Original Research Article

Diagnostic yield of cartridge based nucleic acid amplification test in *Mycobacterium tuberculosis* in a tertiary care medical college and hospital of Southern Odisha, 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.

Methods: Author conducted a retrospective study in Department of Pulmonary Medicine SLN Medical College, Koraput, Odisha to analyze the utility and yield of CBNAAT. Study period was from April 2018 to March 2019. Inclusion criteria was all patients whose samples were subjected to CBNAAT were included in our study. Sputum samples from pulmonary tuberculosis patients, and extra pulmonary samples (pleural fluid, ascitic fluid, CSF, synovial fluid and gastric lavage etc. were included in our study population. Exclusion criteria was patients who were under anti tubercular therapy for pulmonary, extra pulmonary and MDR TB were excluded from this study. Data were collected from Pulmonary Medicine Department, ART center, DOTS center and CBNAAT center. Total number of samples tested for CBNAAT, different sample collection sites, age and sex distribution of patients, HIV status of all patients, result of smear microscopy for AFB and CBNAAT and Rifampicin resistance status were analyzed. The detail statistical analysis was done in tabulation form.

Results: A total of 2621 samples were tested in CBNAAT during the study period. Mean age of the study population was 38.03 years. 1881 tested were negative and 740 samples were positive for CBNAAT. Of these 2621 samples, 2526 were pulmonary samples (sputum, pleural fluid samples) and 95 were extra pulmonary samples. Author found rifampicin resistance rate of 0.54% (4/740)) in pulmonary tuberculosis cases. There was no rifampicin resistance detected in extra pulmonary samples. CBNAAT could identify 536 cases (23.2%) that were smear negative. Author found TB- HIV co-infection rate of 6.22%.

Conclusions: CBNAAT is an important diagnostic modality especially in sputum negative patients for early diagnosis and treatment. In our study it detected *Mycobacterium tuberculosis* in 23.2% of patients with negative smear for microscopy. Rifampicin resistance rate detected was very low compared to other studies.

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

INTRODUCTION

Tuberculosis (TB) is a major communicable disease-causing significant mortality and morbidity worldwide especially in India. It is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS.[1] Incidence of Tuberculosis in year 2016 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. Smear microscopy and conventional cultures have been used as diagnostic modality for pulmonary tuberculosis since many years. It has variable sensitivity (45-80%) in patient with pulmonary TB and there are issues related to quality control and conventional solid culture techniques take long turnaround time of 2-6 weeks and is costly.[3-5] For faster diagnosis, liquid culture (Mycobacterium Growth Indicator Tube) techniques were developed but the mean turnaround time is still long of 21 days.[6] Delays in diagnosis can lead to increase in morbidity and mortality that further predisposes to secondary resistance and transmission of resistant strains.

Recently Nucleic Acid Amplification Tests (NAAT) were developed for rapid detection of TB and identification of drug resistance, but it requires 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, semi nested real-time PCR that detects MTB and tests every positive sample for rifampicin sensitivity using molecular beacons.[9]

Thus, results of presence of MTB and rifampicin resistance, are available within 2 hours with good sensitivity and specificity. This Cartridge Based Nucleic Acid Amplification Test (CBNAAT) have very less pre-requites for its set-up and requires very less technical training. Further, reagent used for processing is bactericidal and tubercle bacilli are inactivated in vitro, eliminating bio-safety risks, enabling its use as a rapid point-of-care diagnostic test. So, with this background present study was undertaken to explore utility of CBNAAT in diagnosis of *Mycobacterium tuberculosis* in Institute.

METHODS

The study was retrospective and observational. It was conducted in Department of Pulmonary Medicine SLN MCH, Koraput after obtaining permission from Institutional Ethic Committee.

Study period was from April 2018-March 2019.

Inclusion criteria

- All patients whose samples were subjected to CBNAAT were included in our study. Sputum samples from pulmonary tuberculosis patients, and extra pulmonary samples (pleural fluid, ascitic fluid,

CSF, synovial fluid and gastric lavage etc.) were included in this study population.

Exclusion criteria

- Patients who were under Anti tubercular therapy for Pulmonary, extra pulmonary and MDR TB were excluded from this study.

Statistical analysis

Data was collected from Pulmonary Medicine Dept, ART centre, DOTS centre and CBNAAT centre. Reports of all patients who were subjected to CBNAAT during the study period were analyzed in respect to patient age, sex, MTB detected or not, Rifampicin sensitivity and resistant status. HIV status result of smear microscopy for AFB was also analyzed by percentage calculation by using latest Microsoft Excel software. Total number of tests showing invalid results was also analyzed. Specimen subjected to CBNAAT was either sputum or extra pulmonary fluid sample (Pleural fluid, pus, synovial fluid, ascitic fluid, Cerebrospinal fluid, gastric aspirate). Tissues were not subjected to CBNAAT due to non-availability of homogenizer at institute. A minimum of 2.5 ml 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.

RESULTS

A total of 2621 samples were tested in CBNAAT during the study period. Mean age of the study population was 38.03 years. Figure 1 showing sex distribution of all samples subjected for CBNAT. Figure 2 showing age wise distribution of all patients. 31.4% samples belong to 31-45-year age group, followed by 28.3% (15-30 years), 24.08% (46-60 years), and 8.85% (1-15 years).
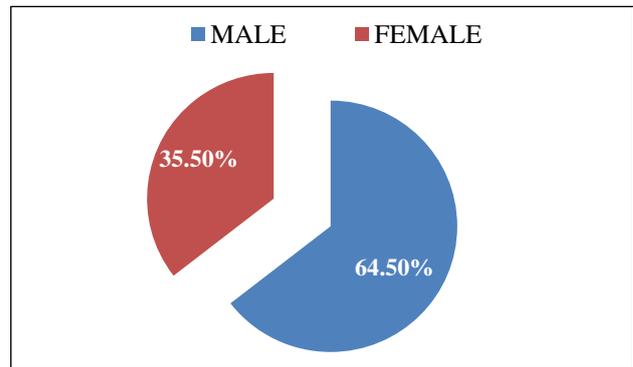
Figure 1: Percentage of male and female patient exposed to CBNAAT test.

Patient over 61 years constitutes 7.37% of total sample. Out of 2621 samples, 1881 tested were negative and 740 samples were positive for CBNAAT. 95 samples

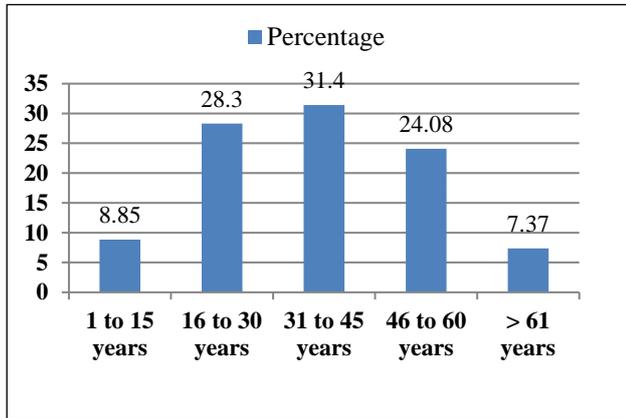processed were extra pulmonary samples and rest were pulmonary samples.

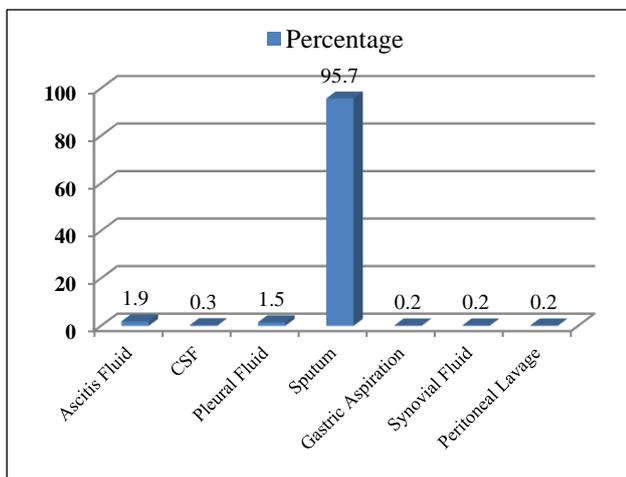**Figure 2: Age wise distribution of patients exposed to CBNAAT.**

Figure 3: Sample site percentage.

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

Sample	Site	Number of samples (total = 2621)	Number (with %) of positive CBNAAT result
Pulmonary samples N= 2526	Smear negative sputum	2255	522(23.2%)
	Smear positive sputum	214	214(100%)
	Pleural fluid	57	0(0%)
Extra pulmonary samples	Ascitis fluid	63	0(0%)
	CSF	23	2(8.7%)
	Gastric aspirate	4	2(50%)
	Peritoneal lavage	3	0(0%)
	Synovial fluid	2	0(0%)

Figure 3 showing site percentage of different samples collected. We found rifampicin resistance rate of 0.54% (4/740) in pulmonary tuberculosis cases. There was no rifampicin resistance detected in extra pulmonary samples. Table 1 depicts nature of specimen tested and yield of CBNAT. As evident from Table 1, CBNAAT could identify 522(23.2%) positive tuberculosis cases from 2255 smear negative sputum. Among the extra pulmonary samples, CBNAT positive results were obtained from 50% Gastric aspirate and 8% CSF samples. TB and HIV co-infection rate of 6.28% was also observed.

DISCUSSION

India contributes to one fifth of global TB cases worldwide. Early diagnosis and treatment are critical to cut transmission of TB. CBNAAT is one diagnostic modality that has been endorsed by WHO recently for diagnosis of TB. In our study CBNAAT detected around 23.2% of patients who were smear negative. The rate of rifampicin resistant TB detected by CBNAAT was 0.54% and among HIV patients it was 0.25%. TB is the leading cause of death among people living with HIV (PLHIV), including those taking antiretroviral therapy (ART). In 2016, around 374,000 Patients with HIV-TB co-infection died in India. 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 to 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.[10] Another study done by Dewan et al., done in Delhi found rifampicin resistance of 10.[11] 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 after China.[12] Incidence of MDR TB in India is about 11/1 lakh population.[2] The prevalence of MDRTB 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.[13]

There are several limitations in our study. First it was retrospective study, so we could not get details of previous treatment, 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.

CONCLUSION

CBNAAT can be an important diagnostic modality especially in sputum negative patients for early diagnosis and treatment. It could detect *Mycobacterium tuberculosis* in most patients with negative smear for

microscopy. It is also an important tool for diagnosis of *Mycobacterium tuberculosis* in PLHIV. CBNAT is also helpful in determination of rifampicin resistance rate, which is very useful in modification of future line of treatment.

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