MOLECULAR CHARACTERIZATION OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATES OBTAINED FROM PULMONARY AND EXTRAPULMONARY CASES OF TUBERCULOSIS

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(ABSTRACT)

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Tuberculosis (TB) is one of the most widespread, lethal infectious diseases in the world and declared as a '**Global Emergency**' by WHO in 1993. The role of laboratory in containment of TB and in prevention of multidrug resistant tuberculosis can't be under estimated. The different parameter to achieve the target are:

(A) Need of rapid, sensitive and specific method for the detection of *Mycobacterium tuberculosis*.

(B) Prompt detection of anti-TB drug resistance in *Mycobacterium tuberculosis*.

(C) Differentiation of the mycobacterial isolates to the species level to know the genetic relatedness.

(D) Adequate therapy and restriction close contacts to arrest further transmission among the population at risk.

In the present research work we have taken the first three aspect as our objective and discussed them one by one.

The use of PCR in the detection of *Mycobacterium tuberculosis* has produced varying results, especially in relation to the sensitivity of the test. To overcome this problem, various target sequences, such as insertion sequence 6110(IS6110), 65kDa (GroEL), 38kDa(Pho S, CIE Ag78 or Pab) and 85 B antigen coding gene have therefore been used. Among these, IS6110 is more commonly used because it is a repetitive sequence in the *Mycobacterium tuberculosis* genome. This characteristics helps in increasing the sensitivity of PCR over that obtained in the amplification of single DNA sequences. However, the IS6110 sequence has been reported to be absent from strains of *Mycobacterium tuberculosis* isolated in India and not much PCR studies have been done targeting 65kDa, 38kDa and 85 B antigen coding gene.

These facts led us our first objective of the present research work of evaluating "**Diagnostic potential of IS6110, 38kDa, 65kDa and 85 B sequence specific polymerase chain reaction for diagnosis of *Mycobacterium tuberculosis* in clinical samples of diversified origin"**.

Another important aspect in tuberculosis is the prompt detection of MDR cases of tuberculosis. Rifampicin and isoniazid are two key anti TB drugs and resistance to these drugs is most likely to influence patient care and utilization of hospital resources. The rifampicin resistance in nearly all the resistant strains is due to missense mutation or less commonly small deletions or insertion within an 81-bp hypervariable region (RRDR) of the *rpo B* gene. Majority of the mutation in the *rpo B* gene involve missense mutation at codon position 516, 526 or 531. There is no information on the prevalence of specific mutations within the *rpo B* gene in rifampicin resistant *Mycobacterium tuberculosis* strain from India.

Unlike rifampicin resistance, INH resistance is apparently controlled by a more complex genetic system that involves several gene. However, extensive studies have demonstrated that INH resistance is most frequently associated with a single mutation in *kat G*, a gene that encodes the catalase peroxidase enzyme in *Mycobacterium tuberculosis*. One particular substitution in codon 315, AGC→ACC (Ser→Thr), was reported to be the most frequent. Other genes associated with INH resistance is *Inh A*, *kas A* and *ahp C* gene, but reported to be less than 10% cases of INH resistance.

Accordingly the present study was undertaken with the second objective of evaluating **(a)PCR-RFLP test to detect mutation at codon 315, AGC→ACC in *kat G* gene associated with isoniazid resistance and (b) Line Probe Assay for characterization of mutation in the *rpo B* gene responsible for rifampicin resistance.** The result of drug resistance to INH and RMP by PCR-RFLP and Line Probe Assay were further validated and extended by DNA sequencing and BACTEC 460 TB system.

Another important aspect in tuberculosis diagnosis is the identification of clinical isolates of mycobacteria to the species level. Accordingly, in the third objective of the present research work, "**a new PRA method is developed that is easier to perform and more precise for mycobacterial species identification than currently available PRA techniques**".

In the present research work 172 clinical samples of diversified origin were obtained with a strong clinical/radiological/histopathological evidence of TB (both pulmonary and

extrapulmonary cases) including clinical response to antitubercular treatment referred from different hospitals of Delhi.

All the clinical samples as mentioned above were subjected to Ziehl Nielsen (ZN) smear examination, radiometric BACTEC culture and PCR test by amplifying specific sequences of IS6110, 65kDa, 38kDa and 85 B complex of *Mycobacterium tuberculosis* genome. All the four PCR protocols were standardized and checked for sensitivity and specificity in 159 clinical samples. PCR targeting 123bp of IS6110 showed the highest sensitivity and specificity in diagnosis of tuberculosis in clinical samples of diversified nature.

As far as drug sensitivity in Mycobacterium tuberculosis is concerned the role of Inno-LiPa assay in the early diagnosis of rifampicin drug resistance was evaluated. It was found that the result of Inno-LiPa assay and DNA sequencing of rifampicin resistance determining region (RRDR), corresponding to codon 507-533 were concorded by 94.73% with phenotypic drug susceptibility testing. Majority of the resistant isolates (8 of 19, 42.1%) showed mutation at only codon position 531 of *rpo B* gene. Mutation were also observed at codon position 516, 522 and 526. Mutation at codon position 522(S522W, TCG→TGG) relatively a rare type of mutation as described in a few studies was also found in 3 isolates.

For the first time in India PCR-RFLP test was developed and standardized for detection of transversion at codon position 315 ACG→ACC(Ser→Thr) in *kat G* gene associated with INH drug resistance. This mutation was found in 74.19% in INH drug resistant isolates of *Mycobacterium tuberculosis* from Delhi.

Further RFLP of *rpo B* gene was used to differentiate the mycobacterial isolates at species level. By this test, *Mycobacterium tuberculosis, Mycobacterium avium, Mycobacterium fortuitum, Mycobacterium smegmatis, Mycobacterium kansasii* and *Mycobacterium gordonae* were differentiated.