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# EVALUATION OF CATRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST IN DIAGNOSIS OF TUBERCULOSIS IN PATIENTS ATTENDING TO GOVERNMENT GENERAL HOSPITAL, ANANTHAPURAMU

**Dermatology**

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## ABSTRACT

**Background:** Diagnosis of Pulmonary tuberculosis is mostly relied on sputum smear microscopy and sputum for mycobacterium culture and chest radiographs. In recent years, CBNAAT has been recommended by World Health Organization diagnostic test.

**Materials and Methods:** All retreated cases of Pulmonary and extra pulmonary TB, newly detected smear-negative and all PTB-HIV cases were recruited for the study and sputum for CBNAAT was performed in all of them.

**Results:** Out of 2561 suspected cases of TB, 447 were sputum smear negative and 2114 cases were sputum smear positive. Overall Sensitivity of CBNAAT was 64%, Specificity 94%, PPV and NPV 70% and 87% respectively. TB-HIV co-infected patients attributed to 9.36%. 95.36% were Rifampicin sensitive, 3.96% were resistance & 0.67% were rifampicin intermediate sensitive.

**Conclusion:** CBNAAT is found to be better diagnostic method and has an additional advantage of identifying rifampicin resistance with high sensitivity and specificity.

## KEYWORDS

Pulmonary tuberculosis, extra Pulmonary tuberculosis, CBNAAT.

## INTRODUCTION

Tuberculosis is the deadliest communicable disease and most common opportunistic infection among the PLHIV (Persistent Lymphadenopathy Human Immunodeficiency Virus) and HIV TB coinfected individuals are at high risk of death [1]. In PLHIV cases, there is scanty sputum production, lack of caseous necrosis leading to decreased number of bacilli in sputum and high incidence of non tubercular mycobacterial infection [2]. These factors decrease the specificity of sputum microscopy as diagnostic tool.

Tuberculosis (TB) still continues to be one of world's most important infectious causes of morbidity and mortality among adults. Diagnosis of Pulmonary tuberculosis is mostly relied on sputum smear microscopy and sputum for mycobacterium culture and chest radiographs. In recent years, CBNAAT has been recommended by World Health Organization diagnostic test.

Catridge based nucleic acid amplification test (CBNAAT) is a recently introduced PCR (Polymerase chain reaction) based method for detection of TB. It also detects rifampicin resistance as it targets the rpoB gene of Mycobacteria. CBNAAT is an M.tb specific automated, catridge based nucleic acid amplification assay, having fully integrated and automated amplification and detection using real time PCR [3].

Its role in diagnosing TB in PLHIV has not been studied widely in India. With this back ground, this study was carried out to evaluate the role of CBNAAT in early diagnosis of TB in smear positive, smear negative patients, detection of M.tuberculosis in sputum by CBNAAT compared to conventional sputum microscopy in pulmonary TB and Coinfection of TB & HIV and also to detect Rifampicin resistance in confirmed TB cases.

## MATERIALS AND METHODS

The study group consisted of 2561 subjects, who were suspected of TB among patients attending to Chest OPD (Out Patient Department) at Government General Hospital, Ananthapuramu.

**Study period:** January 2018 to June 2019

**Inclusion criteria:** All Adult patients suspected of M.tuberculosis infection who were clinically and radiologically proven and both smear positive and negative samples were included.

**Exclusion criteria:** Patients with less than 12 yrs age were excluded from the study group.

**Sample:** Sputum, Body fluids (pleural fluid, CSF)

**Sputum analysis:** Early morning deeply coughed out sputum sample was collected. 2 samples of at least 1 ml sputum were sent for microscopy 24 hours apart to the chest and TB center at GGH, Ananthapuramu in a sterile container. Sputum microscopy for Acid fast bacilli (AFB) was evaluated. Sputum smears after Ziehl-Neelson staining was examined under oil immersion microscopy. A minimum of 1 slide positive even for single AFB/100 fields were taken as positive for Mycobacterium tuberculosis and a minimum of two sputum negative for AFB evaluate for 100 fields were declared as negative.

**CBNAAT:** Sputum for CBNAAT – one sputum sample of 1 ml was collected in a sterile container and was analyzed by CBNAAT on Xpert MTB/RIF manufactured by Cepheid, endorsed by WHO (2016). The sample was diluted with three times with the reagent, incubated at room temperature and loaded into the catridge for automated analysis with results in 100 minutes. Detection of Mycobacteria and rifampicin resistance was carried-out in the same setting.

**Statistical analysis:** Data analysis was done using SPSS (Statistical package for social sciences) software version (22.0.0.0) and Diagnostic efficacy with McNemar test.

## RESULTS

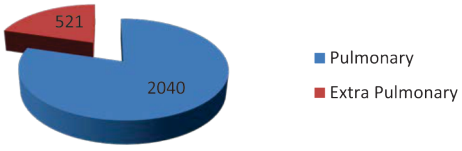
The mean age of study population was 34±7 years. Most patients (69.9%) were in the age group of 21-40 years (Table 1). Majority patients in the present study were men with 74%, 23% were women and remaining 3% were transgenders.

**Table 1. Age distribution of Study Population**

| Age in years | Total No. of patients | Percentage |
|---|---|---|
| **18-20** | 202 | 7.8% |
| **21-40** | 1792 | 69.9% |
| **41-60** | 563 | 21.9% |
| **61 & Above** | 4 | 0.15% |
| **Total** | 2561 | 100% |

Out of 2561 TB suspected samples, 2040 (79.6%) samples were related to Pulmonary and remaining 521 (20.3%) were Extra Pulmonary cases (Fig 1).

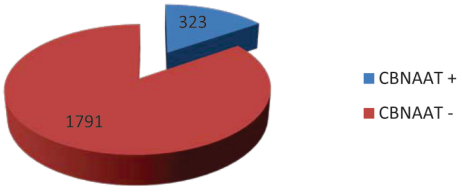
**Fig 1.Distribution of pulmonary and extra pulmonary samples**




A total of 2561 suspected M.Tb patients were screened by microscopy. Out of 2561 samples, 2114 (82.5%) were smear negative and 447 (17.4%) were smear positive. Sputum microscopy was found to be 20% sensitive and 90% specific with PPV and NPV 85% and 87% respectively.

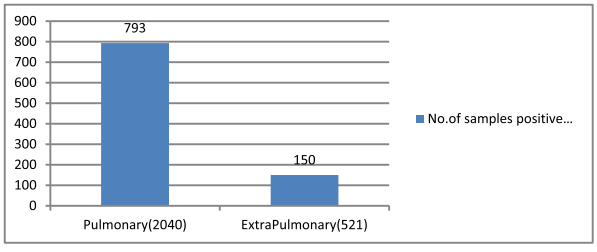
The incidence of TB found in our community was 36.8% i.e., 943 positives among 2561 suspected population. All smear positive cases were CB NAAT positive. Among Smear negative 2114 cases, CBNAAT showed positive in 496 (23.4%) cases and negative in 1618 (76.5%) cases Percentage of CBNAAT positivity in smear negative cases was 23.4% (Fig 2). CBNAAT was found to be 47% sensitive and 100% specific with PPV and NPV 76% and 80% respectively.

**Fig 2. Positivity of CBNAAT in Smear negative cases**




Out of 2040 Pulmonary TB suspected cases, 793 (38.9%) were confirmed and out of 521 (28.9%) extrapulmonary TB suspected cases, 150 were confirmed. Out of 943 CBNAAT positive cases, 84.09% had pulmonary TB and 15.9% had extrapulmonary TB (Fig 3).

**Fig 3. CBNAAT positive in Pulmonary and Extrapulmonary cases**




Out of 943 positives identified by CBNAAT, 899 (95.36%) were sensitive to RIF (rifampicin) while RIF resistance was detected in 36 (3.96%) samples, 08 (0.67%) samples had shown intermediate sensitive (Table 2).

**Table 2. Percentage of Rifampicin Resistance detected by CBNAAT**

| Drug Resistance | No. of Samples | Percentage (%) |
|---|---|---|
| Rifampicin Sensitive | 899 | 95.36 |
| Rifampicin Resistance | 36 | 3.96 |
| Rifampicin Intermediate Sensitivity | 08 | 0.67 |
| Total | 943 | 100 |

Out of 2561 TB suspected cases, 1943 (75.86%) were HIV positive and 618 (24.13%) were HIV negative. Out of 1943 HIV positives, 182 were CBNAAT positives (9.36%) and 1761 were CBNAAT negative. No TB detected in HIV negative patient specimens tested by CBNAAT (Table 3).

**Table 3. Percentage of CBNAAT in HIV positives**

| | No. of HIV positive samples | Percentage |
|---|---|---|
| CBNAAT +ve | 182 | 9.36 |
| CBNAAT -ve | 1761 | 90.64 |
| Total | 1943 | 100 |

## DISCUSSION

India accounts for around one fourth of the global tuberculosis cases [4]. Detection of AFB in sputum smear is a simple, rapid, inexpensive and very specific for diagnosis for PTB,its limitation is its low sensitivity [5,6]. There was a need for a newer rapid diagnostic test for PTB with improved sensitivity and specificity. WHO has endorsed the use of CBNAAT as a rapid diagnostic test for diagnosis of tuberculosis and preprioritized areas like drug resistant tuberculosis, pediatric tuberculosis, TB-HIV coinfection, Extrapulmonary and sputum smear negative PTB for use of CBNAAT [7].

In this study, mean age of PTB patients was 35 years+/-7 years with slight Male predominance (52%). Dewan et al [3] have also reported that mean age of patients in their study was 35±9years and 76% were Male. Sputum smear negative PTB was found to be more common in female. HIV infection was seen in 75% of cases with Male predominant. 7.3% were CBNAAT positive. Dewan et al [3] and Theron G et al [8] have reported a higher value of TB-HIV co-infection, out of 40% and 27% respectively in their studies.

Sensitivity of sputum smear for AFB was very low 20%, Specificity 90%, PPV 85%, NPV 27% in PTB in this study. Geleta DA et al [9] have also found very low sensitivity in their study (9.3%) of sputum smear Microscopy by ZN staining. CBNAAT showed an advantage of increase in diagnosis of microbiologically confirmed PTB cases by 44% over and above the cases diagnosed by sputum smear for AFB. Dewaan et al [3] have also mentioned an increase in microbiologically confirmed PTB diagnosis by 29% and 31% respectively by CBNAAT compared to sputum smear microscopy.

In majority of the studies, microscopy sensitivity ranges from 22 to 41%, specificity from 95 to 100% which is similar to our study findings [10-12].

Overall CBNAAT was positive in 61% PTB cases in this study, but its results varied between 100% in sputum smear positive PTB and 15% in smear negative PTB. Similar results of High sensitivity of CBNAAT in smear positive cases was noted by most of studies ,but sensitivity of CBNAAT in smear negative cases is varied between 35-75 % among several studies.

Specificity of CBNAAT was 94% in this study. Most other studies have also reported a very high specificity of CBNAAT in diagnosis of PTB.

Sensitivity of sputum smear microscopy was20% in this study Patients infected with HIV, TB-HIV coinfected patients 1943 out of 2561 immunocompetent sputum smear positive on Microscopy.

However, no satisfactory significant difference in sensitivity of CBNAA T between sputum smears negative PTB cases with or without HIV coinfection. These findings were similar with observations of Van R et al and Caryquiry G et al [13] in their respective studies. In this study, sputum for CBNAAT resulted in an Increase in Microbiologically confirmed cases by 9% in HIV infected cases. Dewan R et al [3] have also reported a 29% increase in diagnosis of PTB by Application of sputum CBNAAT over sputum smear Microscopy in HIV infected cases.

In present study sensitivity, specificity, PPV, NPV of smear microscopy were 20%, 90%, 85%, 27%.Where as CBNAAT were 64%, 94%, 70%, 86%. Our study results correlated with study results of many other researches. In majority of studies Microscopy Sensitivity ranges from 20 -40%,and specificity ranges from 95-100%.In present study rifampicin sensitivity as detected in 95%,Resistanse in 3.9% of total cases ,Which is similar to study conducted by Hebte et al [14] where the resistance was 4.2%.

The present study showed Nucleic acid based testing is also important in smear negative cases. In Extrapulmonary cases and HIV cases it is often difficult to identify the pathogens by using Acid fast staining method. In such cases CBNAAT plays important role in the detection of pathogen and also drug resistance to rifampicin.

## CONCLUSION

CBNAAT has greater efficacy than conventional sputum microscopy in the diagnosis of TB especially in HIV patients, so can be used for

screening M.Tb and Rifampicin resistance.

Main advantage of CBNAAT lies in its ability in rapid diagnosis and early detection of rifampicin resistance. Sputum for CBNAAT should be sent in all cases of TB-HIV coinfection, sputum-negative PTB for an early and confident diagnosis of PTB and to look for rifampicin resistance.

## REFERENCES


1. Corbette EL, Watt CJ, Walker N et al. The growing burden of tuberculosis:global trends and interactions with the HIV epidemic. Arch intern Med 2003;163 (9): 1009-21.
2. Hopwell P, Pai M, Meher D et al.International standards for tuberculosis care. Lancet infect Dis 2006; 6:710-25.
3. Dewan R, Anuradha S, Khanna A, Garg S, Ish P, Agarwal S, Narayan A. Role of catridge based nuclic acid amplification test (CBNAAT) for early diagnosis of pulmonary tuberculosis in HIV. JIACM 2015; 16(2):114-7.
4. Annual Status Report. Central TB Division. Official website of the Revised National TB Control Programme, Directorate General of Health Services, Ministry of Health & Family Welfare Government of India. 2015. http://www.tbcindia.org.
5. Hopewell PC, Pai M, Maher D, etal. International standards for tuberculosis care. Lancet Infect Dis 2006;6(11):710-25.
6. Getahun H, Harrington M, O'Brien R, et al. Diagnosis of smear negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. Lancet 2007;369(9578):2042-9
7. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children: policy update. Geneva: World Health Organization, 2013. http://www.who.int/tb/laboratory/policy_statements /en/)
9. Theron G, Peter J, van Zyl-Smit RV, et al. Evaluation of the Xpert MTB/RIF Assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. Am J Respir Crit Care Med 2011;184(1):132-40.
10. Geleta DA, Megerssa YC, Gudeta AN, et al. Xpert MTB/RIF assay for diagnosis of pulmonary tuberculosis in sputum specimens in remote health care facility. BMC Microbiology 2015;15:220.
11. Le Pauld P,Cattoir V,Malbruny B,et al, Retrospective observational study of diagnostic accuracy of the Xpert MTB/RIF assay on fibreoptic bronchoscopy sampling for early diagnosis of smear-negative or sputum-scarce patients with suspected tuberculosis. BMC Pulm Med2014:14:137.
12. Barnard DA, Irusen EM,Bruwer jW,et al.The utility of Xpert MTB/RIF performed on bronchial washings obtained in patients with suspected pulmonary tuberculosis in a high prevalence setting,BMC Pulm Med 2015;15:103.
13. Khalil KF, Butt T. Diagnostic yield of bronchoalveolar lavage gene Xpert in smear-negative and sputum-scarce pulmonary tuberculosis J Coll physicians Surg Pak 2015:25(2):115-8.
14. Carriquiry G, Otero L, Gonza´lez-Lagos E, etal. A diagnostic accuracy study of Xpert (®)MTB/RIF in HIV-positive patients with high clinical suspicion of pulmonary tuberculosis in Lima, Peru. PLoS One 2012;7(9):e44626.
15. Sen T, Joshi SR, Udwadia ZF. Tuberculosis and diabetes mellitus: merging epidemics. J Assoc Physicians India 2009;57:399-404.