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Original Research Article

Prevalence of primary drug resistance to rifampicin and isoniazid in newly diagnosed sputum smear positive pulmonary Tuberculosis

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ABSTRACT

Background: To determine the prevalence of primary drug resistance to either rifampicin or isoniazid alone or both in newly diagnosed sputum smear positive pulmonary tuberculosis patients.
Method: A prospective study 100 newly diagnosed sputum smear positive pulmonary TB patients was conducted. The patients with an age of ≥15 years and who had either not taken anti TB treatment or who had taken ATT for less than 1 month were enrolled in this study. Two sputum samples (5ml each), including one early morning sample as per the RNTCP guidelines were collected and subjected to line probe assay (LPA).
Results: Out of 100 cases 6 were having resistance to both rifampicin and isoniazid, 9 has resistance to INH alone and 1 had resistance to rifampicin alone.
Conclusion: The prevalence of primary drug resistance is high. For early and rapid detection of DR-TB newer modality should be used for the detection of primary drug resistance in sputum smear positive TB patients.

Keywords: Multi-drug-resistant tuberculosis, Prevalence, Resistance

INTRODUCTION

Tuberculosis remains a major global health problem affecting millions of people every year. In 2015, there were an estimated 10.4 million new (incident) TB cases worldwide, of which 5.9 million (56%) were among men, 3.5 million (34%) among women and 1.0 million (10%) among children. People living with HIV accounted for 1.2 million (11%) of all new TB cases. Six countries accounted for 60% of the new cases: India, Indonesia, China, Nigeria, Pakistan and South Africa.[1]

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 both TB and MDR TB based on estimates reported in Global TB Report 2016.[2]

The emergence and spread of drug resistant TB are threatening to destabilize global TB control.[3] One of the major contributors to TB related death is drug resistance. WHO recently described the global effects of multi drug resistance TB (MDR TB, resistance to Rifampicin and Isoniazid) as a public health crisis. Furthermore world is witnessing the emergence of extensively drug resistant TB (XDR TB) classed as resistance to Rifampicin, Isoniazid, one Flouroquinolone and one second line injectable. The prevalence of MDR TB is increasing throughout the world both among new TB cases as well as previously treated ones.[4]

The acquisition or emergence of drug resistance may occur from previous exposures to Quinolones, use of inferior regimens, poor adherence to anti- TB drug, previous TB treatment and high human immunodeficiency virus (HIV) co-infection. Although previous treatment for TB is the strongest risk factor for development of drug resistant TB, new patients are also at risk due to spontaneous mutation or transmission of resistant strains.[4-7]

When drug resistance is demonstrated in a patient who has never received anti TB treatment previously, it is termed primary resistance. Acquired resistance is that which occurs as a result of specific previous treatment. The level of primary resistance in the community is considered to reflect the efficacy of control measures in the past, while the level of acquired resistance is a measure of on-going TB control measures.

Isoniazid (INH) is a first-line anti-tuberculosis antibiotic used for treatment of tuberculosis since its introduction in 1952. Over the time mycobacterium TB strains have developed resistance to almost all antibiotics, however, resistant frequency to INH is higher than other drugs. INH resistance develops as a result of gene mutations involving catalase-peroxidase gene (katG), inhibin alpha (inhA), kasA, ahpC and ndh genes.

Rifampicin resistance is growing, largely due to particular genomic mutations in the rpoB gene of Mycobacterium tuberculosis. The rpoB gene encodes the β subunit of RNA polymerase, which is involved in chain initiation and elongation.

The diagnosis of drug resistance require patients to be tested for drug susceptibility to anti TB drugs either by conventional (phenotypic) drug susceptibility testing (DST) or rapid molecular diagnostic test (genotypic method).

Currently, one of the best ways to detect TB as well as multi-drug resistant TB is through a platform called Line probe assay (LPA). Rather than relying on visualizing bacteria under a microscope (as done in acid-fast smear), LPA indirectly detects presence of Mycobacterium tuberculosis by amplifying DNA present in the sputum by polymerase chain reaction (PCR). Two commercial assay are available INNO- Lipa test a geno type MTB complex assay use for rpoB gene mutation for Rifampicin. InhA and KatG mutation for Isoniazid, these also can be used for disease differentiation form Mycobacterium other than Mycobacterium tuberculosis (MOTT). LPA are highly sensitive (≥97%) and specific (≥99%) for the detection of Rifampicin resistance, alone or in combination with Isoniazid (sensitivity ≥90%; specificity ≥99%), on isolates of MTB and on smear-positive sputum specimens. WHO annual report 2017 shows estimated percentage of TB cases in India with MDR/RR-TB among new cases and among previously treated cases are 2.5%(2.1-3.1) and 16%(14-18). Studies from different parts of India show various results. Information regarding primary drug resistance TB in Punjab is limited. So we conducted this study to predict the magnitude of prevalence of primary drug resistance to RIF,INH or both, in patients attending our department.

METHODS

100 newly diagnosed sputum smear positive patients for acid fast bacilli (AFB) who presented in our institute over one year were enrolled in this prospective study. This study was conducted after obtaining the ethical approval by the Institutional Review Board and ethics committee. A detailed history was elicited regarding, previous anti tubercular treatment, smoking, any MDR tuberculosis in the family and co-morbidities. Patients who were newly diagnosed, were more than 15 years of age and who had either not taken ATT or who had taken anti tubercular treatment for less than one month were enrolled. Patients who had previously taken anti tubercular treatment more than one month, extra pulmonary TB, sputum smear negative and patients less than 15 years of age were excluded from the study.

Two sputum samples (5 ml each), including one early morning sample, were collected in Falcon tubes as per the Revised National Tuberculosis control programme (RNTCP) guidelines. The samples were sent to the integrated reference laboratory (IRL) situated in our hospital and were subjected to line probe assay (LPA). LPAs are WHO-approved tests for rapid detection of drug resistance to first- and second-line agents. They are a family of DNA strip-based tests that determine the drug resistance profile of a MTBC strain. They can be used for testing of culture isolates (indirect testing), as well as direct testing of acid fast bacilli (AFB) smear microscopy positive specimens.

Routine investigations including haematology (complete blood count), chest X ray, blood sugar level and HIV were done. The data was analyzed with SPSS software and then evaluated statistically.

RESULTS

All the 100 patients enrolled in the study were sputum smear positive for AFB. The most frequent age group in the study was 21-30 and 41-50 years (21%), mean age was 43.6±17.6 years. Majority of patients were males (62%). The main complaints were dry cough (72%), fever (66%), breathlessness (43%), followed by cough with expectoration, loss of appetite, loss of weight, hemoptysis, night sweats, chest pain and vomiting. As expected, most symptoms were mutually overlapping. Majority of patients (42%) weighed 41-50 kg with a mean weight of 46.2±9.7 kg. 19% patients were diabetic and 38% were smokers. Household contacts of MDR-TB were 3% and HIV reactive patients were 5%. 49% patients had sputum grading 3+, followed by 2+(23%), 1+(21%) and scanty (7%).

Table 1: Prevalence of drug resistance.

Drug	Resistance rate (%)
Isoniazid monoresistance	9%
Rifampicin monoresistance	1%
Isoniazid and Rifampicin	6%

It was found that 16% were resistant to at least one drug. Isoniazid resistance was present in 15% with INH mono-resistance of 9%.

Total RIF resistance was 7% with RIF mono-resistance of 1% and MDR-TB 6%. Resistance to both INH and RIF (MDR-TB) was 6% (Table 1).

Table 2: Prevalence of rifampicin resistance among HIV reactive patients.

HIV	Rifampicin sensitive		Rifampicin resistance		p value	Sig.
	No. of Patients	%Age	No. of Patients	%Age		
Reactive	3	3.2%	2	28.6%		
Non- reactive	90	96.8%	5	71.4%	0.038	S
Total	93	100%	7	100%		

This correlation between prevalence of isoniazid resistance among rifampicin resistance was found to be statistically significant by Fischer exact test.

There was a statistically significant relation between the prevalence of RIF resistance among HIV reactive patients ($p<0.038$) (Table 2).

DISCUSSION

All the 100 patients newly diagnosed sputum positive tuberculosis patients were enrolled for this prospective study to know the prevalence of prevalence of primary drug resistance to rifampicin and isoniazid. Authors used line probe assay to do this study as it has been shown to have be a robust technique for diagnosis of drug-resistant TB and it has also provided the basis for rapid and effective control of drug-resistant TB in India.[9] This test has also proved highly accurate in the rapid detection of R resistance in a multi-site validation in India though in a study from Punjab 2.7% of the specimens were found to be invalid.[10,11]

LPA was also associated with a major reduction in the delay between identification of patients suspected for MDR-TB and initiation of treatment, attributed mainly to a reduction in diagnostic time.[12] WHO policy statement also shows that LPA is not a replacement for conventional culture and drug sensitivity testing and these are still required for sputum negative specimens.[13] The mean age of the patients is 43.6±17.6 years and age range was 15-90 years. Majority of patients were presented with cough (96%), and fever (66%) which has been seen to be very common symptoms in patients with TB. Majority of the patients were non-smokers (62%). Diabetes was seen in 19% of the patients and 5% were HIV positive. Majority of patients were having 3+ grading of sputum (49%) followed by 2+(23%), 1+(21%) and scanty(7%).

In the present study any resistance to Isoniazid was 15%, Isoniazid mono resistance was 9%. In a similar study conducted, resistance to Isoniazid was 14%, Isoniazid mono resistance was 9.5%. In a study conducted any resistance to Isoniazid was 13.2%, Isoniazid mono resistance was 7.4%.

In the present study any resistance to Rifampicin was 7%, Rifampicin mono resistance was 1%. In a similar study conducted, any resistance to Rifampicin was 7.7%.

Out of 7 rifampicin resistant patients 28.6% were HIV reactive and 71.4% were HIV non-reactive. Out of 93 rifampicin sensitive patients 3.2% were HIV reactive and 96.8% were HIV non-reactive. This correlation between HIV and rifampicin resistance is statistically significant. In a similar study conducted, found that Rifampicin nonresistant's was associated with HIV. In a similar study conducted by, found statistically significant association between any drug resistance and HIV infection.

Authors found that prevalence of INH resistance among RIF resistant cases were statistically significant. So we can use RIF resistance as a surrogate marker for detection of MDR-TB. In a study conducted similar relation.

Prevalence of MDR-TB in the present study were 6%. In a similar study conducted, MDR were 4.2%. In a similar conducted, MDR TB were 7.2%. Both the molecular tests for the detection of TB have been endorsed by WHO but which is better has not been ascertained.[14,15]

CONCLUSION

The prevalence of primary drug resistance to Rifampicin, Isoniazid or both were high in the present study. The high prevalence of DR-TB in the small population studied, warrants accurate nation-wide multi-centric drug

resistance surveillance in the country. So rapid detection of DR-TB using WHO approved newer modality should be highly advocated in new and previously treated pulmonary tuberculosis patients.

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