

## THE STUDY OF UTILITY OF CBNAAT IN DIAGNOSING PULMONARY TUBERCULOSIS IN HIV POSITIVE PATIENTS IN NORTH KARNATAKA

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### ABSTRACT

#### BACKGROUND

Tuberculosis being a global burden, there is a need for early detection and treatment of pulmonary TB. CBNAAT is used as rapid diagnostic test and it is a highly efficient. Twenty five percent of global annual TB incidents occur in India making it the highest Tuberculosis burdened country. We wanted to study the role of CBNAAT in early the diagnosis of tuberculosis/multi-drug resistant tuberculosis in HIV seropositive subjects and compare the detection of M. tuberculosis in sputum by CBNAAT with conventional sputum microscopy in pulmonary tuberculosis.

#### METHODS

Patients who presented with persistent cough for more than two weeks, chest X ray suggestive of PTB were subjected to sputum examination by routine microscopy and cartridge based nucleic acid amplification technique (CBNAAT).

#### RESULTS

CBNAAT had a sensitivity of 100%, specificity of 79.31%, PPV of 41.94% and NPV of 100% and diagnostic accuracy of 82%, whereas sputum microscopy showed only 41.94% sensitivity.

#### CONCLUSIONS

CBNAAT helps in early detection of tuberculosis, takes less than 2 hours, and is more sensitive in detecting tuberculosis in HIV sero-positive cases compared to sputum microscopy and reduces the false negative rates. Drug resistance can concomitantly be identified.

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#### BACKGROUND

Tuberculosis (TB) continues to be one of the greatest killers in the world, claiming over 1.4 million deaths in 2011. In the global tuberculosis report (2014).<sup>1</sup>

Twenty five percent of global annual TB incidents occur in India making it the highest Tuberculosis burden country.<sup>2</sup>

In December 2010, WHO recommended use of a new Cartridge Based Nucleic Acid Amplification test (CB-NAAT), named GeneXpert system.<sup>3</sup> The Xpert MTB/RIF test exhibits high sensitivity and specificity for detecting pulmonary TB disease. An in-vitro study demonstrated a limit of detection

of as few as 131 colony-forming units/mL of MTB, compared with approximately 10,000 colony-forming units/mL with conventional smear microscopy. The sensitivity of the MTB/RIF test on single sputum sample has been observed to be 92.2% for culture-positive TB; 98.2% for smear-positive and culture-positive cases; and 72.5% for smear-negative, culture-positive cases, with a specificity of 99.2%.<sup>4</sup>

As compared to people without HIV, people living with HIV (PLWH) have a 20-fold higher risk of developing TB and the risk continues to increase as CD4 cell counts progressively decline.<sup>5</sup>

Although ART can reduce the incidence of TB both at the individual and population level, PLWH on ART still have higher TB incidence rates and a higher risk of dying from TB.<sup>6</sup> This may be due to delayed initiation of ART or the fact that patients present with advanced TB or both.<sup>7</sup> ART is a crucial component of case management of HIV associated TB,<sup>8</sup> reducing mortality risk by 64%–95%.<sup>9</sup>

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**Aims and Objectives**

1. To study the role of CBNAAT in early diagnosis of Tuberculosis/Multi-drug resistant tuberculosis in HIV seropositive Subjects and
2. Detection of M. Tuberculosis in sputum by CBNAAT compared to conventional sputum microscopy in pulmonary Tuberculosis.

**METHODS**

Source of data: Observation Method of Primary Source of information on the Sputum samples of HIV seropositive registered subjects attending KIMS, HUBLI, during November 2015 to January 2017 Total 100 HIV seropositive subjects of all genders, aged above 18 years, who present with-

1. Productive cough for more than 2 weeks and/or
2. Chest X-ray findings suggestive of pulmonary tuberculosis are included in the study.

A detailed history was taken and clinical examination of all the subjects included in the study will be done. History of presenting complaints, past illnesses, mode of transmission of HIV and high-risk behaviour will be taken. Clinical history regarding current complaints of fever, cough, sputum production, haemoptysis, weight loss will be taken. History regarding previous treatment for tuberculosis will also be taken.

- Those subjects who fulfil the inclusion criteria are subjected to: Sputum Examination by routine microscopy using direct Ziehl–Neelsen staining of sputum and cartridge based nucleic acid amplification technique (CBNAAT).

**Statistical Analysis**

Data was entered into Microsoft excel data sheet and was analysed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test was used as test of significance for qualitative data.

**Graphical Representation of Data**

MS Excel and MS word was used to obtain various types of graphs such as bar diagram and Pie diagram. p value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

**Statistical Software**

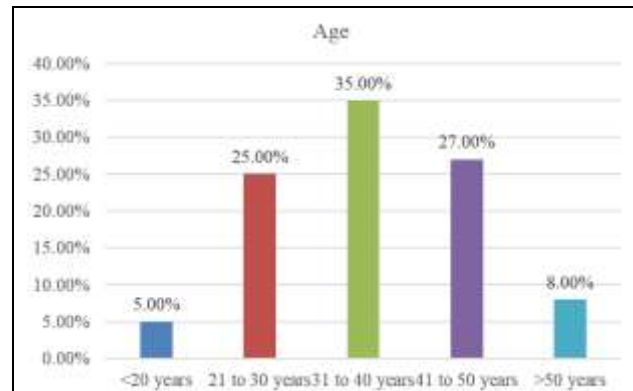
MS Excel, SPSS version 22 (IBM SPSS Statistics, Somers NY, USA) was used to analyse data.

**RESULTS**

		Count	%
Age	<20 years	5	5.0%
	21 to 30 years	25	25.0%
	31 to 40 years	35	35.0%
	41 to 50 years	27	27.0%
	>50 years	8	8.0%
	Total	100	100.0%

**Table 1. Age Distribution of Subjects**

In the study majority of subjects were in the age group 31 to 40 years (35%), followed by 41 to 50 years (27%), 21 to 30 years (25%). Hence majority of subjects were in the middle age group.

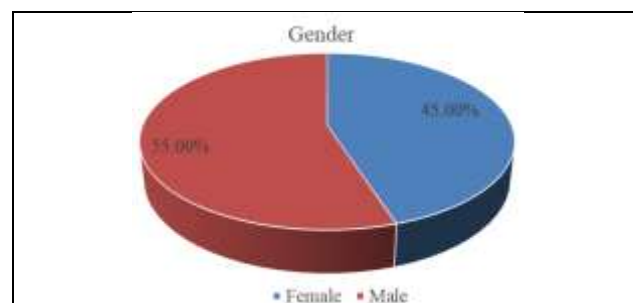


**Figure 1. Bar Diagram Showing Age Distribution of Subjects**

		Count	%
Gender	Female	45	45.0%
	Male	55	55.0%
	Total	100	100.0%

**Table 2. Gender Distribution of Subjects**

Majority of subjects were males 55% and 44% were females.



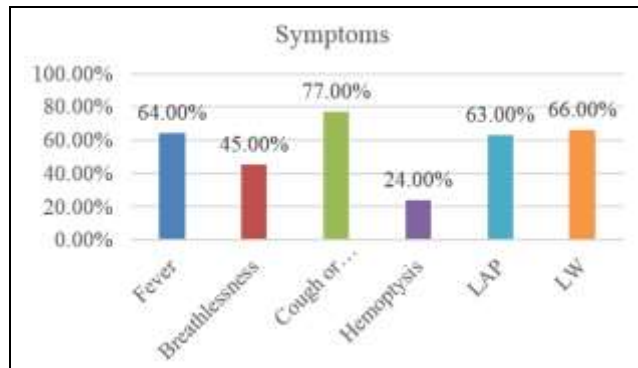
**Figure 2. Pie Diagram Showing Gender Distribution of Subjects**

**Symptoms**

		Count	%
Fever	No	36	36.0%
	Yes	64	64.0%
Breathlessness	No	55	55.0%
	Yes	45	45.0%
Cough or Expectoration	No	23	23.0%
	Yes	77	77.0%
Haemoptysis	No	76	76.0%
	Yes	24	24.0%
LAP	No	37	37.0%
	Yes	63	63.0%
LW	No	34	34.0%
	Yes	66	66.0%

**Table 3. Symptoms on Presentation Among Subjects**

Most common symptoms among the subjects was cough 77%, followed by fever in 64%, 63% with LAP, 66% with LW, 45% with breathlessness and 24% had haemoptysis.



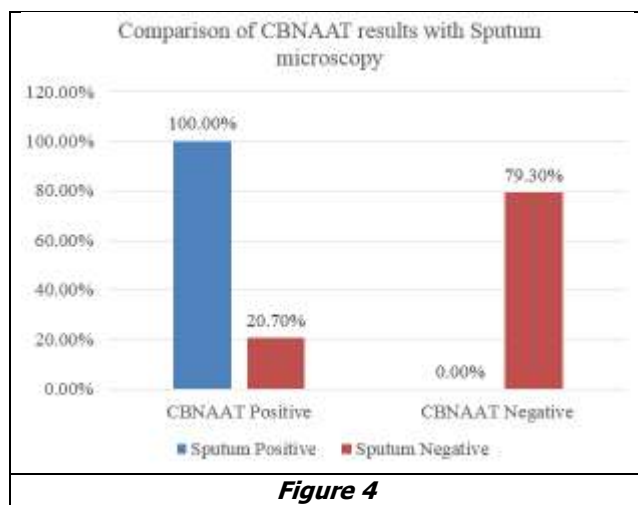
**Figure 3. Bar Diagram Showing Symptoms on Presentation Among Subjects**

		Sputum for AFB			
		Positive		Negative	
		Count	%	Count	%
CBNAAT	Positive	13	100.0%	18	20.7%
	Negative	0	0.0%	69	79.3%

**Table 4. Comparison of CBNAAT Results with Sputum Microscopy**

$\chi^2 = 33.26, df = 1, p < 0.001^*$

In the study significant association was observed between CBNAAT findings and Sputum microscopy findings in detecting MTB. Out of 13 subjects with sputum positivity, 100% were detected by CBNAAT and out of 87 subjects with sputum negativity, 20.7% were positive for MTB by CBNAAT.



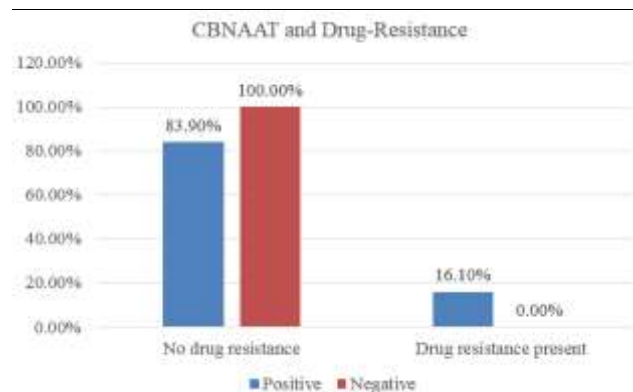
**Figure 4**

		CBNAAT			
		Positive		Negative	
		Count	%	Count	%
Drug-Resistance	No	26	83.9%	69	100.0%
	Yes	5	16.1%	0	0.0%

**Table 5. Association Between CBNAAT and Drug-Resistance**

$\chi^2 = 11.71, df = 1, p = 0.001^*$

In the study there was significant association between CBNAAT and drug resistance. Out of 31 subjects with CBNAAT positivity, 16.1% had drug resistance and out of 69 subjects with CBNAAT negative, 100% did not have drug resistance.

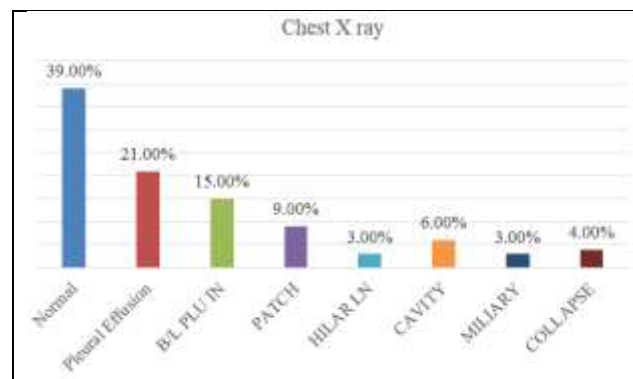


**Figure 5**

Chest X Ray	Finding	Count	%
		Normal	39
Chest X Ray	Pleural Effusion	21	21.0%
	b/l Plu in	15	15.0%
	Patch	9	9.0%
	Hilar LN	3	3.0%
	Cavity	6	6.0%
	Miliary	3	3.0%
	Collapse	4	4.0%
	Total	100	100.0%

**Table 6. Chest X-Ray Findings Among Subjects**

Most common chest x ray finding was pleural effusion in 21%, 8% were sputum positive and 13% were CBNAAT positive, bilateral pleural effusion in 15%, 15% had parenchymal involvement (9% had Patch, 6% had cavity).



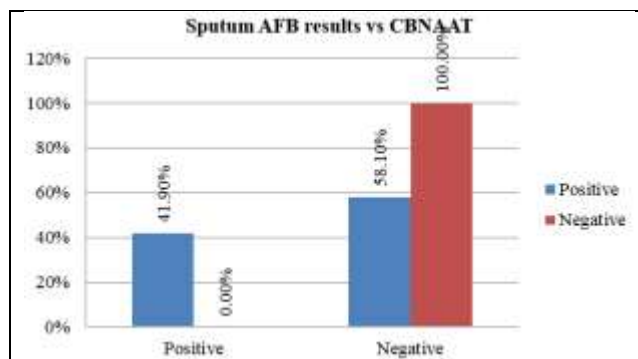
**Figure 6. Bar Diagram Showing Chest X-Ray Findings Among Subjects**

		CBNAAT			
		Positive		Negative	
		Count	%	Count	%
Sputum	Positive	13	41.9%	0	0.0%
	Negative	18	58.1%	69	100.0%

**Table 7. Comparison of Sputum AFB Results with CBNAAT**

$\chi^2 = 33.25, df = 1, p < 0.001^*$

Among 31 subjects with CBNAAT positivity, 41.9% were positive in Sputum AFB, 58.1% were negative in Sputum AFB (False negative). Among 69 subjects who were Negative in CBNAAT (The rest 69 cases were not considered for BAL for CBNAAT), 100% were negative in Sputum. This observation was statistically significant. Hence sputum smear had 58.1% false negative rate in comparison with CBNAAT. This observation was statistically significant.



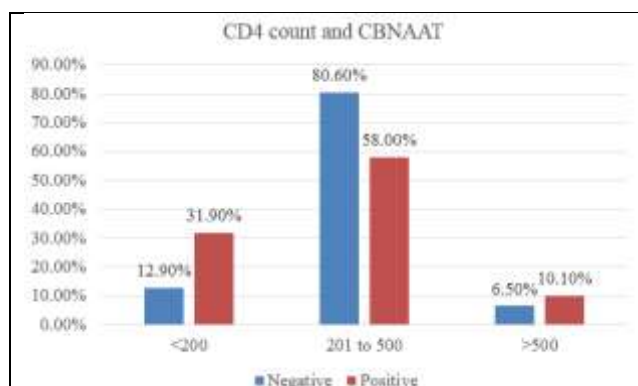
**Figure 7. Bar Diagram Showing Comparison of Sputum AFB Results with CBNAAT**

		CBNAAT			
		Negative		Positive	
		Count	%	Count	%
CD4 Count	<200	4	12.9%	22	31.9%
	201 to 500	25	80.6%	40	58.0%
	>500	2	6.5%	7	10.1%

**Table 8. Association Between CD4 Count and CBNAAT**

$\chi^2 = 4.98, df = 2, p = 0.083$

There was no significant association between CD4 count and CBNAAT findings. Among 31 subjects with CBNAAT negative, 12.9% had CD4 <200, 80.6% had b/w 201 to 500 and 6.5% had CD4 >500. Among 69 subjects with CBNAAT positive, 31.9% had CD4 <200, 58% had CD4 b/w 201 to 500 and 10.1% had CD4 >500.



**Figure 8. Bar Diagram Showing Association Between CD4 Count and CBNAAT**

**DISCUSSION**

In India the burden of Tuberculosis is found to be around 28 lakhs in the year 2015 as indicated by WHO global TB report.<sup>1</sup> Deaths due to TB was 4.8 Lakh in the same year. This is one of the significant concerns in our country. Further the rise in incidence of HIV creates a pavement for further increase in TB incidence. Around 1.1 lakh patients who are HIV positive in our country are co infected with TB further raising the disease burden.

One of the most convenient way of detecting pulmonary TB is by sputum examination with 2 samples as per RNTCP.<sup>10</sup> The sensitivity of this test is variable as it depends on several factors such as sample quality, the load of bacilli and the immune status of the patient. Based on different studies the sensitivity of sputum examination is around 40-80% because of which significant number of patients can be missed on routine sputum examination,<sup>10</sup> this contributes to open cases in community facilitating spread of bacilli to susceptible hosts. The sensitivity further drops to almost 20-40% in HIV positive patients. Thus, we need a test with higher sensitivity to pick up the false negatives in high clinical suspicion of TB.

Following the availability of CBNAAT for the detection of TB which has higher sensitivity, it has been efficiently utilized to detect sputum negative case of PTB. This contributes to increase in the TB detection in HIV patients.<sup>10</sup> KIMS HUBLI is one of the nodal centres for diagnosis and management of TB, also it is nodal centre for HIV. In our study we have evaluated the contribution of CBNAAT in case detection in HIV patients to reinforce the significance of CBNAAT.

In our study with total of 100 cases who were HIV sero-positive 11 were newly diagnosed HIV cases, presented with features suggestive of pulmonary tuberculosis (All are Newly diagnosed pulmonary TB) and all the patients were subjected to sputum AFB and CBNAAT. A significant association was observed between CBNAAT findings and Sputum microscopy findings in detecting MTB. Out of 13 subjects with sputum positivity, 100% were detected by CBNAAT. Out of 87 subjects with sputum negativity, 18(20.7%) were positive for TB by CBNAAT. Out of 31 subjects with CBNAAT positivity, 16.1% had drug resistance (Newly diagnosed). Thus, it was seen that the increase in case detection was almost 18% in our study thus facilitating early initiation of treatment.

Several other studies also have shown similar findings. In one other study conducted by D. Pragati Rao, K. Lakshmi Sowjanya<sup>11</sup> which was done for 2 months from February to March 2016. Out of 231 HIV positive patients, 59 cases (25.54%) had tuberculosis. Sputum smear for AFB negative and GeneXpert positive were 45(76.27%). 8(13.55%) cases were Rifampicin resistance and 51 (86.44%) were sensitive out of all tuberculosis patients.

Another study conducted by Deepak Bansal, Sanjay Avashia, Mitesh Karothiya<sup>3</sup> from January 2015 to December 2015. A total of 3033 pulmonary specimens were included in the study undergoing Gene Xpert; out of which 604 specimens were from HIV positive patients. In 85 (14.07%) HIV positive patients (out of 604) MTB was detected and RIF was sensitive (14.07%). In 06 (0.99%) HIV positive patients MTB, was detected and RIF resistance was found.

Study	Sample Size	Positive for Tuberculosis	AFB Positive	CBNAAT Positive	Resistant Cases
Present Study	100	31	13 (13%)	31 (31%)	5 (5%)
D Pragati Rao et al	231	59	14 (6.06%)	59 (25.54%)	8 (3.4%)
Deepak Bansal et al	604	91 (15.06%)	-	91 (15.06%)	6 (0.99%)
Prem Parkash Gupta et al	30	25 (83.33%)	8 (26.66%)	17 (56.66%)	2 (6.66%)
R Dewan et al	100	40 (40%)	11 (11%)	40 (40%)	9 (9%)

**Table 9. Comparison of Results of Present Study with Previous Studies**

In the study conducted by Prem Parkash Gupta, Adarsh B Mynalli, Aparna Yadav screened 211 patients with clinical symptoms suggestive of pulmonary tuberculosis. 30 subjects were found to be seropositive for HIV. LED-Fluorescent Microscopy sputum examination was positive for acid fast bacilli in 8/30 HIV seropositive patients and 131/181 subjects who were sero-negative for HIV. The data suggested patients who were HIV positive were less likely to have sputum smear positive results. CBNAAT detected M. tuberculosis in 17/30 HIV-seropositive patients and in 133/181 HIV sero-negative subjects. 56.7% of the patients who were positive for HIV were also positive for MTB and two of them had rifampicin resistance detected by CBNAAT.

Another study conducted by R Dewan et al<sup>12</sup> (January 2013 and December 2013) included 100 patients. Eleven patients (11%) were positive by sputum microscopy for acid-fast bacilli and 40 (40%) were positive by CBNAAT. This difference was statistically significant (p value <0.001). Mean time taken for detection of TB was 2 days for microscopy and less than 2 hours for CBNAAT. Out of the 40 patients positive by CBNAAT, rifampicin resistance was detected in 10 patients (25%) out of which 9 had multi-drug resistant tuberculosis (MDR-TB) as detected by LPA. The mean CD4 count of the patients was 230 cells/ml and there was no statistically significant difference in CD4 counts between CBNAAT positive and negative patients (p value = 0.264).

### Summary

1. Our study was conducted on 100 patients with HIV sero-positivity with features suggestive of pulmonary tuberculosis presenting to Karnataka Institute of Medical Sciences, Hubballi. The study was done to detect the role of CBNAAT in diagnosis of tuberculosis and MDR-TB. Mean age of the study population was in group of 31 to 40 years.
2. Out of 100 patients, 55(55%) patients were males and 45(45%) patients were females.
3. The most common symptom was Cough or expectoration in 77 cases (77%) followed by loss of weight 66 cases (66%), fever (64%), Loss of appetite in 63% cases.
4. 65 cases (65%) had their CD4 count in the range of 201-500 cells and 9% cases had CD4 count above 500.
5. Out of 13 subjects with sputum positivity, 100% were detected by CBNAAT and out of 87 subjects with sputum negativity, 20.7% were positive for MTB by CBNAAT.

6. CBNAAT had a sensitivity of 100%, specificity of 79.31%, PPV of 41.94% and NPV of 100% and Diagnostic accuracy of 82% whereas sputum microscopy showed only 41.94% sensitivity.
7. Out of 31 subjects with CBNAAT positivity, 16.1% had drug resistance and out of 69 subjects with CBNAAT negative none had drug resistance
8. Most common chest x ray finding was pleural effusion in 21%, bilateral pleural effusion in 15%, 9% had Patch, 6% had cavity.
9. There was no significant association between CD4 count and CBNAAT findings also with CD4 count and sputum microscopy.

### CONCLUSIONS

CBNAAT helps in early detection of tuberculosis and it takes less than 2 hours. CBNAAT was more sensitive in detecting tuberculosis in HIV sero-positive cases compared to sputum microscopy. It reduces the false negative rates. Drug resistance can concomitantly be identified.

### Limitations

1. The study has small sample size and is done at a single tertiary care centre.
2. The study has not included comparison with standard methods of MTB culture and sensitivity.
3. The incidence and prevalence of disease in different geographical areas affect the conclusion.

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