International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 8 No. 2 Sep. 2014, pp. 482-488

http://www.ijias.issr-journals.org/

The Emergence of Multi – drug resistant tuberculosis in HIV Patients

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ABSTRACT: In imuuno-compromised patient with tuberculosis, drug-resistant tuberculosis (DR-TB) is an emerging problem that adversely affects treatment results and public health in the developing countries. Objective of this study was the emergence of Multi – drug resistant tuberculosis (MDR-TB) in human immune-deficiency virus (HIV) patients. The present study showed the laboratory finding of 250 sero-positive HIV patients carried out of department of Microbiology, Medical University, Lucknow. Their HIV status confirmed by Enzyme linked immune Sorbent Assay (ELISA) test in antiretroviral therapy (ART) centre Medical University, Lucknow. The study consisted with HIV-positive patient, clinically diagnosed for tuberculosis cases. All sputum smear positive cases were subjected to culture and drug-susceptibility testing by 1% proportion method on Lowenstein-Jensen (LJ) medium. A total of 250 diagnosed HIV-positive and sputum smear positive for Acid-fast Bacilli (AFB) in pulmonary tuberculosis were subjected to culture and drug-sensitivity test. Among 250 isolates, 54(21.6%) were culture positive and 182(72.8%) were culture negative. Of these 54 isolates, 50(20%) were *Mycobacterium tuberculosis (M.tb)* and 4(1.6%) were *Mycobacterium* other than tuberculosis (MOTT). Out of 20.0% *M. tuberculosis* positive isolates, 11(22%) were resistant to at least one drug. Resistance pattern of 50(20.0%) strains of *M. tuberculosis* showing resistance to single, double, triple, and quadruple drugs were 11(22%), 8(16%), 4(8%) and 1(2%) respectively. MDR was observed in 7(14%) isolates. The present study highlights the high rate of drug resistance pattern among the sputum smear positive pulmonary tuberculosis patients and also high MDR tuberculosis.

KEYWORDS: Acid-fast bacilli, mycobacterium, human immune-deficiency virus, antiretroviral therapy, tuberculosis.

1 INTRODUCTION

M. tb and HIV co-infection is common, particularly in the developing countries. TB is the commonest co-infection in HIV-positive individuals, who are at increased risk of both reactivation of latent infection and acquisition of new infection. As the degree of immune-suppression increases, the risks of developing TB disease also increase [1].

TB has been reported to be responsible for 1.7 million deaths annually; however, tuberculosis programs face tremendous challenges in reducing multidrug-resistant tuberculosis (MDR-TB). WHO 1994 reported, only 59% of all countries globally have been able to collect high-quality representative data on drug resistance [2]. More recent data suggest that outcomes can be improved if patients promptly start to receive two or more drugs that have in vitro activity against the multidrug-resistant isolates [3]. Resistance of *M.tb* to drugs is a man-made amplification of spontaneous mutations in the genes of the tubercle bacilli [4]. Initial drug resistance develops in a patient, who is denied history of previous chemotherapy. In reality, it consists of true primary resistance and an undisclosed acquired resistance [5]. Treatment with a single drug due to irregular drug supply, inappropriate prescription, or poor adherence to treatment permits the multiplication of drug-resistant strains.

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Since drug resistance develops because of inadequate use of drugs, anti-tuberculosis drug resistance surveillance is, together with the monitoring of treatment outcome, an essential tool for evaluating the quality of tuberculosis control programmes, lack of laboratory resources and rapid accurate point-of care tests [6],[7]. Present study analyzed the MDR pattern in HIV sero-positive pulmonary tuberculosis patients in three years study.

2 MATERIAL AND METHOD

2.1 STUDY DESIGN AND SETTING

A cross-sectional study was conducted between January 2011 to January 2014 at the Department of Microbiology, Medical University, Lucknow district of Uttar Pradesh.

2.2 STUDY POPULATION

The study consisted of 250 HIV-positive for AFB pulmonary tuberculosis patients of both sex and between the age group of < 10 to > 50 years at the time of interview and were about to be registered for treatment. Patients were excluded if they having any cardiac and metabolic problems and not willing to participate in the study**.**

2.3 DATA COLLECTION

2.3.1 PERSONAL INTERVIEW AND CLINICAL EXAMINATION

Interviews using structured questionnaires were used to collect the data on patient history. Subsequently, patients were thoroughly examined by medical doctors at hospital.

2.3.2 ASSESSMENT OF CLINICAL OUTCOMES

Clinical outcomes were assessed with the following symptoms and clinically assessed including fever, cough expectoration, chest pain, breathlessness, wheezing, haemoptysis, dyspnoea, night sweat, loss or improve of appetite and weight loss or gain.

2.4 SPECIMEN COLLECTION

The diagnosis of TB was done in accordance with the RNTCP [8]. At the time of enrolment, three sputum specimens on two consecutive days from each patient were collected in properly labeled screw capped, sterile disposable plastic bottles after oral gargling with normal water. Thus, there were three samples: SPOT - EARLY MORNING – SPOT [8]. Specimens contained mucoid or mucopurulent material with minimum amounts of oral or nasal material into the McCartney bottles and volume was of approximately 5ml.

2.5 ASSESSMENT OF BACTERIOLOGICAL OUTCOMES

Bacteriological outcomes were assessed by RNTCP guidelines, 2006 [8], included AFB smear examination and grading, AFB culture and drug susceptibility test. All specimens were carried to the accredited Intermediate Reference Laboratory (IRL) at the Department of Microbiology, Medical University, Lucknow where further processing was done.

2.6 AFB SMEARS EXAMINATION AND GRADING

AFB smear examination was carried out by direct microscopy using the Ziehl-Neelsen (ZN) method. Sputum smear result was examined and interpreted according to the AFB grading [9].

2.7 AFB CULTURE AND DRUG-SUSCEPTIBILITY TEST

Culture examinations were done on all diagnostic specimens, regardless of AFB smear positivity. Sputum specimens from each patient were processed with sodium hydroxide (NaOH) method- Modified Petroff's procedure and cultured on LJ slopes [10]. All inoculated LJ drug and control media were incubated at 37ºC. All cultures were examined 48-72 hours after inoculation to detect gross contaminants. Thereafter, cultures were examined weekly, up to eight weeks on a specified day of

the week. Typical colonies of *M .tb* were rough, crumbly, waxy, non-pigmented (buff-coloured) and slow growers, i.e., only appeared two to three weeks after inoculation. The colony was confirmed by ZN staining. Detection time for MOTT was 25 days. *M. tb* positive strains were culture negative when they grew on p-nitro benzoate (PNB) containing medium. Only a few colonies of MOTT (MOTT – often pigmented, with smooth morphology or PNB positive) were grown as visible colonies on PNB containing medium [9]. Drug resistance was expressed in proportion method, where a strain was considered to be drug resistant if the number of colonies that grew on a drug containing medium was 1% or more of the colonies that grew on a control drug free medium. The control (drug free) medium showed good growth at least 50 to 100 colonies.

2.8 LABORATORY DEFINITION

MDR was defined as resistance to both isoniazid (INH) and rifampicin (RMP) with or without resistance to other drugs as per RNTCP, 2010 [10].

2.9 STATISTICAL ANALYSIS

The data collected was entered into Microsoft Excel and checked for any inconsistency. The descriptive statistics such as percentage calculated. The descriptive statistics such as percentage and mean(±SD) were calculated.

3 RESULT

A total of 250 HIV positive diagnosed patients with pulmonary tuberculosis were recruited, in which 60.8% were males and 39.2% were females. (Table -1) The most frequent age group in the present study was 31- 40 years consisting of 39.2% patients, followed by 29.6% patients in the age group of 41-50 years. (Table -1) Sputum positivity grade 1+ was most prevalent (10.4%). (Table - 2)

Table 1: Characteristics presentation of the HIV-patient

Variable	n =250	Percent (%)
Age		
<10	10	4.0
11-20	18	7.2
21-30	38	15.2
31-40	98	39.2
41-50	74	29.6
>50	12	4
Gender		
Male	152	60.8
Female	98	39.2
Religion		
Hindu	116	46.4
Muslim	134	53.6
Family history of TB		
Yes	52	20.8
No	198	79.2
Smoking		
Yes	141	56.4
No	109	43.6

*Data were expressed as *mean ± standard deviation*

Table -2 AFB-smear findings from sputum samples

Smear results	No. (n=250)	Percent (%)
Positive	48	19.2
1+	26	10.4
2+	12	4.8
3+	06	2.4
Scanty	04	1.6
Negative	202	80.8

During clinical assessment, most of the patients had persistent fever (99.9%), chronic cough (91.5%), weight loss (93.4%), and appetite loss (99.6%); other frequent symptoms were chest pain (58.8%), breathlessness (82.4%) and haemoptysis (38.6%).

Among 250 HIV positive cases 48 (19.2%) isolates were sputum smear positive for AFB. 54 (21.6 %) were culture positive, 5.6% were contaminated and 182 (72.8%) isolates indicated no growth of *Mycobacteria*. Among 21.6% culture positive isolates, 1.6% was MOTT. (Table 3)

Table- 3 Mycobacterium culture positivity in sputum

	No. (n=250)	Percent (%)
Total culture positive	54	21.6
Culture negative	182	72.8
Contaminated	14	5.6
	n=54	
MOTT	4	1.6
M. tuberculosis	50	20

Remaining 20% *M.tb* positive isolates were subjected to DST. (Table- 4) In the DST among 50 strains, 52% strains were sensitive to all four anti-tubercular drugs and 22% strains were resistant to one or more drugs. Highest resistance was found in INH (8%) either alone or in combination with other drugs. (Table 4 and 5) Among all cases, four most frequent drug resistance patterns of 22% strains of *M.tb* from mono drug, double drug, triple drug and quadruple drug resistance were 22%, 16%, 8% and 2% respectively. MDR was observed in 14% isolates. (Table 5)

Table 4: Sensitivity pattern of M. tuberculosis to four anti-tuberculosis drugs in LJ medium by proportion method (n=50)

	No. (n=50)	Percent (%)
Streptomycin		
Resistant	10	20%
Sensitive	40	80%
Isoniazid		
Resistant	13	26%
Sensitive	37	74%
Rifampicin		
Resistant	12	24%
Sensitive	38	76%
Ethambutol		
Resistant	07	14%
Sensitive	43	86%

Table 5: Resistance pattern of 50 drug resistant strains of M. tuberculosis to four anti-tuberculosis drugs

Drug-resistant pattern	Name of Drug	Number of resistant Strain (%)	Total
Mono resistant	Streptomycin (SM)	3 (6%)	11(22%)
	Isoniazid (INH)	4 (8%)	
	Rifampicin (RMP)	2 (4%)	
	Ethambutol (EMB)	2 (4%)	
Double Resistant	SM+INH	2 (4%)	8 (16%)
	INH+RMP	4 (8%)	
	RMP+EMB	1 (2%)	
	SM+EMB	1 (2%)	
Triple Resistant	SM+RMP+EMB	1 (2%)	4 (8%)
	SM+INH+RMP	2 (4%)	
	INH+RMP+EMB	1 (2%)	
Quadruple drug Resistance	SM+INH+RMP+EMB	1 (2%)	1 (2%)
MDR			7 (14 %)

SM-*Streptomycin,* ***INH-****Isoniazid,* ***RMP-****Rifampicin, ,* ***EMB-****Ethambutol* ****MDR:*** *Multi-drug resistance: Resistance to both isoniazid and rifampicin with or without resistance to other drugs.*
Single resistant = mono resistant, More than one resistant = poly resistant

4 DISCUSSION

Emergence and spread of drug resistant *M.tb* is a serious threat to tuberculosis control programme because patients with drug-resistant bacilli respond less readily to therapy than those with sensitive bacilli, resulting in preferential spread of drug resistant bacilli in the community [11].

Swaminathan, *et al* [12] reported that HIV-positive patients resistance was found to INH (27%) and to RMP (18.9%), while MDR-TB was seen in 13.5% patients. This is about similar to our results where INH (26%), RMP (24%) and MDR –TB (14%).

Matthew et *al*, 2012 [13] determined TB prevalence including MDR-TB among a cohort of high risk patients. Among *M. tb* isolates from 47.2% were resistant to first line anti-TB drugs and 13.2% were MDR-TB.

Gupta et *al*, 2013 [14] reported the drug-resistance pattern of 21.3% strains of *M. tb* showed resistance to single, double, triple, and quadruple drugs were 5.9%, 10.7%, 2.4% and 2.4% respectively. MDR was observed in 4.7% isolates.

Prasad *et al*, 2012 [15] found the drug-susceptibility pattern in Category-II failure patients of pulmonary tuberculosis under Revised National Tuberculosis Control Programme (RNTCP) of India. Among 208 patients, culture was positive in 81.7% cases, negative in 8.1% cases and contaminated in 10% cases. Among 208(92.8%) patients, culture was positive in 81.7% cases, negative in 8.1% cases and contaminated in 10% cases. The drug sensitivity pattern of culture positive cases of Category-II failure patients revealed that, 58.2% had MDR tuberculosis and 40.5% were resistant but were non-MDR tuberculosis and 1.1% cases were sensitive to all first line anti-tuberculosis drugs.

The Tuberculosis Research Centre, Chennai [16] reported that primary resistance to INH was 15.0%, 11.8% to SM and 7.7% to both INH and SM; resistance to INH was reported varying by 3.2% in Pune and 32.9% in Kolar. The present study estimated that the levels of resistance to INH, RMP, SM and EMB were 18.3%, 4.7%, 10.1% and 10.7% respectively. A study among the primary ATT drugs showed that the highest resistance was seen in pyrazinamide (PZA) (4.6%), followed by 3.5%, 2.9%, 3% and 2.1% respectively in RMP, INH, EMB and SM which entirely differ from our results. The overall incidence of initial drug resistance was 1.7-9% and MDR was reported only in 1.6% cases. Another study revealed that the maximum overall resistance was seen in PZA (6.6%), followed by 5.8%, 5.8%, 4.5% and 4.3% in RMP, EMB, SM and INH respectively. Initial drug resistance to first line anti-tubercular drugs was found in 24.1% cases: single drug resistance in 21.3% and poly-drug resistance in 2.8% cases. There was no case of MDR in this study.

Severine *et al*, 2003 [17] observed that a previous history of TB treatment was a risk factor associated with MDR TB in Haiti. However, an assessment of risk factors (sex, HIV positivity, previous treatment, and drug-resistance) showed that none

was significantly associated with the active transmission of TB. Haji and Imran, 2011 [18] determined the resistance patterns of *M. tb* isolates among category I and II patients of pulmonary tuberculosis. The DST for INH, RMP, EMB, PZA and SM were performed. The DST showed drug-sensitive and drug-resistant isolates in 28.05% and 71.92% cases. The drug-resistant TB rates for individual drugs; INH, RMP, EMB, PZA and SM were 51.2%, 15.4%, 13.3%, 9%, and 3.8% respectively. The MDR-TB isolates were detected in 42.1% cases.

Aguiar *et al*., 2009 [19] analyzed 350 treatments, in which 62 were for patients with previous TB. HIV status was positive in 31.2% of cases. Resistance was found in 15.7% and MDR in 4.3% of cases. A study evaluated 54.6% were treatment failure cases and 45.4% were relapse cases, were culture-positive and 58.64% were resistant to one or more drugs [20]. Resistance to one drug was observed in 10.46%, two drug in 18.13%, three drug in 14.8% and to four drug in 15.21%. Single drug resistance was most commonly seen with INH 7.5%, followed by SM 1.4%, RMP 0.9% and EMB 0.4%. Resistance to INH with RMP alone was seen in 9.2% .

In a study of SRM Hospital and Research Centre of Kanchipuram District [21] found 12.1% patients were smear-positive for AFB. Among these 54.4% were positive for *Mycobacterium* species cultured on LJ medium. Out of 89.4% were *M. tb* and 10.5% were MOTT. 4.1% *M. tb* isolates were resistant to RMP, INH and EMB. All the three MDR - TB strains were isolated from pulmonary tuberculosis patients. Nagaraja *et al*., 2011 [22] assessed various resistance patterns among confirmed drug-resistant pulmonary TB patients and MDR pattern was observed in 72%, in which 6.4% had resistance only to INH and RMP, 18.7% were resistant to INH, RMP, and either of the other first line drugs SM or EMB and 47.2% were resistant to all first line drugs. Poly drug-resistance pattern was observed in 23.3% and Mono drug resistance in 4.2%. Paramasivan CN, 1998 [23] observed the resistance to INH and RMP has been increase over the past four decades. It is reported in 2010 [24], 27 high MDR-TB burden countries, 2.3% MDR were estimated in new cases. In WHO, 2011 [25] 2.1% was reported (1.5–2.7%) of new TB cases with MDR-TB in India.

In the present study, a total of 250 diagnosed HIV-positive and sputum smear-positive for AFB in pulmonary tuberculosis patients were subjected to culture and drug sensitivity test. Among 250 isolates, 182(72.2%) isolates were culture negative and 54(21.6%) were positive. Of these 54 isolates, 50(20%) were *M. tb* and 4(1.6%) was MOTT. Out of 20.0% *M. tb* positive isolates, 45(90%) were resistant to at least one drug. Resistance pattern of 45(90%) strains of *M. tb* showing resistance to single, double, triple, and quadruple drugs were 11(22%), 8(16%), 4(8%) and 1(2%) respectively. MDR was observed in 7(14%) isolates.

5 CONCLUSION

This study reveals a high rate of drug-resistance and MDR among the HIV positive co-infected pulmonary TB patients. HIV-infected TB patients should be given antiretroviral therapy in addition to the recommended treatment for TB. The testing of all HIV-positive patients for tuberculosis will help to identify the co-infected patients who need treatment for both infections. This require coordination and communication between the TB and AIDS control programs, in India.

ACKNOWLEDGEMENT

We gratefully acknowledge Dr. K.P Singh Microbiology Department Medical University Lucknow, and Dr. R.C Pande of V.P.I.M.S, Lucknow, U.P. for their valuable support in sample collection and useful suggestions. We appreciate the efforts of the workers (Mr. Sudheer Awasthi, Mr. Santosh Kumar), the cooperation of the patients and the staff.

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