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Utility of CBNAAT and LPA in the Implementation of PMDT Services in a Tertiary Care Hospital

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ABSTRACT

Background: Diagnosis of TB has been a challenging aspect since many years. WHO has endorsed the line probe assay (genotype MTBDR plus, Hain Life science) and the Xpert MTB/RIF assay (Cepheid, Sunnywale, USA) for rapid diagnosis of DRTB. Aim: The aim of this study is to know the prevalence of TB and its resistance pattern, by using CBNAAT and LPA. **Methods:** This is a prospective study done during the period from Jan 2018 to October 2018; all the samples were tested as per the RNTCP guidelines by using CBNAAT and LPA. **Results:** Out of 3465 TB suspected samples, 865 (24.96%) were positive to Mycobacterium tuberculosis. Out of 865 TB confirmed cases, 59 (6.82%) showed rifampicin (R) resistance. Among rifampicin resistant TB cases, 25 were presumptive TB cases and 34 were presumptive DR TB cases. The percentage of rifampicin resistance by CBNAAT was 6.82%, whereas by LPA detection rate of MDR -TB was 14.7%. **Conclusion:** Molecular technologies like LPA and Xpert MTB/RIF are the most promising technologies to detect these mutations. The LPA test detects RIF as well as INH resistance due to mutations in the inhA and katG genes, while the Xpert MTB/RIF can detect only RIF resistance. In such cases LPA has greater role to play.

Keywords: CB NAAT, LPA, Rifampicin resistance.

INTRODUCTION

Tuberculosis (TB) remains as a major global public health problem, affecting millions of people each year and TB is the second leading cause of death among infectious diseases. Worldwide incidence of TB was 10.4 million in 2015, among them 5.9 million (56%) were males, 3.5 million (34%) were females and 1.0 million (10%) among children.[1] India accounts for one fourth of the global TB burden. An estimated incidence of TB cases occurred was 28,00,000 and 4,80,000 people died due to TB.

Diagnosis of TB has been a challenging aspect since many years. Using sputum microscopy, detecting TB bacilli is very low, because of paucibacillary TB in few cases and intermittent shedding of bacilli. Sputum culture and sensitivity is a gold standard method, but it is a slow test usually taking 4-8 weeks, which delays treatment to patients and not economical for screening purposes.[2]

According to the latest Revised National TB Control Program (RNTCP) and Indian Academy of Pediatrics (IAP) guidelines, all attempts should be made to obtain a bacteriological diagnosis. Early detection is the key to successful treatment and reduction of the disease transmission. RNTCP has recently developed National strategic plan (NSP) 2017-25 to work towards achieving the goal of eliminating TB by 2025.[3] Drug resistant TB (DRTB) is one of the major impediments to achieve NSP goal of ending TB in India. India has noted with highest burden of both TB and MDR TB (Multi Drug Resistant Tuberculosis). Worldwide, the estimated MDR TB cases were 0.48 million.[4]

In 2017, the epidemiology of DRTB (Drug Resistant Tuberculosis) estimated by WHO that, there were 558 000 new cases with resistance to rifampicin – the most effective first-line drug – of which 82% had MDR-TB. The MDR-TB burden largely falls on 3 countries – India, China and the Russian Federation – which together account for nearly half of the global cases. About 8.5% of MDR-TB cases had extensively drug-resistant TB (XDR-TB) in 2017.[5]

WHO has endorsed the line probe assay (genotype MTBDR plus, Hain Life science) and the Xpert MTB/RIF assay (Cepheid, Sunnywale, USA) for rapid diagnosis of DRTB. Both methods target the same 81 bp Rifampicin resistance determining region (RRDR) in the gene encoding bacterial RNA polymerase B subunit(rpo B) for detection of rifampicin associated mutations using DNA probes.

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So there is a correspondence of the probes of each other and expected similarity of probe binding.

Early diagnosis of drug resistance among all diagnosed TB patients need to be ensured by providing rapid molecular technology (CBNAAT and LPA) at more decentralized level. Cartridge based nucleic acid amplification test (CB-NAAT/GeneXpert) is an automated cartridge based molecular technique which detects TB bacilli as well as resistance pattern of rifampicin with in a short span of two hours.[6] Line Probe Assay is done by extraction of DNA using Genotype MTBDR plus for detection of MTB complex and rifampicin and/or INH resistance, test run takes up to 72 hours.[7]

We have taken this study to show bacteriological confirmation importance for accurate and early treatment. The aim of this study is to know the prevalence of TB and its resistance pattern, by using CBNAAT and LPA and to stratify the patients based on the variables like age, sex, sample type, drug resistance and HIV status.

MATERIALS AND METHODS

This is a prospective study done during the period from Jan 2018 to October 2018; all the samples were tested as per the RNTCP guidelines by using CBNAAT and LPA. Samples from Presumptive TB and Presumptive DR-TB were collected in the DTCO office, Kurnool and processed by CBNAAT at Department of Microbiology, Government Medical College, Kurnool.

Samples which were positive in CBNAAT were transported in cold chain to Damien Foundation Urban Leprosy and TB centre, Nellore which is operated by DFIT for I line and II line LPA. The lab is accredited as an IRL for LPA testing. Since the observations were made as a part of national TB control program, a separate ethics clearance was not required.

The samples were processed using NALC-NaOH method. Samples were decanted following centrifugation and the sediments were resuspended in phosphate buffer solution. The LPA was performed according to the manufacturer's protocol. Results were obtained by e-mail to the DTCO office within a week.

Xpert MTB/Rif or Gene Xpert:

It is a semi nested PCR based assay which uses five fluorescent wild type probes(Molecular beacon probes) to scan the 81 bp region. The specificity and sensitivity for detection of Rif resistance are more than 98 and 99% respectively. The molecular beacon probes are highly specific to its target sequence than any other type of hybridization probes.

Line probe assay:

The test is based on DNA strip technology and has three steps: DNA extraction, multiplex PCR amplification of the RRDR region and reverse hybridization of PCR products to the specific oligonucleotide probes immobilized on the strip. It employs 6 control probes for verification of the test procedures, 8 WT probes and 4 mutation specific probes (MUT), that target the RRDR. The six control probes include a conjugate control (C), an amplification control (AC) an MTB complex-specific control (TUB), a rpo B amplification control, a kat G amplification control, an inhA amplification control. In order to give a valid result all control probes should be positive. Drop out of WT probes or hybridization of MUT bands indicates Rif resistance.

RESULTS

CBNAAT Results:

Total number of samples included in this study was 3465, which are tested by CBNAAT. Among these samples, 3207 (92.5%) were pulmonary cases and 258 (7.5%) were extra pulmonary cases.

Table 1: TB suspected cases investigated by CBNAAT

No. of Presumptive TB cases – 1345		No. of Presumptive DR- TB cases – 2120		Total no. of cases
PTB	EPTB	PTB	EPTB	
1189	156	2018	102	3465

Among 3465 specimens, most of them were belongs to the age group of 40 -60 years (49.78%), followed by 20-40 years (32.55%), 0-20 years (10.67%) and >60 years (6.98%) [Figure 1].

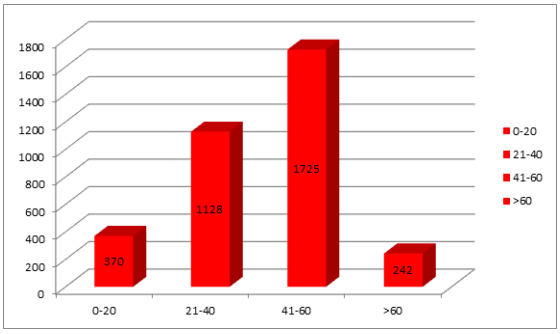

Figure 1: Age distribution of TB suspected cases.

Out of 865 TB confirmed cases, 59 (6.82%) showed rifampicin (R) resistance. Among rifampicin resistant TB cases, 25 were presumptive TB cases and 34 were presumptive DR TB cases [Table 2].

Table 2: Prevalence of TB and rifampicin resistance detection by CBNAAT

MTB detected: 865 (24.96%)			
Presumptive TB: 285 (32.9%)		Presumptive DR-TB: 580 (67.05%)	
R sensitive	R resistant	R sensitive	R resistant
260	25	548	34

Out of 865 samples, 513 samples were positive among men and 352 were among females. Out of 865 TB positive samples, 513 (59.3%) patients were diagnosed as HIV TB co- infection, among HIV TB

co-infected patients rifampicin resistance was observed in 8 (15.5%) patients.

Table 3: Sex wise distribution of TB cases

Total no. of samples tested: 3465					
Males: 2280 (65.80%)			**Females: 1185(34.19%)**		
MTB detected		**MTB not detected**	**MTB detected**		**MTB not detected**
513 (59.30%)		1767 (77.5%)	352 (40.69%)		833 (70.29%)
R sensitive	R resistant		R sensitive	R resistant	
475 (92.60%)	38 (64.40%)		331 (94.03%)	21 (35.5 %)	

Table 4: Treatment regimen for DR TB Cases

Drug Resistant Pattern	**No.of cases**	**Intensive Phase**		**Continuation Phase**	
		Drugs	**Duration**	**Drugs**	**Duration**
H mono resistance	59	Lfx Km R E Z	3-6	Lfx REZ	6
MDR TB (H+R resistance) - Shorter	47	Mfx Km Eto Cfz Z Hh E	4-6	Mfx Cfz Z E	5
MDR/RR + resistance to FQ	24	Km Eto Cs Z Lzd Cfz + (6)Bdq	6-9	Eto Cs Lzd Cfz	18
XDR TB	1	Cm Eto Cs Z Lzd Cfz E + (6)Bdq	6-12	Eto Cs Lzd Cfz E	18

*H-Isoniazid, R-Rifampicin, Z-Pyrazinamide, E-Ethambutol, S-Streptomycin, Lfx – Levofloxacin, Km – Kanamycin, Mfx – Moxifloxacin, Eto – Ethionamide, Cfz-Clofazimine, Hh – High dose Isoniazid, Cs – Cycloserine, Lzd – Linezolid, Bdq – Bedaquiline.

Among the 865 TB positive cases, New cases were 285 (32.9%) and among them R resistance was seen in 17 cases (5.96%). Previously treated cases were 580 (67.05%) among them R resistance was seen in 42 (7.2%).

LPA results and treatment given to different categories of patients:

865 TB confirmed cases by CBNAAT, were again processed by LPA for detection of MDR TB. Among 865 TB cases, 128 (14.7%) were confirmed as resistant bacteria.

Out of 128 resistant TB, 59 (46.09%) cases had Isoniazid (H) mono resistance, 47 (36.7%) cases had MDR TB (H+R resistance), 21 (16.4%) cases had MDR/RR + resistance to FQ and 1 (0.78%) case had XDR-TB. Treatment regimens followed for all 109 drug resistant TB cases mentioned in [Table 4].

Those on conventional DR-TB regimen were continued on the same. Bedaquiline has been started in 15 cases since April 2018. For initiating Bedaquiline patients are reffered to Bathanapalli, Anantapur, Nodal DR-TB centre. There the patients are subjected to all investigations and evaluated as per the inclusion and exclusion criteria for initiating Bedaquiline. After 2 weeks if the patient is an eligible candidate for intiating Bedaquiline they are sent back to DTCO office, Kurnool.

DISCUSSION

World Health Organization (WHO) endorsed the use of molecular-based CBNAAT and LPA for screening of MDR-TB, following which these two assays are being used routinely in different countries.[8] CBNAAT has been endorsed by WHO as an initial diagnostic test in children suspected of having tuberculosis both in pulmonary and specific forms of extra pulmonary tuberculosis.

Still in many countries, reporting of TB to government is based on clinical diagnosis even after rapid advancement and availability of molecular method detection globally. This delays initiation of anti-tubercular treatment especially for drug-resistant forms of TB, increases risk of transmission of (drug-resistant) TB in the community. These rapid molecular tests help to identifying the pathogen quickly and effective management.

MoH & FW, GOI (Government of India) laid down revised guidelines on PMDT (Programmatic management of Drug resistant Tuberculosis) in 2017 for DR-TB diagnosis, treatment and cure. As per the new guidelines, six types of DR-TB have been defined for the purpose of treatment.[9,10]

Types of Drug resistant Tuberculosis (DR-TB):[9,10]

Mono-resistance: resistance to one first-line anti-TB drug only

Poly-resistance: resistance to more than one first-line anti-TB drug, other than both isoniazid and rifampicin

Multidrug resistance (MDR): resistance to at least both isoniazid and rifampicin

Extensive drug resistance (XDR): resistance to any fluoroquinolone, and at least one of three second-line injectable drugs (capreomycin, kanamycin and amikacin), in addition to multidrug resistance

Rifampicin resistance (RR): resistance to rifampicin detected using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs. It includes any resistance to rifampicin, in the form of mono-resistance, poly-resistance, MDR or XDR.

Mixed pattern DR-TB: Multi drug resistance or Rifampicin resistance TB plus resistance to Fluoroquinolone or Second line injectable drug and Linezolid or more.

Among rifampicin resistant TB cases, 25 were presumptive TB cases and 34 were presumptive DR TB cases. Among the 865 TB positive cases, New cases were 285 (32.9%) and among them R resistance was seen in 17 cases (5.96%). Previously treated cases were 580 (67.05%) among them R resistance was seen in 42 (7.2%). Rifampicin resistance was comparatively higher probably

because most of the samples were received from previously treated cases. Among 865 TB cases, 128 (14.7%) were confirmed as resistant bacteria. Out of 128 resistant TB, 59 (46.09%) cases had Isoniazid (H) mono resistance, 47 (36.7%) cases had MDR TB (H+R resistance), 21 (16.4%) cases had MDR/RR + resistance to FQ and 1 (0.78%) case had XDR-TB as per this study.

Syed Beenish Rufai et al,[11] observed out of 285 smear-positive samples were subjected to LPA. Seventy-two (25.8%) samples showed multidrug resistance, 62 (22.2%) showed rifampin monoresistance, 29 (10.3%) showed isoniazid mono resistance, and 116 (41.5%) were pan-susceptible. Six (2.1%) of the samples gave invalid results.

In our study, the percentage of rifampicin resistance by CBNAAT was 6.82%, whereas by LPA detection rate of MDR -TB was 14.7%.

Our study correlated with national observation of rifampicin resistance of 12-17% in MDR suspects.[12] In relation to the present study Kumar et al,[13] and Sharma et al,[14] also reported 25.8% and 22% of MDR respectively. In contrast to this study Singhal et al,[15] and Raizada et al,[16] reported higher percentage of rifampicin resistance i.e., 55.2% and 51% respectively. Tripathi R et al,[17] did a study on 1253 patients; noted 54.4% had rifampicin resistant. They did a comparative study of CB NAAT and LPA; observations was Rifampicin resistance has seen to be (57.7%) in LPA and (53.0%) in CBNAAT.

Kumar P et al,[18] stated that Isoniazid resistance is more common in high TB burden countries and those isolates may not be resistant to Rifampicin. In contrast this statement, Somoskovi A et al,[19] noted if the isolate is RIF resistant, it is more likely that it is also INH resistant, thus making RIF resistance a surrogate marker for the identification of MDR-TB. In High TB burden countries like India, higher rifampicin mono resistance was observed.[18,20] Whereas, in high TB burden country, South Africa reported with lower rifampicin mono resistance levels (13.5%).[21] In United States which low TB burden country, low rifampicin levels were observed by Ridzon R et al.[22]

CBNAAT is a quick automated procedure which can establish the positivity of TB in AFB smear negative specimens of about 50-80%.[23] A Meta analysis by 18 studies noted the sensitivity and specificity of Xpert MTB/ RIF assay (compared with culture) were 83 and 94 percent respectively in lymph nodes; 81 and 98 percent respectively in cerebrospinal fluid; and 46 and 99 percent respectively in pleural fluid.[24]

Hemant Deepak Shewade et al,[25] did a study on Pre diagnosis attrition among presumptive MDR TB patients at Bhopal. They have noted pre-diagnosis attrition rate was high i.e., 60% (459/770); among those 91% were not identified as presumptive MDR-TB by the programme. Age more than 64 years; those from a medical college; those eligible in quarter 1; patients with presumptive criteria 'previously treated – recurrent TB' , 'treatment after loss-to-follow-up' and 'previously treated-others'; and patients with extra-pulmonary TB were independent risk factors for not undergoing DST. Attrition was more than 40% across all subgroups.

Syed Beenish Rufai et al,[11] observed that out of 62 rifampin- monoresistant samples by LPA, 38 (61.4%) showed rifampin resistance, while 21 (33.8%) were found susceptible to rifampin by Xpert MTB/RIF using cartridge version G4. Three (4.8%) samples gave an error. The 25 discrepant samples were further subjected to MGIT960 drug susceptibility testing. The MGIT960 results showed 100% agreement with LPA results but only 64.4% agreement with Xpert MTB/RIF results. Sequencing analysis of discrepant samples showed 91.3% concordance with LPA but only 8.7% concordance with the Xpert MTB/RIF assay.

Tripathi R et al,[17] documented as among total 360 diagnosed cases by LPA 129 were sensitive and seven patients showed indeterminate for rifampicin. A total of 16/360 (4.4%) showed negative for MTB by LPA. Out of 16 cultures, thirteen cultures turned positive (8 cultures showed positive on plain and PNB, 5 were positive on plain only), two cultures were negative and one was contaminated. On further DNA extraction and LPA of culture positive samples, all showed negative for MTB.

CONCLUSION

CBNAAT and LPA assays have been extremely useful in the diagnosis of DR -TB. Though CBNAAT is very user friendly and is considered the method of choice in identification of TB and detection of R resistance, it should always be kept in mind that H Mono resistance and resistance to II line drugs is also very common. Molecular technologies like LPA and Xpert MTB/RIF are the most promising technologies to detect these mutations. The LPA test detects RIF as well as INH resistance due to mutations in the inhA and katG genes, while the Xpert MTB/RIF can detect only RIF resistance. In such cases LPA has greater role to play.

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