A Study to Evaluate Magnitude of Rifampicin Resistance in Cases of Sputum Positive Pulmonary Tuberculosis in a Tertiary Care Centre in Western U.P.

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ABSTRACT

BACKGROUND

The emergence of drug resistance and development of multidrug resistance tuberculosis (MDR TB) has become a new but significant obstacle for TB control. As rifampicin resistance is an important indicator for drug resistant tuberculosis, rapid diagnosis of tuberculosis and detection of rifampicin resistance are essential for knowing the magnitude of the problem and early management of drug resistant tuberculosis (DR TB). The aim of this study is to determine the magnitude of rifampicin resistance in the sputum positive presumptive drug resistant tuberculosis, by using CBNAAT and thus to focus on magnitude of the problem of drug resistant tuberculosis.

METHODS

This is a cross sectional observational study carried out over a period of 8 months in a tertiary care hospital in western U.P. In this study, 182 sputum positive cases of pulmonary tuberculosis, who were potential presumptive drug resistant tuberculosis, were included. Their sputum samples were collected and tested by CBNAAT (an automated cartridge based nucleic acid amplification test) to detect presence of mycobacterium tuberculosis and also status of rifampicin resistance. The results were analysed.

RESULTS

Out of 182 patients, mycobacterium tuberculosis was detected in all 182 patients and out of these 182 patients, rifampicin resistance was found in 16 cases (8.79%). Male and female ratio was 3:1 among rifampicin resistant cases. Regarding age distribution, maximum no. of patients with rifampicin resistance were in the age group of 40-50 yrs. (50%), followed by 20-30 yrs. (25%).

CONCLUSIONS

Rifampicin resistant cases are found in moderate number of presumptive drug resistant tuberculosis. They were mostly male, and 40-50 yrs. of age.

KEYWORDS

MDR Tuberculosis/RR-TB, Presumptive Drug Resistant Tuberculosis, Rifampicin Resistance, CBNAAT

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BACKGROUND

Tuberculosis (TB) is a communicable disease that is a major cause of ill health, one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS). It is caused by the bacillus Mycobacterium tuberculosis, which is spread when people who are sick with TB expel bacteria into the air; for example, by coughing. It typically affects the lungs (pulmonary TB) but can also affect other sites (extrapulmonary TB). About a quarter of the world's population is infected with M. tuberculosis and thus at risk of developing TB disease. With a timely diagnosis and treatment with first-line antitubercular drugs for 6 months, most people who develop TB can be cured and onward transmission of infection curtailed. The number of TB cases occurring each year (and thus the number of TB-related deaths) can also be driven down by reducing the prevalence of health-related risk factors for TB (e.g. smoking, diabetes and HIV infection), providing preventive treatment to people with a latent TB infection, and taking multisectoral action on broader determinants of TB infection and disease (e.g. poverty, housing quality and undernutrition.[1]

Globally, Countries have made substantial progress in the fight against tuberculosis (TB) in the last decade and a half. However as estimated 10 million people developed tuberculosis in 2018, and 1.2 million people died of the disease in the world. Eight countries accounted for two thirds of the global total: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (6%), Nigeria (4%), Bangladesh (4%) and South Africa (3%).[1] 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.[2] Although previously treated cases of tuberculosis is the strongest risk factor for development of MDRTB, new tuberculosis patients are also at risk due to either spontaneous mutations or transmission of resistant strains.[3,4,5,6,7] The risk of transmission of resistant strains from its close contacts is increasing day by day because of the growing burden of MDR TB patients.[2]

The burden of drug-resistant TB is of major concern at global, regional and country levels. In 2018, there were approximately half a million (range, 417 000–556 000) new cases of rifampicin-resistant tuberculosis i.e. RR-TB (of which 78% had multidrug-resistant TB). The three countries with the largest share of the global burden were India (27%), China (14%) and the Russian Federation (9%). Globally, 3.4% of new TB cases and 18% of previously treated cases had MDR/RRTB, with the highest proportions (>50% in previously treated cases) in countries of the former Soviet Union.[1] Drug-resistant TB threatens global TB care and prevention, and it remains a major public health concern in many countries. Three categories are used for global surveillance and treatment: rifampicin-resistant TB (RR-TB), MDR-TB and extensively drug-resistant TB (XDR-TB). MDR-TB is TB that is resistant to both rifampicin and isoniazid, the two most powerful anti-TB drugs. Both MDRTB and RR-TB require treatment with a second-line drug regimen. With increasing use of Xpert MTB/RIF for simultaneous detection of TB and resistance to rifampicin, a growing number of RR-TB cases (without further testing for isoniazid resistance) are being detected and notified. XDR-TB is defined as MDR-TB plus resistance to at least one fluoroquinolone and a second-line injectable agent. The End TB Strategy calls for universal access to drug susceptibility testing (DST). The focus in this section is on DST for notified TB patients with bacteriologically confirmed TB. These are the TB cases that can be tested for MDR/RR-TB using diagnostic tests recommended by WHO.[1,8]

The rapid detection of M. tuberculosis in infected patients is essential for disease management, because of the high risk of transmission from person to person and emergence of MDR TB/RR TB and XDR TB. Detection of rifampicin resistance if also important because it is an important indicator for detection of drug resistant tuberculosis including MDR TB patients. The CB-NAAT is a semi-quantitative nested real-time PCR which detects both MTB and RIF resistance directly from clinical specimens. It is the WHO-recommended method in 2010 for the diagnosis of both pulmonary and extrapulmonary TB and for diagnosing paediatric TB. Universal drug susceptibility testing (U-DST) for rifampicin resistance has been implemented throughout the country. Testing all tuberculosis patient for resistance to at least Rifampicin constitutes U-DST. (This is achieved by offer of CBNAAT to all patients diagnosed as TB). U-DST has been rolled out across the country since January 2018. CBNAAT is also offered for TB diagnosed in key population such as people living with HIV (PLHIV), children, extra pulmonary cases & have an X-ray suggestive of TB and patient referred from the private sector for early diagnoses and initiating appropriate treatment.[8]

Tuberculosis with multidrug and rifampicin- resistance (MDR/RR- TB) are more difficult to treat than the susceptible tuberculosis. This is considered to be one of the major challenges to progress towards the countries targets to end TB by 2025. In March 2018, India introduced Shorter MDR regimen for country-wide implementation. Shorter MDR-TB regimen (9-11 month) duration is the first choice of the treatment for patient diagnosed with rifampicin resistance that would be continued or switch to longer regimen, based on second line probe assay (LPA) result. In addition to pulmonary MDR/RR TB patient, extra pulmonary disease like lymph node tuberculosis & pleural TB are also eligible for shorter MDR TB regimen.[8] 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 prediction 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 implication techniques.[9]

The genetic basis for RIF resistance in approximately 95% of the cases is due to mutations in an 81-bp RIF resistance-determining region (RRDR) of the rpoB gene, corresponding to codons 507 to 533 (Escherichia coli numbering system), which codes for the beta subunit of the RNA polymerase of M. tuberculosis.[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 MTB DR 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 with 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 perform sample processing and real time PCR analysis in a single hand free step for the diagnosis of tuberculosis and rapid detection of RIF resistant in clinical specimen.[13,15] The MTB/RIF/CBNAAT 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 the region for mutations that are associated with RIF resistance. They assay can generally be completed in less than two hours.[13,15]

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

METHODS

In this cross-sectional observational study carried out over a period of 8 months in a tertiary care hospital, a total of 182 sputum positive cases of pulmonary tuberculosis who were presumptive drug resistance tuberculosis were included. So, presumptive drug resistance inclusion criteria were the following:

- TB patients found positive on any follow up sputum smear examination during treatment with first line drugs including treatment failures;
- Paediatrics TB non-responders;
- TB patients who are contacts on drug resistant tuberculosis;
- Previously treated TB patients;
- New TB patients with HIV co-infection;
- All notified new TB patients.[18]

After identifying presumptive drug resistant tuberculosis patients, their sputum sample was collected in a special falcon tube. The CBNAAT MTB/RIF assay was performed.[13,15] The assay utilizes single–use plastic cartridges with multiple chambers that are preloaded with liquid buffers and lyophilized reagent beads necessary for sample processing, DNA extraction and heminested rt-PCR.[13,15] Clinical sputum samples (or decontaminated sputum pellets) are treated with sodium hydroxide and isopropanol-containing sample reagent (SR). The SR is added to the sample (currently recommended at a 3:1 ratio for sputum pellets and a 2:1 ratio for unprocessed sputum samples) and incubated at room temperature for 15 min. The treated sample is then manually transferred to the cartridge which is loaded into the Gene Xpert instrument.

Subsequent processing is fully automated. The cartridge incorporates a syringe drive, a rotary drive and filter upon which M. tuberculosis bacilli are deposited after being liberated from the clinical material. The test platform employs a sonic horn that inserts into the cartridge base to cause ultrasonic lysis of the bacilli and release of the genetic material. The assay then amplifies a 192 bp segment of the rpoB gene using a hemi-nested rt-PCR reaction. Mycobacterium tuberculosis is detected by the five overlapping molecular probes (probes A-E) that collectively are complementary to the entire 81 bp rpoB core region.[13,15]

M. tuberculosis is identified when at least two of the five probes give positive signals with a cycle threshold (CT) of d"38 cycles and that differ by no more than a prespecified number of cycles. The basis for detection of rifampicin resistance is the difference between the first (early CT) and the last (last CT) M. tuberculosis-specific beacon (ACT). The system was originally configured such that resistance was reported when ACT was >3.5 cycles and sensitive if d"3.5 cycles. Results were noted after 2 hours. After finding results of CBNAAT MTB/RIF assay, rifampicin resistant cases were statistically analysed. All smear negative TB patients were excluded from this study. There were no ethical or financial controversy in this study.

RESULTS

In this study, 182 patients who were presumptive drug resistant tuberculosis patient, were selected and their sputum samples were tested by cartridge based nucleic acid amplification test. CBNAAT (GeneXpert MTB/RIF). Out of 182 patients, mycobacterium tuberculosis was detected in 182 patients, and out of these 182 patients, rifampicin resistance was found in 16 cases (8.79%). Male and female ratio was 3: 1 among Rifampicin resistant cases. Regarding age distribution, maximum no. of patients with Rifampicin resistant were in the age group of 40-50 yrs. (50%), followed by 20-30 yrs. (25%).

	No. of Patients	Percentage
Male	12	75
Female	4	25
Table 1. Sex Distribution among Rifampicin Resistance Cases		
Male:Female ratio is 3:1 and (n=16)		

Age in Years	No. of Patients	Percentage
<20	1	6.25
22-30	4	25
31-40	3	18.75
40-50	8	50
Table 2. Age Distribution of Rifampicin Resistance Patients		

DISCUSSION

In this study, we tried to determine the magnitude of rifampicin resistance in pulmonary tuberculosis patients in a tertiary care hospital. Mycobacterial resistance to rifampicin is also associated with resistance to other antitubercular drug, specially isoniazid in majority (93%) of cases. This makes rifampicin resistance a surrogate marker for diagnosing MDR TB.[19,20,21,22] Detection of MDR/RR-TB requires bacteriological confirmation of TB and testing for drug resistance using rapid molecular tests, culture methods or sequencing technologies.

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 2018, approximately 4,84,000 people developed MDRTB worldwide. Currently, the WHO estimated incidence of Rifampicin Resistance (RR) and MDR TB in India to be around 130000. This translated to around 9.6 patients per 100000 population annually as per the Global TB 2019. The global number of MDR/RR-TB cases notified in 2018 was 39% of the estimated 484 000 (range, 417 000– 556 000) MDR/RR-TB incident cases in 2018. TB affects people of both sexes in all age groups but the highest burden is in adult men, who accounted for 57% of all TB cases in 2018. By comparison, adult women accounted for 32% and children for 11%. Among all TB cases, 8.6% were people living with HIV. The First national anti TB drug resistance survey for 2014-16 (NDRS) concluded recently. In the present study Rifampicin resistance was found 8.79% of patients which is almost similar to The First national anti TB drug resistance survey for 2014-16 (NDRS) 6.19% in all cases.[18] Though some reports very high incidence of rifampicin resistance from Jaipur 28.2% by Malhotra et al,[25] New Delhi 33.7% by Jain et al,[26] Gujarat 37.3% by Trivedi et al[27] and Gujarat 37.4% by Shah et al.[28]

In this study, 75% patients with rifampicin resistance were male. A European study by Faustini et al[29] observed more drug resistant TB cases among men. Regarding age distribution in our study, maximum no. of patients with Rifampicin resistance were in the age group of 40-50 yrs. (50%) followed by 22-30yrs (25%) . In a study done by Aleyamma Thomas et al[30] in TRC Chennai, 70% of drug resistance patients were male and their mean age was 37.

CONCLUSIONS

Rifampicin resistance cases are found in presumptive MDR TB using CBNAAT (GeneXpert MTB/RIF). They are mostly male, between 40-50 yrs. of age. Most of the resistant patients are retreatment failure cases whereas, new smear positive failure patients also contribute to a significant disease burden. Also, HIV seropositive patients should be screened for drug resistance tuberculosis.

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