ORIGINAL ARTICLE

Prevalence of multidrug-resistant tuberculosis among category II treatment failures in North Karnataka

Mohammed Ashraf Ali S Namaji, Shobha D Nadgir, Jnaneshwara KB, Shylendra DS[1], Mahesh Kumar S, Yashwant AM[2]

Departments of Microbiology and [1]Pulmonary Medicine, Karnataka Institute of Medical Sciences, Hubballi, [2]District Tuberculosis Officer, Dharwad, Karnataka, India

ABSTRACT

Introduction: The worldwide emergence of multidrug-resistant tuberculosis (MDR-TB) is a major threat to tuberculosis (TB) control. **Objectives:** This study was undertaken to know the prevalence of MDR-TB among category II patients, who were treatment failures, in North Karnataka. **Materials and Methods:** Category II pulmonary TB includes those patients who are treatment failures, relapsed after treatment or defaulted during previous treatment. Only the patients who had failed previous treatment were included in the present study. Sputum samples obtained from all these patients, received between January 2014 and June 2014, were subjected to microscopy by the Ziehl–Neelsen (ZN) method, as per Revised National Tuberculosis Control Program (RNTCP) protocol. Sputum-positive samples were subjected to drug susceptibility testing by the rapid molecular assay, line probe assay (LPA). **Results:** A total of 379 patients were enrolled. Of these, 355 patients' sputum samples were positive for acid-fast bacilli (AFB) and one sample negative for AFB was culture-positive. All of these were subjected to LPA. The total number of MDR-TB detected was 71 (18.73%) patients. Mono-drug resistance to Rifampicin was detected in 30 (7.91%) and Isoniazid resistance in 32 (8.44%) patients. **Conclusions:** The magnitude of resistance being considerably high among the patients with treatment failures, it is essential to screen these patients for MDR-TB. Rapid diagnostic tests (molecular tests) such as the LPA will facilitate the diagnosis of MDR-TB at an early stage and thus will minimise transmission of the disease.

Key words: Line probe assay (LPA), mono-Isoniazid resistance in North Karnataka, mono-Rifampicin resistance in North Karnataka, multidrug-resistant tuberculosis (MDR-TB)

INTRODUCTION

Tuberculosis (TB) continues to be one of the most important infectious disease threats to human health.[1] Although standard anti-TB regimens have been established for decades, the recrudescence of TB in India has been exacerbated by the emergence and spread of multidrug-resistant tuberculosis (MDR-TB).[2] There are alarming reports of increasing drug resistance from various parts of the world, which potentially threatens to undermine the gains achieved in TB control over the last decade.[3] In 2013, globally 480,000 people developed MDR-TB and there were an estimated 210,000 deaths from MDR-TB.[4]

Category II pulmonary TB includes those patients who had failed previous TB treatment, relapsed after treatment, or defaulted during previous treatment. As such patients have already been exposed to anti-TB agents, they are at high risk for harbouring multidrug-resistant strains. Therefore, it is imperative to know the prevalence of MDR-TB among category II pulmonary TB patients.[3]

A smear-positive patient who is smear-positive at the fourth month or for longer after starting treatment is defined as treatment failure as per Revised National Tuberculosis Control Program (RNTCP) guidelines.

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Address for correspondence: Dr. Mohammed Ashraf Ali S Namaji, E-mail: ashrafnamaji@gmail.com

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The present study was undertaken to find out the prevalence of MDR-TB and the pattern of drug resistance among category II patients from 11 districts in North Karnataka, India who were treatment failures.

MATERIALS AND METHODS

This cross-sectional study was done at the Culture and Drug susceptibility testing (C&DST) laboratory TB unit, Department of Microbiology, Karnataka Institute of Medical Sciences, Hubballi. Sputum samples of all the patients, who were category II treatment failures, received between January 2014 and June 2014 were included in the study.

Sputum samples received were subjected to homogenisation and decontamination by the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method and microscopy by the Ziehl–Neelsen (ZN) method. Handling of samples, decontamination and homogenisation, inoculation on Löwenstein–Jensen (LJ) medium with the sediment and DNA extraction was done in biosafety cabinet (BSC) class II type A2.

Sputum samples positive for acid-fast bacilli (AFB) by ZN staining were subjected to drug susceptibility testing by rapid molecular assay, that is, line probe assay (LPA), whereas samples negative for AFB were subjected to culture on LJ medium. When growth occurred on LJ medium, the colonies were subjected to LPA.

Line probe assay

The GenoType MTBDR*plus* assay is a commercially available LPA from Hain Lifescience, Nehren, Germany, and is designed to detect the most important gene mutations conferring Rifampicin (rpoB genes) and Isoniazid (inhA, katG) resistance in *Mycobacterium tuberculosis*.[5]

Strict adherence to RNTCP protocol was maintained

For quality assurance, external quality control was carried out by retesting and panel culture testing by the National Tuberculosis Institute (NTI) Bangalore, which is a National Reference Laboratory (NRL).

RESULTS

Out of a total of 379 category II patients with treatment failure, who were enrolled, sputum samples of 355 (93.67%) patients were positive for AFB by ZN staining and microscopy. Of the remaining 24 samples, which were inoculated on LJ medium, one was culture-positive.

Of the 355 samples subjected to LPA, the total number of MDR-TB cases detected were 71 (18.73%), 30 (7.91%) showed Rifampicin resistance alone (mono-Rifampicin) and 31 (8.18%) cases of Isoniazid resistance alone (mono-Isoniazid). The remaining 223 (58.83%) sputum samples were sensitive to both Isoniazid and Rifampicin [Table 1].

One sputum sample that was smear-negative and culture-positive was also found to be mono-Isoniazid-resistant. Hence the total number of mono-Isoniazid resistant samples in our study was 32 (8.44%).

While the age of the patients ranged 11-60 years, the majority of MDR-TB cases were in the age group of 21-30 years. Of the 71 MDR-TB cases, 44 (61.97%) were males and 27 (38.02%) were females. The maximum number of MDR-TB cases, that is, 11 each were found in Yadgiri district (40.7%) and Karwar district (27.5%) [Table 2].

DISCUSSION

MDR-TB is defined as resistance to both Isoniazid and Rifampicin, with or without resistance to other first line anti-TB drugs.[4] MDR-TB results from either primary infection with resistant bacteria or may develop in the course of a patient's treatment.

Molecular assays to detect gene mutations that signal drug resistance are widely recognised as being most suited for rapid diagnosis, especially as these assays can be directly used on clinical specimens, such as sputum. Among the molecular assays, LPAs have shown great promise.[1] In a systematic review and meta-analysis of 15 articles performed by Ling *et al.*, they reported a pooled sensitivity of 98.1% and specificity of 98.7% for GenoType MTBDR assays.[1,6]

Table 1: Results of LPA

	Mono-Rif. resistance (%)	Mono-Isoniazid resistance (%)	MDR-TB (%)	Sensitive (%)	LPA not done	Total (%)
AFB- and culture-positive	30 (7.91)	31 (8.18)	71 (18.73)	223 (58.83)	—	355 (93.67)
AFB-negative and culture-positive		1 (0.26)			—	1 (0.26)
AFB- and culture- negatives					23	23 (6.07)
Total	30 (7.91)	32 (8.44)	71 (18.73)	223 (58.83)	23 (6.07)	379 (100)

Our study shows the prevalence of MDR-TB among category II patients who were treatment failures to be 18.73%. It is comparable with the World Health Organization (WHO) Global Report on Surveillance and Response, 2010, which states that MDR-TB prevalence is 17.2% among previously treated cases in India.[3,7] The prevalence of MDR-TB among category II pulmonary TB cases in different parts of India within the past two decades is shown in Table 3.

Among the 379 patients who were enrolled, 24 (6.33%) were smear-negative at C&DST lab. After registering as smear-positive at designated microscopy centers (DMCs), as per the protocol followed, a fresh sputum sample is collected in a sterile container and sent to C&DST lab for detection of drug resistance. Poor quality of such sputum samples, delay in sputum transportation from site of collection to the C&DST lab, and false positive reports due to nonspecific fluorescence (as the LED fluorescent microscopes were newly introduced in DMCs) are the probable reasons for this discrepancy. Except one, all these samples were culture-negative, which confirms the smear negativity report at the C&DST Lab. Evaluation of such patients by repeat sampling is necessary to rule out whether they are wrongly labeled as treatment failures.

The reemergence of TB as a global health problem over the past two decades, accompanied by increasing resistance, represents a serious problem in terms of both TB control and clinical management. The reasons for the mounting prevalence of MDR-TB may be due to underdiagnosis of TB cases and irregular drug consumption related to drug compliance.[12]

The prevalence of mono-Isoniazid resistance was found to be 8.44% and this is comparable with previous studies done in Chennai (10.6%)[8] and Jaipur (10%).[12]

The prevalence of mono-Rifampicin resistance was 7.91%. Different studies have reported prevalence of mono-Rifampicin resistance varying from 1.5-11%.[2,3,5,12] Mono-Rifampicin resistance is considered as a surrogate marker for MDR-TB.

Drug resistance is a dynamic phenomenon, and MDR-TB patients are at high risk of developing extensively drug-resistant TB (XDR-TB) if not properly managed.[3] Growing concerns regarding the spread of MDR-TB and alarm over the emergence of XDR-TB have sparked a great deal of interest in the development and application of rapid diagnostic tests for the detection of drug-resistant *Mycobacterium tuberculosis* disease, especially in settings with high human immunodeficiency virus (HIV) prevalence. Early detection of MDR-TB and XDR-TB is critical to initiate appropriate treatment, reduce morbidity and mortality, and prevent further transmission of drug-resistant strains of TB.[1]

Table 2: District-wise distribution of MDR-TB cases among category II pulmonary TB, treatment failure cases in North Karnataka

District	No. of samples	Resistance pattern		
		MDR-TB (%)	Mono-Rif. (%)	Mono-Isoniazid (%)
Bagalkote	40	7 (17.5)	2 (11.1)	Nil
Belgaum	43	4 (9.3)	3 (6.9)	Nil
Bidar	21	3 (14.2)	5 (23.8)	4 (19.04)
Bijapur	35	5 (14.2)	3 (8.5)	3 (8.5)
Davanagere	23	4 (17.39)	3 (13.04)	1 (4.3)
Dharwad	52	8 (15.3)	4 (7.6)	4 (7.6)
Gadag	28	6 (21.4)	1 (3.5)	8 (28.5)
Gulbarga	30	7 (23.3)	3 (10)	5 (16.6)
Haveri	40	5 (12.5)	2 (5)	1 (2.5)
Karwar	40	11 (27.5)	2 (5)	2 (5)
Yadgiri	27	11 (40.7)	2 (7.4)	4 (14.7)
Total	379	71	30	32

Table 3: Prevalence of MDR-TB among category II pulmonary TB cases in India

Location	Study period	MDR-TB (%)
Tamil Nadu[8]	1996	20.3
Delhi[9]	1996-1998	14
Bangalore[10]	1999-2000	12.8
Ahmadabad[11]	2000-2001	37
AIIMS, Delhi[3]	2005-2008	20.4
Burdwan, West Bengal[13]	2011-2012	80.81
KIMS, Hubballi Present study	Jan 2014-June 2014	18.73

CONCLUSION

The magnitude of drug resistance being considerably high among patients with treatment failures, it is essential to screen all these patients for MDR-TB. Rapid diagnostic tests (molecular tests) such as the LPA will facilitate the diagnosis of MDR-TB at an early stage and thus will minimise transmission of drug-resistant strains of TB.

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Conflicts of interest

There are no conflicts of interest.

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