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Original research article

Genotyping of Multi Drug Resistant TuberculosisAmong the Smear Positive Pulmonary Tuberculosis Cases at a Tertiary Care Hospital

Dr. Gudesh Kumar[1], Dr. Sanjeev Kumar[2], Dr. Manish Kumar[3], Dr. Rakesh Kumar[4], Dr. Namrata Kumari[5]

[1]Junior Resident, Department of Microbiology, IGIMS Patna

[2]Junior Resident, Department of Microbiology, IGIMS Patna

[3]Junior Resident, Department of Microbiology, IGIMS Patna

[4]Additional Prof. Department of Microbiology, IGIMS Patna

Corresponding Author: Dr. Sanjeev Kumar

Abstract

Background: Mycobacterium tuberculosis was discovered by Robert Koch on March 24, 1882, and every year this day is remembered as the World Tuberculosis (TB) Day.

Objectives: To detect MDR-TB among new cases of smear positive pulmonarytuberculosis by Genotyping method.

Materials and methods: Prospective study was conducted in patients with sputum-positive pulmonary tuberculosis to detect resistance to Rifampicin and Isoniazid using Genotyping by Hain's technique at RNTCP in IGIMS, Patna , Bihar. 78 patients were study duration of eighteen months. The data was collected after obtaining permission from Institutional Ethical Committee,

Conclusion: LPA- Hain's technique identifies sensitivity to Rifampicin and Isoniazid in the clinical isolates and provides results in 2-3 days which in turn helps the patient to initiate early treatment and community at large to prevent the spread of MDR-TB.

Keywords: LPA-HAIN's technique; MDR-TB, Smear positive pulmonarytuberculosis.

Introduction

Mycobacterium tuberculosis was discovered by Robert Koch on March 24, 1882, and every year this day is remembered as the World Tuberculosis (TB) Day. Despite many new advents in TB management - diagnosis and treatment, TB remains as the major cause of morbidity and mortality worldwide for more than a century. Due to its high incidence rate TB still stands among the top 10 causes of death globally [1] As per World Health Organization (WHO) 2017 Global TB Report, a total of 10.4 million cases of TB and 600,000 cases of Multidrug-resistant (MDR) TB and rifampicin-resistant TB were reported. These seven countries accounted for 2/3rd ofTB burden in the world by 7 countries: India, Indonesia, China, Philippines, Pakistan, South Africa, and Nigeria.[2,3], The Revised National Tuberculosis Control Programme (RNTCP) Annual Report 2016 states that one-fourth of the global TB cases occur in India annually. Out of 10.4million global cases of TB, India accounts for 2.8 million TB cases (WHO Global TB Report 2017) and 4.8 lakh people had died due to TB.[4] Worldwide, 3.3% of new cases and 20% of previously treated cases were estimated tohave MDR-TB. According to „End TB Strategy", by 2020, there should be a 35% reduction in the number of TB deaths compared

with 2015percentage and 95% by the end of 2035. In India, the prevalence of MDR-TB was estimated to be about 2.2% in new cases and 15% in re-treatment cases.[5] MDR-TB represents the main burden/challenge and the major cause of death, and it symbolizes the antimicrobial resistance.[1] It accounts for 1.3 lakh cases in India.[4] It is a major menace and accounts for more than 50% of the annual budget of national TB programs.[6] The, End TB Strategy‟ of RNTCP‟s Vision 2020 is to reduce the TB Burden in India50% in incidence and 75% in TB related deaths by 2025.[7] For detection of resistance, Conventional Drug Susceptibility Testing (DST) by culture requires a minimum of eight weeks to obtain a result. However, molecular methods for identification and detection of drug resistance have reduced the turn over time to 48-72 hours. Two popular molecular methods include, Cartridge based nucleic acid amplification technique (CBNAAT) & Line probe assays (LPA) are recommended by WHO.[8,9] Cartridge based nucleic acid amplification technique (CBNAAT) detects only the presence of *Mycobacterium tuberculosis* and Rifampicin (RIF) resistance. LPA identifies Mycobacterium tuberculosis complex (MTBC), atypical mycobacteria,as well as sensitivity to RIF, high and low level sensitivities to Isoniazid (INH), in the clinical isolates simultaneously. It can be performed directly from smear-positive sputum or on culture isolates and provides results in 2-3 days.

Objectives

To detect MDR-TB among new cases of smear positive pulmonary tuberculosis by Genotyping method. determine the proportion of mono drug resistance and MDR among the study samples.

Review of Literature

TB is one of leading cause of death due to infectious disease next to Human Immunodeficiency Virus (HIV). Analysis reveals that globally one third of the world‟s population are infected with *Mycobacterium tuberculosis*. Among the infected individuals, about one-tenth of the patients develop active TB during their lifetime. MDR-TB represents one of the greatest hazards for TB control.[10]MDR-TB is associated with a 2-to 4-fold period of treatment, psychological problems, economic wastage, poor treatment adherence, and consequently treatment failure, psychological problems, economic wastage, poor treatment adherence, and consequently treatment failure.[11] It is essential to have an understanding on the factors responsible for the development of MDR-TB as this helps in formulating the effective control strategies for national programs.[12] Even clinical epidemiological data on risk factors of MDR-TB is also an important factor to be considered for risk factors of MDR-TB. Clinical studies at Ethiopia and Pakistan showed that there is 1.19 times more risk of developing MDR- TB in patients who are affected with TB and has a history of >2 times anti-TB treatment.[11] Resistance to one drug is not associated with resistance to an unrelated drug. A granuloma of macrophage generally contains 10^7 - 10^9 TB bacilli. Resistance to isoniazid occurs in about 1 in 10^6 replications of bacteria and to rifampicin occurs in about 1 in10^8 replications of bacteria. So, the probability of spontaneous mutations causing resistance to both isoniazid and rifampicin would be 10^6 x 10^8 = 1 in 10^{13}.The basic mechanism of MDR-TB is due to perturbations in the individual drug targetgenes.[14] Tan Y et al., conducted a multi center study of the diagnostic accuracy of the MTBDR plus 2.0 assay and compared with conventional and molecular reference standard in 4 TB specialized centres of China. In smear-positive/culture-positive cases, the sensitivity was 97.7% and 86.7% in smear-negative/culture-positive cases. The agreement rate between MTBDR plus 2.0 and Xpert MTB/RIF was 97.7% for smear- positive cases and 97.0% for smear-negative cases. As compared with phenotypic DST, the MTBDR plus 2.0 accurately detected 94.6% of patients with rifampicin- resistance. MTBDR plus 2.0 assay was considered to be rapid, accurate and increases sensitivity for detecting smear-

negative TB patients as well as an alternative fordetecting both rifampicin and isoniazid resistance in persons with presumptive TB.[15] Addo KK et al., conducted a prevalence survey which aimed to evaluate the use of LPA to differentiate mycobacterial isolates (n=361) obtained from TB prevalence survey in Ghana and to determine their drug resistance patterns. In the study, 45.7% MTBC and 33.2% non-tuberculosis Mycobacterium were identified to the species levels while 21.1% could not be completely identified. The MTBC comprised 97.6% M. tuberculosis and 2.4% *M. africanum*. Isoniazid and rifampicin monoresistant MTBC isolates were 10.9% and 1.2% respectively while 6.7% were resistant to both drugs. Study showed that resistance against isoniazid and rifampicin are commonly associated with mutations in the katG (Ser315Thr) and rpoB (Asp516Val) respectively.[16]

Materials and methods

Prospective study was conducted in patients with sputum-positive pulmonary tuberculosis to detect resistance to Rifampicin and Isoniazid using Genotyping by Hain's technique at RNTCP in Patna , Bihar. 78 patients were Study I Indira Gandhi institute of medical sciences, patna, Bihar. study duration of eighteen months. The data was collected after obtaining permission from Institutional Ethical Committee,

Inclusion criteria

Sputum sample of patients who have smear positive for acid fast bacilli diagnosed for the first time, Sputum sample of patients in high-risk categories including treatment failure, defaulters, relapse cases and delayed converters and who are sputum smear positive for acid fast bacilli

Exclusion criteria

Extra pulmonary tuberculosis cases, Patients with sputum smear negative for acid fast bacilli
After obtaining permission from Institutional Ethical Committee, written informed consents (in their local language) were taken from participants, and for children (<18 years) consents were obtained by their parent/guardian.
Specimen collection:
Specimens: Sputum, Bronchoalveolar Lavage (BAL) fluid
Gastric lavage in children
Collected in sterile containers
Method: Genotyping by Hain‟s Technique It is a qualitative in vitro test for identification of Mycobacterium tuberculosis complex and it resistance to rifampicin (RIF) and/or isoniazid (INH) from pulmonarysmear-positive or negative clinical specimens and cultivated samples.M. tuberculosis, M. africanum, M.bovis subsp. bovis, M.bovis subsp. caprae, M.bov is BCG, M. microti, M. canettii and M. pinnipedii. **Wild type probes** the wild type probes comprise the most important regions of the respective genes. When all wild type probes of a gene stain positive, there is no detectable mutation. This indicates that the test strain tested is sensitive for therespective antibiotic. In case of a mutation, the respective amplicon cannot bind to thecorresponding wild type probes. The absence of a signal for at least one of the wild type probes indicates a resistance to that antibiotic. (CHECK). Each pattern deviating from the wild type pattern indicates a resistance of the tested strain. The banding pattern obtained with the rpo B probes concludes about an RMPresistance the kat G and the inhA banding pattern indicates about an INH resistance.
The data was collected Ethical clearance was obtained from IGIMS, Patna. Samples were collected as a part of routine diagnostic workup and patient management. No animal experiments were required for the study.

Results

The present study was a prospective study carried out in patients with sputum-positive pulmonary tuberculosis to detect resistance to Rifampicin and Isoniazid using Genotyping by Hain‟s technique. Study performed on 40 samples; mutations in the rpoB gene for RIF and katG and inhA genes for INH were analysed. Study aimed to determine the proportion of mono drug resistance and Multidrug Resistance (MDR) among the study samples. Analyses were done at Department of Microbiology, Indira Gandhi Institute of medical sciences Patna, Biahr. Only patients fulfilling the inclusion criteria a total of 40 patients were studied.

Data Analysis

Seventy-eight patients who attended DMC of RNTCP at Patna, Bihar. were included in the study, Out of these 78 patients, 35 patients. Of the 40 subjects in the study, 20 (50%) were unemployed and 20 (50%) wereemployed. Employment status does not have any effect on the incidence of TB. The mean monthly income of the subjects were Rs, 7685 (SD=375.2). In the study, 7 (17.5%) subjects were above the poverty line and 33 (82.5%) were living below the poverty line.. This incidence elucidates that, low income groups are having more proportion of TB than high income groups. Twenty three (57.5%) subjects were smokers and 17 (42.5%) were non-smokers. Of the 40 subjects, 16 (40%) subjects had a previous exposure to patients with pulmonarytuberculosis and the other 24 (60%) subjects had no such previous exposure. All patients were on DOTS category I regimen for pulmonary tuberculosis. Out of the 40 study subjects, 16 (40%) used chewable tobacco and 24 (60%) did not have any habit of tobacco use. Alcohol use was present in 15 (37.5%) subjects, and 25(62.5%) subjects were non-alcoholic. In the study population, 21 (52.5%) subjects were diabetic and 19 (47.5%) were non-diabetic. Among the study subjects, 4 (10%) were HIV positive and 36 (90%) were HIV negative. Out of the 40 study subjects, 23 (57.5%) suffered from Chronic Obstructive Pulmonary Disease (COPD) and the rest 17 (42.5%) did not have COPD. Of the 40 study subjects, 24 (60%) had a history of asthma and the rest 16 (40%) had no history of asthma. Among the 40 subjects, 20 (50%) were vegetarians and the rest 20 (50%) were non-vegetarians Mutations in the *rpoB* gene for RIF and *katG* and *inhA* genes for INH were seen by Line Probe Assay (LPA), that is Genotyping by Hain‟s technique are seen in 16 (40%) subjects meaning positive for resistance to both Rifampicin and Isoniazidwhile in 24 (60%) subjects were sensitive both

Table 1: Drug Resistance Status

Variables	N (%)
Mutation(*rpoB* gene and *katG* *and inhA gene*) Present	16 (40%)
Mutation(*rpoB* gene and *katG* *and inhA gene*)Absent	24 (60%)

Of the 40 study subjects, 16 (40%) were MDR positive for both Rifampicin andIsoniazid, while 24 (60%) were sensitive to both Rifampicin and Isoniazid. When we look at the occupational status, 12.5% were unemployed MDR positive patients, 27.5% were employed MDR positive patients, 37.5% were unemployedMDR negative patients and 22.5% were employed MDR negative patients. Comparing economic status and MDR, 2.5% were MDR positive patients abovepoverty line, 37.5% were MDR positive patients below the poverty line, 15% were MDR negative patients above poverty line and 45% were MDR negative patients below poverty line. Comparing total income status and MDR status, most of the patients who were MDR positive had low mean income of Rs. 6962 (2543.7) than the patients who were MDR negative which was Rs. 8166 (4356.0). Our study also compared the concurrent medication usage status and the MDR status. 12.5% were MDR positive patients using

medication for COPD, 7.5% for asthma, and15% were using medications for both COPD and asthma, but 5% were with none of the medications. Similarly 15% were MDR negative patients using medications for COPD, 22.5% for asthma, and 15% were using medications for both COPD and asthma, but 7.5% were with none of the medication. Comparison of defaulter status of the study population to the MDR status , 20% were MDR positive patients among defaulters and non-defaulters, 17.5% were MDR negative among defaulters and 42.5% were MDR negative patients who were non- defaulters.

Discussion

In this study, 40 patients with smear positive pulmonary tuberculosis patients were included. Patients were assessed for MDR-TB among new cases of smear-positive pulmonary tuberculosis by Genotyping method. Study also determined the proportion of mono drug resistance and MDR among the study samples, The present study estimated the burden of MDR- TB based on the incidence and prevalence. In this study, we have determined the prevalence of TB and its association with sex, age, type of treatment, HIV status, COPD, asthma status and exposure to TB history of the participants. Maru M et al., conducted a cross-sectional study on samples of a smear-positive newly diagnosed and retreatment pulmonary TBpatients. In the study, a total of 144 smear-positive TB cases were studied. Out of 144 cases, 44.4% (n=64) were females and 55.6% (n=80) were males. The median age ofthe patients was 27.5 years (range 10–78 years). In our study, a total of 65% weremales and the mean age in the study was 40.96 (14.42) years. The study by Maru Met al., and our study concluded that TB incidence were more in males than females.[18] Sharma SK, et al conducted a prospective, observational study in newly diagnosedcases of sputum-positive pulmonary TB cases in India. Of a total 218 sputum-positivepulmonary tuberculosis cases, the mean age of the patients was 27.8 ± 10.2 years. Atotal of 27% (n=59) patients were females and 73% were males. Even the study bySharma SK et al. was consistent with our study. [19] showed that highest frequency of MDR-TB infection was found among the age group 31–45 years. Same study showed that females were more affected than males, 7 (63.6%) and 4 (36.4%), respectively.[17] The study by Workicho A et al., revealed that subjects with HIV infection were three times at higher risk of development of MDR-TB than those patients without HIV infection. This association has a marginal statistical significance showing that HIV infection is not a strong predictor of MDR- TB infection in TB patients.[20] In our study, among the 16 MDR-TB cases, 50% were defaulters to treatment and the other 50% were non-defaulters to treatment. Eldirdery MM et al., study showed 9.09% cases defaulters to treatment.[17] This was not consistent with our study.

In the present study with 16 MDR-TB cases, 56.25% were with previous exposurestoTB patients and the other 43.75% were not previously exposed to TB patients. In the cross-sectional study by Eldirdery MM et al., 63.6% were previously exposed to TB patients and the rest 36.4% were not previously exposed to TB patients. This was absolutely similar to our study with more number of cases previously exposed to TB patients. For an effective and successful treatment, there is a need for the accurate and efficientTB detection methods. This further decreases the morbidity, complications, unnecessary treatment and isolated false positive cases.[21] The major limitation of the present study and as that of seen in most of the studies of MDR-TB is the small sample size, and therefore, it is not representative of thepopulation at large.
methods

Conclusion

TB detection which in turn decreases the morbidity, complications, unnecessary treatment and isolated false positive cases. LPA identifies Mycobacterium tuberculosis complex (MTBC), atypical mycobacteria, as well as sensitivity to Rifampicin (RIF), high and low level sensitivities to Isoniazid (INH) and also second line antiTB drugs in the clinical isolates simultaneously. It can be performed directly from smear-positive sputum oron culture isolates and provides results in 2-3 days. One of the major pitfalls was that among the newly emerging MDR-TB cases, only 3% get serious treatment globally.

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