ORIGINAL ARTICLE

A STUDY TO EVALUATE PATTERN OF RIFAMPICIN RESISTANCE IN CASES OF SPUTUM POSITIVE PULMONARY TUBERCULOSIS

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ABSTRACT: AIMS: The emergence of drug resistance and development of multidrug resistant tuberculosis (MDR TB) has become a new but significant obstacle for TB control. As Rifampicin resistance is an important indicator for drug resistant TB, rapid diagnosis of tuberculosis and detection of Rifampicin (RIF) resistance are essential for knowing the magnitude of problem & early management of drug resistance TB. The aim of this study is to determine the pattern of rifampicin resistance in the sputum positive MDR TB suspects by using GeneXpert MTB/RIF and thus to focus on magnitude of the problem on drug resistance tuberculosis. **STUDY DESIGN:** A cross sectional observational study carried out over a period of 2 years in a tertiary care hospital. **SUBJECTS & METHODS:** In this study 428 sputum positive cases of pulmonary tuberculosis who were potential MDR suspect were included, there sputum samples were collected and tested by GeneXpert MTB/RIF assay, which is an automated cartridge based nucleic acid amplification test to detect presence of mycobacterium tuberculosis and status of Rifampicin resistance. The results are statistically analyzed. **RESULTS:** Out of 428 patients, mycobacterium tuberculosis was detected in 328 patients (76.63%) & out of these 328 patients, Rifampicin resistance was found in 98 cases (29.87%). Male and female ratio was 6:1 among Rifampicin resistant cases. Regarding age distribution, maximum no. of patients with Rifampicin resistance were in the age group of 21-30 yrs (26.53%) followed by 31-40 yrs (22.44%). In this study among cases of Rifampicin resistance, 23.47% of cases were new smear positive failure patients, retreatment cases smear positive at 4 months were 8.16%, 22.44% of cases were retreatment cases smear positive at diagnosis, 41.83% of cases were retreatment failure cases, 1.03% patient had history of contact with MDR TB and 3.06% patient was HIV seropositive. **CONCLUSIONS:** Rifampicin resistance cases are found in significant no. of MDR TB suspects. They are mostly male, 21-30 yrs. of age, predominantly retreatment failure cases whereas, new smear positive failure patients also contributes a significant disease burden. Also, HIV seropositive patients should be screened for drug resistance tuberculosis.
KEYWORDS: MDR tuberculosis, Rifampicin resistance, GeneXpert MTB/RIF.

INTRODUCTION: Tuberculosis (TB) is a major cause of illness and death in Asia and Africa.[1] There are 22 high burden countries which constitute 80% of all incident cases worldwide. India ranks first of them with two million estimated TB cases as against estimated global incidence of 9.4 million accounting for 21% of new cases.[2]

The emergence and spread of multi-drug resistant tuberculosis (MDR-TB) is another new threat upon existing one to destabilize global tuberculosis control. The prevalence of MDR-TB is increasing throughout the world both among new tuberculosis cases as well as among previously-treated ones.[3]

ORIGINAL ARTICLE

Although previous treatment for TB is the strongest risk factor for development of MDR-TB, [4-6] treatment-naïve patients are also at risk due to either spontaneous mutations or transmission of resistant strains.[7,8] The risk of transmission of resistant strains from close contacts is increasing day-by-day because of the growing burden of MDR-TB patients.[3]

The rapid detection of M. tuberculosis in infected patients is essential for disease management, because the high risk of transmission from person to person and emergence of MDR-TB and extensively drug resistant tuberculosis and detection of rifampicin resistance is also important because it is an important indicator for detection of drug resistant tuberculosis including MDR-TB patients. Culture is the "gold standard" for final determination, but it is time consuming and may take up to 2 to 8 weeks. Although smear microscopy for acid-fast bacilli (AFB) is rapid and inexpensive, it has poor sensitivity and a poor positive predictive value (PPV). Thus, rapid identification, which is essential for earlier treatment initiation, improved patient outcomes, and more effective public health interventions, relies on nucleic acid amplification techniques.[9]

Collectively, DNA sequencing studies demonstrate that more than 95% of RIF-resistant M. tuberculosis strains have a mutation within the 81-bp hot spot region of the rpoB gene.[10,11,12] Several molecular methods have been developed in recent years for the diagnosis of tuberculosis and rapid detection of drug resistance in clinical specimens, including line probe assays (Geno Type MTBDR plus [Hain Life science GmbH, Nehren, Germany], INNO LIPA Rif. TB [Innogenetics, Ghent, Belgium]) and real-time PCR (GeneXpert MTB/ RIF; Cepheid, Sunnyvale, CA). Molecular assays have been established to allow the prediction of drug resistance in clinical specimens within 1 working day and are potentially the most rapid methods for the detection of drug resistance.[10,11,13-17]

The GeneXpert MTB/RIF assay is a novel integrated diagnostic device that performs sample processing and real-time PCR analysis in a single hands-free step for the diagnosis of tuberculosis and rapid detection of RIF resistance in clinical specimens.[13,15] The MTB/RIF assay detects M. tuberculosis and RIF resistance in same setting by PCR amplification of the 81-bp fragment of the M. tuberculosis rpoB gene and subsequent probing of this region for mutations that are associated with RIF resistance. The assay can generally be completed in less than 2 h.[13,15]

The aim of this study is to determine the pattern of rifampicin resistance in the sputum positive patients who are MDR suspects by using GeneXpert MTB/RIF and thus to focus on magnitude of the problem on drug resistance tuberculosis.

SUBJECTS AND METHODS: In this cross sectional observational study carried out over a period of 2 years in a tertiary care hospital, a total of 428 sputum positive cases of pulmonary tuberculosis who were potential MDR suspect were included. So, potential MDR suspect /Inclusion criteria were the following:

- New treatment failure.
- Retreatment case sputum positive at 4 month.
- Retreatment case, smear positive at diagnosis.
- Retreatment case failures.
- Smear positive, H/o contact with MDR TB.
- HIV positive, smear positive TB.

After identifying potential MDR TB suspect patients, their sputum sample was collected in a special falcon tube. The GeneXpert MTB/RIF assay as described previously was performed then.[13,15,18]

ORIGINAL ARTICLE

Briefly, sample reagent was added at a 3:1 ratio to clinical specimens. The closed specimen container was manually agitated twice during a 15-min period at room temperature, before 2 ml of the inactivated material (Equivalent to 0.5 ml of decontaminated pellet) was transferred to the test cartridge. Results were noted after 2 hours.

After finding results of GeneXpert MTB/RIF assay, Rifampicin resistant cases were statistically analyzed. All smear negative TB patients were excluded from this study. There was no ethical or financial controversy in this study.

RESULTS: In this study, 428 patients who were potentially MDR suspect, were selected and their sputum samples were tested by cartridge based nucleic acid amplification test- CB NAAT (GeneXpert MTB/RIF). Out of 428 patients, mycobacterium tuberculosis was detected in 328 patients (76.63%) & out of these 328 patients, Rifampicin resistance was found in 98 cases (29.87%). Male and female ratio was 6:1 among Rifampicin resistant cases. Regarding age distribution, maximum no. of patients with Rifampicin resistance were in the age group of 21-30 yrs (26.53%) followed by 31-40 yrs (22.44%). In this study among cases of Rifampicin resistance, 23.47% of cases were new smear positive failure patients, retreatment cases smear positive at 4 months were 8.16%, 22.44% of cases were retreatment cases smear positive at diagnosis, 41.83% of cases were retreatment failure cases, 1.03% patient had history of contact with MDR TB and 3.06% patient was HIV seropositive.

DISCUSSION: In this study, we tried to determine the pattern of rifampicin resistance in pulmonary tuberculosis patients in a tertiary care hospital. Mycobacterial resistance to rifampicin is also associated with resistance to other antitubercular drugs, specially isoniazid in majority (93%) of cases. This makes rifampicin resistance a surrogate marker for diagnosing MDR TB.[19,20,21,22]

In India, prevalence of MDR TB is about 1-3% in new cases and around 12% in previously treated cases.[23,24] Global Drug resistance surveillance data indicate that in 2013, approximately 4, 80, 000 people developed MDRTB worldwide. Among TB patients reported by national TB programs in 2013, there were an estimated 3, 00, 000 cases of MDR-TB. More than half of these cases were in India, China and the Russian Federation.[25] In drug resistance surveillance studies in India, Rifampicin resistance was observed in all the centers studied except Gujarat.[26] In the present study, Rifampicin resistance was found in 29.87% of patients which is similar to that reported from Jaipur 28.2% by Malhotra et al,[27] New Delhi 33.7% by Jain et al,[28] Gujarat 37.3% by Trivedi et al[29] and Gujarat 37.47% by Shah et al[30] though Bombay reports a very high incidence of Rifampicin resistance of 66.8% (Chowgule et al).[31]

In this study, 85.71% patients with rifampicin resistance were male. A European study by Faustini et al[32], more drug resistant TB cases were among men. Regarding age distribution in our study, maximum no. of patients with Rifampicin resistance were in the age group of 21-30 yrs (26.53%) followed by 31-40 yrs (22.44%). In a study done by Aleyamma Thomas et al[33] in TRC, Chennai, 70% of the drug resistant patients were male and their mean age was 37. In another study by Robert J et al,[34] the age and the sex distribution were similar to this study.

In our study, 23.47% cases of new treatment (previously known as category I) failure cases was found among Rifampicin resistance cases. A study from South India found 17% MDR-TB among failures of the Category I regimen.[35] Another study done by R Singla et al[36] found that 14% of CAT I failure developed drug resistant TB. Retreatment cases, smear positive at diagnosis was 22.44%

ORIGINAL ARTICLE

among rifampicin resistant. In a study by S K Sharma et al[37] drug resistance was found in 20% of Retreatment cases at diagnosis which is similar to our study. Retreatment (Previously CAT II) failure cases were found to be 41.83% among Rifampicin resistant in our study. In a study done by Anil M Gupta et al,[38] it was found that 34% of cat II failures were drug resistant. A high prevalence of MDR-TB in Cat-II failure is not unique to India and has been documented in Vietnam,[39] Thailand,[40] and Rowanda.[41] We found only one resistant case (1.02%) with history of contact with MDR TB. This is consistent with a study done by N Singla et al[42] in which only 0.66% of contacts developed MDR TB. Three patients (3.06%) among rifampicin resistant cases were found to be HIV seropositive. In study done by Deivanayagam CN et al,[43] HIV seropositivity observed in 4.42% of MDRTB patients.

So, from this study we conclude that Rifampicin resistance cases are found in significant no. of MDR TB suspects using CB NAAT (GeneXpert MTB/RIF). They are mostly male, between 21-30 yrs. of age. Most of the resistant patients are retreatment failure cases whereas, new smear positive failure patients also contribute a significant disease burden. Also, HIV seropositive patients should be screened for drug resistance tuberculosis.

	No. of patients	Percentage
Male	84	85.71
Female	14	14.28
Table 1:Distribution of sex among Rifampicin resistant cases		

Male: female ratio is 6:1

Age in years	No. of patients	Percentage
<20	18	18.36
21-30	26	26.53
31-40	22	22.44
41-50	14	14.28
51-60	10	10.20
>60	8	8.16
Table 2: Distribution of Rifampicin resistant patients among different age group		

Category	No. of patients	Percentage
New smear positive failure patients	23	23.47
Retreatment cases who are smear positive at 4 months	8	8.16
Retreatment cases smear positive at diagnosis	22	22.44
Retreatment failure cases	41	41.83
Smear positive with H/o contact with MDR TB	1	1.02
Smear positive patient who was HIV seropositive	3	3.06
Table 3: Distribution of Rifampicin resistant patients according to patients' category		

ORIGINAL ARTICLE

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ORIGINAL ARTICLE

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