibrated to room temperature; addition of insufficient reagents, improper mixing of reagents, addition of reagents in incorrect amounts; improper immersion of strips in the reagents during incubation; improper washing of strips and improper sampling, storage, transport or preparation of specimen [3]. **Table 4** enables us to interpret the FLLPA result.

Table 4. FLLPA interpretation.

Target region	MTBR plus probe	Mutation or region interrogated	Result Interpretation	Additional diagnostic action	Clinical implication
KatG WT	kat G MUT 1 or MUT 2 developed	S315T1/S315T2	Mutation associated high level increase in MIC detected	No additional diagnostic action required	Isoniazid is unlikely to be effective even at high dose.

	kat G WT, MUT 1 or MUT 2 not developed	Mutation(s) at codon 315	Mutation associated high level increase in MIC inferred	Optional :Perform sequencing of Kat G to identify the specific mutation.	Isoniazid is unlikely to be effective even at high dose
inhA WT1	inhA MUT1 developed	c-15t	Mutation associated with atleast low level increase in MIC detected. Resistance to Eto/Pto detected.	Optional: Perform sequencing of inh A coding region and Kat G gene. No additional diagnostic action for Eto/Pto.	Isoniazid at high dose is likely effective. Ethionamide /Prothiamide(Pto) are not effective.
	inhAMUT2 developed	a-16gd	Mutation likely associated with atleast low-level increase in MIC (Minimum Inhibitory Concentration) detected. Resistance to Eto/Pto detected.	Optional : Perform sequencing of inh A coding region and Kat G gene . No additional diagnostic action for Eto/Pto.	Isoniazid at high dose is likely effective. Ethionamide /Prothiamide are not effective
inhA WT2	Inh A MUT 3A Developed	t-8cd	Mutation likely associated with atleast low-level increase in MIC detected. Resistance to Eto/Pto detected.	Optional: Perform sequencing of inh A coding region and Kat G gene. No additional diagnostic action for Eto/Pto	Isoniazid at high dose is likely effective. Ethionamide /Prothiamide are likely not effective
	Inh A MUT 3B Developed	t-8ad	Mutation likely associated with atleast low-level increase in MIC detected. Resistance to Eto/Pto detected.	Optional: Perform sequencing of inh A coding region and Kat G gene. No additional diagnostic action for Eto/Pto	Isoniazid at high dose is likely effective. Ethionamide /Prothiamide are likely not effective

	Inh A WT2, MUT3A and MUT 3B not developed	Mutation in the -8 region	Mutation likely associated with atleast low-level increase in MIC detected. Resistance to Eto/Pto likely inferred	Recommended: Repeat FL-LPA to confirm the result. Optional: Perform sequencing to identify specific mutation Perform Phenotypic DST for H, Eto/Pto	Isoniazid at high dose is likely effective. Ethionamide/Prothiamide are likely not effective

In the study we have observed that resistance was more due to *Kat G* rather than *Inh A*.

Locus Control for Kat G was absent for four cases where we needed to infer that the particular sample was resistant, which programmatically was considered Isoniazid resistance.

Most of the times in Kat G resistance was mostly due to expression of MUT1.MUT2 expression was noticed very low.

In following instances **(Table 5)**, Isoniazid resistance has to be inferred.

Table 5. FLLPA interpretation for Kat G Resistant.

No. of samples	***Kat-G***				**Result**
	Kat-G	***Kat G WT***	***kat G MUT1***	***kat G MUT2***	Isoniazid resistance to *kat G* inferred.
1	present	absent	absent	present	
1	absent	present	absent	absent	
5	present	absent	absent	absent	
1	present	absent	present	present	

In special cases of *InhA* resistance, the following was inferred **(Table 6)**.

In one typical case where Isoniazid resistance was due to both *Kat G* and *InhA*, where resistance was inferred as follows **(Table 7)**.

Resistance to Isoniazid is prevalent with substantial geographical variation [3].

As observed in many studies conducted by Charan et al. [4] our study too showed that Kat *G* resistance of Isoniazid was mainly due to absence of Wild type (S315T).

Increasing the dose of Isoniazid in resistance due to *Kat G* may not be helpful. Hence in all guidelines it was mentioned to remove the drug once resistance is noticed [5].

Table 6. FLLPA interpretation inferred for *Inh A*.

Inh A						
Inh A	Inh A WT1	Inh A WT2	Inh A MUT1	Inh A MUT2	Inh A MUT3 A	Inh A MUT 3B
P	p	P	P	A	A	A
P	A	P	A	P	A	A

Table 7. FLLPA interpretation for both *Kat G* and *Inh A*.

Kat-G				**Inh A**						
Kat-G	*KatG WT*	*Kat G MUT1*	*Kat G MUT2*	*Inh A*	*Inh A WT1*	*Inh A WT2*	*Inh A MUT1*	*Inh A MUT2*	*Inh A MUT3 A*	*Inh A MUT 3B*
P	P	P	P	P	P	P	A	P	A	A

In many studies conducted in world on Isoniazid resistance the results were as follows **(Table 8)** and similar results were reported in our study too.

Table 8. Comparative results of Mutation patterns reported from various studies.

S. No	**Study /Publication**	**Number of Patients**	**Kat G**	**InhA**	**Both Kat G and InhA**
1	Yao et al. [6]	50	41(82%)	9 (18%)	nil
2	Huyen et al. [7]	251	227(75.3%)	28.3%	
3	Alagappan et al. [8]	1821	1297(71%)	528 (29%)	nil
4	Charan et al. [4]	192	125(65.1%)	54 (28.1%)	13 (6.7%)
5	Abraham et al. [9]	603	435(72.13%)	99 (16.41%)	69 (11.4%)
6	Present study	185	123(66.5%)	55 (29.7%)	7 (3.8%)

The presence of mutations in *Kat* G alone or in combination with *Inh* A signifies a high degree of resistance to INH. The addition of even high doses of INH for these patients is unlikely to increase the effectiveness of a regime. A mutation limited only to *Inh* A, on the contrary, is usually associated with a low degree of INH resistance, and these individuals are likely to be benefitted with high doses of INH (10-15 mg/kg/day) [10].

CONCLUSION

The above study concludes that most common mutation in Isoniazid monoresistance is *Kat G.* Among *Kat G* mutations, absence of Wild type (C15T) and presence of *MUT 1*(S315T1) is observed more frequently. Ethionamide is also not effective in *Inh A* mutation of Isoniazid monoresistance. In order to design the regimen, gene patterns are important to study.

CONFLICT OF INTERESTS

None declared.

REFERENCES

1. Donnell M (2018) Isoniazid Monoresistance: A precursor to multidrug-resistant tuberculosis? Ann Am Thorac Soc Mar 15: 306-307.
2. Farooqi JQ (2012) Line probe assay for detection of Rifampicin and Isoniazid Resistant Tuberculosis in Pakistan". J Pak Med Assoc 62: 767-772.
3. Desikan P (2017) Line probe assay for detection of Mycobacterium tuberculosis complex. Indian J Med Res 145: 70-73.
4. Charan AS, Gupta N, Dixit R (2020) Pattern of InhA and KatG mutations in isoniazid monoresistant Mycobacterium tuberculosis isolates. Lung India 37: 227231.
5. Tiemersma EW, van der Werf MJ, Borgdorff MW, Williams BG, Nagelkerke NJ (2011) Natural history of tuberculosis: Duration and fatality of untreated pulmonary tuberculosis in HIV negative patients: A systematic review. PLoS One 6: e17601.
6. Yao C, Zhu T, Li Y, Zhang L, Zhang B, et al. (2010) Detection of rpoB, katG and inhA gene mutations in Mycobacterium tuberculosis clinical isolates from Chongqing as determined by microarray. Clin Microbiol Infect 16: 1639-1643.
7. Huyen MN, Cobelens FG, Buu TN, Lan NT, Dung NH, et al. (2013) Epidemiology of isoniazid resistance mutations and their effect on tuberculosis treatment outcomes. Antimicrob Agents Chemothe 57: 3620-3627.
8. Alagappan C, Shivekar SS, Brammacharry U, Kapalamurthy CVR, Sakkaravarthy A, et al. (2018) Prevalence of mutations in genes associated with isoniazid resistance in Mycobacterium tuberculosis isolates from re-treated smear-positive pulmonary tuberculosis patients: A meta-analysis. J Glob Antimicrob Resist 14: 253-259.
9. Abraham JN, Mlisana K, Gandhi NR, Mathema B, James CM (2015) High Prevalence of inhA Promoter Mutations among Patients with Drug-Resistant Tuberculosis in KwaZulu-Natal, South Africa. PLOS One 10: e0135003.
10. World Health Organization (2018) WHO treatment guidelines for isoniazid resistant tuberculosis: Supplement to the WHO treatment guidelines for drug resistant tuberculosis. Available online at: https://www.who.int/tb/publications/2018/WHO_guidelines_isoniazid_resistant_TB/en/