Original Article

Pattern of Drug-resistance and Treatment Outcome in Multidrug-resistant Pulmonary Tuberculosis

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ABSTRACT

Aims and Objectives. To study the pattern of drug-resistance and treatment outcomes among patients with confirmed multidrug-resistant pulmonary tuberculosis (MDR-PTB).

Methods. A prospective study was conducted at Rajiv Gandhi Institute of Chest Diseases, Bengaluru, Karnataka, India. Between January 2005 and December 2008, 224 confirmed MDR-PTB cases were studied for various drug-resistance patterns, and their treatment outcomes were analysed until November 2010. Sputum culture and drug sensitivity tests (DST) were carried out at National Tuberculosis Institute, Bengaluru; DST was done for all first-line drugs except pyrazinamide.

Results. Of the 224 MDR-PTB patients, 146 (65.2%) were resistant to all first-line drugs, 39 (17.4%) to isoniazid, rifampicin and streptomycin; 19 (8.5%) to isoniazid, rifampicin and ethambutol; and 20 (8.9%) to isoniazid and rifampicin. Among them, 145 (64.7%) patients were cured, 5 (2.2%) had treatment-failure, 10 (4.4%) died, and 64 (28.5%) defaulted. Among 145 cured cases, 100 (69%) were resistant to all first-line drugs, 23 (16%) to isoniazid, rifampicin and streptomycin, 11(8%) to isoniazid, rifampicin and ethambutol, and 11(8%) to isoniazid and rifampicin.

Conclusions. The most common pattern observed in this study was resistance to all four first-line drugs followed by resistance to isoniazid, rifampicin and streptomycin. Patients resistant to all first-line drugs had early sputum culture conversion and better cure rate as compared to other resistance patterns. **[Indian J Chest Dis Allied Sci 2012;54:23-26]**

Key words: MDR-TB, Drug resistance, Treatment outcome.

INTRODUCTION

Tuberculosis (TB) is a major cause of morbidity and mortality in India. India accounts for 1/5th of global TB incidence. According to the World Health Organization (WHO), each year an estimated 9.4 million new cases of TB are detected leading to nearly 2 million deaths. In India, the numbers of TB patients are 1.96 million per year, and among them 0.8 million are new smear-positive cases comprising of 75 new sputum smear-positive cases per lakh annually with 0.33 million deaths per year. There is a rising trend of drug-resistant TB in different parts of the world, India being next only to China, both contributing more than 50% of global multidrug-resistant (MDR-TB) cases. Frequency of MDR-TB is less than 3% in new cases and 12% to 17% among re-treatment cases as per the recent studies.[1, 2] An MDR-TB patient is the one whose sputum culture is positive for *Mycobacterium tuberculosis* that is resistant *in vitro* to isoniazid (INH) and rifampicin with or without resistance to other anti-tuberculosis drugs based on drug sensitivity testing.[3] Since second-line anti-tuberculosis treatment (ATT) is more toxic and less efficacious than first-line drugs, treatment of MDR-TB is more challenging and requires judicious use of various regimens as per resistance patterns in specialised and designated centers. Hence, this study was done to assess the pattern of drug-resistance among confirmed multidrug-resistant pulmonary tuberculosis (MDR-PTB) patients and their treatment outcomes.

MATERIAL AND METHODS

This study was carried out at the Department of Pulmonary Medicine, Rajiv Gandhi Institute of Chest Diseases, Bengaluru. Between January 2005 and December 2008, 224 confirmed MDR-PTB cases were recruited and their treatment outcomes were analysed until November 2010.

All sputum smear-positive category II failures aged more than 15 years with confirmed reports of MDR-

[Received: March 8, 2011; accepted after revision: August 19, 2011]

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PTB from the National Tuberculosis Institute (NTI), Bengaluru, were included in the study. Culture and sensitivity reports from other non-accredited laboratories were not considered. Sputum culture for acid-fast bacilli (AFB) and DST were carried out at NTI, Bengaluru, a Revised National Tuberculosis Control Programme (RNTCP)-accredited laboratory. DST was done for all first-line drugs except pyrazinamide (PZA). DST for second-line drugs were carried out in a few MDR failure cases at Tuberculosis Research Centre (TRC), Chennai, the only south Indian centre with DST facility for second-line drugs. Category I and III failures were not included in this study as they were not considered as MDR-suspects when the study started.

Sputum specimens were collected in sterile wide mouthed bottles from sputum smear-positive patients of pulmonary TB. The collected specimens were processed by modified Petroff's method. For each specimen, two Lowenstein-Jensen (LJ) slopes were inoculated each with one 5 mm loopful of the centrifuged sediment, distributed over the surface. All cultures were incubated at 35-37 °C for up to 8 weeks. The tests were done in a biosafety class II cabinet.

Various regimens followed depending on the DST for the treatment of MDR-PTB in this study are given below.

The initial regimen contained at least three newer drugs that were not used previously and included at least one injectable aminoglycoside and PZA, as this combination has good bactericidal activity. The number of newer drugs varied in the intensive phase depending on the resistance pattern. One or more drugs, preferably weaker drugs (injectable and cycloserine), were not used in the continuation phase after sputum conversion. The total duration of the treatment was 18 to 24 months.

The patients were followed up every month during the entire period of treatment with clinical assessment and sputum smear examination for AFB. Sputum culture was done thrice during the entire course of treatment — one at sputum smear conversion, second at the end of intensive phase, and third at one month before completion of treatment. Five patients that failed treatment for MDR-PTB were subjected for sputum AFB culture and sensitivity to first- and second-line drugs at TRC, Chennai. Chest radiographs were done once in three months in all the patients. Blood urea and serum creatinine estimation were carried out monthly during the intensive phase in all the patients. Liver function tests were done at baseline in all the patients. Other relevant investigations were carried out based on clinical assessment.

Resistance Pattern	Intensive Phase Regimen
HR	Streptomycin + Ofloxacin/Levofloxacin + Ethionamide + Ethambutol + Pyrazinamide
HRS	Kanamycin + Ofloxacin/Levofloxacin + Ethionamide + Ethambutol + Pyrazinamide
HRE	Streptomycin + Ofloxacin/Levofloxacin + Ethionamide + Cycloserine/Para-aminosalicylic acid + Pyrazinamide
HRSE	Kanamycin + Ofloxacin/Levofloxacin + Ethionamide + Cycloserine + Pyrazinamide + Para-aminosalicylic acid

H=Isoniazid, R=Rifampicin, S=Streptomycin, E=Ethambutol

Conventional proportion method - LJ media with the recommended drug concentrations of 4mg/L for streptomycin, 0.2mg/L for INH, 40mg/L for rifampicin, 2mg/L for ethambutol and plain LJ medium for control were prepared. The growth of an isolate from culture was scraped with a loop and bacterial suspension was made in sterile distilled water, vortexed and matched with McFarland opacity tube No. 1. Dilutions of 10^{-2} and 10^{-4} were made and inoculated on both control and drug containing media and incubated at 37 °C. The first reading was taken after 28 days of incubation and if the isolate was susceptible a second reading was taken on 42nd day. Colonies were counted only on the slopes seeded with the inoculums that have produced exact readable counts or actual counts (up to 100 colonies on the slope). Ratio of the number of colonies in drug containing slopes by that in drug free slopes was considered as the percentage resistance (R). If R >1%, the isolate was considered resistant. H37Rv strain was used as standard control.

RESULTS

In total, 224 patients with confirmed MDR-PTB were studied. None of the patients were tested for PZA sensitivity. Of the 224 patients (158 males), 18 patients had diabetes mellitus, 5 had human immunodeficiency virus (HIV) co-infection, 3 had hypertension, 1 had ischaemic heart disease and diabetes mellitus, 2 had chronic obstructive pulmonary disease, and 1 had Hansen's disease. One hundered and forty-eight patients had cavitary lesions, while the remaining 76 had only infiltrates. Bilateral lesions were seen in 175 patients, right-sided

lesions in 27 patients and left-sided lesions in the remaining 22 patients.

Table 1 shows the pattern of drug-resistance in the study sample. Majority of them (65%) showed resistance to all the first-line drugs tested. The mean duration of sputum culture conversion according to the resistance pattern is presented in table 2. Treatment outcomes of various MDR-PTB patterns are shown in table 3.

Table 1. Various resistance patterns seen in the study sample

Resistance Pattern	Number of Patients
HRSE	146 (65.2)
HRS	39 (17.4)
HR	20 (8.9)
HRE	19 (8.5)

H=Isoniazid, R=Rifampicin, S=Streptomycin, E=Ethambutol
Figures in parenthesis indicate percentage.

Table 2. Time to culture conversion in the study sample

Resistance Pattern	Mean Duration of Sputum Culture Conversion (Days)
HRSE	40.3
HRS	48.2
HRE	51.3
HR	55.0

Table 3. Treatment outcomes in 224 patients with MDR-PTB

	HRSE	HRE	HRS	HR	Total
Cured	100 (69)	11 (58)	23 (59)	11 (55)	**145**
Defaulter	37	5	15	7	**64**
Failure	4	1	0	0	**5**
Death	5	2	1	2	**10**
Total	**146**	**19**	**39**	**20**	**224**

Figures in parenthesis indicate percentage.

Most common adverse drug reactions observed in this study were gastrointestinal disturbances mainly due to para-aminosalicylic acid (PAS), which subsided with routine management and assurance. Nine patients developed ototoxicity such as tinnitus, hard of hearing, positional imbalance due to streptomycin/kanamicin toxicity (confirmed by audiometry), necessitating us to stop the drug. Five patients complained of acne attributed to ethionamide; but, the drug was not stopped, and eventually the acne subsided in most of them. Nine patients developed cycloserine-induced psychosis requiring anti-psychotic medications. Cycloserine was stopped in two patients. Three patients developed swelling of the thyroid gland. Investigations confirmed hypothyroidism in them, and they improved following replacement therapy. Twelve patients developed arthritis along with elevated serum uric acid levels which warranted us to stop the drug temporarily; they were treated with non-steroidal anti-inflammatory drugs. Uric acid level estimation was done monthly and PZA was re-started once the uric acid levels normalised.

DISCUSSION

An appropriate assessment of various patterns of drug-resistance among patients with confirmed MDR-PTB is required to initiate a proper regimen as per DST to improve the treatment outcome. A mere diagnosis of MDR-PTB and initiation of second-line ATT without proper regimens based on DST may not help achieve a good treatment outcome. As there is an increasing trend of MDR-PTB in India, proper formulation of treatment regimens consisting of newer drugs based on various drug resistance patterns in confirmed MDR-PTB cases is very much required as evident from the present study.

In the present study, 65.2% were resistant to all first-line drugs, 91.2% were resistant to at least one other first-line drug apart from INH and rifampicin. Rao *et al*[4] showed that of the 577 proven MDR-TB patients, 56.5% had isolates resistant to all first-line drugs, 88% cases had MDR plus resistance to at least another first-line drug. In the present study, MDR plus streptomycin resistance was 17.4% and MDR plus ethambutol resistance was 8.5%, while Kudzawu *et al*[5] reported it to be 25% for MDR plus streptomycin resistance and 21.4% for MDR plus ethambutol resistance.

Isolates resistant only to INH and rifampicin were 8.9% in the present study as compared to 10.7% in a study by Chowgule and Dheodhar,[6] 25.2% in a study by Dheodhar *et al*,[7] 21.4% in a study by Janmeja *et al*,[8] and 35.7% in the study by Kudzawu *et al*.[5]

The mean sputum culture conversion was analysed in MDR-PTB patients with various drug-resistance patterns. The patients with resistance to all first-line drugs showed a mean sputum conversion of 40 days and the conversions observed in other resistance patterns were 48 days for MDR plus streptomycin, 51 days for MDR plus ethambutol and 55 days for isolated MDR. In the present study, the mean time to culture conversion observed in all 224 patients was 48.6 days. Joseph *et al*[9] found that of the 38 cases, 82% had culture conversion in two months or less. Shin *et al*[10] in a study of 230 patients found a culture conversion of 95% after a median period of two months.

The treatment outcomes were also analysed in the present study. In a total of 224 cases, 145 (64.7%) were cured, 5 (2.2%) had treatment failures, 10 (4.4%) patients died, and 64 (28.5%) patients defaulted.

Masjedi *et al*[11] in a study of 43 cases, documented that 29 (67.5%) had a successful outcome (cured), 6 (14%) had treatment failures, and 8 (18.6%) patients died with no defaulters. In the study by Shin *et al*,[10] 77% were cured, 5% died, 7% failed, and 12% defaulted. Patients with resistance to all first-line drugs showed an early sputum culture conversion and a better cure rate compared to other resistance patterns. These observations were probably due to judicious use of more newer drugs in the initial regimen which were not used previously in those patients and is attributed to the bacilli being fully sensitive to these new drugs.

In the present study, 28.5% of patients defaulted despite good pre-treatment counselling and providing the drugs for free under supervision, probably due to a sense of well-being after a few months of treatment or due to social stigma. Inability to collect drugs from the centre due to costs involved in travel and loss of earnings for that particular day was observed more commonly in males as they were the only earning members of the family and also due to broken families. Proper counselling, education, and motivation are needed to improve the adherence to treatment and cure rates. The cure rate (64.7%) in our study was good mainly because of formulation of appropriate treatment regimens based on the various drug-resistance patterns.

In conclusion, close monitoring of drug-resistance patterns in confirmed MDR-PTB isolates is required to formulate different regimens as per the drug-resistance pattern. The commonest pattern observed in this study was resistance to all four first-line drugs followed by resistance to isoniazid plus rifampicin plus streptomycin. Patients resistant to all first-line drugs showed a better cure rate compared to other resistance patterns. Hence, early diagnosis of MDR-PTB and treatment under supervision by formulating appropriate regimens based on resistance pattern are the keys to success in treating MDR-PTB.

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