Association of Risk Factors and Drug Resistance Pattern in Tuberculosis Patients in North India

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Abstract

Context: India is one of the high tuberculosis (TB) burden countries in the world. Improper implementation in the guidelines for the management of TB and high rate of defaults on the part of the patients are most important risk factors for the development of multi-drug resistant TB. **Aims:** This study examines the drug resistance profile and the effect of demographic, clinical and behavioral risk factors on the prevalence of TB and multidrug resistance (MDR) in north India. **Settings and Design:** This was a prospective, observational study carried out from May 2012 to February 2014 in tertiary care hospital of Varanasi. **Subjects and Methods:** The study was performed on 721 pulmonary and extrapulmonary specimens of suspected TB patients based on history, was subjected for the Ziehl–Neelsen staining and culture on Lowenstein–Jensen (LJ) media. **Statistical Analysis:** The features of groups were compared by Chi-square (χ^2) and odds ratio. **Results:** Out of 721 clinically suspected pulmonary and extrapulmonary TB patients, 222 (30.8%) patients were smear positive for acid-fast bacilli and 244 (38.3%) were positive for *Mycobacterium* species cultured on LJ medium. The prevalence of resistance to at least one anti-TB drug was 71.1% and MDR was 53.5%. Age, gender, HIV status, nature of TB, smoking, and alcohol consumption risk factors were significantly associated with TB prevalence; while prior history of TB infection, pervious household exposure, smoking, and alcohol consumption were significantly associated with MDR. **Conclusion:** This study showed a high prevalence of drug resistance TB in this region. It also provides evidence in our circumstance, of the role of prior history of TB infection, alcohol and smoking in increasing the risk of developing TB and MDR-TB. Therefore, it is necessary for the public health community to incorporate and strengthen alcohol and smoking nonparticipation interference in TB control program.

Keywords: Drug regimen, drug susceptibility test, Lowenstein–Jensen medium, multidrug resistant, *Mycobacterium tuberculosis*, risk factors

INTRODUCTION

Tuberculosis (TB) is a major global health problem and it ranks alongside the human immunodeficiency virus (HIV) as a leading cause of death worldwide (WHO. 2015) especially in developing countries.[1] The recognition of the emergence and dissemination of drug-resistant TB especially multi-drug resistant TB (MDR-TB), defined as *Mycobacterium tuberculosis* strain that is resistant to both isoniazid (INH) and rifampicin (RIF), the two most powerful first-line anti-TB treatment drugs, and extensively drug-resistant TB (XDR-TB) is of great concern. XDR-TB is defined as MTB strains resistant to both INH and RIF (i.e., MDR-TB), as well as further resistant to any fluoroquinolone and second-line injectable drugs (kanamycin, amikacin or capreomycin). In fact, the threat of drug resistance emergence may compromise the effectiveness of TB control program.[2]

The frequency of MDR-TB is increasing among new and previously treated cases worldwide and WHO estimated that 480,000 MDR-TB cases occur each year.[1] MDR-TB most commonly develops due to inappropriate prescription of regimens, patients missing doses or failing to complete their treatment, delay in the diagnosis, transmission of drug-resistant MTB strains in the community, epidemic coinfection with HIV and spontaneous chromosomal mutations.[3] This situation has unfavorably affected the control of TB efforts being made by different countries with limited access to second-line anti-TB drugs.[4]

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According to WHO out of the estimated global annual incidence of 9.6 million TB cases, 3.2 million were estimated to have occurred in India.[1] An emergence of MDR-TB in several regions of the world including India has been one of the major causes for declaring TB control program as a global emergency.[5] A number of outbreaks of MDR-TB required the continuous surveillance of drug resistance for effective treatment of TB patients and also for initiating adequate public health assessment. Current estimates reported the prevalence of primary and acquired MDR-TB in India as 3.5% and 20.5%, respectively.[1]

It is essential to appreciate the risk factors and demographic characteristics of the disease at the population level.[6,7] Various studies have focused on the effect of HIV on TB, but there are limited data on the effect of clinical, demographical as well as behavioral risk factors on the prevalence of TB and multidrug resistance (MDR) TB. This study was undertaken to perform drug resistant profile in patients of pulmonary and extrapulmonary TB and to describe clinical, demographic, and behavioral risk factors associated with the prevalence of TB and MDR TB in a tertiary referral hospital of north India.

SUBJECTS AND METHODS

Study design

This was a prospective, observational study carried out from May 2012 to February 2014 in the Department of Microbiology, Institute of Medical Sciences and Sir Sundarlal Hospital in Banaras Hindu University, Varanasi, India, which is a tertiary referral hospital. The hospital is providing super specialty services to health-care needs of about 20 crores population of eastern Uttar Pradesh, Western Bihar, including Madhya Pradesh, Chhattisgarh and Jharkhand, as well as neighboring areas of Nepal. The duration of the study was from May 2012 to February 2014.

Patients and sample collection

The samples were collected from different departments and wards based on the maximum frequency of patients attending these centers such as Department of TB and Respiratory Diseases, antiretroviral therapy centers of Sir Sundar Lal Hospital of Banaras Hindu University (BHU), Integrated Counseling and Testing Centre of the Department of Microbiology and Department of Pathology, Institute of Medical Sciences, BHU.

Case inclusion

All clinically suspected TB cases were included in this study. Samples were collected on the basis of clinical signs and symptoms suggestive of TB with/without radiology, previous and family history. The following risk factors were examined demographic status (gender and age category), clinical factors (history of previous antitubercular treatment, HIV status, contact history of TB and pulmonary and extrapulmonary TB of TB), and behavioral factors (smoking history and alcohol consumption). All the cases were categorized as primary and acquired drug resistance as per Revised National Tuberculosis Control Programme.[8]

Ethical approval

The study was approved by the Ethical Committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Laboratory methods

Specimen microscopy and culture

A total of 666 sputum, 10 bronchoalveolar lavage (BAL) and 45 other (pus, cerebrospinal fluid [CSF], pleural fluid [PF], fine needle aspirate [FNA], urine and bone marrow) nonduplicate clinical specimens were included in this study. All clinical samples were screened for acid-fast bacilli (AFB) through the standard Ziehl–Neelsen's staining method.[8] The specimens of sputum, pus, urine, and BAL were decontaminated, digested and homogenized using modified Petroff's method.[9] The samples of tissue biopsy were ground well with 5 ml sterile distilled water. All the samples were concentrated by centrifugation at 3000 g for 15 min at 25°C. The pellet was used for AFB staining, and 0.5 ml homogenate was inoculated on Lowenstein–Jensen (LJ) slant. The bone marrow and FNA samples were collected aseptically and directly inoculated onto a pair of LJ slants. The slants were incubated at 37°C and inspected weekly for mycobacterial growth for 8 weeks.

Any suspected growth was confirmed by biochemical tests such as growth rate on solid media, colony morphology, pigmentation, nitrate reduction, catalase production at 68°C and sensitivity to PNB (*p*-nitrobenzoic acid)[10,11] and differentiated from nontuberculous mycobacteria (NTM).

Drug susceptibility testing for antitubercular drugs

Drug susceptibility testing (DST) was performed for first line antitubercular drugs, namely, RIF (40 µg/ml), INH (0.2 µg/ml), streptomycin (SM) (4.0 µg/ml), and ethambutol (EMB) (2 µg/ml) (Sigma, St. Louis, USA) using conventional 1% proportion method.[8,10] The tested MTB isolate was considered resistant if the proportion of the tested isolate was >1% of the control isolates.

Quality control

Mycobacterium tuberculosis H37Rv ATCC27294 and a known MDR strain were used as quality controls.

Statistical analysis

The features of groups were compared by Chi-square (χ^2) test for the assessment of statistical significance. A $P < 0.05$ was considered significant. Odds ratio (OR) has been calculated using online software MEDCALC.

RESULTS

Characteristics of the study participants

A total of 721 participants were included, comprising 429 males and 292 females. The majority (46%) of the participants were between the age groups from 21 to 45 years (mean age 35.7 ± 12.5). Among 721 participants, 230 were newly

diagnosed cases while 302 were previously treated cases. TB was significantly prevalent in males (*P* = 0.04), patients with HIV positive (18.9%, *P* = 0.0000) and pulmonary TB patients (82.5%, *P* < 0.0001), and among those who smoked (25.8%, *P* = 0.0000) and consumed alcohol (45%, *P* = 0.004) [Table 1].

Culture and identification

Among 721 clinical specimens, 45 were extrapulmonary specimens. From these, 15 (33.3%) were AFB smear and culture positive (including 3 pus, 8 PFs, 3 CSF, and 1 FNA) and 8 (17.8%) were AFB smear negative culture positive (including 2 pus, 3 PFs, 2 CSF, and 1 bone marrow sample) [Table 2]. Among 676 pulmonary specimens, 14 sputum specimens (2.1%) were AFB smear negative but culture positive, whereas 207 (30.6%) specimens (including 5 BAL and 202 sputum) were both smear and culture positive and remaining 455 (67.3%) specimens were both smear and culture negative [Table 2]. Of the 721 specimens, 222 (30.8%) were found to be positive for AFB smear, whereas 244 (33.8%) were culture positive and 20 (2.8%) AFB-positive specimens were contaminated. From 244 culture positive isolates, 235 (96.3%) culture isolates were identified as *M. tuberculosis* strains and remaining 9 (3.7%) isolates were identified as NTM.

Drug resistance pattern

With respect to overall DST pattern, 167 (71.1%) of the 235 MTB positive patients evaluated were observed as resistant to at least one anti-TB drug, whereas 68 (28.9%) were found to be sensitive to all drugs. The prevalence of overall resistance pattern to at least one drug was highest in INH 145 (61.7%) followed by RIF 134 (57.0%), SM 119 (50.6%), and EMB 96 (40.8%) and 124 (52.8%) were MDR. Drug resistance pattern was shown in Table 3.

Initial resistance to at least one drug among the newly diagnosed cases were 70.9% (73/103), with resistance to INH being the most common (40/103, 38.8%) drug. Acquired resistance to one drug among the previously treated cases were 71.2% (94/132), with the resistance to INH being the most common (105/132, 79.5%). The prevalence of MDR was found to be highest in previously treated cases (98/132, 74.2%) than newly diagnosed cases (26/103, 25.2%). In addition, one MTB strain isolated from bone marrow sample was found to be multidrug resistant, i.e., resistant to INH and RIF. Resistance to more than two drugs was (other than MDR) observed to be highest in previously treated patients (14/132, 10.6%) than new patients [2/103, 1.9%; Table 4].

Risk factors associated with prevalence of tuberculosis and multidrug resistance tuberculosis

Among the 235 patients, 152 (64.7%) were males and 83 (35.3%) females. The male to female ratio was 1.8:1. One fifty two patients were positive for TB infection with the mean age of 26.5 (SD ± 10.6). Among 312 HIV tested cases, 59 (18.9%) were HIV infected. Age (*P* < 0.0001), pulmonary TB (*P* < 0.0001), HIV status (*P* = 0), smoking (*P* = 0.0000), and alcohol consumption (*P* = 0.004) showed a significant association with the prevalence of TB while previous household exposure of TB cases and prior history of treatment

Table 1: Demographic, clinical and behavioral characteristics of study participants

Risk factors	Total numbers (*n*)	Category, *n* (%)	Occurrence, *n* (%)	Present TB positive patients			MDR patients		
				Positive, *n* (%)	Negative, *n* (%)	*P*	Positive, *n* (%)	Negative, *n* (%)	*P*
Demographic									
Gender	721	Male	429 (59.5)	152 (35.4)	277 (64.6)	0.04	56 (36.8)	96 (63.1)	>0.05
		Female	292 (40.5)	83 (28.4)	209 (71.6)		39 (47.0)	45 (54.2)	
Age group (years)	721	5-20	186 (25.8)	49 (26.3)	137 (73.6)	<0.0001	19 (20)	30 (61.2)	0.0001
		21-45	332 (46.0)	155 (46.7)	177 (53.3)		52 (33.5)	103 (66.4)	
		>50	203 (28.1)	32 (15.8)	171 (84.2)		24 (75)	8 (25)	
Clinical history									
HIV status*	312	Positive	59 (18.9)	15 (25.4)	44 (74.6)	0.0001	6 (40)	9 (60)	>0.05
		Negative	253 (81.1)	220 (86.9)	33 (13.0)		89 (40.4)	131 (59.5)	
Prior history of TB treatment	532	Yes	302 (57.7)	146 (48.3)	280 (92.7)	>0.05	32 (21.9)	114 (78.1)	0.03
		No	230 (43.2)	89 (38.7)	127 (55.2)		31 (34.8)	58 (65.2)	
Previous household exposure	58	Yes	36 (62.1)	12 (33.3)	24 (66.7)	>0.05	3 (25.0)	9 (75.0)	>0.05
		No	22 (37.9)	3 (13.6)	19 (86.4)		2 (66.7)	1 (33.3)	
Nature of TB	721	Pulmonary	595 (82.5)	212 (35.6)	383 (64.4)	<0.0001	83 (39.1)	129 (60.8)	>0.05
		Extrapulmonary	126 (17.5)	23 (18.2)	103 (81.7)		12 (52.2)	11 (47.8)	
Behavioral									
Smoking	389	Yes	256 (65.8)	66 (25.8)	190 (74.2)	0.0001	31 (47.0)	35 (53.0)	0.01
		No	133 (34.2)	85 (63.9)	48 (36.1)		45 (52.9)	22 (25.9)	
Alcohol use	496	Yes	209 (42.1)	94 (45)	115 (55.0)	0.004	32 (34.0)	62 (65.9)	0.0004
		No	287 (57.9)	44 (15.3)	243 (84.7)		29 (65.9)	15 (34.1)	

*More number of data unavailable. MDR: Multi drug resistant, TB: Tuberculosis

Table 2: Types of pulmonary and extrapulmonary samples

Specimens of tuberculosis patients	Number of specimens	Smear negative culture positive, *n* (%)	Smear positive culture positive, *n* (%)	Smear negative culture negative, *n* (%)
Extrapulmonary samples (*n*=45)				
Pus	6	2 (33.3)	3 (50)	1 (16.7)
Pleural fluid	14	3 (21.4)	8 (57.1)	3 (21.4)
Fine needle aspirates	10	-	1 (10)	9 (90)
Cerebrospinal fluid	6	2 (33.3)	3 (50)	1 (16.7)
Bone marrow	1	1 (100)	-	0
Urine	8	-	-	8 (100)
Pulmonary samples (*n*=676)				
Sputum	666	14 (2.1)	202 (30.3)	450 (61.7)
BAL	10	0	5 (50)	5 (50)
Total	721	22 (3.0)	222 (30.8)	481 (67.6)

BAL: Bronchoalveolar lavage

Table 3: Resistance pattern to first-line antituberculosis drugs among the 235 *Mycobacterium tuberculosis* isolates (*n*=235)

Drugs	Total number of drug resistant strains (%)	Total number of drug susceptible strains (%)
INH	145 (61.7)	90 (38.3)
RIF	134 (57.0)	101 (43.0)
SM	119 (50.6)	116 (49.4)
EMB	96 (40.8)	139 (59.1)
MDR	124 (52.8)	-

MDR: Multi drug resistant, RIF: Rifampicin, EMB: Ethambutol, SM: Streptomycin, INH: Isoniazid

Figure 1: Prevalence of multi-drug resistant tuberculosis by gender, age groups, TB treatment, co-infection status, Nature of TB, smoking history and alcohol consumption

were not significantly associated with the prevalence of TB [Tables 1 and 5]. On the other hand, age (*P* < 0.0001), previous antitubercular treatment (*P* = 0.03), smoking (*P* = 0.01), and alcoholism (*P* = 0.0004) were significantly associated with the development of MDR-TB, whereas gender (*P* = 0.15), HIV status (*P* > 0.05), previous household exposure (*P* = 0.2), and pulmonary and extrapulmonary TB (*P* = 0.2) were not significantly associated with the development of MDR-TB [Tables 1 and 5, Figure 1].

DISCUSSION

In this study, the TB was predominant in the patients (65.9%) of 21–45 years age groups. Similar findings have been reported in other studies which have estimated that 22% and 56% of patients were found in this age group.[12,13] This age group is mostly exposed to open cases of TB which may be the reason to make this age group more vulnerable. The male to female ratio was found to be 1.8:1. The previous studies have shown 56.0% and 84.8% of the patients were males, respectively.[12,14] WHO reported that male to female ratio in India was 2:1 in 2013.[1]

This study demonstrated a high prevalence of drug resistance among pulmonary and extrapulmonary TB isolates of *M. tuberculosis* from north India. Resistance to one or more first line anti-TB drugs was found to be 71.1% which is relatively higher than the previous reports from this tertiary
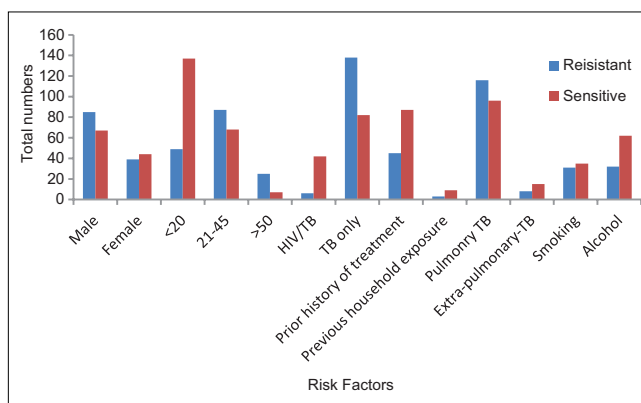
care center (21.5%)[15] and (56.1%).[16] This study reflects that there is continuous increase in the level of drug resistance in MTB strains in this tertiary care center.

The study found that the frequency of drug resistance in previously treated TB is higher than those of newly diagnosed patients for a single drug as well as for all first line anti-TB drugs, as stated earlier in other studies.[6,17] This study showed a higher prevalence of acquired resistance to INH and RIF (INH, 57.5% and RIF, 49.3%) than primary resistance. Similarly, the previous study from this tertiary care center has reported the high prevalence of acquired resistance to INH and RIF (INH - 56.2%, RIF - 68.6%)[15] and (INH - 49.7%, RIF - 39.5%)[16] in comparison to primary resistance cases. Sethi *et al*., in India, have also reported a high prevalence of resistance (46.9% to INH and 27.65 to RIF) in previously treated cases than newly diagnosed cases (26.4% to INH and 9.9% to RIF).[18] The message from these studies is clear that the level of acquired drug resistance is in progress due to irregular or improper use of anti-TB drugs, which have led to accumulation and multiplication of drug-resistant strains.[3]

Interestingly, we have isolated a MDR (resistance to INH and RIF) strain of MTB in bone marrow sample from a patient

Table 4: Drug resistance in new and previously treated patients against first line antitubercular drugs

Variables	New cases		Previously treated		Total	
	n (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI
Total DST results	103		132		235	
Sensitive to all drugs	30 (29.1)	20.3-37.9	38 (28.9)	21.1-36.5	68 (28.9)	23.1-34.7
Resistant to all drugs	73 (70.9)	62.1-79.6	94 (71.2)	63.5-78.9	167 (71.1)	65.3-76.9
Resistance to INH	40 (38.8)	29.4-48.2	105 (79.5)	72.7-86.4	145 (61.7)	55.5-67.9
Resistance to RIF	36 (35.0)	25.7-44.1	98 (74.2)	66.8-81.7	134 (57.0)	50.7-63.3
Resistance to SM	28 (27.2)	41.8-61.1	91 (68.9)	61.0-76.8	119 (50.6)	44.2-57.0
Resistance to EMB	24 (23.3)	15.1-31.5	72 (54.5)	46.0-63.0	96 (40.8)	34.6-47.1
Resistance to only INH	4 (3.9)	0.1-7.6	5 (3.9)	0.1-7.0	9 (3.8)	1.4-6.3
Resistance to only RIF	3 (2.9)	−0.3-6.1	4 (3.0)	0.1-5.9	7 (3.0)	0.8-5.1
Resistance to only SM	1 (1.0)	−0.9-2.9	3 (2.7)	−0.3-4.8	4 (1.7)	0.4-3.3
Resistance to only EMB	2 (1.9)	−0.7-4.6	0	0-0	2 (0.8)	−0.3-2.0
Total monoresistance	10 (9.7)	3.9-15.4	12 (9.1)	4.2-13.9	22 (9.4)	7.0-15.5
INH + RIF	6 (5.8)	1.3-10.3	16 (12.1)	6.5-17.7	22 (9.4)	5.6-13.1
INH + RIF + SM	8 (7.8)	2.6-12.9	11 (8.3)	3.6-13.0	19 (8.1)	4.6-11.6
INH + RIF + EMB	3 (2.9)	−0.3-6.2	8 (6.1)	2.0-10.1	11 (4.7)	2.0-7.4
INH + RIF + EMB + SM	24 (23.3)	15.1-31.5	49 (37.1)	28.9-45.3	73 (31.1)	25.1-36.9
Total MDR	26 (25.2)	16.8-33.6	98 (74.2)	66.8-81.7	124 (52.8)	46.3-59.1
INH + EMB	0	0-0	1 (0.7)	−0.7-2.2	1 (0.4)	−0.4-1.2
SM + EMB	0	0-0	2 (1.5)	−0.6-3.6	2 (1.0)	−0.3-2.0
INH + SM + EMB	1 (1.0)	−0.9-2.9	5 (3.9)	0.5-7.0	6 (2.5)	0.5-4.6
SM + RIF + EMB	0	0-0	1 (0.7)	−0.7-2.2	1 (0.4)	−0.4-1.2
RIF + SM	1 (1.0)	−0.9-2.9	1 (0.7)	−0.7-2.2	2 (1.0)	−0.3-2.0
RIF + EMB	0	0-0	0	0-0	0	0-0
Total poly-resistance other than MDR	2 (1.9)	−0.7-4.6	14 (10.6)	5.3-15.8	16 (6.8)	3.6-10.0

DST: Drug susceptibility testing, INH: Isoniazid, RIF: Rifampicin, EMB: Ethambutol, SM: Streptomycin, MDR: Multi drug resistance, CI: Confidence interval

without any treatment history. Among the resistant cases, the prevalence of MDR-TB in newly diagnosed and previously treated patients was 25.2% and 74.2%, respectively, which is similar to the previously reported studies.[18,19] The findings are similar to a previous survey conducted at this center which has reported higher prevalence (35.7%) of MDR-TB in previously treated cases.[16] Another study also reported higher rate of MDR-TB (57.8%) in previously treated patients than newly diagnosed (18.9%) patients.[17] The prevalence of primary drug resistance observed in different studies from India varies from 7.9% to 27.7%.[15,16,20-22] Similarly, the prevalence of acquired drug-resistance ranges from 60% to 85% in Indian studies.[23-27] The resistance in previously treated cases is indicator of poor compliance, lack of treatment supervision, and ineffective TB control program whereas in new cases is due to the dissemination of disease with resistant bacilli.[28]

From 312 cases, there was a seropositivity of 18.9% for HIV cases, whereas among 235 patents with TB there were 6.4% (15/235) seropositive cases. Some other parts of country such as Chennai, Pune, and Chandigarh have shown higher seroprevalance of HIV in TB cases such as 17%, 20.4%, and 9.23%, respectively.[18,29,30] A previous study at this center has recorded HIV seropositivity rate of 10.3% in TB patients.[16]

Globally, few studies have reported the strong confounding effects of demographic factors such as age groups and gender

on the prevalence of TB.[18,31] In this study, we have undertaken the study of various risk factors associated with the isolation of MDR as compared to susceptible TB patients. We found that there was a significant association between gender and occurrence of TB. Males had higher OR (1.38, 95% confidence interval [CI]: 1.00–1.91) than females in the age group of 21–45 (4.68, 95% CI: 3.03–7.73) years which demonstrated that males were more infected than females in the age group of 21–45 years. In contrast, one study have reported that females were more infected than males in the age group of 18–29 years (OR, 2.31, 95% CI: 1.70–3.15).[7]

This study showed highest TB prevalence ($P > 0.05$) and MDR ($P = 0.03$) among those individuals who had previous history of antitubercular treatment. It has been reported that patients who have history of house hold TB contact are at a high risk of becoming infected with MTB and develop into active TB.[32] Similar to these findings, in this study, participants who had previous house hold exposure of TB were more likely to have TB (OR 3.17, 95% CI: 0.78–12.85) than those without exposure.

The prevalence of TB was significantly associated ($P = 0.0000$) in those who were HIV infected. Our data suggest no association between MDR-TB and HIV status because only 43.3% of the patients in our study were tested for HIV and MDR, we cannot make a conclusion on this. The coexistence

Table 5: Association of demographic, clinical history and behavioral factors with present tuberculosis positive status and multi drug resistance patients

Risk factors	Category	Present TB positive patients		MDR patients	
		OR (95% CI)	*P*	OR (95% CI)	*P*
Demographic					
Gender	Male	1.38 (1.00-1.91)	0.05	1	0.15
	Female	1		0.67 (0.39-1.16)	
Age group (years)	5-20	1.91 (1.06-3.15)	<0.0001	1	<0.0001
	21-45	4.68 (3.03-7.23)		1.25 (0.64-2.44)	
	>50	1		0.21 (0.08-0.56)	
Clinical history					
HIV status	Positive	0.05 (0.03-0.10)	<0.0001	0.98 (0.34-2.85)	>0.05
	Negative	1		1	
Prior history of TB treatment	Yes	0.74 (0.53-1.04)	>0.05	0.52 (0.29-0.94)	0.03
	No	1		1	
Previous household exposure	Yes	3.17 (0.78-12.85)	0.10	0.17 (0.01-2.56)	0.2
	No	1		1	
Nature of TB	Pulmonary	2.48 (1.53-4.01)	0.002	0.59 (0.25-1.40)	>0.05
	Extra-pulmonary	1		1	
Behavioral					
Smoking	Yes	0.19 (0.12-0.31)	<0.0001	0.43 (0.21-0.87)	0.01
	No	1		1	
Alcohol use	Yes	4.51 (2.96-6.88)	<0.0001	0.27 (0.12-0.57)	0.0006
	No	1		1	

OR: Odd ratio, MDR: Multi drug resistance, CI: Confidence interval, TB: Tuberculosis

of HIV infection is another risk factor for the development of MDR-TB. Studies in Latvia and Donetsk Oblast have reported an association between HIV and MDR-TB.[33] Some study showed that there was no significant association between HIV and MDR-TB.[6,7]

We found that alcohol use (4.51, 95% CI: 2.96–6.88) and smoking (0.19, 95% CI: 0.12–0.31) were associated with a high risk of TB prevalence than those who had not smoked and used alcohol. In addition, smoking ($P = 0.01$) and alcohol consumption ($P = 0.0006$) had significantly affected the development of MDR-TB. Among TB patients alcoholism has been associated with high treatment failure and poor treatment outcome.

The major limitations are the study being conducted at a tertiary referral center may not be representative of the population at large. However, culture and sensitivity is usually performed in clinically doubtful samples or in cases exposed to anti-TB treatment previously. Hence, there may be a slight overestimation of MDR-TB in our study. Nevertheless, previous drug exposure is an important risk factor for the development of drug resistance and mandates drug sensitivity testing.

Conclusion

The prevalence of MDR was found to be high in previously treated patients as compared to new cases which re-enforced the fact that routine mycobacterial culture and DST of clinically suspected cases should be done in different health sectors during initial phase of therapy which gives base line data

to formulate effective anti-TB drug policy for guidance and patient treatment. This study demonstrated that risk factors such as smoking, alcohol consumption, and selection pressure from previous treatment factors increasing the development of MDR and it is more commonly in people, positive for HIV. Immediate therapeutics and more surveillance are necessary to combat the threat of MDR-TB. It is also necessary for public health community to incorporate and strengthen alcohol and smoking nonparticipation interference in TB control program.

Conflicts of interest
There are no conflicts of interest.

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