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Clinical Utility of CBNAAT in the Diagnosis of Pulmonary Tuberculosis – Our Experience

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Abstract
Introduction: *WHO End TB Strategy calls for the early diagnosis of TB and universal drug susceptibility testing (DST). Molecular assays based on nucleic acid amplification techniques such as polymerase chain reaction have been developed for rapid TB diagnosis and are being implemented in developing countries. CBNAAT is cartridge based nucleic acid amplification test an automated polymerase chain reaction test utilizing the GeneXpert platform.*
Aim and Objectives: *The study aims to determine the clinical utility of CBNAAT in the diagnosis of pulmonary TB in a tertiary care center.*
Materials and Methods: *129 cases OF PRESUMPTIVE PTB CASES were included in the study after exclusion criteria. All patients were subjected to routine blood investigations and Sputum, BAL specimens for AFB smear, CBNAAT and liquid cultures. Results were analysed using SPSS software.*
Results: *Of the 129 cases males were 82 (63.5%), and females were 47(36.5%).*
The sensitivity, specificity, PPV and NPV of all samples for AFB smear were 54.79%, 98.43%, 95.24% and 79.11% respectively. The sensitivity, specificity, PPV and NPV of all samples for CBNAAT were 84.93%, 92.13%, 86.11% and 91.41% respectively.
The sensitivity and specificity of smear positive, culture positive cases was (26/29) 96.30% and 100% with a positive predictive value 100% and negative predictive value of 66.67%. The sensitivity and specificity of smear negative culture positive cases was 70%, 92.5% respectively with a PPV of 70% and NPV of 92.5%.
Conclusions: *CBNAAT has good clinical utility in smear negative culture positive cases both in pulmonary cases. CBNAAT has higher diagnostic efficacy than AFB smear in all pulmonary samples.*
Keywords: *Pulmonary Tuberculosis, Molecular Diagnostics, CBNAAT, Genexpert.*

Introduction

Tuberculosis (TB) remains a large-scale public health problem. Key global priorities for tuberculosis (TB) care and control include improving case-detection and detecting patients earlier, including patients with smear-negative. The WHO End TB Strategy calls for the early diagnosis of TB and universal drug susceptibility testing (DST), highlighting the critical role of laboratories

in the post-2015 era for rapidly and accurately detecting TB and drug resistance. Molecular assays based on nucleic acid amplification techniques such as polymerase chain reaction (PCR) have been developed for rapid TB diagnosis and are being implemented in developing countries[(1-8)].

India has the highest number of TB cases in the world, with over 2 million active TB cases every year[9]. One fourth of the global incident TB cases occur in India annually[10]. Early and accurate diagnosis is the first critical step in controlling TB. The control of TB is hampered by slow, insensitive diagnostic methods, particularly for the detection of drug resistant forms and in patients with human immunodeficiency virus infection. Early detection is essential to reduce the death rate and interrupt transmission, but the complexity and infrastructure needs of sensitive methods limit their accessibility and effect.

The sensitivity of smear microscopy and its inability to detect drug resistance limits its impact on TB control. Culture methods and drug susceptibility testing is complex, time consuming, taking around 6-8 weeks. While patients await diagnosis, they may be inappropriately treated leading to their disease progression. This results in an increased chance of morbidity from tuberculosis. They continue to transmit drug-resistant TB to others, especially family members and resistance may become amplified[11, 12].

To respond to the urgent need for simple and rapid diagnostic tools in high-burden countries, a new diagnostic test has been developed by Cepheid & FIND, a rapid, fully automated test based on PCR which detects DNA directly from clinical specimens and also detects rifampicin resistance. This test is designed to purify, concentrate, amplify and identify targeted rpo B nucleic acid sequences, and delivers answers from unprocessed samples in 120min, with minimal hands on time[13, 14,15,16].

Xpert MTB/RIF is cartridge based nuclic acid amplification test (CBNAAT) an automated polymerase chain reaction test utilizing the GeneXpert platform. Xpert MTB/RIF is a single test that can detect both Mycobacterium tuberculosis complex and rifampicin resistance within 2 hours after starting the assay with minimal hands-on technical time[17].

The test procedure may be used directly on clinical specimens, either fresh sputum samples or sputum pellets, which are obtained after decontaminating and concentrating the sputum. In both cases, the test material is combined with the reagent and incubated at room temperature for 15 minutes. After incubation, 2 ml of the treated sample are transferred to the cartridge and the run is initiated[17].

Aims

The study aims to determine the clinical utility of CBNAAT in the diagnosis of pulmonary TB in a tertiary care center.

Objectives

1. To compare the sensitivity and specificity of CBNAAT with AFB smear in the diagnosis of pulmonary tuberculosis.
2. To determine sensitivity and specificity of CBNAAT in smear positive culture positive cases (SPCP) and smear negative culture positive cases (SNCP).

Materials & Methods

It is a single center observational study done at a tertiary care hospital where IRL lab facility is there.

We have facilities for CBNAAT, LPA, LIQUID CULTURES are available.

Both inpatients and outpatients attending Government chest hospital with presumptive PTB (symptoms of cough more than 2 weeks, fever, chest pain, hemoptysis, loss of weight, loss of appetite) were enrolled in the study after taking informed written consent.

About 129 cases were included in the study after exclusion criteria during a period of 12 months.

Demographic data noted for all patients and subjected to following routine investigations.

- Complete blood Profile
- Chest x-ray
- Liver function tests, renal function tests
- ICTC ((Integrated counseling and testing centre)

Sputum **Sample collection**- was done as per the standard protocol of RNTCP (Revised national tuberculosis control programme) from patients who has productive cough. And who does not have sputum production induced sputum was collected with 3% Nacl neubulizations. Sputum samples were sent for AFB smear, sputum CBNAAT and liquid culture- considering it as gold standard. For patients with sputum smear negative bronchoscopy was done and bronchial washings taken and sent for AFB staining, CBNAAT and liquid culture.

The study did not affect the treatment of patients in any manner. Confidentiality was maintained during and after the study owing to the stigma associated with the diseases.Data analysis was done using SPSS software.Statistical analysis done using percentages, mean,standard deviation, sensitivity and specificity formulas.

Results

Of the 129 cases males were 82 (63.5%), and females were 47(36.5%). (FIG-1) Maximum number of cases fall in the age group of 20 to 50 years 85 (65.89%), most economically productive age group.(TABLE-1)

Among 129 cases only 29 cases were (both sputum, BAL) smear positive and 100 cases (were sputum, BAL) smear negative. Of the 29 smear positive cases 26 cases have shown positive results with (sputum, BAL) CBNAAT and culture positivity in 27 cases. Only in 2 cases sputum culture was negative. Among the 100 smear negative cases 20 cases were culture positive and CBNAAT could be able to detect M-TB in 20 cases of which only 14 cases were culture positive and 6 cases were culture negative.

Of the total 129 cases Culture positivity was seen in 47/129(36.43%) cases of which 40 (85.1%)cases were detected by CBNAAT and only 26 (20.15%)cases were detected by smear for AFB. Culture was negative in 82 cases of which 6 cases (false positives) were CBNAAT positive and 76 cases were true negatives. (TABLE-2)

The sensitivity of CBNAAT in bronchial washings is 91.3% compared to 52.7% of AFB smear. The specificity of CBNAAT is 93.06% where as it is 100% for AFB smear. The sensitivity, specificity and positive predictive value of CBNAAT in sputum are 78.26%, 88.89% and 94.74% respectively which are higher than that of AFB smear which are 60.87%, 77.78% and 87.5% respectively. (table -3).

The sensitivity, specificity, PPV and NPV of all samples for AFB smear were 54.79%, 98.43%, 95.24% and 79.11% respectively. The sensitivity, specificity, PPV and NPV of all samples for CBNAAT were 84.93%, 92.13%, 86.11% and 91.41% respectively. (Table- 4).

The sensitivity and specificity of smear positive, culture positive cases was (26/29) 96.30% and 100% with a positive predictive value 100% and negative predictive value of 66.67%. The sensitivity and specificity of smear negative culture positive cases was 70%, 92.5%respectively with a PPV of 70% and NPV of 92.5%. (Table- 5).

Table-1: Age Distribution

Age Group	No. of Cases
0-10 years	1
10-20 years	10
20-30 years	20
30-40 years	35
40-50 years	30
50-60 years	20
60-70 years	12
>70 years	1

Table-2: Xpert MTB/RIF Assay versus AFB and Culture Status in all Pulmonary Samples

PULMONARY SAMPLES		AFB - ve		AFB + ve	
		Culture Positive	Culture Negative	Culture Positive	Culture Negative
Xpert MTB /RIF	MTB Detected	14	6	26	0
	MTB Not detected	6	74	1	2

Table-3: Comparision of Sensitivity, Specificity, PPV and NPV OF CBNAAT and AFB Smear in Individual Pulmonary Samples

Pulmonary Samples	BRONCHIAL WASHINGS		Sputum	
	AFB Smear	CBNAAT	AFB Smear	GeneXpert
Sensitivity	52.17 % (30.59%-73.18%)	91.3 % (71.96%-98.93%)	60.87 % (38.54%-80.29%)	78.26 % (56.3%-92.54%)
Specificity	100 % (95.01%-100%)	93.06 % (84.53%-97.71%)	77.78 % (39.99%-97.19%)	88.89 % (51.75%-91.72%)
PPV	100% (73.54%-100%)	80.77 % (60.65%-93.45%)	87.5 % (61.65%-98.45%)	94.74 % (73.97%-99.87%)
NPV	86.7 % (77.52%-93.19%	97.1 % (89.92%-99.65%)	43.78 % (19.75%-70.12%)	61.54 % (31.58%-86.14%)

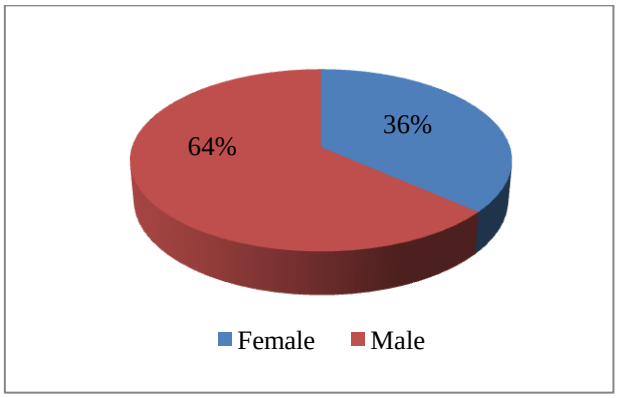
Table-4: Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value In AFB SMEAR AND CBNAAT

	AFB Smear	CBNAAT
Sensitivity	54.79 % (42.70%-66.48%)	84.93 % (74.64%-92.23%)
Specificity	98.43 % (94.43%-99.81%)	92.13 % (86.00%-96.16%)
PPV	95.24 % (83.84%-99.42%)	86.11 % (75.94%-93.13%)
NPV	79.11 % (71.94%-85.17%)	91.41 % (85.14%-95.63%)

Table-5 Comparision of Sensitivity, Specificity, PPV and NPV of CBNAAT in Smear-Postive, Cultutre-Positive and Smear-Negative, Culture - Positive Pulmonary Samples

	PULMONARY SAMPLES			
	Sensi-tivity	Speci-ficity	PPV	NPV
Smear Positive Culture Positive cases	96.30% (81.03%-99.91%)	100 % (15.81% -100%)	100 % (86.77%-100%)	66.67 % (9.43%-99.16%)
Smear Negative Culture Positive cases	70 % (45.72%-88.11%)	92.5 % (84.39%-97.2%)	70 % (45.72%-88.11%)	92.5 % (84.39%-97.2%)

Fig 1: Gender distribution of all subjects



Discussion

India has the highest TB burden in the world. Early detection of tuberculosis, determining drug resistance and prompt treatment is essential to decrease the spread of disease. CBNAAT, a fully automated test based on PCR, has been approved by WHO for early detection of tuberculosis. In this study, the clinical utility of CBNAAT in the diagnosis of pulmonary tuberculosis has been investigated.

In the present study majority (65.9%)of the patients belong to the age group of 20 to 50 years most economically productive age group. Gender and age distribution in the present study were similar to other studies by Arzu et al, Bodmer et al and Rachow et al.

In a study on utility of CBNAAT in bronchial washings by Theron G et al.,[18] in which only 58% were detected by smear microscopy compared with 93% by CBNAAT, the specificity of CBNAAT in bronchial washings was 96%. In this study the sensitivity of CBNAAT in bronchial washings is 91.3% compared to AFB smear which is only 52.17% and the specificity of AFB smear and CBNAAT are 100% and 93.06% respectively which are compatible with the above study.

In this study the overall sensitivity of CBNAAT was 84.93% and the specificity was 92.13%, where as for AFB smear, they are 54.79% and 98.43% respectively. The overall positive predictive value of CBNAAT and AFB smear are 86.11% and 95.24% respectively. The negative predictive value of CBNAAT and AFB smear are 91.41% and 79.11 % respectively.

In the present study, the sensitivity of CBNAAT is 96.3% in smear-positive, culture-positive pulmonary specimens and 70% in smear-negative, culture-positive pulmonary specimens. The specificity of the test is 100% in smear-positive, culture-positive cases and 92.5% in smear-negative, culture-positive cases. In a study done by Arzu NZ et al.,[19] the sensitivity of CBNAAT test was 100% in smear-positive, culture-positive pulmonary specimens and 74.2% in smear-negative, culture-positive pulmonary specimens. In another study done by Maynard-Smith L et al.,[20] the pooled summary estimates of sensitivity when testing smear positive and smear negative samples were 95% and 69% respectively where as specificities were typically very high up to 98%. In a study done by Bodmer et al[21], the sensitivity of the test was 99.8% in smear-positive, culture-positive pulmonary specimens and 90.2%in smear- negative, culture-positive pulmonary specimens.

In the previous studies, the sensitivity of the MTB/RIF test for detecting RIF resistance was between 94.4 to 100%and the specificity was 98.3 to 100%. In our study, CBNAAT detected 12 rifampicin resistance cases.

Conclusion

CBNAAT has higher sensitivity (84.93%) than AFB smear (54.79%) in all pulmonary samples. The sensitivity of CBNAAT in smear-positive, culture-positive and smear-negative, culture-positive pulmonary samples is 96.3% and 70% respectively. This shows that CBNAAT has good clinical utility in smear negative culture positive cases both in pulmonary cases. In bronchial washings, CBNAAT showed a sensitivity of 91.3% when compared to AFB smear (52.17%).In sputum, CBNAAT showed a sensitivity of 78.26% when compared to AFB smear (60.87%). The study concludes that CBNAAT has higher diagnostic efficacy than AFB smear in all pulmonary samples.

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