

## Utility of CBNAAT in diagnosis of mycobacterium tuberculosis in a tertiary care teaching hospital in South India

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### Abstract:

**Background:** Tuberculosis is the ninth leading cause of death worldwide. India contributes to about one fifth of global TB burden. It is very important to diagnose early and treat Tuberculosis to cut down transmission of Tuberculosis.

**Material and Methods:** We conducted a retrospective study in department of Pulmonary medicine to analyze the utility and yield of CBNAAT from Jan- Dec 2017. We included all patients who were subjected to CBNAAT in the study period. Data was collected from ART centre, DOTS centre and CBNAAT centre. We collected total number of samples tested for CBNAAT, indication for CBNAAT, HIV status, result of smear microscopy for AFB and CBNAAT.

**Results:** A total of 1703 samples were tested in CBNAAT during the study period. Mean age of the study population was 35.5±10.2 years. 1366 tested were negative and 290 samples were positive for CBNAAT. Of these 290 positive samples, 267 were sputum/BAL samples and 23 were extra pulmonary samples. We found rifampicin resistance rate of 2.4% (8/329) in pulmonary tuberculosis cases, There were no rifampicin resistance detected in extra pulmonary samples. CBNAAT could identify 184 cases (13.3%) that were smear negative. We found TB- HIV coinfection rate of 10.02%.

**Conclusions:** We found CBNAAT to be an important diagnostic modality especially in sputum negative patients for early diagnosis and treatment. We could detect Mycobacterium tuberculosis in 13.4% of patients with negative smear for microscopy. In PLHIV, CBNAAT detected Mycobacterium Tuberculosis in 9.3% of patients. We found rifampicin resistance rate of 2.4% (8/329) in pulmonary tuberculosis cases.

**Keywords:** CBNAAT; Tuberculosis; Smear negative; PLHIV.

### Introduction:

Tuberculosis is a major communicable disease causing significant mortality and morbidity worldwide especially in India. TB is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS [1]. In 2016, the incidence of Tuberculosis in India was 2.79 million with mortality rate of 32/lakh population [2]. India constitutes 24% of the total TB burden [2]. Early detection of TB cases is the key to successful treatment and reduction of disease transmission and most deaths from TB could be prevented with early diagnosis and appropriate treatment.

Since past many years, smear microscopy and conventional cultures have been used as diagnostic modality for pulmonary tuberculosis. Smear microscopy has variable sensitivity (45-80%) mainly in patient with smear negative TB, extra pulmonary TB and there are issues related to quality control [3,4] and conventional solid culture techniques take long turnaround time of 2-6 weeks and is costly [5]. For faster diagnosis, liquid culture (Mycobacterium Growth Indicator Tube) techniques were developed but the mean turnaround time is still long of 21 days [6]. Such delays in diagnosis increase morbidity and mortality that predispose to secondary resistance and cause transmission of resistant strains. Recently nucleic acid amplification tests (NAAT) were developed for rapid detection of TB and identification of drug resistance.

However, conventional NAATs require well-trained technical staff and sophisticated equipments [7].

WHO recommended use of a Cartridge Based Nucleic Acid Amplification test (CB-NAAT), for diagnosis of TB in December 2010. In 2013, WHO endorsed conditional recommendation for Xpert MTB/RIF as the initial diagnostic test in all adults with presumptive tuberculosis and MDR TB [8]. Xpert MTB/RIF is an automated, seminested real-time PCR that detects MTB and tests every positive sample for rifampicin sensitivity using molecular beacons [9]. Thus, results for both, presence of MTB and rifampicin resistance, are available within 2 hours with good sensitivity and specificity. It is a cartridge based nucleic acid amplification test (CBNAAT) that does not have any specific pre-requisites for its set-up and does not require much technical training. Further, as the reagent used for processing is bactericidal and tubercle bacilli are inactivated in vitro, biosafety risks are eliminated, thus enabling its use as a rapid point-of-care diagnostic test.

### Methodology:

We conducted a retrospective study in the department of Pulmonary medicine to analyze the utility and yield of CBNAAT from January to December 2017. We included all patients who were subjected to CBNAAT in the study period. Data was collected from ART centre, DOTS centre and CBNAAT centre. We 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 extrapulmonary fluid sample (Pleural fluid, pus, synovial fluid, ascitic fluid, Cerebro spinal fluid). Tissues were not subjected to CBNAAT due to non-availability of homonizer at our institute. A minimum of 2.5ml of sample was considered adequate for analysis and bloody specimen was rejected. Specimen was collected in Falcon tubes and analysis was done on the same day and results were given within a day.

**Statistical Methods:** Descriptive data are presented as frequencies (percentages) for discrete variables and as means (SDs) for continuous variables. All statistical tests were 2-tailed, and factors were considered statistically significant at  $p < 0.05$ . IBM SPSS version 22 and CDC Epi Info version 7 was used for analysis.

### Results:

A total of 1703 samples were tested in CBNAAT during the study period. Mean age of the study population was  $35.5 \pm 10.2$  years. Of 1703 samples, 1366 tested were negative and 290 samples were positive for CBNAAT. 192 samples processed were extra pulmonary samples and rest was pulmonary samples (Fig. 1). Of these 290 positive samples, 267 were sputum/BAL samples and 23 were extra pulmonary samples. We found rifampicin resistance rate of 2.4% (8/329) in pulmonary tuberculosis cases, of which 2 were HIV positive. Mycobacterium Tuberculosis was detected in 11.97% of extrapulmonary samples subjected to CBNAAT. There was no rifampicin resistance detected in extra pulmonary samples. CBNAAT could identify 184 cases (13.3%) that were smear negative (Table 1). We received 168 samples from private sector, of which 29 were positive for MTB and 1 case was rifampicin resistant.

We found TB and HIV co-infection rate of 10.02%. Mean age of the population was  $39.9 \pm 12.2$  years and 56 were males. There were 17 Extra Pulmonary TB samples and rest pulmonary sample (Table 2). Two cases of MDR TB were detected and both were pulmonary Koch's. Only 1 patient had

recurrent TB while rest was new cases. Ninety six percent of PLHIV were on Cotrimaxazole prophylaxis.

**Discussion:** India contributes to one fifth of global TB cases worldwide. Early diagnosis and treatment is critical to cut transmission of TB. CBNAAT is one diagnostic modality that has been endorsed by WHO in the recent past for diagnosis of TB. We found in our study CBNAAT detected around 13.4% of patients who were smear negative. The rate of rifampicin resistant TB detected by CBNAAT was 2.4% and among HIV patients it was 0.25%

TB is the leading cause of death among people living with HIV (PLHIV) including in those taking antiretroviral therapy (ART). In 2016, around 374,000 patients with HIV-TB co-infection died in India.<sup>2</sup> 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 our study [10].

Another study done by Dewan et al., done in Delhi found rifampicin resistance of 10%.<sup>11</sup> This high resistance to rifampicin compared to our study may be due to higher prevalence of MDR TB in north India and also referral from multiple states.

India has second highest number of TB cases in India after China.<sup>12</sup> Incidence of MDR TB in India is about 11/1 lakh population [2]. The prevalence of MDR TB is 2-3% among new cases and 12-17% among retreatment cases. A study done by Sharma et al found prevalence of MDR TB to be 1.1% in new cases and 20% in retreatment cases.<sup>13</sup> 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 [12].

There are several limitations in our study, First it was retrospective study so we could not get details on previous treatment, if taken. Second, details of associated risk factors and comorbidities like smoking, alcoholism, diabetes, and hypertension couldn't be fetched. Thirdly we have no data on tissue yield for Tuberculosis in CBNAAT. Finally there were many invalid results (21 samples) due to electric failure.

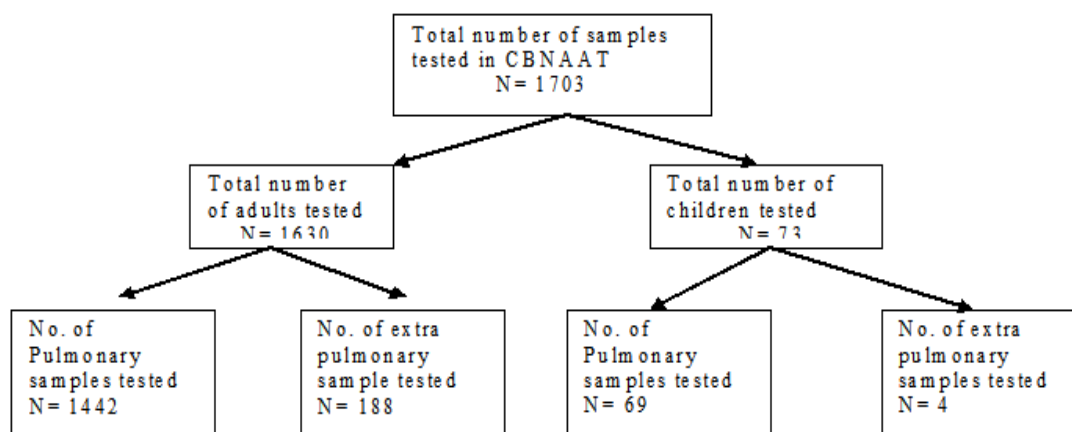
**Table 1: Table depicting nature of specimen tested and yield of CBNAAT**

Sample	Site	Number of samples	Number of positive CBNAAT result, n (%)
Pulmonary samples N= 1511	1. Smear negative sputum	1379	184 (13.34)
	2. Smear positive sputum	70	70 (100)
	3. BAL	40	13 (32.5)
	4. Gastric aspirate	12	0 (0)
Extra pulmonary samples N= 192	1. Pleural fluid	101	9 (8.9)
	2. Ascitic fluid	17	1 (5.8)
	3. Synovial fluid	4	0 (0)

4. Pus	31	11 (35.4)
5. Cerebro spinal fluid	39	2 (5.12)

**Table 2:** Table depicting nature of the specimen subjected to CBNAAT and yield of CBNAAT in patients with HIV

Sample sent for CBNAAT, n=778	Site	Number (%)	Number of positive CBNAAT result, n (%)
Pulmonary samples N=761	1. Sputum	761	71 (9.3)
	2. BAL	0	0 (0)
	3. Gastric aspirate	0	0 (0)
Extra pulmonary samples N= 17	1. Pleural fluid	37	5 (13.5)
	2. Ascitic fluid	2	0 (0)
	3. Synovial fluid	0	0 (0)
	4. Pus	10	3 (30)
	5. Cerebro spinal fluid	5	3 (60)



**Fig. 1:** Figure depicting flow of cohort subjected to CBNAAT

### Conclusions:

We found CBNAAT to be an important diagnostic modality especially in sputum negative patients for early diagnosis and treatment. We could detect *Mycobacterium tuberculosis* in 13.4% of patients with negative smear for microscopy. In PLHIV, CBNAAT detected *Mycobacterium Tuberculosis* in 9.3% of patients. We found rifampicin resistance rate of 2.4% (8/329) in pulmonary tuberculosis cases.

**Conflicts of Interest:** None declared

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