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Original Research Article

An Epidemiological Assessment of Prevalence of INH and Rifampicin Resistance in the Treatment Naïve New Tuberculosis Cases

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Abstract

Aim: The present study was planned to find the prevalence of INH and Rifampicin resistance in the treatment naïve new tuberculosis cases. We also tried to find a correlation between sputum grading, sociodemographic factors, and resistance patterns.

Methods: It was a cross-sectional study done conducted in the Department of TB and Chest, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India for the period of 2 years. 100 patients were included in the study. All Adult patients attending Department of TB and Chest who were diagnosed with new sputum smear-positive tuberculosis and have not received ATT drugs for more than 2 weeks were included in the study after taking informed written consent.

Results: A total of 100 newly diagnosed microbiologically confirmed cases of pulmonary tuberculosis were recruited in the study. Most of the patients were male (n=80, 80%) and a mean age of 45.435 (SD ±12.18) years. Based on the Modified Kuppuswamy socioeconomic scale 55% cases belonged to the lower socioeconomic group, whereas 35% belonged to the upper lower group. 20% were smokers and 80% were non-smokers. 85% patients presented with cough as chief complaint followed by Fever in 35%, loss of appetite in 50%, and loss of weight in 45% cases. Among comorbidities, Diabetes mellitus was most common followed by asthma. None of the cases had a previous history of anti-tubercular drug intake. All cases were HIV-Sero-negative.

Conclusion: INH resistance was found to be low (5.6%) with negligible MDR in the current study. Regular and large studies are needed to quantify and tackle the problem of primary MDR TB.

Keywords: Primary MDR TB, Tuberculosis, MGIT, Treatment naïve TB.

Introduction

According to the World Health Organisation (WHO) global report 2016, among 10.4 million incident TB cases worldwide, 3.9% are estimated to have had rifampicin- or multidrug-resistant tuberculosis (MDR/RR-TB) in 2015. In addition, 21% of previously treated TB cases were estimated to have had MDR/RR-TB in the same year. MDR-TB is caused by strains of M. tuberculosis that is resistant to both isoniazid and rifampicin. Drug-resistant TB (DR-TB) patients require prolonged and expensive treatment using second-line medications that are less effective and more toxic. [1] Tuberculosis is a major public health problem. India accounts for one fourth of the global TB burden. In 2015, an estimated 28 lakh cases occurred and 4.8 lakh people died due to TB. India has highest burden of TB and MDR TB based on estimated report 2016, an estimated 1.3 lakh incident multi-drug tuberculosis patients emerge annually in India which includes 7900 MDR TB patients estimated among notified pulmonary cases. [2] Drug resistance tuberculosis is a major public health problem that threatens progress made in TB cases and control worldwide. According to global tuberculosis report 2015, globally 3.3% of new and 20% of previously treated tuberculosis cases was multi-drug resistant (MDR-TB) in 2014. [3]

Drug resistant tuberculosis is caused by genetic mutation of bacilli, an inadequate or poorly administered treatment regimen and weak services programme that lead to delay detection and effective treatment of drug resistance and are unequipped to support patients to keep adherence to treatment. [4] Multidrug resistance tuberculosis (MDR-TB) is defined as disease caused by mycobacterium tuberculosis which is resistant to at least isoniazid and rifampicin with or without other first line anti-tubercular drugs. [5] The rapid detection of mycobacterium tuberculosis is essential for early diagnosis and disease management because the high risk of transmission from person to person and emergence of MDR-TB

and XDR-TB (extensively drug resistant tuberculosis). Culture is the "gold standard" for final determination but it is time consuming and may take up to 2-8 weeks. In 2011, WHO introduced the wide use of Xpert MTB/Rif assay, it is fully automated diagnostic molecular test using real-time polymerase chain reaction (PCR) technology to simultaneously detected M. tuberculosis and rifampicin resistance mutation in the rpo B gene. [6] The Xpert assay is highly rapid, sensitive and specific in diagnosis of both pulmonary and extra-pulmonary tuberculosis. [7-9]

The emergence of drug resistance to M. tuberculosis has become a significant obstacle for TB control. [10] The emergence and spreading of multidrug (MDR) and extensively (XDR) drug-resistant M. tuberculosis complex (MTBC) strains poses significant challenges to TB control. [11] Despite low sensitivity in detection of M. tuberculosis, acid-fast staining remains the main diagnostic method in resource-limited settings. Mycobacterial culture is the gold standard and the most sensitive method for TB diagnosis; however, its use in clinical practice is limited due to a slow turnaround time, biosafety requirements, and high cost. [12,13] In 2011, WHO introduced the wide use of Xpert MTB/RIF assay. It is a fully automated diagnostic molecular test using real-time polymerase chain reaction (PCR) technology to simultaneously detect M. tuberculosis and rifampicin resistance mutations in the rpoB gene. [14] One of the promising method, well evaluated and accepted in varying settings is the fluorimetry based liquid culture detection system, Mycobacterial Growth Indicator Tube (MGIT 960) (Becton and Dickinson, USA). [15]

With the above problem of primary drug resistance among newly diagnosed tuberculosis cases the present study was planned to find the prevalence of INH and Rifampicin resistance in the treatment naïve new tuberculosis cases. We also tried to find a correlation between sputum grading, sociodemographic factors, and resistance patterns.

Materials and Methods

It was a cross-sectional study done conducted in the Department of TB and Chest, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India for the period of 2 years. 100 patients were included in the study. All Adult patients attending Department of TB and Chest who were diagnosed with new sputum smear-positive tuberculosis and have not received ATT drugs for more than 2 weeks were included in the study after taking informed written consent.

Inclusion Criteria: Treatment naive new tuberculosis cases (those cases who have received anti-tuberculosis drugs for less than 2 weeks)

Exclusion Criteria

- Subjects who were previously treated for pulmonary or extrapulmonary tuberculosis for more than 2 weeks
- Subjects not willing to participate in the study

Suspected cases of tuberculosis were given a widemouth bottle for collection of sputum. Two sputum specimens were collected (spot & early morning). Sputum was subjected to ZN and/or fluorescent stains (as per RNTCP/NTEP guidelines) and studied under light & Fluorescent microscopy respectively. Results were recorded and documented as per RNTCP guidelines. Patients with positive results were screened as per inclusion and exclusion criteria. Those patients with positive smears were asked to participate in the study and details of the study explained both verbal and documents. After taking informed consent willing patients were asked to give 2 more samples in a sterile sputum collection bottle in Falcon tube for MGIT culture and DST.

Sputum samples were processed as per instructions by MGIT manufacturer FIND Diagnostics. The culture was done in MGIT 960 and positive cultures were recorded and subjected to drug sensitivity testing for INH (0.1 µg/ml) and Rifampicin (1 µg/ml) and a parallel nondrug MGIT was run as a control. Data were recorded and analysed.

Culture and drug susceptibility in liquid media (BACTEC MGIT 960): The samples were processed as per the guideline For BACTECTM MGIT 960TM TB system given by the manufacturer FIND diagnostics.[16]

NaOH-NALC Procedure: The equal volume of sputum sample and NaOH-NALC Sodium citrate solution was added and vortexed lightly around 15-30 seconds. NALC-NaOH solution was added to the mixture and allowed for 15-20 minutes (25 minutes max). Vortex was done lightly in every 5-10 minutes. Phosphate buffer (pH 6.8) was added up to the top ring and mixed well and centrifuged at 3000 rpm for 15-20 minutes and allowed the tubes to stand for 5 minutes to settle down. The supernatant was discarded carefully into the container containing a mycobactericidal disinfectant. Phosphate buffer (pH 6.8) 1-2 ml was added and resuspended the sediment with the help of a pipette or vortex mixer. Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim, and Azlocillin (PANTA) was reconstituted according to the manufacturer's description and then used in MGIT tubes.

Inoculation into MGIT Medium: 7 ml MGIT tubes were aseptically added with 0.8 ml of MGIT growth supplement and PANTA and then 0.5 ml of a well-mixed processed/concentrated specimen and

mixed by inverting the tube several times. The tubes and caps were wiped with a Mycobactericidal disinfectant and inoculated tubes were kept at room temperature for 30 minutes. Inoculated MGIT (7 ml) tubes were then entered in the BACTEC MGIT 960 instruments after scanning. Tubes were incubated at 37◦C. MGIT tubes were kept incubated until the instrument flagged them positive. [16] Once a MGIT tube is flagged positive, the tubes were manually observed, and smear was made and stained with Auramine Rhodamine for AFB to rule out any contamination. After a maximum of 6 weeks, the instrument flags the tube negative if there is no growth. The second Sample was also subjected to the Line Probe Assay for cross-checking the resistance pattern, the sample was processed as per the manufacturer's guideline by Hain's MTBDR plus for LPA. [17,18]

Statistical analysis

Outcome variables: Resistance to INH and Rifampicin. Distribution of data on categorical variables related to gender, age, weight, sputum grade, sputum smear results, symptoms like cough, fever, haemoptysis, loss of appetite and loss of weight, presence and absence of HIV seropositivity, co- morbidities, smoking history, history of ATT, household contact and resistance to INH and Rifampicin etc. were expressed in frequency and percentage. The distribution of the variables was revealed via the Kolmogorov-Smirnov test. The correlation of Resistance and various variables like age, sex, symptoms, socioeconomic status (based on Modified Kuppusamy Scale 2019),[19] smoking, diabetes, and household contact history were analyzed by one-way Anova with Bonferroni correction using IBM-SPSS program (SPSS version 19.0; SPSS Inc, Chicago, IL) All statistical analysis was carried out at 5% level of significance and p-value <0.05 was considered as significant.

Results

Table 1: General characteristics of the cases

Characteristics	**N%**
Male	80 (80)
Female	20 (20)
Smokers	20 (20)
Non-Smoker	80 (80)
HIV Seropositive	0
Household Contact	5 (5)
Diabetes	15 (15)
Asthma	5 (5)
CKD	4 (4)
Hypertension	4 (4)
Previous history of ATT intake	0
Socioeconomic status(category)	
Lower(V)	55 (55)
Upper Lower (IV)	35 (35)
Lower Middle (III)	8 (8)
Upper Middle (II)	2 (2)
Upper (I)	0

A total of 100 newly diagnosed microbiologically confirmed cases of pulmonary tuberculosis were recruited in the study. Most of the patients were male (n=80, 80%) and a mean age of 45.435 (SD ±12.18) years. Based on the Modified Kuppuswamy socioeconomic scale 55% cases belonged to the lower socioeconomic group, whereas 35% belonged to the upper lower group. 20% were smokers and 80% were non-smokers.

Table 2: Correlation of resistance with categorical variables

Categorical Variables	**Result**	**INH Resistance n (%)**		**p value**
		Absent n (%)	**Present n (%)**	
Gender	Male	75	5	0.545
	Female	20	0	
Rifampicin Resistance	Absent	90	10	0.055
	Present	0	0	
Cough	Absent	15	0	1.00
	Present	80	5	
Fever	Absent	32	3	1.000

	Present	60	5	
Haemoptysis	Absent	75	5	0.314
	Present	20	0	
Weight loss	Absent	50	5	0.634
	Present	40	5	
Loss of appetite	Absent	45	5	0.715
	Present	47	3	
Household Contact	Absent	45	5	1.000
	Present	50	0	
Jaundice	Absent	90	5	1.000
	Present	5	0	
Asthma	Absent	92	3	1.000
	Present	5	0	
DM	Absent	80	5	1.000
	Present	12	3	
Smoking	Absent	85	8	0.343
	Present	6	0	
Socioeconomic Status	Lower	52	3	0.625
	Upper Lower	28	2	
	Lower Middle	8	0	
	Upper Middle	2	0	

85% patients presented with cough as chief complaint followed by Fever in 65%, loss of appetite in 50%, and loss of weight in 45% cases. Among comorbidities, Diabetes mellitus was most common followed by asthma. None of the cases had a previous history of anti-tubercular drug intake. All cases were HIV-Sero-negative.

Discussion

The Global TB Report 2017 published by World Health Organization (WHO) estimates that India contributes 27% (2.79 million) and 25% (147 000) of the global burden of TB and multi-drug resistant TB (MDR-TB), respectively. [1,20] A male predominance of pulmonary tuberculosis with Male to Female ratio of 5.25:1 was noted in our study which probably reflects the study population distribution as a whole. Similar male gender predominance has also been noticed in other studies. [21,22] This gender difference may be due to the high prevalence of tuberculosis in the male population due to their exposure to external environment for their occupation and other related work.

Pressure to continue working in spite of poor health could lead to decreased compliance toward regular medication thereby increasing the threat of developing drug resistance. A national drug resistance survey found 58% males in the age group of 25-55 years and females more common in the younger age group (72% in 15-45 years). [23] In the present study the most common co-morbidity found as Diabetes, followed by asthma, chronic kidney disease and hypertensive. Similar results have been found in previous studies also. In the study done by Myneedu et al most common comorbid condition found was diabetes (2%). [21]

A total of 100 newly diagnosed microbiologically confirmed cases of pulmonary tuberculosis were recruited in the study. Most of the patients were male (n=80, 80%) and a mean age of 45.435 (SD ±12.18) years. Based on the Modified Kuppuswamy socioeconomic scale 55% cases belonged to the lower socioeconomic group, whereas 35% belonged to the upper lower group. 20% were smokers and 80% were non-smokers. In the present study 20% cases were smokers, similar findings are also found in several studies. Harshita Gupta et al. in their study from Lucknow reported 64.4% smokers. [24] In a study done in New Delhi by Myneedu et al., found 49 out of 453 cases (10.8%) smokers. Household contact to TB and MDR TB is a well-documented risk factor for developing Initial MDR TB.38 In our study, only four patients had household contact as tuberculosis. All were sensitive to both INH and Rifampicin. Gupta et al., in their study from Lucknow reported 23.3% of cases with a history of household contact of tuberculosis patients. They also found higher primary MDR and other combinations of drug resistance rates (4.7%). [24] All 200 (100%) samples showed growth of mycobacterium tuberculosis and on sensitivity 7 (7%) showed resistance to INH, and none of the samples showed resistance to Rifampicin. This outcome is lower in contrast with national drug resistance survey of 2.2% of primary MDR. In our study, mono-resistance to rifampicin was found to be zero; which is similar to the recently concluded first National Drug resistance survey (2014-16) of India. [23] 85% patients presented with cough as chief complaint followed by Fever in 35%, loss of appetite in 50%, and loss of weight in 45% cases. Among comorbidities, Diabetes mellitus was most

common followed by asthma. None of the cases had a previous history of anti-tubercular drug intake. All cases were HIV-Sero-negative.

The limitation of the study was less number of cases. A large number of patients were from other states and did not give consent for the study due to logistic problems. Further none of our patients had HIV seropositivity which denied opportunity to study the impact of HIV on primary drug resistance. Similarly, very few patients had a history of household contact with tuberculosis patients and none of them had resistance, we could not study the impact of the same on primary drug resistance.

Conclusion

Primary drug resistance in treatment naïve cases of tuberculosis is not rare. Mono-resistance to Isoniazid is common (7%). Mono-resistance to Rifampicin is negligible. Primary MDR is also very less but present. The proper history of ATT exposure is of utmost importance to rule out primary drug resistance. Newer diagnostic techniques like CB-NAAT should be offered upfront to a newly diagnosed tuberculosis case to rule out primary drug resistance and proper treatment. A regular survey of drug resistance is very important for the knowledge of the problem and preparation for the same.

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