Original Research Article

Role of cartridge-based nucleic acid amplification test to diagnose tuberculosis at tertiary care teaching hospital in Rajasthan, India

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ABSTRACT

Background: Tuberculosis is one of the top 10 causes of death worldwide as per the Global TB report 2017, the estimated incidence of TB in India was approximately 28,00,000 cases accounting for about a quarter of the world's TB cases (10 million). It is of utmost important to diagnose early and treat it to reduce disease transmission. GeneXpert MTB/RIF, an automated cartridge-based molecular technique detects *Mycobacterium Tuberculosis* and rifampicin resistance within two hours, has been recommended by WHO for rapid diagnosis of TB.

Methods: Author conducted a retrospective study in the Department of TB and Chest, of tertiary care center at Jhalawar Medical College (JMC), Jhalawar to evaluate and analyze the role of CBNAAT to diagnose tuberculosis from 1st January 2018 to 31st December 2018. Author included all patients who came to department of TB and Chest of JMC, Jhalawar either new/ relapsed/ defaulters/ referred cases from ART/ ICTC center, Pediatric Department; Gynaecology and Obstetrics Department, peripheral Government Health Care Facilities and Private Hospitals of Jhalawar District catering about 15.5 lac population were subjected to both ZN staining/ Fluorescent microscopy and CBNAAT in the study period.

Results: A total of 3078 samples (pulmonary 2739+EP 339) were tested for ZN staining / Fluorescent microscopy and CBNAAT during the study period. Mean age of the study population was 36.5±10.3 years. 1873 tested were negative and 1205 samples were positive for CBNAAT. Of these 1205 positive samples, 1174 were sputum/ BAL samples and 31 were extra pulmonary samples. Authors found rifampicin resistance rate of 6.98% (82/1174) in pulmonary tuberculosis cases, 3 rifampicin resistance cases were detected in extra pulmonary samples. CBNAAT could identify 255 cases (14.01%) that were smear negative. Author found TB-HIV coinfection rate of 18.75%.

Conclusions: Author found CBNAAT to be an important diagnostic modality especially in smear negative patients for early diagnosis and treatment. Author could detect *Mycobacterium Tuberculosis* in 14.01% of patients with negative smear microscopy for AFB. In PLHIV, CBNAAT detected *Mycobacterium Tuberculosis* in 18.75% (12/64) of patients. Author found rifampicin resistance rate of 6.98% (82/1174) in pulmonary tuberculosis cases.

Keywords: Cartridge-based nucleic acid amplification test, Extra-pulmonary, People living with HIV, Smear negative, Tuberculosis

INTRODUCTION

Tuberculosis is a major communicable disease-causing significant mortality and morbidity worldwide, especially in India. It is one of the top 10 causes of death worldwide. In 2017, 10 million people fell ill with TB and 1.6 million died globally from the disease (including

0.3 million among people with HIV). TB is a leading killer of HIV-positive people.

In 2017, an estimated 1 million children became ill with TB and 2,30,000 children died of TB (including children with HIV associated TB). Multidrug-resistant TB (MDR-TB) remains a public health crisis and a health security threat. WHO estimates that there were 5,58,000 new cases with resistance to rifampicin-the most effective first-line drug, of which 82% had MDR-TB. Globally, TB incidence is falling at about 2% per year. This needs to accelerate to a 4-5% annual decline to reach the 2020 milestones of the End TB Strategy. An estimated 54 million lives were saved through TB diagnosis and treatment between 2000 and 2017. Ending the TB epidemic by 2030 is among the health targets of the Sustainable Development Goals.[1]

In 2016, the incidence of tuberculosis in India (including HIV) was 2.79 million with mortality rate of 211/ lac population. Mortality due to TB (Excluding HIV) was 4.23 million with mortality rate of 32/lac population. India accounting for about a quarter of the world's TB cases.[2] Early detection of TB cases is the key to successful treatment and reduction of disease transmission.

Most of the deaths from TB could be prevented with early diagnosis and appropriate treatment. To reduce the incidence and prevalence, India introduced National Sample Survey (NSS) in 1958, National Tuberculosis Control Programme (NTP) in 1962, followed by Revised National Tuberculosis Control Programme (RNTCP) 1993-1996 and with Directly Observed Treatment Short-Course Chemotherapy (DOTS) strategy in 1997.

WHO released STOP TB STRATEGY in 2006. India adopted it in 2007. There are continuous efforts made to decrease the incidence and prevalence of tuberculosis, continuous change in the strategies under RNTCP are made. Further there was adoption of Goals of NSP with a vision of TB Free India in 12th five-year plan in (2012-17). The current adoption of END TB STRATEGY has a vision of world free of TB.

TB affecting other sites-known as extra-pulmonary TB, is rarely smear-positive; Extra-pulmonary tuberculosis forms a significant proportion of the total TB cases and is a major health problem in both developing and developed countries. Diagnosing EPTB is challenging due to its varied clinical presentations and paucibacillary nature of the disease. AFB smear hasn't proved to be much useful in diagnosing EPTB.[3] For several decades smear microscopy and conventional culture techniques have been the mainstay of diagnostic testing for pulmonary tuberculosis. While smear microscopy has poor sensitivity and issues related to quality control, conventional solid culture techniques have the limitation of long turnaround time of several weeks.

Liquid culture techniques were developed for early detection of *Mycobacterium Tuberculosis* growth, but the mean turnaround time of 21 days is still long for a diagnostic test to be effective in curbing transmission. Such delays in diagnosis increase morbidity and mortality, predispose to secondary resistance and cause transmission of resistant strains. Nucleic acid amplification tests (NAAT) such as in-house polymerase chain reaction (PCR) for TB and line probe assay were developed for rapid detection of TB and identification of drug resistance. However, the conventional inhouse NAATs require well-trained technical staff and sophisticated equipment. Also, for these PCR, there are no validation studies done in large sample size. As the conventional NAATs have various steps from DNA isolation to amplification, there are also chances of cross-contamination from environmental factors or carry-over contamination from other samples.[4]

Cartridge-based nucleic acid amplification test (CBNAAT) is a recently introduced polymerase chain reaction (PCR) based method for detection of TB. It also detects rifampicin resistance as it targets the rpoB gene of mycobacteria. CBNAAT is a *Mycobacterium tuberculosis*-specific automated, cartridge based nucleic acid amplification assay, having fully integrated and automated amplification and detection using real-time PCR, providing results within 100 minutes. It is a highly specific test as it uses 3 specific primers and 5 unique molecular probes to target the rpoB gene of M. tuberculosis, which is the critical gene associated with rifampicin resistance.

No cross-reactions have been observed with many other bacterial species tested, including a comprehensive panel of mycobacteria, thereby excluding non-tubercular mycobacteria (NTM). Being a PCR based method, clinical validation trials done in four distinctly diverse settings have shown that 92.2 per cent of culture-positive patients were detected by a single CBNAAT test with a specificity of 99 per cent as compared to the sensitivity of a single direct sputum smear of 59.5%.[5]

The GeneXpert system was launched in 2004, and the development of the GeneXpert test, based on the GeneXpert platform, was completed in 2008.[6] The first clinical validation studies were carried out in 2009.[7] Data from these studies were then submitted to the World Health Organization (WHO) for evaluation in September 2010. In December 2010 WHO endorsed the GeneXpert technology and released a recommendation and guidance for countries to incorporate the new test into their programs.

WHO recommended that the test should be used as the initial diagnosis test in individuals suspected of having MDR TBor HIV associated TB. They also suggested that it could be used as a follow-on test to microscopy in settings where MDR TB and/or HIV is of lesser concern, especially in smear negative specimens, because of the lack of accuracy of smear microscopy.

They did however say that they recognized the major resource implications of using it in this second way.[8] WHO did also emphasize that the test does not eliminate the need for conventional microscopy, culture and drug sensitivity testing, as these are still required to monitor treatment progress and to detect other types of drug resistance. The GeneXpert MTB/ RIF cannot be used for treatment monitoring, as it detects both live and dead bacteria.

The present study was conducted at laboratory of department of TB and Chest of tertiary care center of Hadoti region at JMC, Jhalawar with the objective of evaluating the role of GeneXpert MTB/RIF assay in diagnosing pulmonary TB/ EPTB and detecting resistance to rifampicin, taking culture as the gold standard for confirmed diagnosis and drug sensitivity.

METHODS

Author conducted a retrospective study in the department of TB and Chest of tertiary care center at JMC, Jhalawar to analyze the role of CBNAAT from 1st January 2018 - 31st December 2018.

In Cohort we have taken total 3078 samples for CBNAAT after exclusion. Among these cohort, 2751 were adults and 327 were children . Out of 2751 adults we have tested 2448 pulmonary and 303 extra-pulmonary samples. Out of 327 children we have tested 291 pulmonary and 36 extra-pulmonary samples (Figure 1).

Inclusion criteria

Author included all patients who were subjected to CBNAAT in the study period. Data were collected fromnew/ relapsed/ defaulters/ currently on anti TB treatment coming for follow-up investigation approaching/ or referred to department of TB and Chest of JMC from ART/ ICTC center, Pediatric department, Gynaecology and Obstetrics Department, peripheral Government Health Care Facilities and Private Hospitals of Jhalawar District and were subjected to both ZN staining/ fluorescent microscopy and CBNAAT in the study period. Author collected total number of samples tested for CBNAAT, indication for CBNAAT, HIV status, result of smear microscopy for AFB and CBNAAT specimen subjected to CBNAAT was either sputum, gastric lavage, BAL or extra-pulmonary fluid sample (Pleural fluid, pus, synovial fluid, ascitic fluid, CSF, Endometrial tissue fluid). A minimum of 2.5 ml of sample was considered adequate for analysis.

Exclusion criteria

Blood, stool, blood mixed with sputum, tissue and fluid sample were rejected. Specimens were collected in Falcon tubes; analysis was done on the same day and results were given within a day.

Statistical methods

Data are entered in MS-Excel and analyzed through SPSS 20.0 (trial version) software. Descriptive data are presented in the form of frequency and percentage. Chi-square test and sensitivity and specificity are used to find the inference of the result and p-value <0.05 considered as significant.

RESULTS

A total of 3137 samples were collected during the study period, out of them 59 samples were rejected due to error (pulmonary 36 and EP 23). A total of 3078 samples (pulmonary 2739+EP 339) were tested for ZN staining+ fluorescent microscopy and CBNAAT during the study period. Mean age of the study population was 36.5±10.3 years. When subjected to ZN staining+Fluorescent microscopy of 3078 samples, 919 pulmonary samples (sputum 908+gastric lavage 11) were positive, No EP sample was detected positive on microscopy. Gastric lavage samples were obtained from most of pediatric patient and considered as pulmonary samples (Table 1).

Table 1: Nature of specimen tested and yield of smear microscopy (ZN* staining + fluorescent microscopy).

Sample	Nature of samples	Number of samples	Smear positivity for AFB, n(%)
Pulmonary samples (N= 2739)	Sputum	2503	908(36.27%)
	Gastric lavage	236	11(4.66%)
	BAL	00	0(0%)
Extra pulmonary sample (N= 339)	Pleural fluid	217	0(0%)
	CSF	46	0(0%)
	Pus	41	0(0%)
	Ascitic fluid	17	0(0%)
	Endometrial	18	0(0%)

Of 3078 samples, 1873 tested were negative and 1205 samples were positive for CBNAAT. Out of 1205 positive samples, 1174 were sputum/ BAL samples and 31 were extra pulmonary samples. Author found rifampicin resistance rate of 6.98% (82/ 1174) and 9.67% (3/ 31) in pulmonary and EP tuberculosis cases respectively, of which 12 were HIV positive (Table 3). *Mycobacterium Tuberculosis* was detected in 14.01% of smear negative pulmonary and 9.14% of smear negative extra-pulmonary samples subjected to CBNAAT.

CBNAAT could identify 255 cases (14.01%) that were smear negative (Table 2). Author received 161 samples from private sector, of which 31 were positive for MTB and 03 case was rifampicin resistant. Author found TB and HIV co-infection rate of 18.75%. Mean age of the population was 36.5±10.3 years. There were 07 Extra Pulmonary TB samples and rest pulmonary sample (Table 4). No case of RR was detected. Ninety six percent of PLHIV were on Co-trimoxazole prophylaxis.

Table 2: Nature of specimen tested and yield of CBNAAT to detect *Mycobacterium Tuberculi*.

Sample	Smear microscopy results (pos / neg)	Site	No. of samples	CBNAAT positivity results, N (%)
Pulmonary samples (n=2739)	Negative	Sputum (1595)	1820	255(14.01%)
		Gastric lavage (225)		
	Positive	Sputum (908)	919	919(100%)
		Gastric lavage (11)		
Extra-pulmonary samples (n=339)	Negative	Pleural fluid	217	15(6.91%)
		C.S.F.	46	02(4.34%)
		Pus	41	14(34.14%)
		Ascitic fluid	17	0(0%)
		Endometrial	18	0(0%)

Table 3: Nature of specimen tested by CBNAAT and yield of rifampicin resistance (RR).

CBNAAT positive samples	Site	No. of samples	Rifampicin resistance (RR) by CBNAAT
Pulmonary samples (n= 1174)	Sputum and Gastric lavage (919 + 255)	1174	82(6.98%)
Extra-pulmonary samples (n=31)	Pleural fluid	15	1(6.66%)
	C.S.F.	02	1(50%)
	Pus	14	1(7.14%)
	Ascitic fluid	0	0(0%)
	Endometrial	0	0(0%)

Table 4: Nature of specimen tested and yield of CBNAAT to detect *Mycobacterium Tuberculi* in HIV patients.

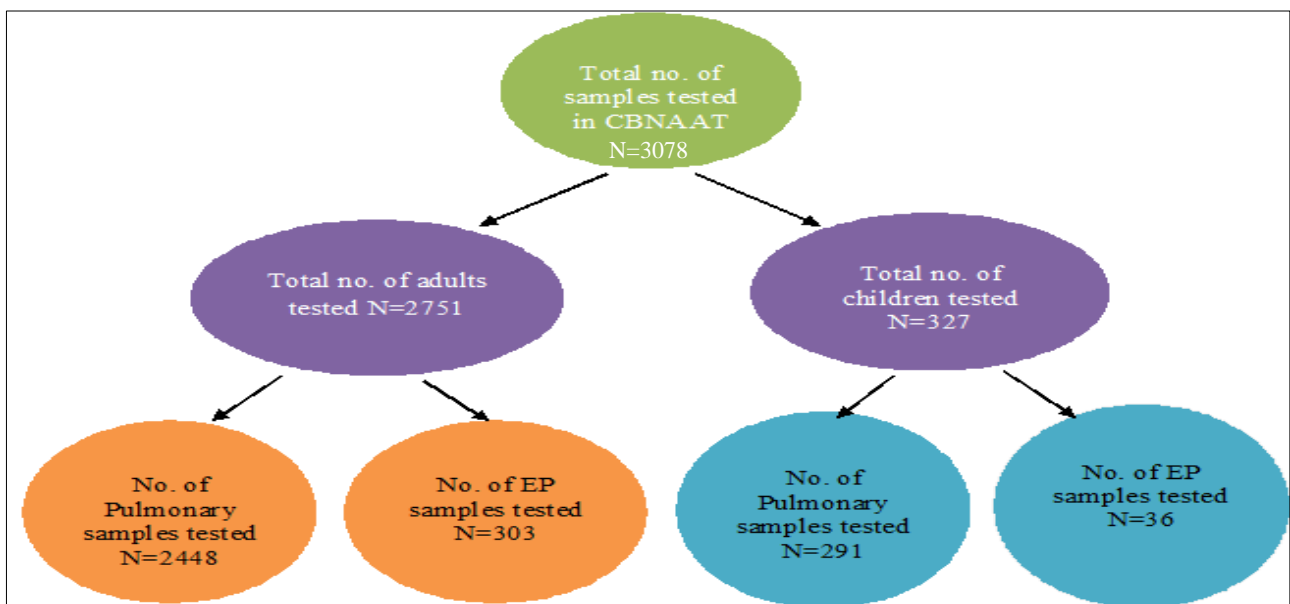| Samples sent for CBNAAT, n=64	Site	No. of samples	CBNAAT positivity results, N (%)
Pulmonary samples (n=57)	Sputum and Gastric lavage	57	10(17.54%)
Extra-pulmonary samples (n=07)	Pleural fluid	05	1(20%)
	C.S.F.	0	0(0%)
	Pus	02	1(50%)
	Ascitic fluid	0	0(0%)
	Endometrial	0	0(0%)

Figure 1: Flow of cohort subjected to CBNAAT.

DISCUSSION

India contributes to a quarter (28,00,000) of global TB cases worldwide. Early and rapid diagnosis and treatment is of utmost importance to hamper transmission of TB. CBNAAT is one diagnostic modality that has been endorsed by WHO in the recent past for diagnosis of TB by which Author can get results within 2 hours and can achieve the aim of rapid diagnosis and treatment. Author found in Author study that CBNAAT detected around 14.01% of pulmonary patients who were smear negative. The rate of rifampicin resistant TB detected by CBNAAT was 6.98% in pulmonary and 9.67% in EP tuberculosis cases and among HIV patients it was 0.0 in pulmonary and EP TB. TB is the leading cause of death among people living with HIV (PLHIV) including in those taking antiretroviral therapy (ART).

In 2017, around 0.3 million people with HIV-TB co-infection died in India.[1] Apart from diagnostic difficulties due to lack of caseous necrosis there is high prevalence of MDR TB. Hence early diagnosis and treatment is of paramount importance to cut the transmission of MDR TB and decrease mortality in PLHIV. A study done by Arora et al, found rifampicin resistance of 15.7% in PLHIV which was higher than this study.[9] A study done by Stephen Oluwasegun et al, found rifampicin resistance of 12% in PLHIV which was higher than this study.[10] A study conducted by C.K. Vidyaraj et al, in Puducherry found rifampicin resistance of 5.6%.[11] Another study done by Dewan et al, done in Delhi found rifampicin resistance of 10%.[5] This high resistance to rifampicin compared to this study may be due to higher prevalence of MDR TB in north India because of referral from multi-states and Nigeria.

India has the highest number of TB cases around the world. China is the next to India who has the highest number of TB cases. TB affects all countries and all age groups, but overall the best estimates for 2017 were that 90% of cases were adults (aged ≥15 years), 64% were male, 9% were people living with HIV (72% of them in Africa) and two thirds were in eight countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%). Only 6% of cases were in the WHO European Region and the WHO Region of the Americas, each of which had 3% of cases.[12] Incidence of MDR TB in India is about 11/1 lakh population.[2] A study done by Sharma et al, found prevalence of MDR TB to be 1.1% in new cases and 20% in retreatment cases.[13] Another multi-centric study done by Sukhdev et al, found prevalence of MDR TB to be 2-3% in new cases and 12-17% among retreatment cases.[14]

There are several limitations in this study, first it was retrospective study so author could not get details on previous treatment, if taken. Second, details of associated risk factors and co-morbidities like smoking, alcoholism, diabetes, and hypertension couldn't be fetched. Finally, there were many invalid results (59 samples) due to multiple reasons.

CONCLUSION

Author found CBNAAT to be an important diagnostic modality especially in sputum negative patients for early diagnosis and treatment. Author could detect *Mycobacterium Tuberculosis* in 14.01% of pulmonary patients and 9.14% of extra-pulmonary samples with negative smear for microscopy. In PLHIV, CBNAAT detected *Mycobacterium Tuberculosis* in 17.54% of pulmonary and 28.57% of EP samples. Author found rifampicin resistance rate of 6.98%(82/1174) and 9.67%(3/31) in pulmonary and EP tuberculosis cases respectively, of which 12 were HIV positive.

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