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Research Paper

Evaluation of Drug Susceptibility Profile Among Category Ii Pulmonary Tuberculosis Patients (Relapse, Failure, Defaulter) at Tirunelveli

Medical Science

KEYWORDS : Mycobacterium tuberculosis, Multidrug resistant tuberculosis, Drug susceptibility testing

Amudha VP	Amudha VP.M.D., Department of Microbiology, Tirunelveli Medical College, Tirunelveli - 627 011, Tamil Nadu, India
Ramnath R	Ramnath R, DTCD., Consultant in Thoracic Medicine Department, ESI hospital, Tirunelveli-627011
Palaniappan N	Palaniappan N, M.D., Department of Microbiology, Tirunelveli Medical College, Tirunelveli - 627 011, Tamil Nadu, India

ABSTRACT

The present study was undertaken at Tirunelveli Medical College aiming to find the drug susceptibility profile among previously treated pulmonary tuberculosis cases and identify the risk factors of acquiring drug resistant TB. Early morning sputum specimens were collected, cultured and drug sensitivity testing was performed. Of the 108 samples processed, 78.7% showed a positive growth for M. tuberculosis, 13.9% were culture negative, 3.7% were contaminated and 3.7% had a growth of non tuberculous mycobacteria. Among the 85 culture positive cases, 50 (58.8%) were sensitive to the first line drugs and 35 (41.2%) were resistant to one or more drugs. Resistance to one drug was noted in 14 (16.6%), to two drugs in 9 (10.6%), to three drugs in 10 (11.8 %) and to all four drugs in 2 (2.4%) cases. Monodrug resistance was most commonly seen with Isoniazid and Streptomycin (6 patients each, 7.1%), followed by Rifampicin and Ethambutol (1 patient each, 1.2%). Among the 35 drug resistant cases, cumulative resistance was highest to Isoniazid (27 patients 31.7%) followed by Streptomycin (21 patients, 24.7%), Rifampicin (19 patients, 22.3%) and Ethambutol (3 patients, 3.5%). 18 were MDR TB (21.2%) among the culture positive cases. No XDR TB cases were present. Age, sex, residence, education, income, diabetes were not risk factors for multidrug resistant tuberculosis. Smoking and taking irregular and interrupted treatment was a significant risk factor among MDR cases.

Introduction

"I have no business to live this life if I cannot eradicate this horrible scourge from the mankind,"-Robert Koch, delivering a lecture at Berlin University on his discovery of tuberculosis bacilli, 1882.

It has been 125 years since Robert Koch first discovered the tuberculous bacilli and the world is still fighting hard to control the disease. The poor and developing countries are still in the grip of TB. Almost 40 years after introduction of combination chemotherapy for TB, and with the accumulated knowledge of the mechanisms leading to development of drug resistance, drug resistant TB, particularly MDR forms, remain a barrier to TB control.

Today, with the greatly expanded efforts to strengthen tuberculosis prevention and control programmes worldwide, there is growing concern about the currently reported and potential future rates of drug-resistant tuberculosis. The resistant cases must be identified as swiftly as possible when they present at health care facilities so that they do not pose a threat to the community.

The present study was undertaken at Tirunelveli Medical College aiming to find the Drug susceptibility profile among previously treated pulmonary tuberculosis cases and identify the risk factors of acquiring drug resistant TB.

Material and methods

The present study was conducted at the Department of Thoracic Medicine, Tirunelveli Medical College, Tirunelveli for a period of one year from May 2007 to April 2008 to assess the drug susceptibility profile of Category II patients registered under RNTCP.

The study population constituted smear positive patients previously treated under RNTCP, comprising cases of Failure, treatment after Default and Relapse started on the CAT-II regimen. Extra pulmonary cases of Category II and cases that had been previously treated privately were not included for the study.

Socio demographic and clinical characteristics such as smear status, type of case, type of disease, category, treatment details such as drug regularity, number of doses taken by the patients and reasons for default were obtained from patient. Information on patient's literacy, occupation, and personal habits like smoking, other diseases like diabetes and HIV were also obtained.

Study Samples

Early morning sputum specimens were collected in a sterile container from the study group who were smear positive by Ziehl Neelsen method. All the laboratory works were carried out as per standard laboratory procedures and Bio-safety norms in Class II Biosafety cabinet. The smears were graded according to the RNTCP guidelines[1].

Culture of Sputum Specimens [2]

The specimens were subjected to a proper digestion and decontamination procedure by Sodium Hydroxide (Modified Petroff's Method) or Cetyl Pyridinium Chloride (CPC) and Sodium Chloride (Nacl) method.

Culture Examination

Typical colonies of *M. tuberculosis* are rough, crumbly, waxy, non-pigmented (buff colored) and slow-growers having the appearance of breadcrumbs or cauliflower. All cultures were examined within 48-72 hours after inoculation to detect gross contaminants. Thereafter cultures were examined weekly, up to 8 weeks on a specified day of the week.

Biochemical methods for identification of mycobacteria

Identification of Mycobacteria species were done by biochemical tests like susceptibility to p-nitro benzoic acid (PNB) and niacin production test.

Drug Susceptibility Testing 3

The drug sensitivity testing for the positive cultures were carried out at Tuberculosis Research Centre (ICMR), Chetput, Chennai. The inoculum was prepared by using a representative sweep of the entire surface of the growth on the slope. The absolute concentration method uses a standardized inoculum grown on drug-free media and media containing graded concentrations of the drugs to be tested. Resistance is expressed in terms of the lowest concentration of the drug that inhibits growth; i.e., minimal inhibitory concentration (MIC).

Results

108 cases of retreatment pulmonary tuberculosis were included for the study for whom the drug susceptibility profile and risk factors for acquiring drug resistant TB were analysed. 85

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sputum samples (78.7%) showed a positive growth for M. tuberculosis, 15 samples (13.9%) were culture negative, 4 samples (3.7%) were contaminated and 4 samples (3.7%) had a growth of Non tuberculous mycobacteria. Among the 85 culture positive cases, 50 were sensitive to the first line drugs (58.8%). The remaining 35 were resistant to one or more drugs (41.2%).

Among the total culture positive cases cumulative drug resistance was most commonly seen to Isoniazid (27 patients 31.7%) followed by Streptomycin (21 patients, 24.7%). Resistance to Rifampicin was seen in 19 patients (22.3%) and to Ethambutol in 3 patients (3.5%).

Mono drug resistance was noted in 14 patients (16.6%). It was most commonly seen with Isoniazid and Streptomycin (6 patients each, 7.1%), followed by Rifampicin and Ethambutol (1 patient each, 1.2%).

Poly-drug resistance was observed in 21 patients (24.7%). Resistance to any two drug combination was seen in 9 patients (10.6%) and to any three drugs in 10 patients (11.8%). Resistance to all four drugs was seen in 2 patients (2.4%).Most of the Rifampicin resistant cases were also resistant to Isoniazid. A high degree (21.2 %) of MDR-TB was observed among the study group.

Of the 18 MDR cases, 9 cases were sensitive to all the second line drugs tested. Of the 9 cases that were resistant to the drugs, resistance to Ethionamide alone was seen in 5 cases, and Ofloxacin alone in 1 case. The Kanamycin and Ethionamide combination was resistant in 2 cases and Ethionomide plus Ofloxacin resistance was seen in 1 case.

8 cases were HIV reactive in the total study group. HIV and MDR co-infection was seen in only one case and Rifampicin monoresistance was observed in a case.

Table -1. Percentage distribution of drug resistance.

Particulars	Cases	
	N	%
Total patients recruited in the survey	108	100
Total patients with DST results(n=108)	85	78.7
Total patients with susceptible isolates(n=85 henceforth)	50	58.8
Total patients with drug resistance	35	41.2
Any resistance to H	27	31.7
Any resistance to R	19	19
Any resistance to E	3	3.5
Any resistance to S	21	24.7
Total patients with mono-resistance	14	16.6
Resistance to H only	6	7.1
Resistance to R only	1	1.2
Resistance to E only	1	1.2
Resistance to S only	6	7.1
Total patients with poly resistance	21	24.7
Resistance to any 1 drug	14	16.6
Resistance to any 2 drugs	9	10.6
Resistance to any 3 drugs	10	11.8
Resistance to any 4 drugs	2	2.4
Total patients with MDR TB	18	21.2

Discussion:

Among the 85 culture positive cases, 50 were sensitive to the first line drugs (58.8%). The remaining 35 were resistant to one or more drugs (41.2%). Resistance to one drug was noted in 14 patients (16.6%), to two drugs in 9 patients (10.6%), to three drugs in 10 patients (11.8 %) and to all four drugs in 2 patients (2.4%). The similar pattern of resistance was observed by *Shah et al*[4] and *Sophia Vijay et al*[5] except for all four drugs which was 15.21% in the former and 4% in the latter.

Single drug resistance was most commonly seen with Isoniazid and Streptomycin (6 patients each, 7.1%), followed by Rifampicin and Ethambutol (1 patient each, 1.2%). Resistance to Isoniazid plus Rifampicin alone was seen in 6 patients (7.1%) similar to study of *Shah et al*[4] which showed 9.2 % and 4.9% by *Sophia Vijay et al*[5].

Most of the Rifampicin resistant cases were also resistant to Isoniazid. This has also been observed in studies of *Trivedi et al*[6] and *Shah et al*[4]. Drug resistance to Rifampicin in the present study was 22.3% which is similar to that reported from Jaipur 28.2% by *Malhotra et al*[7], New Delhi 33.7% by *Jain et al*[8], Gujarat 37.3% by *Trivedi et al*[6] and Gujarat 37.47% by *Shah et al*[4] though Bombay reports a very high incidence of Rifampicin resistance of 66.8% *(Chowgule et al).* [9]

Ethambutol was the least resistant drug in all the studies as in the present study, although the percentage was very less (3.5%) compared to 6.6% in Bangalore *(Sophia Vijay et al)* [5], Bombay 8.4% *(Chowgule et al)* [9], Indore 22% *(Hemvani et al)* [10], Chennai 28.7% (*Deivanayagam et al)* [11], Gujarat 35.45% (*Shah et al)* [4] and Jodhpur 39.39% (*Mathur et al)* [12].

A high degree (21.2 %) of MDR-TB, in accordance to most of the studies in India was observed. Proportion of MDR- TB in re-treatment cases varied from 100% *Raichur,* Karnataka, 69% North-Arcot study, *(Paramasivan CN et al 2002)*[13], Jodhpur 38.2% *(Mathur et al)*[12], Jaipur 24.3 % *(Malhotra et al)*[7], 20.3 % *(Paramasivan, 1998)*[14], 17.2%-Gujarat *(Anti-tuberculosis drug resistance in the world, WHO Fourth Global Report 2008),* [15] Bangalore 12.8% *(Sophia Vijay et al, 2002*)[5], Indore 8.1%. *(Hemvani et al)* [10].

Of the 18 MDR cases, 9 cases were sensitive to all the second line drugs tested and 9 cases were resistant to the drugs. Resistance to Ethionamide was seen in 8 cases explaining cross-resistance of Ethionamide with INH. However, the fact that all tested isolates were resistant to Isoniazid but one was not resistant to ETH might suggest that the mutations leading to drug resistance are located in different regions of the genome. Kanamycin resistance was seen in 2 cases, both of which had resistance to Streptomycin also. *Rajesh Mondal et al 2007*[16], reported 7.4 % of XDR TB cases, the first ever report from India. A limitation to accurate detection of XDR TB is because; the existing tests for resistance to second line drugs is not yet standardized and are less reproducible than results for first line drugs.

8 cases were HIV reactive in the total study group. HIV reactive Category II failure case was multidrug resistant. A review of the published literature by *Ormerod LP* [17] and *Sharma et al*[18], suggests that, in the early 1990s, several institutional outbreaks of MDR-TB among HIV-infected patients drew attention to the problem. In this study, HIV and MDR co-infection was seen in only one case and Rifampicin monoresistance was observed in a case. Since the HIV cases were not in significant numbers, an attribute could not be made out. Studies of *Barroso et al* [19], *Spellman et al* [20], *Asch et al* [21] have found that MDR-TB is not more common among people infected with HIV. But *Swaminathan et al*[22] in their study have observed MDR-TB in 13.5 % of the re-treatment HIV cases. *Sharma et al* [23] and *Jasmin Johnson et al*[24] have demonstrated Rifampicin monoresistance in HIV patients.

Risk factor analysis:

Because of the incompliance of more male than female cases being registered for re-treatment were noted, gender issues are significant for development of drug resistance also though a statistical association could not be found in the present study. An European study by *Faustini et al* [25] observed more MDR cases among men. *Barroso et al* [19] and *Pande et al* 26 did not associate gender and MDR TB.

There was no significant difference in drug resistance among the rural and urban cases under study. But the study of *Deepak Almeida et al* [27] highlights an alarmingly high percentage of MDR TB in an urban area (51%) than a rural center (2%). For epidemiological reasons, there may be less onward transmission of multidrug-resistant strains in rural areas with low population densities.

The association known for centuries between TB and poverty

also applies to MDR-TB but we found no significant association between MDR-TB and family income. Poor nutritional status results in a decrease in the plasma drug concentration time curve and an increased renal clearance of unbound drug. In effect, low serum levels of anti-TB drugs result in patients being administered fewer anti-TB medications or, in some cases, even monotherapy. The latter regimen could then lead to acquired drug resistance as observed by *Byrd RP* [28].

Though the educated study groups were in less numbers for retreatment and development of drug resistance, there was no association observed in the present study. *Al Jarad et al*[29], *London* and *Murray et al*[30], *South Africa* found no association with MDR-TB but *Barroso et al*[19] revealed an association between MDR-TB and lack of school education.

There was a significant association between smoking and multidrug resistant tuberculosis in the present study (62% vs. 17%). *Barroso et al* [19] (60 % vs. 40 %) identified that smoking was associated with MDR-TB in their analysis. However *Pande et al* [26], India observed that smoking had no relation to infection with MDR organism.

There was no evidence for an increased risk of MDR TB among people with diabetes in the present study. *Mona Bashar et al* [31] in his study found out that diabetic patients were more than five times as likely to have infection with a multi drug resistant strain of tuberculosis. In a study by *Subhash et al*[32] CMC, Vellore on patients with diabetes and Tuberculosis, only 26% of the diabetic subjects with tuberculosis had MDR TB, which ruled out an association between both as observed by *Barroso et al* [19](8%).

Among those who had taken irregular treatment, 89% developed multi-drug resistant tuberculosis which was statistically significant. There is virtually a consensus among researchers regarding the fact that the number of previous treatments is a risk factor for MDR TB and our study confirmed this association. *Pande et al* [26] observed the prevalence of MDR-TB in patients with past history of ATT at two centres in Delhi to be 44.7% and 20% which was statistically significant in their study. 84.8% of MDR TB cases had irregular and interrupted treatment as reported by *Vasanthakumari et al* [33]. Poor past compliance to treatment was associated with MDR TB in the study of *Sharma et al* [18]. *Barroso et al* [19] observed that number of previous treatments and irregular treatments were significant risk factors for MDR TB.

Conclusion

Age, sex, residence, education, income, diabetes were not risk factors for multidrug resistant tuberculosis. Smoking was a significant risk factor among MDR cases. There is a need to devise effective strategies for counseling patients about the impact of smoking on their cure. Irregular and interrupted treatment was a risk factor among multidrug resistant cases. Ensuring adherence to a full course of treatment is the key to cure TB patients and prevent the emergence of drug resistance. HIV reactivity was noted among MDR cases.

Though registered for retreatment, most of the isolates were sensitive to all the first line drugs and hence can be successfully treated with a category II regimen if they are compliant enough. Drug resistance was more among prior treatment failure cases, necessitating the need for timely culture and sensitivity testing for those who remain sputum positive during the course of treatment to curb the spread of multidrug resistant strains.

Continuous monitoring of the trends of drug resistance is essential to assess the current interventions and their impact on the TB epidemic. Above all ensuring adherence to a full course of treatment is the key to cure TB patients and prevent the emergence of drug resistance.

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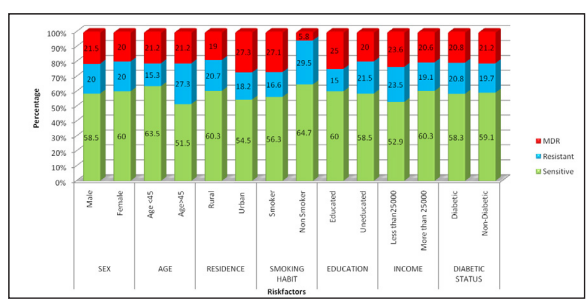

Figure-1. Risk factors among multi drug resistant cases

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