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STUDY OF MDR AND XDR TB IN PATIENTS DIAGNOSED WITH PULMONARY TUBERCULOSIS: A HOSPITAL BASED STUDY, SIKKIM

Microbiology

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ABSTRACT

Background: The increasing number of tuberculosis cases and an alarming rate of MDR cases in the state of Sikkim has raised concerns calling for an effective awareness generation among the masses regarding MDR and XDR TB and for innovative ideas and dedicated service to tackle such challenges. Hence, the researcher aims to study MDR and XDR TB cases among the newly diagnosed cases of pulmonary tuberculosis.

Methods: A hospital based cross-sectional study was conducted from January 2016-May 2017 among 300 new clinical suspects of PTB. For all the clinical suspects, two sputum samples were collected and subjected to Auramine-o staining. Further, the samples diagnosed with tuberculosis were subjected to detection of MDR and XDR TB by real time PCR. We performed all the analysis using SPSS software (Statistical package, SPSS Inc., Chicago, IL, USA, version 13.0).

Result: A total of 300 clinical suspects enrolled for the study, of which 82(27.33%) were diagnosed of pulmonary tuberculosis by fluorescence microscopy. On further diagnosis for MDR and XDR TB cases; 1(7%) was mono resistant to rifampicin, 13(93%) showed point mutations in genes responsible for drug resistance to one or more drugs, 2(14%) cases showed resistance to both *katG* and *inhA* for isoniazid and 1(7%) case showed resistance to *katG* gene. All the diagnosed cases were resistant to *rpoB* gene for rifampicin resistance; further, only one case was found to be resistant to *gyrA* and *eia-10* genes, thus determined to be an XDR TB case. Socio demographically, MDR TB cases were predominant in male and it was determined that the house-hold status, living condition, cross ventilation, overcrowding, history of contact were significantly correlated.

Conclusion: Detecting MDR and XDR TB in the initial stage helps in initiating the treatment quickly and effectively, further providing the benefit of better management of MDR and XDR TB cases in the diagnosed patients. The study also provides information on the socio demographic elements determining the burden of MDR and XDR TB among the suspected patients of Sikkim.

KEYWORDS

Tuberculosis, Fluorescence microscopy, Drug resistance.

INTRODUCTION:

Drug resistance in MTB occurs by random, single step spontaneous mutation at a low but predictable frequency, in large bacterial population. [1] From a microbiological perspective, the resistance is caused by genetic mutation that makes a drug ineffective against the mutant bacilli**.** [1] An inadequate or poorly administered treatment regimen allows drug resistant mutants to become the dominant strain in a patient infected with MTB. Factors like poor case detection, inadequate/irregular treatment, use of anti-TB drugs for indications other than TB, laboratory delays in identification and susceptibility testing of MTB, massive bacillary load in patients, non-compliance, illiteracy and low socio-economic status of patients play an important role in the emergence of drug resistance (DR). [2] The conventional methods for diagnosis are time-consuming and are of low sensitivity. Early diagnosis, besides effective treatment of infected cases can help control the transmission of this devastating disease. The probability of incidence of drug resistant mutants is 10^{-8} for rifampicin, while for isoniazid and some of the other commonly used drug it is 10^{-6}. [3]

These consequences thus, make it very clear to detect, diagnose and treat MDR and XDR TB in the first place; fast, efficiently and directly from the obtained sample. Molecular methods are genetic procedures that make use of the genetic material (DNA/RNA) to detect specific proteins or genes of the organism using specific probes or short stranded oligonucleotides (primers) complementary to the test DNA strand. [3] These molecular methods are not only useful in conformation of identity of the isolates, direct detection of gene sequence from the clinical specimen but also for molecular detection of drug resistance. [4] Real time PCR helps in early detection of minimal load of bacteria in the sample when amplified with the target region of MTB complex. HELINI™ MTB-MDR-XDR detection kit uses the specific gene probes; *rpoB* (18 mutations), *katG* (4 mutations), *inhA* (3 mutations) for the detection of MDR TB and *gyrA* (7 mutations), *rrs* (3 mutations) and *eia* promoter (2 mutations) for the detection of XDR TB.

METHODS:

Study setting and study population:

A hospital based cross-sectional study was performed in a tertiary care central referral hospital, Sikkim from 2015 to 2017. The institutional ethical clearance was obtained prior to the study. Only new clinically suspected cases of PTB, not on any anti-TB drug regimen and providing consent were included in the study.

Collection of data and interview: Personal interview was conducted with each of the suspects and in the case of child and aged patients with their parents/guardian; with a pre-designed questionnaire comprising of a set of 11 questions with multiple choice answers. We performed all the analysis using SPSS software (Statistical package, SPSS Inc., Chicago, IL, USA, version 13.0).

Sputum smear microscopy:

Two sputum samples; one spot and one early were collected in a sterile, leak proof 5-10ml container and were transported to the Microbiology Tuberculosis laboratory. At the laboratory Auramine-O staining was performed and the slides were examined under a fluorescence microscope to identify sputum positive and sputum negative samples.

Real time PCR.

All the collected sputum samples were processed by N-acetyl L-cysteine- sodium hydroxide (NALC-NaOH) method. [5] After decontamination the samples were re-suspended in sterile phosphate buffer solution (pH 6.8). Extraction was carried out using HELINI™ PureFast Bacterial Genomic DNA Minispin prep Kit (HELINI Biomolecules, Chennai) (Cat. No. 200218-100 purifications) as per the user guidelines. The eluted purified DNA was then stored at -20 with the volume adjusted to 100µl. For detection HELINI™ MTB-MDR-XDR Real time PCR kit (HELINI Biomolecules, Chennai) (Cat. No. 8344-50 purifications) was used and the samples were processed as per the instructions provided in the user's manual. As stated in the user guidelines CT values between 24-34 were interpreted within the normal range and was considered a positive result considering the extraction efficiency, the quality of elite added to the PCR reaction and the individuals machine settings.

RESULT:

Study population: A total of 300 patients fulfilling the inclusion criteria were included in the study. Amongst the total study population 82(27.33%) cases were diagnosed of PTB.

TABLE 1: STUDY OF MDR AND XDR TB IN SAMPLES DIAGNOSED WITH PULMONARY TUBERCULOSIS (N=82)

N	No. of MDR TB Positive Cases	% of MDR TB Positive Cases with Respect to (N)	No.of XDR TB Positive Cases	% of XDR TB Positive Cases with Respect to (N)
Male Patients (40)	9	22.50	0	0
Female Patients (42)	5	11.90	1	2.38
Total No. of Cases (82)	14	17.07	1	1.20
Sample Size (300)	14	4.67	1	0.33

Diagnostic outcome: Of the total diagnosed cases 14(22%) were MDR TB and 1(2%) case was XDR TB, among which 1(7%) was mono resistant to rifampicin, 13(93%) showed point mutations in genes responsible for drug resistance to one or more drugs, 2(14%) cases showed resistance to both *katG* and *inhA* for isoniazid and 1(7%) case showed resistance only to *katG* gene. All the 14 cases were resistant to *rpoB* gene for rifampicin resistance. Only one case was found to be XDR TB and was resistant to *gyrA* and *eia-10* genes.

TABLE 2: DRUG SENSITIVITY PROFILING IN MDR AND XDR TB PATIENTS

	Rif-R	INH-R	INH-R	FQ-R	INJECTABLE DRUGS-R	INJECTABLE DRUGS-R	INJECTABLE DRUGS-R
	rpo B	*kat G*	*inh A*	*gyr A*	*rss*	*eia-10*	*eia-37*
PATIENT 1	*		*				
PATIENT 2	*	*	*				
PATIENT 3	*		*				
PATIENT 4	*		*	**		**	
PATIENT 5	*		*				
PATIENT 6	*		*				
PATIENT 7	*		*				
PATIENT 8	*						
PATIENT 9	*		*				
PATIENT 10	*		*				
PATIENT 11	*		*				
PATIENT 12	*	*					
PATIENT 13	*		*				
PATIENT 14	*		*				
PATIENT 15	*	*	*				

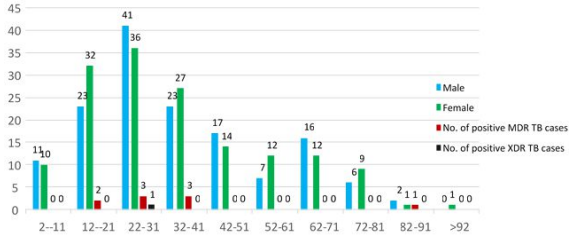
The diagnosed MDR and XDR TB were found to be present only amongst the sputum smear positive cases with the dominance of MDR and XDR TB in +3 AFB graded patients.

TABLE 3: RELATION BETWEEN OCCURRENCE OF MDR AND XDR-TB USING SMEAR GRADING

AFB GRADING (N=82)	MDR TB	XDR TB
+1 (N=42)	7 (16.67%)	0
+2 (N=19)	1 (5.26%)	0
+3 (N=20)	6 (30%)	1 (5%)

MDR cases were found to be persistent in male (60%) than the female (40%) population and an XDR TB case was found in a female (Table1). The age group of 22-41 showed maximum (60%) MDR and XDR TB cases, which is the most productive age groups. Thus, compromising the economical as well as personal growth of an individual and state.

TABLE 4: AGE AND GENDER DISTRIBUTION OF MDR AND XDR TB CASES(N=82).



Further analysis based on the socio demographic profiling of the MDR and XDR TB patients revealed the prevalence to be dominant among the Nepalese (12(80%)MDR cases), people residing in an urban setting (12(80%)MDR cases), poor or no cross ventilation, kutcha house (11(73%)MDR cases), undernourished (10(67%)MDR cases), overcrowding present (13(87%) MDR cases) and in patients with history of contact (12(80%) MDR cases) with p-value<0.05.

TABLE 5: SOCIO DEMOGRAPHIC PROFILING

CATEGORY	CHARACTERISTICS	MDR POSITIVE (N=14)	XDR POSITIVE (N=1)	P-VALUE
MARITAL STATUS	SINGLE	07	00	0.22
	MARRIED	01	01	
	DIVORCED	01	00	
	SEPERATED	01	00	
	WIDOW	03	00	
	WIDOWER	01	00	
RACES	NEPALI	12	00	0.03*
	BHUTIA	01	01	
	BENGALI	00	00	
	MARWARI	00	00	
	BEHARI	00	00	
	MUSLIM	01	00	
	OTHER	00	00	
RESIDENTIAL SETTING	URBAN	12	00	0.04*
	RURAL	02	01	
TYPE OF HOUSE	KUCCHA	11	00	0.03*
	SEMI PUCCA	01	01	
	PUCCA	02	00	
CROSS VENTILATION	YES	02	01	0.04*
	NO	12	00	
NUTRITIONAL STATUS	UNDERNOURISHED	10	00	0.03*
	OBESE	01	01	
	NORMAL	03	00	
TYPE OF FAMILY	NUCLEAR	08	00	0.11
	JOINT	02	01	
	EXTENDED	04	00	
OVERCROWDING	ABSENT	01	01	0.01*
	PRESENT	13	00	
LIVING STATUS	ALONE	06	00	0.27
	WITH FAMILY	08	01	
EDUCATION	ILLITRATE	04	00	0.53
	LITERATE	10	01	
H/0 TB IN FAMILY	YES	12	01	0.04*
	NO	02	00	

DISCUSSION:

Presence of MDR TB gene in the non-responders is a serious challenge not only from the public health point of view but also in context of its economic burden, especially in the absence of diagnostic and treatment facilities for MDR TB at national level programmes in most of the countries. From the patients point of view, presence of MDR TB prolongs the treatment duration from the standard 6-9 months to 18-24 months; with a possible chance of developing XDR TB in the process. The second line drugs used in the treatment of MDR TB cases have serious side effects that may need hospitalization. These drugs are 50-200 times more expensive than the first line ATT drugs.[6] Furthermore, patients with MDR TB have lower cure rates and high mortality than patients with drug susceptible TB.[7] It is known that some strains of MDR TB e.g. Beijing Strain are more infectious and is likely to cause large outbreaks of TB.[8],[9] Patients infected with MDR TB continue to have active TB despite optimal treatment and may even die.[3]

As reported in the Hindu; 4th July 2016, in Sikkim 11% of the new tuberculosis cases were MDR TB.[10] This study revealed a total of 27.34% cases amongst the clinical suspects diagnosed with TB, indicating 1/4th of the new suspected cases suffering from the disease, with 17% MDR and 1% XDR TB case, thus implying a load that cannot be overlooked. Detection of MDR and XDR TB directly from the sputum sample along with the determination of the specific gene causing resistance in the individual at focus, helps in understanding the nature and the type of resistance in each patient. Thus, helps in better treatment and prognosis.

One of the study reported that the majority of the MDR-TB cases (30.23%) were in the younger age group (21-30 years); mean age was 32.52 years. In the same study it was also found that majority of the cases were male (61.62%).[11] Other researchers have also reported the male predominance among MDR TB cases.[12],[13] One of the study done in a TB unit in Mumbai, also reported that the majority of the cases (67.6%) were in the age group 15-35 years with a mean age of 31years.[14]- [16] Majority of our cases were male (61.62%). Male predominance among MDR TB cases has been also reported by other authors.[17], [18] Our study also revealed the prevalence of MDR TB to be dominant in the age group of 21-41, and to be maximum in the male population. Undernutrition among MDR-TB cases was also reported from another study done in a tertiary care setting in New Delhi.[11],[16] Undernutrition was one of the determining features of MDR TB in our study also.

In our study we also determined the prevalence of MDR and XDR TB to be dominant among patients residing in an urban setting with no cross ventilation and overcrowding present. It was also determined that the high occurrence of MDR and XDR TB to be associated with history contact and undernourishment and in the Nepalese race. Studies have shown that people living in the same house hold as that of the TB patient have a high risk of becoming infected and developing TB within themselves, particularly if their immune defences are at all impaired.[19] Our study the prevalence of XDR TB to be 1.20%. Some recently published reports from various other parts of India has reported the prevalence of XDR TB to be 2-3% in their study population. [11], [15], [20], [21] However some other Indian authors have reported a higher prevalence of XDR-TB among MDR-TB cases. [12],[14]

There were some limitations; the small number of suspected TB patients were present as it is a central referral hospital and patients included were only the ones who attended CRH for their treatment. Therefore, the clinical findings of our study may also vary depending on the load of the disease in this region. Hence, forcing the need of large meta-centric studies in the area.

CONCLUSION:

Identification of the genetic mutations responsible for MDR and XDR TB cases amongst the suspected individuals helps in the initial and early management of MDR and XDR TB cases before starting the DOTS therapy which would not have cured him, given the MDR and XDR tb status also limiting the wastage of time and side-effects of drugs therapy that a patient has to undergo before the detection of the potentially lethal MDR and XDR TB. Molecular tests which offer high accuracy and are available through a network of quality assured laboratories plays an important role in determining MDR and XDR TB. There has always been a need to rely on affordable and accurate tests. Accelerating the access to new diagnostic tools that will make a difference in the fight against antimicrobial resistance. Gap still exists in understanding which social determinants are involved in the current MDR and XDR TB epidemic, the underlying processes linking social determinants to TB and how to address it. Thus, a better understanding of such gaps is the necessity of the hour to curb the mortality and morbidity of this epidemic disease throughout.

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