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Original Research Article.

To Assess the Diagnostic Accuracy of CBNAAT and Sputum Microscopy Against Gold Standard Sputum Culture Among Symptomatic HIV Patients: An Institute Based Study

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ABSTRACT

Background: Tuberculosis remains the most common opportunistic infection among PLHIV, and HIV-TB co-infected individuals are at high-risk of death. Cartridge-based nucleic acid amplification test (CBNAAT) is a recently introduced polymerase chain reaction (PCR) based method for detection of TB. This study was carried out to evaluate the role of CBNAAT in early diagnosis of TB in PLHIV and detection of *M. tuberculosis* in sputum by CBNAAT compared to conventional sputum microscopy in pulmonary TB.

Materials & Methods: This is a hospital based observational, descriptive study on 100 subjects who were diagnosed as HIV infected by ELISA rapid and simple (ERS) test according to NACO guidelines with history of productive cough for 2 weeks and/or chest X-ray findings suggestive of pulmonary tuberculosis. We assessed HIV positive adults referred to with pulmonary symptoms suggestive of tuberculosis by sending two sputum samples for microscopy using ZN stain, one sputum sample for CBNAAT and one sputum sample for BACTEC based culture in each patient. Sputum smears after Ziehl-Neelsen 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 samples negative for AFB evaluated for 100 fields were declared as negative.

Results: The mean age of patients was 38.67 years. The positive sputum culture was seen in 63% of cases & 37% cases had negative sputum culture. Out of 120 screened patients, 100 included with adequate sputum volume, 22 were microscopy positive and 78 were microscopy negative. The sensitivity, specificity, PPV, NPV of Sputum microscopy was 22.22%, 78.38%, 63.64% & 37.18% respectively. The Test accuracy as represented by AUROC (Area under receiver operator characteristics) was significantly higher for CBNAAT compared to Sputum microscopy [0.9189 vs 0.7838, mean diff 0.13, p<0.001).

Conclusion: We concluded that CBNAAT performs better than sputum microscopy in diagnosis of pulmonary tuberculosis in HIV patients.

Keywords: CBNAAT, Tuberculosis, Sputum Culture, Sputum Microscopy.

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INTRODUCTION

Tuberculosis related deaths are rising in the world in an alarming trend. No wonder that this chronic infection is setting the major brakes in human resources and economy of a nation particularly the developing ones. Tuberculosis (TB), an infectious disease caused by Mycobacterium tuberculosis, has an estimated global annual incidence of 9.6 million with 2.2 million cases in India according to World Health Organization (WHO) Global TB Report (2015) Thus,23% of global annual TB incidents occur in India making it the highest TB burden country.[1]

Tuberculosis remains the most common opportunistic infection among PLHIV, and HIV-TB co-infected individuals are at high-risk of death.[2] Standard sputum-based methods to detect pulmonary tuberculosis include sputum microscopy and culture. However, in PLHIV, 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. These factors decrease the sensitivity and specificity of sputum microscopy as a diagnostic tool for tuberculosis. Cartridge-based nucleic acid amplification test (CBNAAT) is a recently introduced polymerase chain reaction (PCR) based method for detection of TB. It also detects rifampicin resistance as it targets the rpoB gene of mycobacteria. CBNAAT is a *Mycobacterium tuberculosis*-

specific automated, cartridge based nucleic acid amplification assay, having fully integrated and automated amplification and detection using real-time PCR, providing results within 100 minutes. It is a highly specific test as it uses 3 specific primers and 5 unique molecular probes to target the rpoB gene of *M. tuberculosis*, which is the critical gene associated with rifampicin resistance.

The specificities and sensitivities of the polymerase chain reaction (PCR) based diagnostic tests are quite variable.[3,4] Further, these tests involve multiple manual steps and long turnaround time, making them unsuitable for decentralized deployment. A series of meta-analyses have shown cartridge based nucleic acid amplification test (CBNAAT)/ Xpert MTB/ RIF to have a high specificity with variable sensitivity in different type of specimens for TB diagnosis.[5-7] CBNAAT, a tool with a quick turn-around time, which simultaneously detects TB and rifampicin resistance, offers a promising solution to achieve the global objective of improved TB care and control and early TB case detection.

Diagnosing tuberculosis (TB) in people living with HIV/AIDS (PLHIV) is challenging as sputum microscopy is negative in more than half of the patients due to lack of caseous necrosis. Sputum culture is a slow method which takes 4 - 8 weeks for growth of the mycobacteria. Delayed treatment for TB in PLHIV is associated with increased mortality. The role of a newly launched cartridge based nucleic acid amplification test (CBNAAT) with a potential to diagnose TB and rifampicin resistance within 2 hours in PLHIV is promising. It's role in diagnosing TB in PLHIV has not been studied widely in India. This study was carried out to evaluate the role of CBNAAT in early diagnosis of TB in PLHIV and detection of *M. tuberculosis* in sputum by CBNAAT compared to conventional sputum microscopy in pulmonary TB.

MATERIALS & METHODS

This is a hospital based observational, descriptive study on 100 subjects who were diagnosed as HIV infected by ELISA rapid and simple (ERS) test according to NACO guidelines and who presented to the TB clinic, and collaboration with department of microbiology in Dr. S.N. Medical College & Attached Group of Hospitals, Jodhpur with history of productive cough for 2 weeks and/or chest X-ray findings suggestive of pulmonary tuberculosis.

Inclusion Criteria

A HIV positive patient with–

1. Complaint of fever
2. Cough > 2 weeks ± Sputum ± Haemoptysis
3. Weight loss
4. Night sweat

Exclusion Criteria

1. Age <18 years and Age >55 years
2. Pregnant woman
3. Extra pulmonary case
4. People not given consent for study

History and Examination: All patients included in the study underwent a detailed history and clinical examination. History of presenting complaints, past illnesses, mode of transmission of HIV and high-risk behaviour was taken. Clinical history regarding current complaints of fever, cough, sputum production, haemoptysis, weight loss was taken. History regarding previous treatment for tuberculosis was also taken. All patients were evaluated for because of headache, seizures, chest pain, breathlessness and neck swelling or any other evidence of extrapulmonary tuberculosis.

Immune Status Assessment: CD4 lymphocyte counts of all the patients were determined by flow cytometry.

Sputum Analysis: We assessed HIV positive adults referred to with pulmonary symptoms suggestive of tuberculosis by sending two sputum samples for microscopy using ZN stain, one sputum sample for CBNAAT and one sputum sample for BACTEC based culture in each patient.

Sputum Microscopy: Sputum smears after Ziehl-Neelsen 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 samples negative for AFB evaluated for 100 fields were declared as negative.

Sputum for CBNAAT: One sputum sample of 1 ml was collected in a sterile container and was analysed by CBNAAT on *Xpert® MTB/RIF* manufactured by Cepheid, endorsed by WHO (2010). The sample was diluted with three times the reagent, incubated at room temperature and loaded into the cartridge for automated analysis with results in 100 minutes. Detection of mycobacteria and rifampicin resistance was carried-out in the same setting.

Rifampicin resistant samples were further analysed by LPA. The three steps for LPA test included DNA extraction, multiplex polymerase chain reaction (PCR) amplification and reverse hybridisation.

Sputum Culture: Using BACTEC culture positive as gold standard, we determined comparative sensitivity, specificity, Positive Predictive Value, Negative Predictive value, Positive Likelihood Ratio and Negative Likelihood Ratio of CBNAAT and sputum microscopy using SPSS 15.0 software.

RESULTS

The mean age of patients was 38.67 years. Youngest age was 18 years and oldest age was 70 years was seen. Male to female ratio was 3:1. Our study showed that the ART regimen was TL TDF based Ist line regimen mostly in 92.85% of cases.

The table no. 2 represent that the maximum cases (43%) had less than 6 months ART regimen followed by 26% cases had taking 1-2 years ART regimen, 18% cases had taken 6 months-1 year and only 13% cases had more than 2 years taking ART regimen.

Our study showed that the mostly cases (45%) had below 200 cells/cumm CD4 count followed by 16% cases had 301-400 cells/cumm and 14% cases had seen more than 500 cells/cumm CD4 count (table 3).

The positive sputum culture was seen in 63% of cases & 37% cases had negative sputum culture. Out of 120 screened patients, 100 included with adequate sputum volume, 22 were microscopy positive and 78 were microscopy negative. The sensitivity, specificity, PPV, NPV of Sputum microscopy was 22.22%, 78.38%, 63.64% & 37.18% respectively. (table 4)

Our study showed that 55 were CBNAAT positive and 45 were CBNAAT negative. . The sensitivity, specificity, PPV, NPV of Sputum microscopy was 82.54%, 91.89%, 94.55% & 75.56% respectively. (table 5)

The Test accuracy as represented by AUROC (Area under receiver operator characteristics) was significantly higher for CBNAAT compared to Sputum microscopy [0.9189 vs 0.7838, mean diff 0.13, p<0.001).

Table 1: Age Wise Distribution of Cases

Demographic profile	No. of cases (N=100)	Percentage
Age (yrs)		
18-20 yrs	2	2%
21-30 yrs	19	19%
31-40 yrs	38	38%
41-50 yrs	33	33%
51-60 yrs	6	6%
>60 yrs	2	2%
Gender		
Male	**75**	**75%**
Female	**25**	**25%**
Clinical Features		
Fever	70	70%
Cough	72	72%
Night sweats	50	50%
Loss of appetite	65	65%
Hemoptysis	18	18%
Shortness of breath	14	14%
ART regimen		
TLE (TDF based Ist line regimen)	93	93%
ZLE (AZT based Ist line regimen)	6	6%
TL/ATV/R	1	1%

Table 2: Duration of ART Regimen

Duration	No. of cases	Percentage
< 6 months	43	43%
6 months - 1 years	18	18%
1-2 years	26	26%
>2 years	13	13%

Table 3: Distribution of CD4 in cases

CD4/cumm	No. of Cases	Percentage
<200	45	45%
201-300	14	14%
301-400	16	16%
401-500	11	11%
>500	14	14%
Total	**100**	**100%**

Table 12: Sputum microscopy with sputum culture

	Culture P	Culture N	sensitivity	Specificity	PPV	NPV	P-value
Sputum P	14	8	22.22%	78.38%	63.64%	37.18%	0.9442
Sputum N	49	29					
Total	**63**	**37**					

Chi-square test, 1 degree of freedom

Table 14: Results - CBNAAT with sputum culture

	Culture P	Culture N	sensitivity	Specificity	PPV	NPV	P-value
CBNAAT P	52	3	82.54%	91.89%	94.55%	75.56%	<0.0001***
CBNAAT N	11	34					
Total	**63**	**37**					

Chi-square test, 1 degree of freedom

DISCUSSION

Tuberculosis has been a major challenge in countries suffering with a high load of HIV co-infection along with a resource-limited socio-economic scenario.[8] These risks are further increased manifold due to increased probability of presence of multi-drug resistant tuberculosis.

Diagnosing tuberculosis (TB) in people living with HIV/AIDS (PLHIV) is challenging as sputum microscopy is negative in more than half of the patients due to lack of caseous necrosis. Sputum culture is a slow method which takes 4 - 8 weeks for growth of the mycobacteria. Delayed treatment for TB in PLHIV is associated with increased mortality. The role of a newly launched cartridge based nucleic acid amplification test (CBNAAT) with a potential to diagnose TB and rifampicin resistance within 2 hours in PLHIV is promising. The aim of this study to evaluated the diagnostic accuracy of CBNAAT and sputum microscopy against gold standard sputum culture among symptomatic HIV patients.

Our study showed that the majority of cases with tuberculosis in HIV were seen in 31-50 years of age groups. The mean age of patients was 38.67±9.723 years. R Dewan et al (2015)[9] found that the mean age of the study population was 35 ± 9 years. Most patients (69%) were in the age group of 20 to 40 years.

Our study showed that the male was more common (75%) as compared to female (25%). Male to female ratio was 3:1. The mean CD4 count of the patients was 267.9±190.2 cells/mm. Mostly cases (44.99%) had <200 cells/mm CD4 count. R Dewan et al (2015)[9] found that the majority (76%) of the patients was males, there were 21% women and three transgenders. The mean CD4 count of the subjects was 230 cells/ml. Thirty-two patients had CD4 count less than 100 cells/ml, which is consistent with our results.

In the present study out of 100 screened patients taking sputum culture as a gold standard for diagnosis of tuberculosis. 22 were microscopy positive and 78 were microscopy negative.

Only 22 patients were found to be sputum positive for AFB by direct microscopy and 78 cases were missed and reported as sputum smear negative, which was statistical non-significant (p=0.9442). This indicated that sputum microscopy is not a very useful method in diagnosis of pulmonary tuberculosis in HIV patients. The consequences of this can be several, including delayed or misdiagnosed cases, contributing to delayed treatment with increased morbidity and mortality rates and continued spread of TB to contacts. Indian studies show varying degree of sensitivity of sputum microscopy range from 11% to 61.5% in study of R Devan et al[9] 11%, Prem Prakash Gupta et al[10] 26.66%, Sowjanya D et al, Vizianagaram[11] 52.68%, and Anupam Kumar Singh et al[12] 61.5% which are compatible in our study.

Sowjanya D et al, Vizianagaram 2012-2013[11]. An observational study Pulmonary Medicine Maharajah's Institute of Medical Sciences, out of 205 sputum samples from HIV status unspecified patients, 108(52.68%) were ZN AFB smear positive, 96 (47.32%) were negative.

Out of 120 screened patients, 100 were included with adequate sputum volume, of these 55 were CBNAAT positive and 45 were CBNAAT negative. Thus, tuberculosis detection rate increased by more than double using CBNAAT as compare to sputum microscopy, which was statistical significant (p<0.0001***).

In Indian study sensitivity of CBNAAT ranges from 40% to 82.7%. R Dewan et al[9] 40%, Prem Prakash Gupta et al[10] 56.66%, Sowjanya D et al[11] 70.24% and Anupam Kumar Singh et al[12] 82.7% which was compatible to our study.

HIV-TB co-infection has been shown to substantially decrease the sensitivity of sputum microscopy (to 47%), but it does not significantly affect CBNAAT performance.[13] Studies from high HIV endemicity areas in Peru have also shown that HIV status does not affect the performance of CBNAAT.[14] Sensitivity and specificity of CBNAAT were reported to be > 95%.

The WHO policy guidance on the use of CBNAAT was issued in December 2010. The recommendations were that it should be used as the initial diagnostic test in individuals at risk of having MDR-TB or HIV-associated TB (strong recommendation), and that it could be used as a follow-on test to microscopy in settings where MDR and/ or HIV is of lesser concern, especially in smear-negative specimens (this was a conditional recommendation, recognising major resource implications). This recommendation applied to the use of CBNAAT in sputum specimens only, as data on its performance (sensitivity and specificity) for testing of extrapulmonary specimens at that time were limited.[15]

This molecular technique of GeneXpert assay is relatively more expensive than traditional culture methods; however, it makes an important contribution to the modern-day detection of TB with higher sensitivity and provides a more rapid diagnosis than culture and histology.

CONCLUSION

We concluded that CBNAAT performs better than sputum microscopy in diagnosis of pulmonary tuberculosis in HIV patients. CBNAAT is recommended as first line modality for diagnosis of tuberculosis in HIV patients and re-treatment cases.

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