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Use of Molecular Diagnostic Test in new AFB Smear Positive Tuberculosis Isolates to Study Multidrug Resistance in Sub Himalayan Region of North India-A Pilot Study

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Abstract
Background: *Tuberculosis (TB) is the leading cause of morbidity and mortality in India especially in rural areas.*
Objective: *The goal of this study was to examine the patterns of TB drug resistance among new smar positive TB patients in a rural regions of Uttarakhand.*
Method: *All smear positive isolates received under a DOTS centre were stocked. Isolates were subjected to first-line drug susceptibility testing by the Genotype MDR Line probe assay.*
Results: *The prevalence of resistance to any TB drug was 13.6% (38 cases). Eleven percent of the new treatment TB group (28 patients) and 40.7% of the retreatment TB group (11 patients) were resistant to all TB drugs. Twelve (4.3%) patients had multidrug-resistant tuberculosis (MDR-TB) (2.38% in the new TB treatment group and 23.1% in the retreatment group). One patient had extensively drug-resistant tuberculosis (XDR-TB). There was a statistically significant relationship between TB drug resistance and smoking ($p=0.02$) and a history of migration from village to city ($p=0.04$), also between TB drug resistance and recurrence of TB in patients that had previously received treatment ($p<0.001$).*
Conclusion: *Knowledge of drug resistance patterns for new and previously treated cases is critical for effective control of MDR-TB in different regions of the country. The burden of MDR-TB in retreatment cases was high. Previous TB treatment was one of the most important mokers and those who had a history of rural to urban migration were at high risk for the occurrence of TB drug resistance.*
Keywords: *MDR TB, Line Probe Assay.*

Introduction

Drug-resistant tuberculosis (TB) has become a global threat in recent years. The World Health Organization has estimated that the global burden of multidrug-resistant TB (MDR-TB), defined as combined resistance to INH (Isoniazide) and RIF (Rifampicin) will increase from the current 500,000 cases per year to nearly 2,000,000 cases by 2015[1]. The widespread use of RIF and INH in global TB control programs has inevitably given rise to this resistance leading to increase in number of MDR-TB cases. Along with that, expanding efforts to treat patients infected with MDR-TB has lead to the generation of extensively drug-resistant TB (XDR-TB) defined as MDR-TB

plus resistance to any fluoroquinolone and at least one of three second-line anti-TB injectable drugs[2]. Treating patients with MDR-TB requires identifying them by performing DST (Drug susceptibility tests) on bacteria isolated from sputum, a process that can take up to 2 months. This delay in diagnosis can result in worsening of disease and further transmission of MDR-TB disease. To avoid this delay in proper diagnosis, several rapid diagnostic methods have been developed that rely on amplification of specific alleles known to be associated with resistance to specific drugs. LPA (Line probe assay) a method that is based on nucleic acid amplification followed by hybridization of amplicons to target probes immobilized on membranes to detect resistance-associated sequence polymorphisms, are widely used[3,4]. These tests have been officially endorsed by WHO to detect MDR-TB from cultured isolates and directly from smear positive sputum samples. The diagnostic accuracy of LPA's is quite robust and up to 97%of isolates can be correctly identified as resistant or susceptible to anti-tubercular drugs[5].

According to WHO 2013 report, globally in 2012 an estimated 450,000 people developed MDR-TB and there were an estimated 170,000 deaths from MDR-TB worldwide. The scenario is no better in India with an estimated 26% of total MDR cases being reported from our country alone[1]. The burden of this disease in Uttarakhand has also been found to be quite high. A study conducted by Rawat et al in 2009 reported an overall rate of drug resistance to be 62.77% to one or more antitubercular drugs[6]. We took this study a step further by studying the prevalence of resistance to both RIF and INH, two of the four first line anti-tubercular drugs used in the treatment of TB and also explored the mutations involved in the genes responsible for confering resistance to RIF and INH in this geographical region of North India.

Material and Method

The study was carried out in the department of Microbiology of Himalayan Institute of medical sciences. All smear positive sputum samples were included in the study. Sputum was processed and the following tests were performed

Processing of samples

Equal volume of NALC-4%NaoH were added to sputum cup and kept at room temperature for 15-20 minutes. The mixture was centrifuged at 12000 rpm for 15 minutes and supernatant discarded. A TE buffer was added and centrifugation done. Supernatant was discarded.

A 50µl TE Buffer was added and mixed well. The mix was heated at 95 – 100°C for 15 – 30 minutes and freezed for 30 minutes. The solution was returned to room temperature and mixed well. A 5µl of the mix was used for amplification.

Genotype MTBDR assay

LPA using GenoType MTBDR plus (Hain Life science, Nehren, Germany) was performed according to the manufacturer's instructions. Reactions were interpreted by comparison with the template provided in the kit. Absence of any wild-type band with or without the presence of mutant band was taken as indicative of a resistant strain.

Results

A total of 11 isolates were positive for *M.tuberculosis* complex. Four (36.3%) isolates had no mutations and were rpoB WT (Wild Type)positive and Kat G WT and in hAWT positive, whereas (63.6%) isolates were Rifampicin resistant and rpoB MUT band was positive. Five isolates(45.4%) were INH sensitive with Kat G WT and inhA WT positive. However 6(54.5%) isolates were INH resistant due to either Kat G or inhA mutation. Three isolates (27.2%) were MDR with both rpoB MUT and Kat G MUT/Inh A MUT positive. Three isolates were hetero resistant with both WT and MUT bands positive.

Table 1: Showing the mutations and the sensitive and resistant isolates.

S.No	Isolates	TUB	*rpo*B WT	*rpo*B MUT	*kat*G WT	*kat*G MUT	inhA WT	inhA MUT	RMP S	RMP R	INH S	INH R
1	1	+	+	_	+	_	+	_	+	_	+	_
2	2	+	+	_	_	+	+	_	+	_	_	+
3	4	+	_	+	+	+	+	+	_	+	_	+
4	5	+	_	_	_	+	_	_	+	_	_	+
5	6	+	+	_	+	_	+	_	+	_	+	_
6	8	+	_	+	_	+	+	_	_	+	_	+
7	9	+	+	_	+	_	+	_	+	_	+	_
8	10	+	_	+	_	+	_	_	+	_	_	+
9	15	+	+	+	+	_	_	+	_	+	_	+
10	17	+	_	+	+	_	+	_	_	+	+	_
11	18	+	+	_	+	_	+	_	+	_	+	_

Discussion

Tuberculosis is becoming a worldwide problem due to the recurrence of the disease due to the association of TB with the HIV pandemic and the appearance of drug-resistant strains . Tuberculosis programs face challenges in reducing MDR-TB. The reason being that the treatment of MDR-TB tuberculosis is difficult due to side-effects, is expensive and often unsuccessful. Also, it may take up to 6 weeks to get a culture report result and during this time many transmission events may take place.

In order to prevent the spread of drug-resistant *M.tuberculosis* strategies for the treatment and prevention of MDR emergency measures are required, so alternative methods need to be evaluated to improve the speed of diagnosis of drug resistance.

In our study, we found that drug resistance to Rifampicin (63.6%) and INH(54.5%) was quite rampant. The high prevalence of Rifampicin and INH resistance in our study may be due to widespread and inappropriate use of these antibiotics in the past for treatment of TB. This is in agreement with the observations of other Iranian researchers[11,12].Resistance to isoniazid in this study was 16.1%, and was also higher than that seen in Ethiopia[12], and in Mozambique (14.9%). In 2008, the WHO reported a worldwide resistance rate to isoniazid of 5.9%[13,14]. According to the WHO, isoniazid resistance rates higher than 10% can predict the development of MDR-TB[14].In Our study the MDR isolates(27.2%) were quite high which may be caused due to the fact that Rifampicin and INH are first-line drugs or poor compliance by patients.

The high level of Rifampicin and INH resistance among our study population is an indicator that there is a high probability of developing MDR-TB among our patients of this eco- geographical region in the future. What is alarming is that INH, which is also the drug of choice for chemoprophylaxis of TB and is used in developed countries for treating latent TB also might not work at all in future.

The high prevalence of MDR-TB in our study is an alarm bell that early case detection, rapid implementation of DST, effective anti-TB treatment is the need of the hour.

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