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Original Article

Microbiology Section

# Effectiveness of CBNAAT in the Diagnosis of Sputum Negative Tuberculosis

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## ABSTRACT

**Introduction:** Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis*, remains one of the major health problems in India. Early detection of TB and Rifampicin (RIF) resistance are essential for effective disease management. The recent introduction of Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) also known as Gene Xpert MTB/RIF assay has significantly transformed the diagnostics of TB.

**Aim:** To evaluate the role of CBNAAT and smear microscopy by Ziehl-Neelsen (ZN) staining in the diagnosis of Pulmonary Tuberculosis (PTB).

**Materials and Methods:** Sputum samples from 337 patients having symptoms suggestive of PTB were included in this study. Sputum samples for ZN staining and CBNAAT were processed. RIF resistance was detected by CBNAAT.

**Results:** Out of 337 samples, 36 (10.68%) sputum samples were positive by smear microscopy. Samples from 107 cases with high clinico-radiological presumption were subjected to CBNAAT examination, out of which 41 (38.31%) were confirmed positive microbiologically. The mean age was 45±18.30 years, 24% were females and 76% were males. Overall sensitivity of CBNAAT was 38.31%. Sensitivity of CBNAAT was 100% for sputum positive cases and sensitivity was 15.38% for sputum negative cases. Overall RIF resistance was detected in two (1.86%) cases in present study.

**Conclusion:** CBNAAT helps in early detection to diagnose PTB. It can be used for screening MDR-TB for starting anti-tubercular treatment early.


**Keywords:** Acid fast bacilli, Cartridge-based nucleic acid amplification test, Ziehl-neelsen staining

## INTRODUCTION

Pulmonary Tuberculosis (PTB) continues to be an important cause of preventable mortality in both developing and developed nations. Early diagnosis and treatment remains the cornerstone of TB control [1]. According to Global TB Report-2018, 10.0 million people (range, 9.0-11.1 million) developed TB disease in 2017: 5.8 million men, 3.2 million women and 1.0 million children [2]. Smear microscopy is the cornerstone for the diagnosis of TB in resource-limited settings; it has only modest (35-80%) sensitivity and a poor Positive Predictive Value (PPV) [3]. Mycobacterial culture, though is gold standard, usually takes 2-6 weeks for final result and requires technical expertise [4]. Chest X-ray is useful but is not specific for diagnosing PTB. Also, TB may show symptoms and atypical radiologic findings, indistinguishable from those of community-acquired pneumonia [5]. Quick and accurate detection of the pathogen with its drug susceptibility patterns is vital for treatment initiation and disease control [6]. Rapid molecular tests are recent diagnostic tools that can be used to simultaneously test for PTB and RIF resistance with higher sensitivity than sputum smear microscopy and which could replace conventional culture-based drug susceptibility testing [7]. The CBNAAT detects the presence of TB bacilli and also tests for resistance to RIF.

CBNAAT, as it is a very cost-effective and rapid test is likely to revolutionize the diagnosis and treatment of PTB [8]. CBNAAT is a highly specific test as it uses 3 specific primers and 5 unique molecular probes to target the *rpoB gene* of *Mycobacterium tuberculosis*, which is the critical gene associated with RIF resistance [9]. With the above background, the present study was undertaken to evaluate the role of CBNAAT and smear microscopy by ZN staining in the diagnosis of PTB.

## MATERIALS AND METHODS

This was a prospective study conducted at The Oxford Medical College Hospital and Research Centre, Bangalore, Karnataka, India. The study was conducted from October 2018 to May 2019 after obtaining ethical clearance and consent.

A total of 337 sputum samples from all the patients with symptoms and signs, suggestive of PTB, as well as chest X-ray showing features of PTB, during the study period from October 2018 to May 2019 were included in the study. The power of the study was 1. Early morning, deep coughed sputum specimens in sterile containers were considered for the study. Sputa from clinically suspected PTB patients are included in the study. Patients suspected to have Extra Pulmonary Tuberculosis (EPTB) were excluded from the study. Each sputum sample thus received in the laboratory was divided into two parts; one part was subjected for ZN staining to detect Acid Fast Bacilli (AFB). The second part was used to carry out CBNAAT for detection of *M. tuberculosis* and RIF resistance as per Revised National Tuberculosis Control Program (RNTCP) guidelines. Due to resource limited settings, the Isoniazid (INH) resistance could not be performed.

### GeneXpert MTB/RIF

After thorough rinsing of the oral cavity with clean water, all specimens were collected in pre-sterilised Falcon tubes with three layer packing system. Sputum specimens were processed according to the GeneXpert Dx system operator manual given by Central TB Division, Government of India, Guidance Document for Use of CBNAAT under RNTCP [10,11]. The assay is designed for extraction, amplification and identification of *rpoB* gene of *M. tuberculosis*, which accounts for more than 95% of mutations associated with RIF resistance. CBNAAT exhibits high degree of specificity by using three specific primers and 5 unique molecular probes [12].

## RESULTS

ZN staining was done for 337 samples of the patients who were having a history suggestive of PTB. Out of these, 36 (10.68%) sputum samples were AFB positive and 301 (89.3%) were negative [Table/Fig-1]. During the above period, 107 cases with high clinico-radiological presumption were subjected to CBNAAT examination. Of them, 41 (38.3%) were confirmed positive microbiologically. Of the 41 microbiologically confirmed cases, 29 were positive by sputum microscopy and

12 smear negative cases were positive by CBNAAT [Table/Fig-2]. Two cases were rifampicin resistant and 39 cases were rifampicin sensitive. The sensitivity, specificity, PPV and Negative Predictive Value (NPV) were 100%, 84.61%, 71% and 100%, respectively.

| | No. of cases examined | No. of cases diagnosed | % |
|---|---|---|---|
| ZN Stain | 337 | 36 | 10.68 |
| CBNAAT | 107 | 41 | 38.31 |

**[Table/Fig-1]:** ZN stain smears positive vs. CBNAAT positive.

| | Smear positive (29) | Smear negative (78) |
|---|---|---|
| CBNAAT positive (41) | 29 | 12 |
| CBNAAT negative (66) | 0 | 66 |

**[Table/Fig-2]:** ZN stain smear positive vs CBNAAT positive in all CBNAAT cases.

## DISCUSSION

India accounts for around one-fourth of the global TB cases. Detection of AFB in sputum smear is a simple, rapid, inexpensive and very specific diagnostic tool for PTB. However, its major limitation is low sensitivity. The World Health Organisation (WHO) has endorsed the use of CBNAAT as a rapid diagnostic test for the diagnosis of TB and prioritised areas like drug-resistant TB, paediatric TB, TB-HIV co-infection, extra-pulmonary TB, and sputum smear-negative PTB for use of CBNAAT [13]. In this study, mean age of PTB patients was 45±18.30 years (mean±SD) with male preponderance (76%).

Dewan R et al., [9] in their study found that mean age of patients was 35±9 years; 69% of were in 20-40 years age group and 76% were males. Diabetes mellitus (n=35) was a common co-morbid condition in this study population. Sensitivity of conventional sputum smear microscopy by ZN staining was very low (10.68%). Geleta DA et al., [14] have also found a very low sensitivity (9.3%) of sputum smear for AFB. In this study, overall CBNAAT was positive in 38.31% PTB cases. Sensitivity of CBNAAT varied significantly between 100% in sputum smear-positive PTB and 15.38% in sputum smear-negative PTB. In studies conducted by Mukherjee S et al., [13] and Geleta DA et al., [14] showed similar results of very high sensitivity of CBNAAT in smear positive cases have been reported. Mohanty T et al., [15] and Dewan R et al., [9] reported sensitivity of 32% and 32.58% of CBNAAT in smear negative PTB, which correlates with present study.

RIF resistance was detected in two (1.86%) cases of PTB. Sensitivity (100%), specificity (100%), PPV (100%), and NPV (100%) of CBNAAT for identifying RIF resistance were very high in this study. This finding is supported by the study of Sharma SK et al., [1] where sensitivity and specificity of CBNAAT was found to be 94.5%-99% and 97.7%-99.3% respectively. Hence, to summarise, PTB was more common in males of 20-40 years of age and diabetes was the commonest co-morbidity in these patients. Sputum smear microscopy exhibited a low sensitivity, while the sensitivity, specificity, PPV and NPV of CBNAAT were 100%. Resistance to Rifampicin was detected in two patients.

## LIMITATION AND FUTURE RECOMMENDATION

INH resistance could not be performed and a larger number of smear negative samples can be studied in future.

## CONCLUSION

CBNAAT is a very useful and rapid test for diagnosis of PTB, but its limitation is that its sensitivity is modest in smear-negative PTB. Despite its modest sensitivity in smear-negative PTB, CBNAAT adds significantly to the number of microbiologically confirmed PTB in these patients. The main advantage of CBNAAT lies in its rapid diagnostic ability and early detection of RIF resistance. It also helps to avoid injudicious use of anti-TB drugs.

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