

## A role of first line- line probe assay in previously treated cases of pulmonary tuberculosis: A retrospective study

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Received: 12-05-2021 / Revised: 16-05-2021 / Accepted: 11-06-2021

### Abstract

**Introduction:** India has a high tuberculosis burden country with second highest burden of MDR TB (Multidrug Resistant Tuberculosis) in the world. For rapid diagnosis of tuberculosis and drug sensitivity under National Tuberculosis Elimination Programme (NTEP) molecular method like Line Probe assay (LPA) is used in previously treated case of pulmonary tuberculosis at the time of diagnosis. Along with diagnosis, LPA provides result of drug sensitivity of Rifampicin and Isoniazid in same sitting within 72 hours. **Method:** Retrospectively we have collected data from NTEP register from January to December 2019 of first line-line probe assays done in previously treated cases of sputum positive pulmonary tuberculosis at the time of diagnosis. Aims and objectives of this study are to find out drug sensitivity pattern of Rifampicin and Isoniazid based on LPA results in previously treated cases of tuberculosis. **Result:** Out of 196 samples, LPA was positive in 187 samples with male-to-female ratio 2.5:1. Maximum number of patients were in age group 18-40 years followed by 41-64 years. Out of 187 positive LPA reports 6.6 % were Isoniazid monoresistance, 4% Rifampicin monoresistance, 4.5 % resistance to both Isoniazid and Rifampicin seen. Patients were put on antitubercular medications according to drug sensitivity and resistance pattern as per NTEP guidelines. **Conclusion:** By rapid molecular method like LPA, early diagnosis of tuberculosis and drug sensitivity testing in same sitting help in timely and effective management of disease. Early initiation of antitubercular treatment also decreases tuberculous transmission in community.

**Key words:** Previously treated Sputum positive Pulmonary Tuberculosis, Line Probe Assay, Drug Resistance

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### Introduction

Tuberculosis(TB) is major public health problem. As per the global TB report estimate 10 million people diagnosed with TB[1].India has the highest TB burden country in the world, accounting for an estimated of one-fifth of the entire global TB cases worldwide. It has an estimated prevalence of 3 million TB cases, with 2 million new cases occurring each year. About 280,000 people die from TB in India annually[2].

The prevalence of multidrug-resistant TB (MDR-TB), defined as resistance to at least rifampicin (RIF) and isoniazid (INH), is rising in a number of geographic regions. Globally in 2019, an estimated 3.3% of new cases and 18% of previously treated cases had MDR/RR-TB[3].

India has the second highest burden of MDR TB in the world after China, with an estimated 99,000 new cases per year[4].According to national DR TB survey prevalence of MDR is 2.84% in new TB cases and 11.62% in previously treated cases[5].

Drug resistance can develop due to inadequate or poorly administrated regimen, patients noncompliance, delayed in effective TB treatment or genetic mutation that makes drug resistance against bacilli[5].

Earlier in TB programme, MDR TB was suspected in all patients who failed the first-line drug regimen, all patients whose sputum was positive after 4 months of treatment, and all smear-positive contacts of MDR TB patients. Subsequently with availability of rapid diagnostic molecular method under programmatic management, criteria changed over the years as laboratory capacity and personnel expand.

As per guideline in management of DR TB in India 2019, presumptive DR TB includes 1) All notified TB patients 2) Follow up sputum positive on microscopy 3) Any clinical non responder[5].

There are two methods for culture and drug sensitivity testing 1).Phenotypic method involves solid culture like LJ(Lowenstein Jensen) Medium and liquid culture like MGIT(mycobacteria growth indicator tube). 2).Genotypic method like CBNAAT(Cartridge based Nucleic Acid Amplification Test) and LPA(Line Probe Assay). The advantage of the molecular method is that it gives the results faster, 2 hours in CBNAAT and 72 hrs in LPA. Shortening the time for diagnosing MDR-TB has the potential to improve access to appropriate treatment and reduce loss to follow-ups.

Line probe assays are a family of DNA strip-based tests that determine the drug resistance profile of a MTBC (Mycobacteria Tuberculosis Complex) strain through the pattern of binding of amplicons (DNA amplification products) to probes targeting the most common resistance associated mutations to first and second line agents and to probes targeting the corresponding wild-type (WT)

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DNA sequence. LPAs are WHO-approved tests for rapid detection of drug resistance to first and second line agents. They can be used for testing of culture isolates (indirect testing), as well as direct testing of acid fast bacilli (AFB) smear microscopy positive specimens (FL-LPA), and both smear positive and smear negative sputum specimens (SL-LPA)[6,7]. The LPA test only requires an average time of 2-3 days to diagnose MDR-TB, which is vastly shorter than the previous diagnostic methods like L-J Culture and liquid culture which take approximately 84 days and 42 days respectively.

While prevention of drug resistance is of paramount importance for ending TB, early detection and immediate enrolment as well as completion of an effective treatment regime are keys to interrupt the ongoing transmission.

**Materials and Method**

We collected data of all sputum positive previously treated cases of pulmonary tuberculosis underwent First Line LPA from the data register of NTEP from January 2019 to December 2019 from the

DMC laboratory of Sola Civil Hospital, Ahmedabad. Aims and objectives of this study were to find out drug sensitivity pattern of Isoniazid and Rifampicin resistance in sputum positive previously treated cases of pulmonary tuberculosis based on the LPA results.

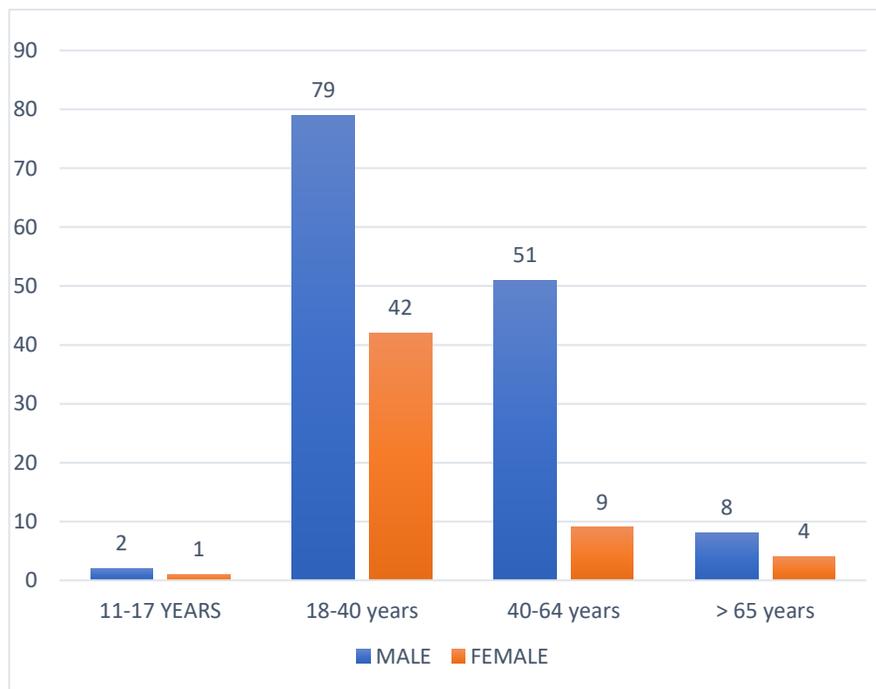
Inclusion criteria includes sputum positive previously treated pulmonary TB and age above 11 years. Exclusion criteria includes sputum negative patients, extrapulmonary Tb and age <11 years.

**Result**

There were a total of 196 previously treated pulmonary tuberculosis patients in which first line LPA was done after the diagnosis of sputum positivity. Out of these 196, 140 (71.46%) were male and 56(28.54 %) were female. Out of 196 cases 121 (61.73%) patients were in age group 18-40 years followed by 60 (30.16%) patients in 41-64 years. So more than 50 % patients were young adults while adult involvement was 30% (Table 1, Figure 1).

**Table 1 : Age distribution of previously treated sputum positive Tb patients.**

	AGE	MALE	FEMALE	TOTAL	PERCENT
ADOLESCENT	11-17 YEARS	2	1	3	1.5%
YOUNG ADULT	18-40 YEARS	79	42	121	61.73%
ADULT	40-64 YEARS	51	9	60	30.16%
ELDERLY	MORE THAN 65	8	4	12	6.1%



**Fig 1:Age distribution of previously treated sputum positive Tb patients.**

Out of 196 cases LPA positive in 187 cases with drug sensitivity seen as per table 2. So out of 187 cases only Isoniazid sensitivity was seen in 3 cases, only Rifampicine sensitivity was seen in 14 cases, both Isoniazid and Rifampicine sensitivity was seen in 140 cases, while isoniazid mono resistance was observed in 13 cases, rifampicine mono resistance in 8 cases and both isoniazid and rifampicine resistance(MDR TB) was seen in 9 cases only. (Table 2)

**Table 2 : Drugs Sensitivity result in LPA positive samples:**

DRUGS	SENSITIVE	RESISTANCE
ISONIAZID	03(1.5%)	13(6.6%)
RIFAMPICIN	14(7.1%)	8(4%)
BOTH H AND R	140(71.42%)	9(4.5%)
TOTAL 187	157	30

As per NTEP guideline drugs sensitive cases were put on first line oral antituberculosis treatment regimen.

Since 2017 for only INH resistance cases, there is separate MONO H regimen available under NTEP so 13 patients of INH mono resistance put on MONO H regimen.

Under NTEP Rifampicin mono resistance patients were also put on short course regimen like MDR cases because Rifampicin resistance is considered as a surrogate marker for MDR TB. So a total of 17 patients (8 only Rifampicin mono resistance and 9 cases of Isoniazid plus Rifampicin resistance) were put on short course MDR TB regimen as per programme guideline.(Figure 2).

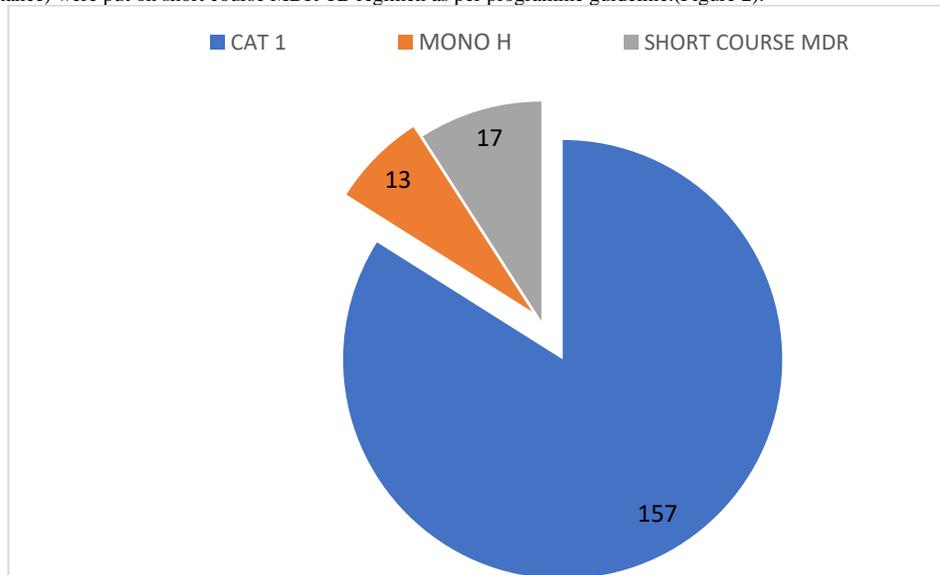


Fig 2: Regime as per NTEP guidelines.

### Discussion

Tuberculosis is caused by *Mycobacterium Tuberculosis* bacilli and it is a highly infective disease. It still remains a major public health problem worldwide. There are many methods for diagnosis of tuberculosis like sputum microscopy by Z-N(Zeil-Nehls) stain or LED (Light Emission Diode) fluorescent microscopy, culture methods like solid culture (L J medium), liquid culture (MGIT), molecular method like CBNAAT and LPA.

Microscopy gives the result whether bacilli are seen or not and by this method drug sensitivity testing is not possible. While the culture method (L J method and liquid culture) detects the growth of bacilli, from that growth, drug sensitivity testing can be done. But this is time consuming and requires expertise. The genotypic methods like LPA and CBNAAT are PCR base molecular methods which detect bacilli and drug sensitivity in same sitting so it is less time consuming than the culture methods with the added advantage of rapid drug sensitivity result[8]. Drug Resistant TB is a major health issue in tuberculosis management because it requires a lengthy treatment course with regimes involving second line drugs which are more toxic as compared to first line drugs. Early detection and timely management of drug resistant TB helps in early recovery, better patient compliance, less morbidity, decreased infectivity to other person and overall decrease in mortality.

Under NTEP, LPA testing is carried out in all previously treated cases of pulmonary tuberculosis at the time of diagnosis. LPA is a DNA stripbased test that gives result within 72 hours and give result of Isoniazid and Rifampicine sensitivity result in same sitting. CBNAAT is another rapid molecular method which gives result within 2 hours but it can detect only rifampicine drug sensitivity not isoniazid drug sensitivity. So LPA has the added advantage detection of isoniazid drug sensitive pattern.

Under the programme guidelines, DST based treatment regimen is available. At present three regimens for management of drug resistance are available 1) Mono H regimen for only isoniazid resistance patients 2) Short course MDR TB regimen for rifampicine and MDR (both isoniazid and rifampicine resistance) case and 3) Oral

longer regimen for MDR TB with any second line drug resistance cases.

In our study out of 196 cases LPA was positive in 187 cases. Out of this only isoniazid resistance is seen in 13 cases, all were put on Mono H regimen. while a total 17 patients (8 rifampicine resistance and 9 rifampicine plus isoniazid resistance) put on short course MDR TB regimen.

In present study we had 6.6% only isoniazid resistance, 4% only rifampicine resistance and 4.5% both isoniazid and rifampicine resistance. While in the study done by Prabha Desikan et al, 19% MDR(both isoniazid and rifampicine resistance) samples, 10.6% rifampicine mono resistance and 8.3% isoniazid mono resistance in MDR suspect patients by LPA method[9].

Study done by Neeta Singla et al shows that introduction of LPA significantly reduced the time taken for diagnosis so total time taken to initiate MDR TB treatment was also reduced[10].

Phenotypic and Genotypic analytical study conducted by Wah Wah Aung et al for newly diagnosed sputum positive pulmonary tuberculosis and it showed 23% isoniazid resistance and 18.3% rifampicine resistance. In this study Genotypic susceptibility results were 99.5% concordant and agreed almost perfectly with phenotypic DST[11].

In a study carried out in Andhra Pradesh, Dorai Deepa et al on isoniazid resistance impact on sputum positive retreatment Of 1,947 TB patients, 1,127 (58%) were tested with LPA—50 (4%) were rifampicine resistant, 933 (84%) were sensitive to INH and rifampicin and 144 (12%) were INH resistant. Out of these 64% had poor outcomes and hence they emphasised the need of a regular Mono H regime[12].

### Conclusion

Line Probe Assay is a molecular method which detects the mycobacteria tuberculosis bacteria along with sensitivity to isoniazid and rifampicin both simultaneously. As it gives results within 72 hrs, it decreases the time between diagnosis and initiation of treatment of drug resistant tuberculosis. From public health perspective it has a major benefit for the patient and community. Early initiation of treatment according to drug sensitivity helps in improving patient's

clinical condition and ultimately decreasing morbidity and improve quality of life of patient and decrease community transmission of TB.

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**Conflict of Interest: Nil Source of support: Nil**