

Development of Multidrug-Resistance among Previously Treated Cases of Pulmonary Tuberculosis: A Public Health Concern

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Abstract

Background: Multidrug-resistant tuberculosis (MDR-TB) has emerged as a significant global health concern. The most important risk factor for the development of MDR-TB is the previous anti-tuberculosis treatment.

Objective: To describe the development of MDR among previously treated cases of Pulmonary Tuberculosis.

Study type, settings and duration: A cross-sectional, descriptive study was conducted at PHRC TB Research Center, King Edward Medical University/Mayo hospital, Lahore by involving previously treated patients diagnosed between March 2015 to March 2016.

Methodology: All sputum-positive previously successfully treated pulmonary TB cases were subjected to mycobacterium culture, GeneXpert and drug-susceptibility testing (DST). MDR-TB was defined as TB caused by bacilli showing resistance to at least isoniazid and rifampicin.

Results: A total of 139 patients were initially selected but later on only 95 cases of sputum-positive previously treated pulmonary tuberculosis patients were included in the study. Of those, 95 previously treated cases, 40 (42.1%) patients had developed MDR-TB. The mean age of MDR-TB patients was 35.5 ± 16.7 years; 20 patients (50%) were male and 20 (50%) were female. The prevalence of MDR-TB among previously treated pulmonary tuberculosis patients was found to be 42.1%.

Conclusion: The prevalence of MDR-TB in previously treated patients of TB patients was significant. Findings of this report suggest that high ratio of previously treated pulmonary TB patients, developed MDR-TB. Therefore, each patient should be screened for MDR-TB using rapid diagnostic tests (molecular tests) such as the line probe assays, GeneXpert at the end of their treatment. This will facilitate the diagnosis of MDR-TB at an early stage and thus will minimize transmission of the disease.

Key words: Tuberculosis, multidrug resistance, previously treated cases.

Introduction

Tuberculosis (TB) is as old as the mankind, and one of the leading causes of death in the world today.¹ The World Health Organization (WHO) estimates that Mycobacterium tuberculosis caused active disease in 9.15 million people across the

globe, killing 1.6 million of them. More people carry the bacillus today (one-third of the world's population) than at any other period in history.¹ Its symptoms, such as hacking, productive cough, chest pain and fever were accurately identified by Hippocrates in the fifth century BC. Its contagiousness was established as early as the eleventh century AD and the bacterium that causes it was isolated by Robert Koch in 1882.² The strains that are resistant to the two main first line anti TB drugs that form the back bone of short course chemotherapy, isoniazid and rifampicin are known as multi drug resistance tuberculosis (MDR-TB). These strains have been found throughout the world and are a significant cause of global TB morbidity and mortality.^{3,4}

In Pakistan, incidence of tuberculosis is estimated as 181 per 100,000 populations and the rising trend of drug resistance is alarming. According to WHO, the amount of drug resistance

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Authors Contribution

AAS conceptualized the project. AAS & RI did the data collection. RI & AI revised the manuscript with intellectual content. MA & TR performed the statistical analysis. Drafting, revision and writing of manuscript was done by TR.

has been trending upward in many parts of the world and reports show that multi drug resistance tuberculosis is increasing by 3 percent among new patients.⁵ The total global burden of multi drug resistance tuberculosis is estimated at almost 490,000 new cases per year or over 4 percent of all TB cases; an estimated 120,000 of these patients die annually.⁶

MDR-TB is called as a man-made phenomenon and it arises due to an inadequate treatment of drug-sensitive TB.⁷ The prevalence of MDR-TB mirrors the functional state and efficacy of tuberculosis control programs in the country.⁸ Previous treatment for TB is considered as a strongest risk factor for the development of MDR-TB.⁸ Previous treatment or category II pulmonary TB includes those patients who had failed previous TB treatment, relapsed after treatment or defaulted during previous treatment.⁹ Since such patients have already been exposed to anti-tuberculosis agents they are at high risk for developing multi-drug resistant strains. Therefore, it is necessary to know the chances of development of MDR-TB among previously treated pulmonary TB patients. The present study focused on the development of MDR-TB among previously treated pulmonary TB patients reporting at a tertiary care hospital in Lahore.

Methodology

This descriptive cross-sectional study involved previously treated sputum positive pulmonary tuberculosis patients. Standard definitions for treatment failure, relapse and default were used⁶. The cases were selected between March 2015 to March 2016 and their respective sputum was taken and tested in PHRC TB Research Center King Edward Medical University/ Mayo hospital, Lahore. All the cases who were coming to the clinic were enrolled and then screened as per protocols of the study. Since Mayo hospital is a tertiary care hospital and the patients came here from all over the province, so the sample size actually represents a large number of community.

A new TB patient was defined as a patient who had never received TB treatment or who had received TB treatment for <1 month. A TB patient previously treated with only first-line drugs was defined as TB patient who had been treated for >1 month with only first-line anti-TB drugs.⁶

A written informed consent was taken from each patient for inclusion in the study. L-J Culture, drug susceptibility testing and GeneXpert were carried out at Pakistan Health Research Council,

Tuberculosis Research Center, Mayo Hospital, King Edward Medical University, Lahore.

Culture of sputum on LJ medium was performed as it is a gold standard in the diagnosis of tuberculosis while GenXpert only detects resistance to rifampicin. A Total of 95 isolates of Mycobacterium tuberculosis, were subjected to GenXpert and LJ culture and later on for drug susceptibility testing on LJ culture.

For Primary isolation of mycobacterium on Lowenstein Jensen (LJ) medium, cultures were grown on LJ medium with pH 6.8 and later they were subjected to drug susceptibility testing on LJ medium containing drugs with the following concentrations using standard proportion method: RIF 40.0 µg/ml medium, INH 0.2 µg/ml, STM 4.0 µg/ml, EMB 2.0 µg/ml and PZA 100.0 µg/ml.

Stock solutions of drugs were made aseptically and added to the medium with pH 6.8 to achieve the desired concentration. Pyrazinamide containing medium was acidified with 1N HCL and pH of the medium was kept at 5.5. A small portion of several colonies was scraped from a culture on Lowenstein Jensen slant and was homogenized using glass beads and vortex mixer. The turbidity of the suspension was adjusted to the McFarland No. 1 turbidity standard. Two dilutions of homogenized bacterial suspension, 103 and 105 were inoculated on each of the drug-containing medium, and on drug free medium which served as a positive growth control.

All the tubes were incubated at 37°C +/- 10°C for a minimum of four weeks. The growth was checked and colonies were counted both on drug containing as well as on control media. The number of colonies on drug-containing medium was compared with those on drug-free medium. The ratio of two was calculated and expressed as percentage. This semi quantitative analysis gave the proportion of mycobacterium population resistant to each drug tested. Any isolate giving more than 1% growth on the medium containing isoniazid, ethambutol and rifampicin as compared with control was labeled as resistant strain. The critical proportion for resistance of streptomycin and pyrazinamide was 10%. The standard reference strain H37Rv was tested in addition with each batch of tests. The inoculated slopes were evaluated for growth after 28 and 42 days of incubation.

For GeneXpert, the sputum samples were taken directly and initially mixed with the reagent provided with the Kit. Then after thorough shaking the samples were loaded in the Cartridge. The cartridge is then placed in the GeneXpert apparatus to check the presence of Mycobacterium

tuberculosis and the presence or absence of Rifampicin resistance.

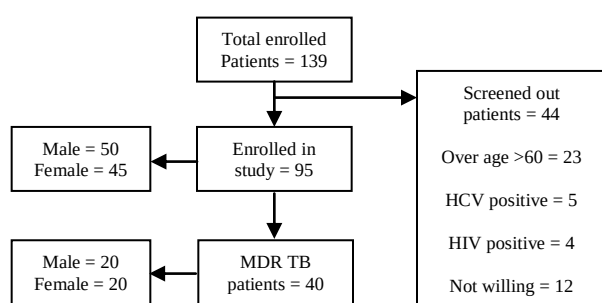
Since in GeneXpert only rifampicin resistance is reported so for the isoniazid resistance normal DST was performed.

MDR patients are defined as patients who are found to be resistant to isoniazid and rifampicin drugs.

Results

A total of 95 out of 139 patients willing to participate in the study were enrolled which is presented in the form of Flow chart.

Flow chart showing the detail of study:



The demographic characteristics of the final 95 patients enrolled in the study are shown in the Table-1.

Table 1: Demographic characteristics of the patients enrolled.

Character	Description	N	%
Gender	Male	50	52.6
	Female	45	47.4
Marital status	Married	63	66.3
	Unmarried	32	33.6
History of contact	Yes	34	35.7
	No	61	64.2
BCG scar	Yes	20	21.0
	No	75	78.9
Educational status of patient	Illiterate	25	26.3
	Primary	24	25.2
	Middle	24	25.2
	High school	5	5.2
	Intermediate	9	9.4
Economic status	Graduation	8	8.4
	< 10000	33	34.7
	10000 – 20000	47	49.4
	20000 – 40000	10	10.5
	40000 – 60000	5	5.2

MDR-TB was found positive in 40 (42.1%) cases out of 95 enrolled Cat-II patients of tuberculosis. Of the 40 patients of MDR-TB, 20 (50%) were male and 20 (50.0%) were female.

Mean age of the patients are found to be 35.5±16.7 years as presented in Table-2.

Table 2: Base line characteristics of the patients enrolled in the study and the development of multi-drug resistance.

Categories	Total (%)	MDR +ve (%)
Microscopy results	1+ 59 (62.1)	22 (37.0)
	2+ 19 (20)	8 (42.1)
	3+ 17 (17.9)	10 (58.8)
Treatment outcomes	Defaulter 6 (6.3)	3 (50.8)
	Failure 29 (30.5)	13 (44.8)
	Relapsed 40 (42.1)	18 (45.0)
	Unknown 20 (21.1)	6 (30.0)

Microscopic results revealed that among 1+ smear positives, at the time of diagnosis, of Cat-II pulmonary tuberculosis patients, 22 (37.3%) were found to have MDR-TB status. Among 2+ smear positive patients 8 (42.1%) were found to have MDR-TB and among 3+ smear positive 10 (58.8%) patients were having MDR-TB.

Among the defaulters 3 (50%) had MDR-TB, 13 (44.8%) cases of treatment failure had MDR-TB, 18 (45%) cases of relapse cases had MDR-TB.

Patients were asked if they had stopped their treatment at any point of their previous TB treatment and in this paper we refer to it as incomplete treatment. Incomplete treatment during previous TB treatment was reported by 60.0% of patients. Reasons for incomplete treatment among the patients with and without MDR-TB are presented in Table-3.

Table 3: Reasons reported by patients behind incomplete treatment.

Variable	Non-MDR-TB (n=55) n (%)	MDR-TB (n=40) n (%)
Treatment completion		
Completed treatment	22 (40.0)	18 (45.0)
Incomplete treatment	33 (60.0)	22 (55.0)
Reason reported by the patient behind incomplete treatment		
Felt Better and left	3 (9.09)	3 (9.09)
Remained positive in microscopy test	16 (48.3)	13 (39.3)
Others	20 (60.6)	6 (18.1)

Discussion

The present study showed the prevalence of MDR-TB among previously treated pulmonary TB patients at the value of 40 (42.1%). This was not comparable to MDR-TB rates published in previous studies¹⁰⁻¹⁸ which clearly indicates drastic increase in

the emergence of MDR-TB in the world. The rates of MDR tuberculosis in Pakistan vary from 2.3% in untreated individuals to an alarming 17.9% in individuals who have been previously treated for the disease¹¹ but in our study quite high value of MDR-TB patients have been reported.

The emergence of multi-drug resistant (MDR) strains of tuberculosis poses an important challenge for the healthcare sector in Pakistan. MDR tuberculosis strains are resistant to a number of first line anti-tuberculosis drugs including "atleast isoniazid and rifampicin".¹¹ Pakistan is amongst the top ten countries in the world with regards to the disease burden of tuberculosis and the high incidence and prevalence of the disease in the country, has been attributed to several factors such as poverty, low awareness, patient non-compliance and suboptimal healthcare infrastructure.^{19,20}

Studies conducted over the past two decades have shown MDR-TB rates varying from 14 to 49 per cent among previously treated cases¹³⁻²⁰ and in the present study the value of MDR-TB among previously treated patients is found to be 42.1%. The World Health Organization fourth Global Project reported MDR-TB prevalence of 17.2 per cent among previously treated cases in India.²¹

Among Smear positivity, 40 (42.1%) patients show MDR-TB status and this value is quite different from the reported ratio of 2.7% in another study.²² Further sub-divisions of the smear results indicate a high rate of MDR-TB among 3+ (58.8%) smear patients than 2+ (42.1%) and 1+ (37.3%). The highest rate of MDR-TB status is found among the relapsed cases 18(45.0%), which is quite high as compared to the reported ratio of (19.7%) and (17.2%).^{21,23} Among defaulters the value of MDR-TB is (44.8%) against the reported ratio of (24.2%).¹⁹ The high value of MDR TB among defaulter carries significant importance as they are also already exposed to rifampicin treatment in the past which is mainly responsible for the development of MDR-TB.²⁴

Our findings carry significant importance because there have been scarce data on the prevalence of MDR-TB among category II pulmonary TB patients from the recent past in Pakistan. Since drug-resistance is a dynamic phenomenon, it is important to monitor the trend of drug-resistance periodically.

In conclusion, our findings showed that the prevalence of MDR-TB in category II TB patients was high and these patients are at high risk of amplified resistance including XDR-TB.²⁵ A large multi-centric study involving patients recruited at primary-care level from different parts of the country is needed to determine the nation-wide prevalence of MDR-TB in category II TB patients. Findings of this report suggest that all category II PTB patients,

given the high prevalence of MDR-TB, should be screened for MDR-TB using rapid diagnostic tests (molecular tests) such as the line probe assays. This will facilitate the diagnosis of MDR-TB at an early stage and thus will minimize transmission of the disease.²⁶ We stress the importance of continuous monitoring of drug resistance trends, in order to assess the efficacy of current interventions and their impact on the TB epidemic.

Conflict of interest: None declared.

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