

ORIGINAL ARTICLE

Assessment of the pattern of drug resistance in sputum positive smear cases of pulmonary tuberculosis using Line probe assay

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ABSTRACT:

Background: The present study was conducted for assessing the pattern of drug resistance in sputum positive smear cases of pulmonary tuberculosis using Line probe assay. **Materials & methods:** Patients with potential symptoms suggestive of pulmonary tuberculosis and history of treatment with antituberculosis drugs reported to Chest and T.B department. Hence, study was conducted on sputum smear positive previously treated pulmonary TB patients and follow up sputum smear positive new pulmonary TB patients. 100 patients were included in this study. All sputum smear positive cases with previous history of treatment with first line antituberculosis drugs were instructed to collect sputum sample in a sterile, leak proof falcon tube. The samples were immediately sent to Intermediate Reference Laboratory, Patiala for Line Probe Assay, as per guidelines. All the data were compiled in Microsoft excel sheet and were analysed by SPSS software. Fischer's exact t test was used for assessment of level of significance. P- value of less than 0.05 was taken as significant. **Results:** Isoniazid (H): Sensitive, Rifampicin (R): Sensitive was found to be present in 48 percent of the patients, while R: Sensitive, H: Resistant was found to be present in 29 percent of the patients. H: Sensitive, R: Resistant was found to be present in 7 percent of the patients while H: Sensitive, R: Sensitive was found to be present in 15 percent of the patients. **Conclusion:** Line probe assay is a rapid and accurate tool for identification of drug resistance in pulmonary tuberculosis patients.

Key words: Tuberculosis, Resistance

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This article may be cited as: Yadav H. Assessment of the pattern of drug resistance in sputum positive smear cases of pulmonary tuberculosis using Line probe assay. J Adv Med Dent Sci Res 2015;3(3):159-162.

INTRODUCTION

The global burden of tuberculosis (TB), particularly with multidrug resistance (MDR), is increasing and has become a major health challenge. The disease caused by *Mycobacterium tuberculosis* resistant to two primary antitubercular drugs, rifampin (RIF) and isoniazid (INH), is known as MDR-TB. Such instances are more common among clinical relapse cases. It has been reported that *M. tuberculosis* that is resistant to RIF is more likely to have concomitant resistance to INH, making RIF resistance a surrogate marker of MDR-TB. Early diagnosis of TB and rapid detection of RIF resistance is important for proper management of drug-resistant TB (DR-TB). But in spite of major efforts that are being done to increase case detection, one-third of new TB cases are still missed due to nonavailability of rapid, low-cost, and accurate diagnostic facilities in high-TB-burden countries.¹⁻³

Over the last 6 years, efforts have been made to improve and develop rapid diagnostic tools and drug susceptibility testing (DST) for TB. During this period, the World Health Organization (WHO) had issued 10 policy statements for improving diagnosis of TB, including the use of commercial and noncommercial DST methods and implementation of molecular methods such as the line probe assay (LPA) and Xpert MTB/RIF (or GeneXpert) assay. These molecular methods are developed to target the

rpoB gene, which consists of a 81-bp hot-spot region from codons 507 to 533, called the rifampin resistance-determining region (RRDR). So far more than 50 mutations have been characterized within this region by DNA sequencing but only point mutations at codons 526 or 531 are known to cause high levels of RIF resistance. In contrast, mutations in codons 511, 516, 518, 522, and 533 cause low-level resistance to RIF. Mutations conferring RIF resistance occur rarely in other regions of the rpoB gene.⁴⁻⁷ Hence; the present study was conducted for assessing the pattern of drug resistance in sputum positive smear cases of pulmonary tuberculosis using Line probe assay

MATERIALS & METHODS

Patients with potential symptoms suggestive of pulmonary tuberculosis and history of treatment with antituberculosis drugs reported to Chest and T.B department. Hence, study was conducted on sputum smear positive previously treated pulmonary TB patients and follow up sputum smear positive new pulmonary TB patients. 100 patients were included in this study. All sputum smear positive cases with previous history of treatment with first line antituberculosis drugs were instructed to collect sputum sample in a sterile, leak proof falcon tube. The samples were immediately sent to Intermediate Reference Laboratory, Patiala for Line Probe Assay,

as per guidelines. All the data were compiled in Microsoft excel sheet and were analysed by SPSS software. Fischer's exact t test was used for assessment of level of significance. P- value of less than 0.05 was taken as significant.

RESULTS

In the present study, a total of 100 patients of pulmonary tuberculosis were analyzed. Mean age of the patients of the present study was 39.5 years. 65 percent of the patients were males while the remaining 35 percent were females. Fever and cough were found to be present in 95 and 98 percent of the patients. Shortness of breath and weight loss was

found to be present in 53 percent and 86 percent of the patients. On radiographic examination, cavitation was found to be present in 66 percent of the patients while opacities were found to be present in 29 percent of the patients. Infiltration was found to be present in 5 percent of the patients. Isoniazid (H): Sensitive, Rifampicin (R): Sensitive was found to be present in 48 percent of the patients, while R: Sensitive, H: Resistant was found to be present in 29 percent of the patients. H: Sensitive, R: Resistant was found to be present in 7 percent of the patients while H: Sensitive, R: Sensitive was found to be present in 15 percent of the patients.

Table 1: Radiographic findings

Radiographic findings	Number of patients	Percentage of patients
Cavity	66	66
Infiltration	5	5
Opacities	29	29
Total	100	100

Table 2: Pattern of H and R resistance

H and R pattern	Number of patients	Percentage	p- value
H: Sensitive R: Sensitive	48	48	0.0001
H: Resistant R: Sensitive	29	29	
H: Sensitive R: Resistant	7	7	
H: Resistant R: Resistant	15	15	
Total	100	100	

R: Rifampicin

H: Isoniazid

DISCUSSION

Rapid laboratory identification of tuberculosis (TB) and Mycobacterium tuberculosis drug resistance are critical to ensure timely initiation of therapy, to inform appropriate TB therapy, and to facilitate infection control. Early diagnosis of TB and drug-resistant disease is of particular importance in human immunodeficiency virus (HIV)-infected individuals, as delay of therapy and drug-resistant TB can be devastating in those with compromised immune systems. Diagnosis of TB in HIV can be a particular challenge, as 24% to 61% of HIV coinfecting individuals with pulmonary TB have acid-fast bacillus (AFB)-smear-negative sputum. Culture-based testing for M. tuberculosis and M. tuberculosis drug resistance requires an unacceptably long turnaround for results, is limited by contamination rates, and requires considerable infrastructure and human resources. Molecular line probe assays (LPA) permit rapid diagnosis of TB, isoniazid and rifampin resistance, and clinically relevant non-M. tuberculosis mycobacteria. In these assays, DNA or RNA is isolated from culture or direct (i.e., sputum) respiratory samples and then amplified and reverse

hybridized onto a nitrocellulose strip with immobilized probes for different mycobacteria or for mutations that confer resistance. These strips can be quickly interpreted using a template, with the entire testing process taking a day or less in most cases.⁶⁻¹⁰ Hence; the present study was conducted for assessing the pattern of drug resistance in sputum positive smear cases of pulmonary tuberculosis using Line probe assay

In the present study, a total of 100 patients of pulmonary tuberculosis were analyzed. Mean age of the patients of the present study was 39.5 years. 65 percent of the patients were males while the remaining 35 percent were females. Fever and cough were found to be present in 95 and 98 percent of the patients. Shortness of breath and weight loss was found to be present in 53 percent and 86 percent of the patients. On radiographic examination, cavitation was found to be present in 66 percent of the patients while opacities were found to be present in 29 percent of the patients. Infiltration was found to be present in 5 percent of the patients. Out A et al analyzed the pattern of drug resistance among pulmonary tuberculosis patients. Sputum samples

from consecutive TB patients in Calabar were subjected to culture on Lowenstein-Jensen (LJ) slopes followed by drug susceptibility testing (DST). The DST was performed on LJ medium by the proportion method. Forty-two of the 100 *Mycobacterium tuberculosis* strains were found to be resistant to at least one drug. Resistance to only one drug (monoresistance) was found in 17 patients. No strains with monoresistance to rifampicin were found. Resistance to two drugs was found in 22 patients, while one patient was resistant to both three and four drugs. MDR TB was seen in 4% (4/100). The independent variables of HIV serology and sex were not significantly associated with resistance. There was a high prevalence of anti-TB drug resistance in Calabar.¹² Singhal R et al detected multi-drug resistant tuberculosis (MDR-TB) among MDR-TB suspects, and common mutations among MDR-TB cases using GenoType MTBDRplus. A total of 553 sputum samples from MDR suspects were received of which, 181 (32.7%) isolates were found to be multi-drug resistant. Missing WT8 along with mutation in codon S531L was commonest pattern for rifampicin resistant isolates (65.1%) and missing WT along with mutations in codon S315T1 of katG gene was commonest pattern for isoniazid resistant isolates (86.2%). Average turn-around time for dispatch of LPA result to these States from cultures and samples was 23.4 and 5.2 days, respectively. The MDR-TB among MDR-TB suspects in North-Eastern States of India was found to be 32.7 per cent. The common mutations obtained for RIF and INH in the region were mostly similar to those reported earlier.¹³

Isoniazid (H): Sensitive, Rifampicin (R): Sensitive was found to be present in 48 percent of the patients, while R: Sensitive, H: Resistant was found to be present in 29 percent of the patients. H: Sensitive, R: Resistant was found to be present in 7 percent of the patients while H: Sensitive, R: Sensitive was found to be present in 15 percent of the patients. Kumar P et al compared the performance of LPA with standard Bactec MGIT 960 system. In phase I, a total of 141 *Mycobacterium tuberculosis* isolates from our routine laboratory were tested by LPA and Bactec MGIT 960 for DST. In phase II, 578 sputum specimens of suspected DR-TB patients were received from the Punjab state of India. Of them 438 specimens or their cultures were subjected to LPA. The presence of mutant bands with their corresponding wild type band was identified as "hetero-resistance". In phase I, LPA showed high concordance with 96.4% positive agreement and 97.6% negative agreement with Bactec MGIT 960-DST. In phase II, 12 (2.7%) specimens were detected as invalid by LPA. Of the remaining 426 specimens, 184 (43.1%) had resistance to RIF and 142 (33.3%) to INH while 103 (24.1%) specimens showed resistance to both INH and RIF (MDR-TB) by LPA. Of the 142 INH resistant, 113 (79.5%) showed mutations in katG and 29 (20.4%) in inhA. A high rate of hetero-resistance

pattern was observed in rpoB gene (28.8%) and katG gene (9.8%). The most frequent mutation was S531L (81.1%) in rpoB region and S315T1 (100%) in katG gene.⁴

CONCLUSION

Line probe assay is a rapid and accurate tool for identification of drug resistance in pulmonary tuberculosis patients.

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