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Pattern of Drug of Resistance in Previously Treated Pulmonary Tuberculosis with Line Probe Assay.

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ABSTRACT

Background: Tuberculosis is global health problem known since ancient times. Drug-resistant TB has been known from the time of anti-TB drugs were first introduced for the treatment of TB. The emergence of drug resistant tuberculosis particularly MDR TB has become significant health problem worldwide and an obstacle to effective tuberculosis control. Line Probe Assay (LPA) is a Nucleic Acid Amplification Test (NAAT) which provides rapid diagnosis of R and H resistance and yield results in 72 hours.Literature on drug resistant pattern in patients who are previously treated for tuberculosis and/or suspected MDR cases with Line Probe Assay method are not studied hence this study was carried out. Aims and objectives: To know the drug resistance pattern of Rifampicin and Isoniazid in previously treated pulmonary tuberculosis cases and correlating with the demographic characteristics of patients. **Methods:** This study was carried out in department of Respiratory Medicine, Mahatma Gandhi Medical College and Hospital, Jaipur in AFB smear positive patients of pulmonary tuberculosis who have previously taken treatment, before reporting at OPD/IPD. The exclusion criterion was new cases of pulmonary tuberculosis. Detailed history, examination and investigations were carried out. The diagnosis of active pulmonary TB was based sputum smear examination by Ziehl - Nelson staining method. Sputum smear positive cases were subjected to line probe assay to detect resistant pattern at RNTCP accredited laboratory (SMS Medical College and Hospital, Jaipur). **Results:** A total of 175 previously treated sputum smear AFB positive patients of pulmonary tuberculosis were taken in this study. Out of which 141(80%) were males and 34(20%) cases were females with male : female ratio 4:1 and maximum cases (43.4%) belonged to 31-45 age group with mean age 38 years. Majority of cases belonged to rural area and lower middle class group. More than 2/3rd cases were smokers (72%) among male. Out of 175 cases, 100(57.1%) cases were drug resistant, 75(42.8%) cases were drug sensitive. Line probe assay with regard to resistant pattern was highest in grade +3 sputum (100%) followed by grade +2(98%) and grade +1(96%), while least in scanty sputum positive cases (3%). Resistance to Isoniazid (H), Rifampicin(R) and Both (H+R) were seen in 27%, 14% and 59% respectively. Half of patients (56%) out of total resistance belonged to default category of previously treated pulmonary tuberculosis cases. Resistance to H (27 cases) were 62.5% in relapse, 33.3% in default and 11% in failure cases. Out of R resistance (14 cases), 63% and 37% were in relapse and default cases. Out of 59 cases of H+R resistance 65% belonged to failure category and 20% default and 15% relapse category. **Conclusion:** Line Probe Assay (LPA) provides accurate and rapid diagnosis of R and H resistance and is recommended for diagnosis of DR-TB in previously treated pulmonary tuberculosis patients.

Keywords: Line Probe Assay, Multi Drug Resistant tuberculosis(MDR-TB)

INTRODUCTION

Tuberculosis is a major health problem worldwide including India. Tuberculosis is the second leading cause of death from an infectious disease worldwide.[1] Drug-resistant TB has been known since introduction of anti-tubercular drugs. First streptomycin was introduced in 1944. Then later in mid 1940s isoniazid, and para aminosalicyclic acid (PAS) was used to treat TB. Later on with advent of rifampicin, pyrazinamide and ethambutol, short course chemotherapy was introduced. Due to indiscriminate use of antibiotics initially and laxity in monitoring of drug regimens led to emergence of multi drug resistant strains of TB. India along with China and Russia accounted almost half of around 5 lakh MDR-TB cases to control MDR-TB in a community, one must rapidly diagnose patients and put them on appropriate treatment thus limiting further spread.[2] Conventional methods of drug susceptibility testing (DST)that include solid media based methods, liquid culture method takes up 2 to 6 weeks to produce definitive results, leading to prolonged infectiousness consequently increase incidence of MDR- TB. They are also more costly,

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complex and specific but not sensitive. It requires sophisticated laboratories and trained personnel and biosafety precautions.

WHO published a policy on Line Probe Assay (nucleic acid amplification technology which allows rapid detection of Mycobacteria tuberculosis complex along with Rifampicin and INH resistance) in 2008 which was further approved by Central Tuberculosis Division (CTD) and Government of India for diagnosis of MDR-TB in RNTCP.[3] DNA is extracted either from sputum specimens or from cultures isolates. It is able to detect mutation associated with lower level of phenotypic resistance. This is automated, standardized and quality assured. The result is obtained within 72 hours. Large number of specimen may be directly applied and processed at the same time. It is highly sensitive and specific for early diagnosis to MDR-TB.

There is paucity of clinical studies of drug resistant pattern in patients who are previously treated for tuberculosis and/or suspected MDR cases with Line Probe Assay modality. This study aims to know the drug resistance pattern of Rifampicin and Isoniazid in previously treated pulmonary tuberculosis cases and correlating with the demographic characteristics of patients.

MATERIALS AND METHODS

This observational prospective study was carried out in department of Respiratory Medicine, Mahatma Gandhi Medical College and Hospital, Jaipur in previously treated sputum smear AFB positive patients of pulmonary tuberculosis reporting at OPD/IPD. The exclusion criteria were untreated pulmonary tuberculosis cases.

Detailed history, examination and investigations were carried out. The diagnosis was based on detection of active pulmonary tuberculosis cases by sputum examination for Acid fast bacilli by using Ziehl - Nelson staining method and further evaluated and sputum sent to RNTCP accredited laboratory(SMS Medical College & Hospital, Jaipur) for Line probe assay for assessing drug resistant pattern.

RESULTS

A total 175 cases that were previously treated sputum smear positive cases were subjected to LPA testing. Out of which males were 141(80%) cases and females were 34(20%) cases. Male: female ratio was 4:1. The age distribution profile showed maximum cases (43.4%) belonged to 31-45 age group with mean age 38 years. No significant gender difference was seen in either age group. Majority of cases belonged to rural area and lower middle class group. More than 2/3rd cases were smokers (72%) in male. Out of 175 cases 100(57.1%) cases were found to be drug resistant and rest 75(42.8%) were drug sensitive.

Resistant pattern highest in grade +3 (100%) sputum followed by grade +2(98%) and grade +1(96%), while least in scanty sputum positive cases (3%). Out of 100 cases resistance to Isoniazid (H), Rifampicin (R) and both (H+R) was seen in 27%, 14% and 59% respectively. Previously treated pulmonary tuberculosis cases were categorized into default, relapse and failure category. Resistance to H was seen in 27 cases, out of which 62.5%, 33.3% and 11% were in relapse, default and failure cases. In R resistance (14 cases), 63% in relapse and 37% in default cases. Out of 59 cases of H+R resistance 65% belonged to failure category and 20% default and 15% relapse category.

Table 1: Gender wise distribution of previously treated active PTB cases.

Gender	Number	Percentage
Male	141	80
Female	34	20
Total	175	100

Table 2: Area wise distribution of previously treated active PTB cases

Area	Number	Percentage
Urban	79	45%
Rural	96	55%
Total	175	100%

Table 3: Socioeconomic status wise distribution of treated active PTB cases

Social status	Number	Percentage
Upper	4	2.5%
Upper middle	16	9%
Upper lower	42	24%
Lower	6	3.5%
Lower middle	106	61%
Total	175	100%

Table 4: Age distribution in previously treated active PTB cases

Age group	Male (141)	Female (34)	Total (175)
15-30	40(28%)	17(50%)	57(32.5%)
31-45	64(46%)	11(33%)	76(43.4%)
46-60	28(20%)	4(12.5%)	32(18.2%)
>60	9(6%)	1(4.2%)	10(5.7%)
Total	141(80%)	34(20%)	175(100%)

Table 5: Smoking pattern distribution of treated active PTB cases

Smoking status	Males	Female	Total
Smoker	101(72%)	6(17%)	107(61%)
Non smoker	40(28%)	28(83%)	68(39%)
Total	141(80%)	34(20%)	175(100%)

Table 6: LPA result vs Bacillary load

Sputum smear grading	Valid result	Invalid result	Total
+3	60(100%)	0	60(100%)
+2	20(98%)	1(2%)	21(100%)
+1	18(96%)	2(4%)	20(100%)
Scanty	2(3%)	72(97%)	74((100%)

Table 7: Drug resistant pattern in relation to sub category of previously treated cases of active PTB

	Mono INH Resistant (27)	Mono Rif Resistant (14)	H+R Resistant (59)	Drug sensitive (75)
Default	16(62.5%)	5(35.7%)	12(20%)	19(25%)
Relapse	9(33.3%)	9(62.5%)	10(15%)	56(75%)
Failure	3(11.1%)	0	38(65%)	0

DISCUSSION

India accounts for more than one-fourth of the global tuberculosis cases. 34016 patients of MDR-TB were detected and put on treatment under RNTCP in 2016.[4] Currently 2017, WHO estimated incidence of Rifampicin(R) and MDR-TB in India is estimated to be around 147000 i.e. 11 patients per 100 000 population annually.[2] Drug resistance has affected mankind sine historical time. From a microbiological perspective, the resistance is caused by a genetic mutation that makes a drug ineffective against the mutant bacilli. In clinical settings, an inadequate or poorly administered treatment regimen allows drug-resistant mutants to become the dominant strain in a patient infected with TB. The resistant strains of MTB are particularly problematic in developing countries because alternative agents are expensive, less effective, and less well tolerated than the standard counterparts.[5-7] Acquired drug resistance of MTB is defined as the acquisition of resistance to anti-tuberculosis drugs by the multiplication of the resistant mutant strains of bacteria as a result of inadequate chemotherapy. People who are at risk for MDR-TB include: previously treated for tuberculosis, contacts of patients previously treated and/or known to have MDR-TB, dwellers from developing countries, and patients with AFB positive sputum after 3 months of therapy.[8]

Detection of AFB in sputum smear is a simple, rapid, inexpensive and very specific for diagnosis for PTB, its limitation is its low sensitivity and lack of resistance pattern detection.[9] Sputum culture for Mycobacterium tuberculosis is highly sensitive and specific, but it takes 2-8 weeks' time depending on the method used.[10] Diagnosis of resistant strain of TB is difficult because of limitations of available mean like culture.

In our study 100 cases were diagnosed as drug resistant by line probe assay, maximum were males. Similar observation was reported by Gupta et al,[11] although Ghafoor,[12] et al showed distribution equal in male and female. Patients ranged from 18- 75 years and majority belonged to reproductive age group with mean age 38 years in present study. Similar results were with Sethi et al.[19] Majority of cases belonged to rural area and lower middle class group in our study. Similar results were seen in Gupta et al.[11] However no significant correlation of drug resistance with gender, age and socioeconomic status was seen. Majority of resistant cases were of rural background probably due to as our centre caters mainly rural population. More than 2/3rd cases were smokers (75%). There was no correlation between smoking and drug resistance among both male and female. Bacillary load showed more positivity of LPA results but it did not show any relation with drug resistance (p value=0.9). Correlation of bacillary load with drug resistance was not analyzed by other authors.

Previously treated patients often constitute a very heterogeneous group including those who have relapse after default, cure and treatment failure. Our study showed relationship between previous anti-TB treatment and drug resistance and majority cases belonged to default category. There are various other Indian studies which also observed similar results.[13]

Limitations of study: The limitations of our study were:

Drug resistance pattern was not correlated with radiology and comparison of resistant pattern between culture and line probe assay was not assessed.

CONCLUSION

Line Probe Assay (LPA) provides accurate and rapid diagnosis of R and H resistance and is recommended for diagnosis of DR-TB in previously treated pulmonary tuberculosis patients.

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