ORIGINAL RESEARCH PAPER

Microbiology

CBNAAT: A RAPID DIAGNOSTIC TOOL IN DETECTION OF PULMONARY TUBERCULOSIS FROM RURAL DISTRICT OF RAJOURI (J&K).

KEY WORDS: CBNAAT , *Mycobacterium* Tuberculosis, RIF resistance, Zeihl Neelson staining

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ABSTRACT

Introduction: Pulmonary tuberculosis remains a major health concern worldwide. Rapid simultaneous detection of *Mycobacterium* Tuberculosis (MTB) and RIF resistance are very essential for effective disease management. Traditional diagnostic protocols include history, chest x-ray and sputum collection and staining for acid fast bacilli along with culture in conventional media. Implementation of cartridge based nucleic acid amplification tests (CBNAATs) in diagnosis has augmented the detection rates. **Aim:** The study was carried out to evaluate the utility of CBNAAT test in early detection of MTB in sputum samples in suspected cases of Pulmonary Tuberculosis from Rajouri district of Jammu and Kashmir. **Material and Methods:** Sputum samples from 2159 patients having symptoms suggestive of PTB were included in this study over a period of 2 years from January 2019 to December 2020. Sputum samples for Zeihl Neelson staining and CBNAAT were processed. RIF resistance was detected by CBNAAT. **Results:** 360 (16.67%) were found to be positive by CBNAAT. Rifampicin resistance was present in 5 (1.38%) of the 360 positive cases. Out of 2159 samples tested, 55 (2.54%) sputum samples were positive by smear microscopy. Males (57.90%) constituted majority of our study population. The most common age group involved in the study was between 41-50 years (29.51%). Cough was the most common symptom presented by 69.75%, followed by fever 56.27%. Consolidation (33.44%) and fibrocavitory lesion (27.79%) were the most common Radiological findings in the present study. **Conclusion:** CBNAAT is an efficient, reliable, and confirmatory technique for MTB. It has an added advantage to assess the rifampicin drug sensitivity. All this contribute hugely in diagnosis and management of Pulmonary Tuberculosis.

INTRODUCTION

Tuberculosis remains the top infectious killer in the world claiming close to 4000 lives a day. Millions of people continue to fall ill with TB- a preventable and curable disease each year. It is estimated that India accounts for 25% of global tuberculosis (TB) burden of the world. According to Global TB Report 2019, 26 lakh 40 thousand developed TB and 4 lakh 45 thousand TB deaths were reported in India. About 1 lakh 24 thousand of drug- resistant TB cases were reported in the year 2019. [1]

Tuberculosis can potentially cause infection in any system or organ of the body. Pulmonary tuberculosis (PTB) is the most common presentation, but even today, the diagnosis of TB remains elusive because no biochemical or serological test is valid and acceptable for diagnosis of PTB.[2] Smear microscopy is the cornerstone for the diagnosis of Pulmonary TB in resource-limited settings; it has only modest (35-80%) sensitivity and a poor Positive Predictive Value (PPV). Mycobacterial culture, though is gold standard, usually takes 2-6 weeks for final result and requires technical expertise. [3]

Early and rapid diagnosis and treatment of the case is the most important step in reducing the TB incidence. The urgent need for accurate, feasible, rapid, and affordable TB diagnostic tests for use in resource-limited settings push WHO to reform the new guidelines and diagnostic methods for TB. In December 2010, WHO endorsed CBNAAT or Gene Xpert MTB/RIF1 for use in TB laboratories and in India it was adopted by RNTCP in 2012. The CBNAAT assay consists of a closed system that is based on real-time polymerase chain reaction (RT-PCR). It requires minimal technical expertise in the diagnosis of TB and detects rifampicin resistance within 2 hours. The GeneXpert utilizes a DNA-PCR technique for simultaneous detection of *M. tuberculosis* and rifampicin resistance-related mutations. [4]

The modern CBNAAT testing equipment has been made available in many District Tuberculosis Centers (DTCs). Samples are sent to these DTCs from different blocks of the district. Drug testing facilities are provided in regional laboratories scattered in different zones of the states. Samples are sent to these reference laboratories from a number of districts and results are dispatched to the source hospitals.[5]

Rajouri is one of the rural district of Jammu and Kashmir with high prevalence of Tuberculosis. With this background in mind, the study was carried out to evaluate the utility of CBNAAT test in early detection of MTB in sputum samples obtained from suspected cases of Pulmonary Tuberculosis.

MATERIAL AND METHODS

This was a retrospective study conducted by Department of Microbiology, Government Medical College, Rajouri in collaboration with District Tuberculosis Centre, Rajouri, J&K. Sputum samples were received from various blocks of Rajouri district (Manjhakote, Thanamandi, Buddhal, Darhal, Lambheri, Sunderbani, Nowshera). The study was carried over a period of 2 years ie, from January 2019 to December 2020.

Inclusion Criteria

Patients with clinical suspicion of pulmonary tuberculosis based on symptoms (e.g., cough more than two weeks, hemoptysis, fever, loss of weight and night sweats) and Radiological features (e.g., nodule, consolidation, cavitation and other opacities) were included in the study.

Exclusion Criteria

Samples macroscopically resembling saliva and blood stained samples were excluded.

Patients suspected to have Extra Pulmonary Tuberculosis (EPTB) were also excluded from the study.

Sample Collection

A total of 2159 sputum samples of the patients with symptoms suggestive of pulmonary tuberculosis including both new cases and on treatment were collected and tested over a period of 2 years.

Early morning, deep coughed sputum was considered for the study. All the details of the patients like Name, Address, Age, Sex, Treatment received and Name of the referring centre

were noted down. Sputum samples were collected in pre-sterilized falcon tubes after thorough rinsing of the oral cavity with clean water.

Sample Processing

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. [6,7] For CBNAAT examination the sample reagent were added at a 3:1 ratio to clinical specimens. The closed specimen container was manually agitated twice during a 15 minute period at room temperature, before 2 ml of the inactivated material (equivalent to 0.5 ml of decontaminated pellet) was transferred to the test cartridge.

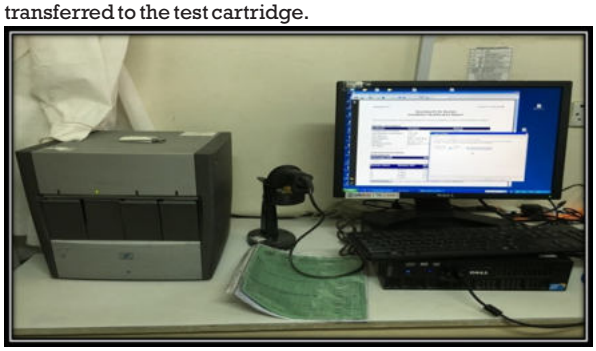

Figure 1: CBNAAT Module in District Tuberculosis Centre, Rajouri, J&K

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 providing results within 100 minutes. CBNAAT exhibits high degree of specificity by using three specific primers and 5 unique molecular probes. [8]

The results were distinguished as MTB detected, MTB not detected, RIF resistance detected; RIF resistance not detected; RIF resistance indeterminate; or invalid with the help of positive beacons, their detection timing and sample processing controls. [9]

RESULTS

During the study period of 2 years, a total of 2159 clinical pulmonary tuberculosis (PTB) cases were enrolled. Of the total samples tested, 360 (16.67%) were found to be positive by CBNAAT. Rifampicin resistance was present in 5 (1.38%) of the 360 positive cases.

ZN staining was performed on all samples. Total number of samples positive by smear microscopy were 55 (2.54 %). CBNAAT thus confirmed more cases of TB than AFB smear (360 vs 55). **(Table 1)**

Table 1: Diagnostic Performance of Sputum CBNAAT versus ZN Stain

Laboratory Method	Result		Total
	Positive	Negative	
CBNAAT	360 (16.67%)	1799 (83.32%)	2159 (100%)
ZN Stain	55 (2.54%)	2104 (97.45%)	2159 (100%)

Majority of the cases included in present study were in the age group of 41-50 years (29.51%) followed by 51-60 years (24.17%) and least were in the age group < 10 years (3.34%). **(Table 2)**

Table 2: Age-wise distribution of study population

Age-group	N	%age
<10 yrs	72	(3.34%)
10-20 yrs	149	(6.90%)
21-30 yrs	168	(7.78%)
31-40 yrs	261	(12.09%)
41-50 yrs	637	(29.51%)
51-60 yrs	522	(24.17%)
>60 yrs	350	(16.21%)
Total	**2159**	**100%**

1250 (57.90%) males and 909 (42.10%) females were included in this study. **(Table 3)**

Table 3: Gender-wise distribution of study population

Gender	N	%age
Male	1250	(57.90%)
Female	909	(42.10%)
Total	2159	(100%)

Among 2159 patients, cough was the most common symptom presented by 1506 patients (69.75%), followed by fever in 1215 patients (56.27%), weight loss was seen in half of the patients (50.94%) and haemoptysis in 355 patients (16.44%). **(Table 4)**

Table 4: Symptomatology of Study Population

Symptom	N	%age
Cough	1506	69.75%
Fever	1215	56.27%
Weight loss	1100	50.94%
Loss of Appetite	912	42.24%
Night sweats	251	11.62%
Hemoptysis	355	16.44%

Radiological features compatible with the diagnosis of tuberculosis included consolidation (33.44%), fibrocavitory lesion (27.79%), nodular opacities (11.25%), miliary shadows (3.6%) and others (16.44%). **(Table 5)**

Table 5: Chest Radiographic findings in study population

Radiological Findings	N	%age
Consolidation	722	33.44%
Fibrocavitory lesion	600	27.79%
Nodules	243	11.25%
Infiltration	526	24.36%
Milliary shadow	78	3.6%
Others	355	16.44%

DISCUSSION

The World Health Organisation (WHO) has endorsed the use of CBNAAT as a rapid diagnostic test for the diagnosis of TB and prioritized areas like drug-resistant TB, paediatric TB, TB-HIV co-infection, extra-pulmonary TB, and sputum smear-negative PTB for use of CBNAAT.[10]

In the present study, the positivity rate of CBNAAT and ZN staining was 16.67% and 2.54% respectively. Similar results were seen in studies conducted by Gaur V *et al* [2] and R.Vanishree *et al* [11] where positivity rate of CBNAAT was significantly higher as compared to ZN staining.

RIF's resistance by CBNAAT is considered to be a surrogate marker of MDR-TB. In our study, Rifampicin resistance was present in 1.38% which was in concordance with Basavaraj V.P *et al* [12] (2.49%). However in comparison to this, study conducted by Ikuabe PO *et al* [13] has reported rifampicin resistance in 14.7% which was significantly higher. Different levels of resistance may be due to variation in mutation, co-infection with HIV and inadequate or inappropriate dosage of anti-TB therapy. [2]

Majority of the cases were in the age group of 41-50 years with male predominance. This was in agreement with studies conducted by Subbarao *et.al* [14] and Desai K *et al* [15]. The most common symptoms in our study were cough (69.75%) and fever (56.27%). Similar findings were seen in study conducted by Avashia *et al* [16] as they found fever (69.4%) and cough (72.2%) as the main symptom. Among radiological finding, consolidation was most common finding (71.91%)

followed by fibrocavitory lesion (30.13%) in our study, which was nearly similar with the study done by Panda RK *et al*[17].

CONCLUSION

This study concludes that in less accessible and resource-limited rural settings where establishing a sophisticated laboratory for culture to the prescribed biosafety levels is difficult, CBNAAT provides a very good detection laboratory method. Extensive use of this assay thereby facilitates early treatment decisions and curbing transmission of Tuberculosis. Our study highlighted the usefulness of the CBNAAT over and above the traditional smear microscopy for significantly higher positive results. It also has an added advantage of detection of multi-drug resistant cases, thus contributing as a milestone in 'End TB' strategy.

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