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Drug Resistance by using the inhA promoter in clinical isolate for *Mycobacterium Tuberculosis* cases

Anjali Rana[1*], Yusra Ahmad[1], Narotam Sharma[2] and Satish Chandra Nautiyal[2]

[1]*Faculty of Pharmacy, Uttarakhand Technical University, Suddhowala, Dehradun, Uttarakhand, India*
[2]*Central Molecular Research Laboratory, Department of Biochemistry, Shri Guru Ram Rai Institute of Medical and Health Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand, India*

Abstract

Tuberculosis is the most significant infectious disease it is caused by *Mycobacterium tuberculosis* (MTB). Bacteria that cause tuberculosis can develop resistance to antimicrobial medicines used to cure the disease. There is a multi-drug resistant tuberculosis TB that does not respond to at least Isoniazid and rifampicin, the two most powerful anti-TB drugs. Isoniazid Resistance Tuberculosis (IRTB) in *M. tuberculosis* is related to loss of catalase and peroxide (CP) activity in TB resistant strains. In other word, INH resistance often happens with the loss and/or decrease of CP or *katG* activity coded by *katG* gene. In fact, INH is a prodrug for which cellular activation is required by *katG* protein, before it's have toxic effect on bacillus. The pulmonary and extra-pulmonary samples were collected from patients for the detection of MTB and the MTB positive samples were undertaken into pre-amplification area for master mix preparation by using forward primer 5'-GTGCCCGACCAACACCCACCATTACAGAAAC-3' and reverse primer 5'-TAAGCGCACGTCGAACCTGTCGA-3'. Subsequently sample were taken to thermal cycler PCR (polymerase Chain Reaction) was used as a tool for detecting mycobacterium *mpb64* gene and *katG* gene in suspected TB patients and for the analyzing of amplicons used Electronic UV trans-illuminator system in which *katG* gene was targeted at 2223 base pair to determine if sample is positive for Isoniazid resistance.

Keywords: TB, *katG* gene, MTB, PCR, Isoniazid, CP.

***Correspondence Info:**
Dr. Narotam Sharma,
Central Molecular Research Laboratory,
Department of Biochemistry,
Shri Guru Ram Rai Institute of Medical and Health Sciences, Shri Guru Ram Rai University,
Dehradun, Uttarakhand, India

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1. Introduction

Antibiotic resistance is a growing threat to the whole world and has been found for every category of antibiotic agents [1]. As reported by the World Health Organization (WHO) it causes disease among 9.6 million people every year [2]. In India every year approx 2 million people develop active disease and up to half a million die [3]. TB is India's biggest health issue, but this issue worsens TDR-TB (Totally Drug-Resistance-Tuberculosis), there is an issue of medicine [4]. TB is an Infectious disease (bacterial disease) generally affecting the lungs (pulmonary TB). Tuberculosis can affect anyone of any age group people [5]. Tuberculosis is a contagious disease that is caused by *Mycobacterium tuberculosis complex* (MTBC) due to several species of positive bacteria which includes *M. bovis, M. canetti, M. microti, M. africanum* [6]. There are two types of TB; Pulmonary TB and Extra pulmonary TB. Pulmonary includes samples obtained from lungs, such as sputum, Pleural fluid, Bronchiolar alveolar lavage, bronchial secretions and extra-pulmonary includes samples obtained from other than lungs, fluid extra pulmonary specimens such as Pus, Urine, Semen, Tissue, Endometrial biopsy, Cerebrospinal Fluid and etc[7]. In case of Acid Fast

Bacilli (AFB) smear microscopy for pulmonary TB, sputum is considered to be the most important sample and it is most widely used method for detection of pulmonary TB [8].

Currently available treatment for tuberculosis;

First line treatment: it involves rifampicin, Isoniazid, Pyrazinamide and ethambutol.

Second line treatment: Amino glycosides (kanamycin and Amikacin) and fluoroquinolones (including ofloxacin, moxifloxacin and levofloxacin) [9].

Isoniazid, also known as isonicotinyl-hydrazide (INH), it is an antibiotic generally used for the treatment of TB [10]. Isoniazid is a prodrug that is activated by the catalase/peroxidase enzyme encoded by the *katG* gene [11]. Isoniazid was introduced in 1952 as an anti-TB agent and remains in conjunction with rifampicin, as the basis for the treatment of the disease [12]. Isoniazid acts by preventing the synthesis of mycolin acid through the NADH-dependent enoyl-acyl carrier protein-reductase encoded by *inhA.* Although simple in its structure, the resistance of this drug has been associated with mutation in many genes, such as *katG, inhA,* and NDH [13]. The first WHO started supporting DOTS Plus (Direct Observation Treatment-short course) programs in 2000 [14]. The Green Light Committee (GLC) was the establishment of medicine for suitable use in TB control programs [15]. Resistance variability and Dose sensitivity are parameter which is use for evolution of resistance [16].

Drug Resistance can be simply defined as the permanent or non-permanent capacity of organisms and drug resistance progeny to continue viable or to multiply in the presence of the concentration of the drug that would normally demolish or inhibit cell growth [17].

Clinically, drug resistance can be classified into 2 types:

Multi-drug resistant TB (MDR-TB): It is defined as the disease caused by *M. tuberculosis* that is resistant to not less than Isoniazid and rifampicin with or without resistance to other anti-TB drugs [18].

Extensive drug resistant-tuberculosis (XDR-TB): It is defined at least as Rifampicin, Isoniazid, a second line injectionable drug (caperomycin, kanamycin) and fluroquinolone resistance [19]. Antibiotics cause cell retardation and cell-death by targeting and inhibiting essential cellular processes.

2. Material and Methodology

The research work was done at Central Molecular Research Laboratory, Department of Biochemistry, Shri Guru Ram Rai Institute of Medical and Health Sciences (SGRRIM & HS), Patel Nagar, Dehradun (U.K).Those patients, who were found positive for TB were recruited for the drug resistance study. Clinical specimen's that include Pulmonary and extra pulmonary specimens were considered for the study. Specimens were collected from patients attending Out Patient Department (OPDs) and In Patient Departments (IPDs) of different Departments of Shri Mahant Indiresh Hospital, Dehradun, (Uttarakhand) India.

Collected samples were undergone for further testing in areas:

Bio-safety Cabinet: Once the sample was collected it was taken for centrifugation process then the DNA was isolated using silica column method.

Pre-amplification area: This area was specific for master mix preparation in which primers were added for PCR by targeting the specific gene (***mpb64 and katG at 2223 and 315 base pair respectively***).

Amplification area: PCR was used for amplification or multiplication of targeted gene.

Post amplification area: After that amplicons (final product) was tested or analyzed by using electrophoresis unit.

3. Results

In this study, the pulmonary and extra-pulmonary sample was collected from patients for the detection of *MTB* infection. PCR was used as a tool for detecting mycobacterium *mpb64* gene and *katG* gene in suspected TB patients. A total of 70 samples were taken from the different Departments in Shri Mahant Indiresh Hospital and processed in Central Molecular Research Laboratory, Department of Biochemistry, Shri Guru Ram Rai Institutes of Medical and Health Sciences, Patel Nagar, Dehradun, Uttarakhand, India out of which 2 were found positive in the process of AFB staining, 9 of positive for *mpb64* (TB), While no sample came positive for Isoniazid. The results are shown in table given below; from table 1 out of 70 received clinical samples, it was found that 30 samples were of female, which was 42.85% of total sample received and 40 were of male which was 57.14%. In case of females, 3 samples (10) were positive for *mpb64* gene well 27(90%) were negative for *mpb64* gene but in case of *katG* gene no females showed positive response towards it, and 100% females negative response. In case of males, 6(15%) were positive for *mpb64* and 34(85%) were negative toward it, while in case of *katG* gene, male also gave 0% positive response drug resistance to Isoniazid and 100% negative response. From table 2 and 3 grouping the sample on the bases of site of infection, it can be divided into two sites, name as pulmonary site and extrapumonary sites. Total clinical sample received were 70 and out these 60 i.e. 85.75% were from extra pulmonary site, well 10 i.e. 14.28% were from pulmonary site. For gene *mpb64,* TB-PCR that was found to be positive was 3(5%) and negative cases were 57(95%). In case of *katG* gene no PCR was found to be

positive, while 3 samples gave 100% negative PCR. The total case of pulmonary infection were 10(14.28%) out of 70 obtained clinical samples. In case of pulmonary samples, PCR positive for *mpb64* gene was 60% while PCR negative for it was 40%.The PCR positive for *katG* gene were 0% that means there was no resistance toward Isoniazid due to mutation in *katG* gene while PCR negative for it was 100%.These whole data indicates that pulmonary samples were found to be more positive in case of *mpb64* gene and here is not show resistivity by *katG* gene. After collection, decontamination, DNA isolation, amplification and post-amplification processes of the 186 clinical isolates, the result were interpreted based on the follow parameters: Gender of patients, Age of patients, Based on site of infection.

3.1 Gender wise positive and negative cases:

Table no. 1: positive and negative cases of *mpb64* gene and *katG* gene in males and females

Gender	Cases	*Mpb64* gene		*katG* gene	
		Positive	Negative	Positive	Negative
Male	40(57.14%)	6(15%)	34(06.85%)	0%	100%
Female	30(42.85%)	3(10%)	27(0.9%)	0%	100%

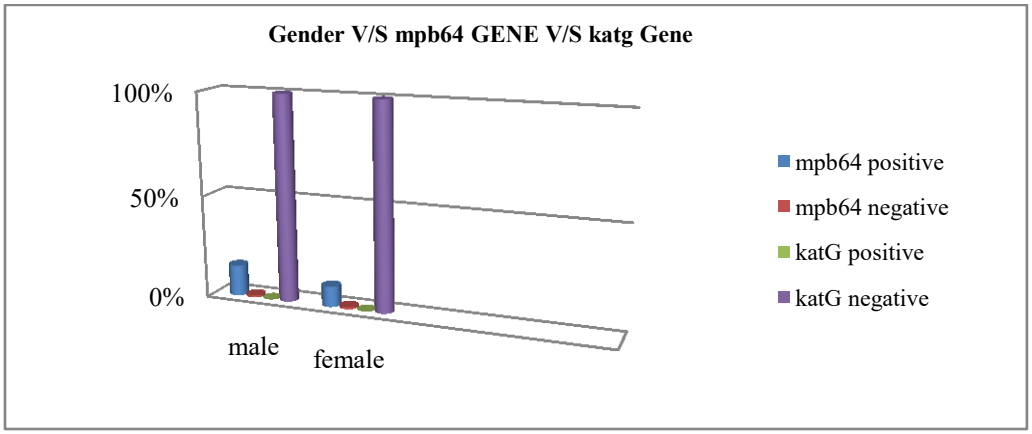

Figure 1: Percentage of positive& negative cases for *katG* and *mpb64* gene in males and female

3.2 Age wise positivity and negativity of samples:

Table 2: positivity and negativity rate of AFB staining and *mpb64 gene*

S/no.	Age range(Yr)	Cases	AFB staining		*mpb64* gene	
			Positive	negative	Positive	Negative
1.	00-25	18(25.71%)	2(11.11%)	16(88.8%)	3(16.66%)	15(83.33%)
2.	26-50	15(21.42%)	0(0%)	15(100%)	1(6.66%)	14(93.33%)
3.	51-75	25(35.71%)	1(4.00%)	24(96%)	2(8%)	23(92%)
4.	76-100	7(10%)	0(0%0	7(100%)	3(42.85%)	4(57.14%)

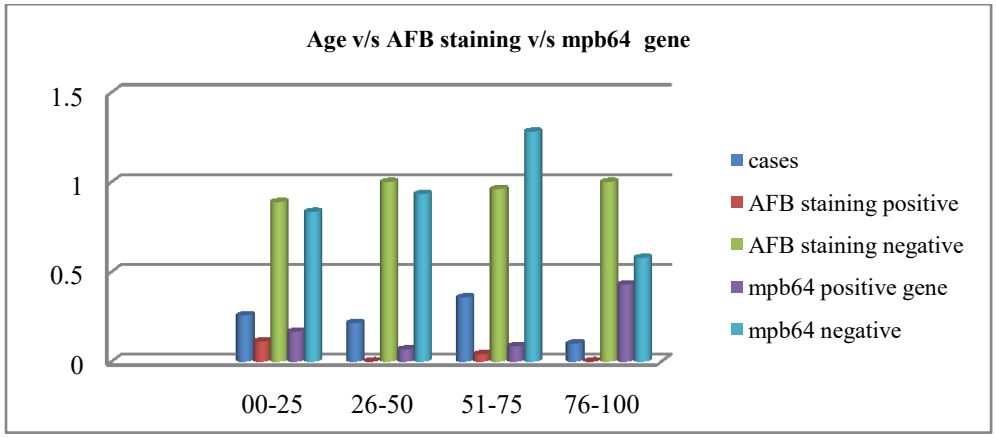

Figure 2: Percentage of positive and negative rate of AFB staining and *mpb64*

3.3 Positivity and negativity based on sites of infection

Extra pulmonary site:

Table 3: Positivity and negativity based on sites of infection

S. No.	Total Cases 60 (85.71%)	Positive	Negative
1.	AFB staining	2(3.33%)	58(96.66%)
2.	PCR (mpb64)	3(5%)	57(95)
3	PCR (*katG)*	0(0%)	100

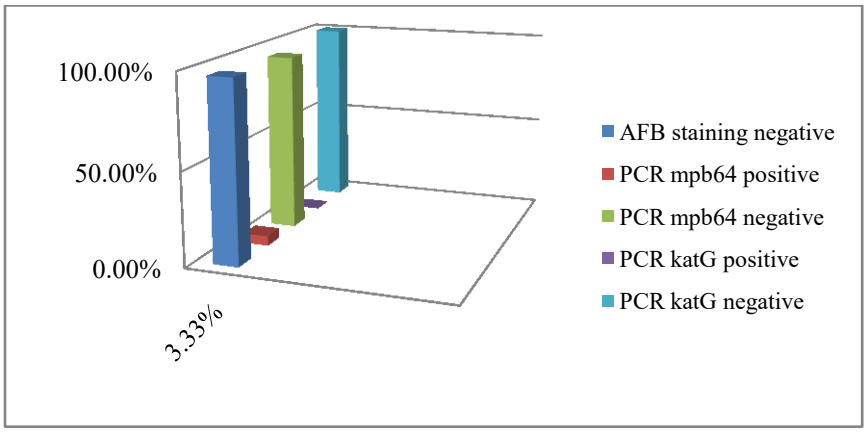

Figure 3: percentage of positive and negative rate of *mpb64* and *katG* gene.

3.4 Pulmonary sites:

Table 3: Positivity and negativity based on sites of infection

S. No.	Total Cases 7(10%)	Positive	Negative
1.	AFB staining	2(20%)	8(80%)
2.	PCR (mpb64)	6(60%)	4(40%)
3.	PCR (*katG)*	0(0%)	100

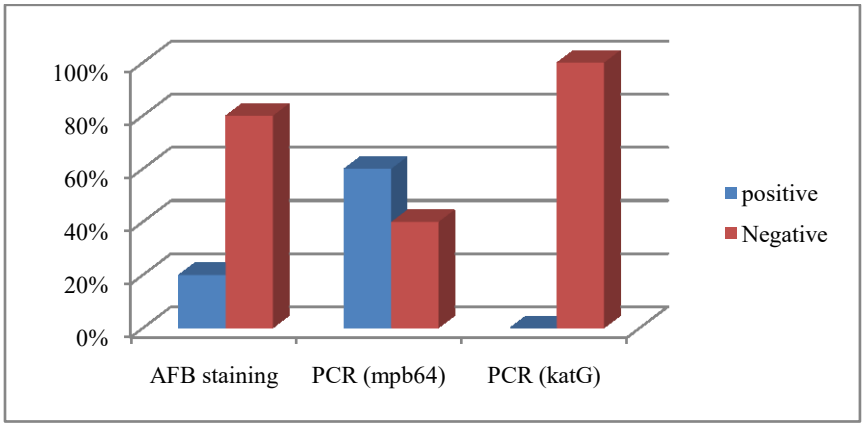

Figure 3: percentage of positive and negative rate of *mpb64* and *katG* gene

4. Discussion and conclusion

Drug resistance in tuberculosis (TB) is a global problem in both developed as well as under developed country. TB is an air born disease that is caused by *Mycobacterium tuberculosis* Gram-positive bacteria that divides every 16 to 20 hours, an extremely slow compared with other bacteria. It is also a major socio-economic burden in India, afflicting 14 million people, mostly in the reproductive age group (20-60years). PCR method has high specificity in identifying *M. tuberculosis* in various extra pulmonary specimens. During our lab scale work and study we came to conclusion after doing 70 samples and we analyzed presence of mycobacterium out of which 9 were found positive in the process of PCR and 2 samples were positive for AFB staining and no positive case for Isoniazid. On the basis of gender we concluded that males (40) were more infected than female (30), as the data shown in the above table 1. On the other hand the age groups belonging

to 51-76 were found to be most infected people. On the basis of the above data we can conclude that the sensitivity of PCR is much more than the AFB staining procedure. From this study we concluded that in Uttarakhand region, no resistance was found for Isoniazid in TB positive patients. This indicates that no mutation has been seen in *katG* gene and this could be the safe drug to be used in case of TB resistance.

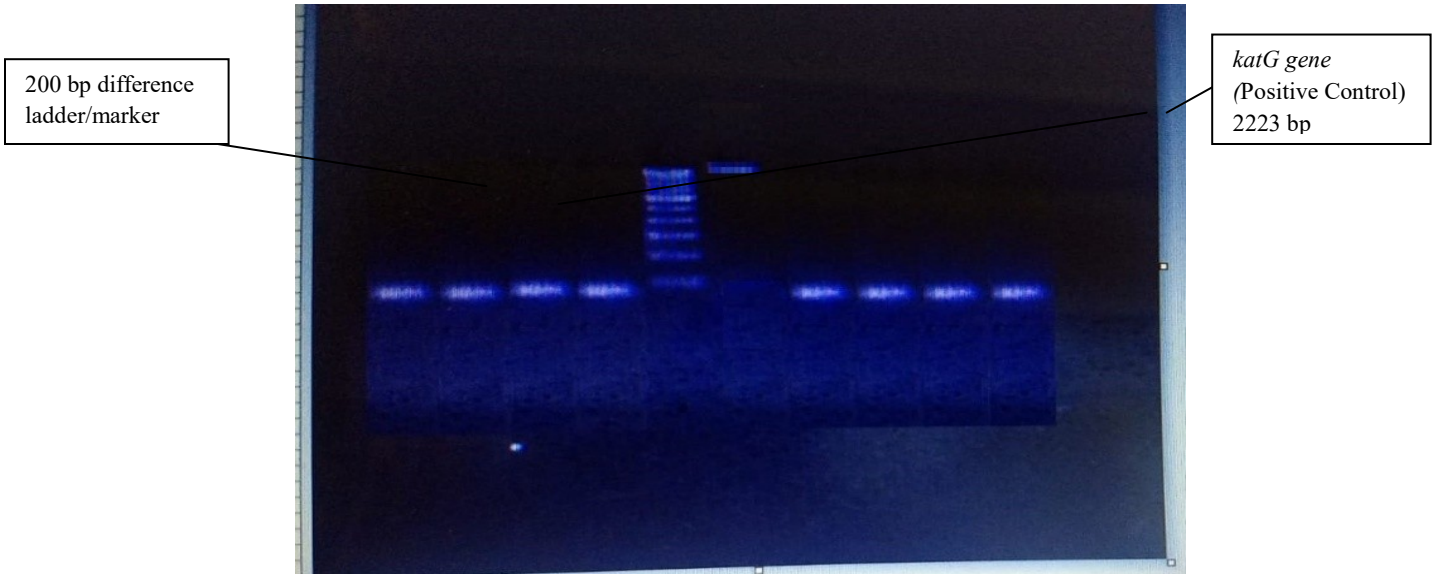

Figure 4: Gel picture showing a PCR product (*katG* gene PC) at 2223bp simultaneously are NEGATIVE

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Conflict of interest: None

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