

Role of cartridge -based nucleic acid amplification test (CBNAAT) for early diagnosis of pulmonary tuberculosis in HIV

D. V. Pratapa Reddy¹, S Laxmi Kumari^{2*}, K Rajendra Kumar³, CH. R.N. Bhushana Rao⁴, P. Hima Sanjana⁵

¹Senior Resident, ²Associate Professor, ³Professor and HOD, ⁴Professor, ⁵Under Graduate, Dept. of Pulmonary Medicine, Guntur Medical College, Guntur, Andhra Pradesh, India

*Corresponding Author: S Laxmi Kumari

Email: laxmikumarisomishetty@gmail.com

Abstract

Background: Pulmonary Tuberculosis (PTB) is the commonest opportunistic infection and leading cause of death among people living with HIV (PLHIV). The role of cartridge based nucleic acid amplification test (CBNAAT) has potential to diagnose PTB and rifampicin resistance within two hours in PLHIV is promising.

Methodology: 100 PLHIV patients with age of >18years having signs and symptoms greater than or equal to two weeks of duration and / or Chest X-ray findings suggestive of pulmonary tuberculosis who are attending to the department of Pulmonary Medicine, Guntur Medical College, Guntur from November 2016 to October 2017 are included in the study. Sputum samples sent for CBNAAT, AFB stain. Positive samples of CBNAAT and AFB stain are sent for conventional culture.

Results: Out of 100 sputum samples, 45 were positive in CBNAAT, and 16 were AFB smear positive. All these 16 smear positive samples have 100% sensitivity in CBNAAT. These positive samples (45) were sent for culture. In culture out of 16 smear positive samples 15 are culture positive 1 is culture negative, which is false positive in CBNAAT also. In culture out of 45 CB-NAAT positive samples 29 culture positive, 16 culture negative. Sensitivity, specificity, PPV, NPV of smear in sputum samples are 46.88%, 98.5%, 93.75%, 79.76%, while that of CB-NAAT are 90.6%, 76.47%, 64.44%, 94.55% respectively. Primary rifampicin resistance was detected in 1 case (2.22%) in CBNAAT and culture.

Conclusions: CBNAAT is a rapid, reliable tool with least bio-safety concern and requires minimally trained staff, is best alternative to conventional methods of tubercular diagnosis and detects rifampicin resistance simultaneously within 2 hours.

Keywords: CBNAAT; Sputum; TB.

Introduction

HIV associated tuberculosis (TB) remains a major global public health challenge, with a 1.4 million patients worldwide. PTB is the leading cause of death among PLHIV. The control of PTB is hampered by slow diagnostic methods with low sensitivity, particularly for the detection of drug resistant forms, so early and accurate diagnosis is the first critical step in controlling PTB to interrupt transmission and reduce the mortality. Individuals who are infected with PTB have an approximately 10% lifetime risk of developing active tuberculosis, compared with 60% or more in persons infected with PLHIV and PTB [1].

India is the world's highest TB burden country with over 2 million active TB cases every year [1]. One fourth of the global incident TB cases occur in India annually [2]. According to WHO Indian statistics, 2016 the incidence of TB with HIV only is 87000 with a mortality of 12,000. Case detection of all forms is 74%. The estimated incidence of MDR-TB is 2% among new cases and 15% among retreatment cases [3].

To respond to the urgent need for simple and rapid diagnostic tools in high burden countries [4], a new diagnostic test (CBNAAT) has been developed which is a rapid, fully automated test based on PCR which detects mycobacterial DNA directly from clinical specimens and also detects rifampicin resistance. This test is designed to purify, concentrate, amplify and identify targeted rpo B gene nucleic acid sequences, and give results from unprocessed samples in 2 hours, with minimal hands on time [5-8].

Materials and Methods

This is an observational study conducted in the department of Pulmonary Medicine, Guntur Medical College, Guntur, from November, 2016 to October, 2017. 100 PLHIV positive patients who have attended (outpatient and inpatient) to the department of Pulmonary Medicine, Guntur Medical College, Guntur, with signs and symptoms and/or chest X-ray findings suggestive of pulmonary tuberculosis (new cases only) were included in the study. Defaulters and retreatment cases were excluded from the study.

Sputum samples collected in falcon tubes and sent for AFB smear to the RNTCP lab at Govt. Hospital/ Department of Pulmonary Medicine, Guntur. One sputum sample for CBNAAT was sent to District Tuberculosis Centre (DTC), Guntur. Positive sputum sample was sent to referral laboratory for culture. CBNAAT is a cartridge based fully automated nucleic acid amplification test currently recommended by WHO and adopted by RNTCP for detection of TB case and RIF resistance by rpo B gene Mutations.

Inclusion Criteria

Age >18 years, living with HIV

Signs and symptoms and with or without chest X-ray findings suggestive of pulmonary TB

New cases

Exclusion Criteria

History of previously treated cases, drug resistant cases, default cases.

HIV negative cases.

Extra pulmonary tuberculosis

Statistical Analysis

Descriptive statistics, mean, frequency, percentage, standard deviation were calculated. Diagnostic accuracy tests including Sensitivity, specificity, Positive predictive value, Negative predictive value were calculated. The significance of differences among groups was assessed by the Student *t* test. A value of *P* of <0.05 was considered significant for all statistical analyses.

Results

Out of 100 sputum samples, 45 are CBNAAT positive, and 16 are AFB smear positive. All these 16 smear positive samples have 100% sensitivity in CBNAAT. Smear positive and CBNAAT positive samples (16+45) were sent for culture. In culture out of 16 smear positive samples 15 are culture positive 1 is culture negative, which is false positive in CBNAAT. In culture out of 45 CB-NAAT positive samples 29 culture positive, 16 culture negative.

In culture out of 32 positive samples 29 true positive and 3 are false negative in CBNAAT. Sensitivity, specificity, PPV, NPV of smear in sputum samples are 46.88%, 98.5%, 93.75%, 79.76% respectively, while that of CB-NAAT are 90.6%, 76.47%, 64.44%, 94.55% respectively.

Primary rifampicin resistance was detected in 1 case. These presumptive MDR- TB cases would have been missed out in the regular diagnostic algorithm of RNTCP and lead to the spread of drug resistant tuberculosis. Total of 100 HIV positive presumptive pulmonary tuberculosis cases studied, rifampicin resistance was detected in 1(2.22%) out of 45 sputum CBNAAT positive cases.

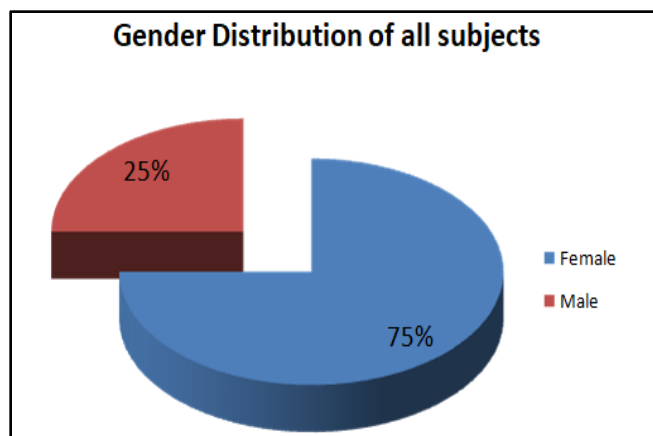


Fig 1: Gender distribution of all subjects

Discussion

TB is a major public health problem in most of the developing countries, it is still bigger threat with the epidemic of HIV and association has been termed as “cursed duet as HIV and TB” have synergistic action. Each accentuates progression of the other. The number of HIV people who develop TB varies widely between countries and regions. In the United States an estimated 4% HIV people have TB. By contrast, in India and

sub Saharan Africa, 50 to 60% of HIV people have TB during the course of illness [9].

TB is the most common opportunistic disease among HIV people. As India has the highest TB burden in the world, early detection of TB, determining drug resistance and prompt treatment is essential to decrease the spread, morbidity and mortality of disease. In the present diagnostic algorithm of RNTCP, smear is the sheet anchor of diagnosis but the main drawback of it is its sensitivity, while culture is the gold standard for the diagnosis of tuberculosis gives delayed results. CBNAAT, a fully automated test based on PCR, is sensitive and gives rapid results, has been endorsed by WHO (2012) for early detection of tuberculosis. Interestingly, in HIV-TB co-infection setting the sensitivity of sputum microscopy has been observed to be substantially decreased, but it does not significantly affect CBNAAT outcome [10].

In this study males are more affected than females. Mean common age group was 21 to 40 years. This is comparable to study by Deivanayagam et al., [11] NACO, Swaminathan et al., [12]. This age reflects the sexually active age group which is commonly affected. Unprotected heterosexual sex was most common mode of transmission. Significantly, a lower number of females living with HIV were found to attend the hospital, reflecting their current health remedy and illiteracy.

In the present study out of 100 sputum samples, 45 were positive in CBNAAT, and 16 were AFB smear positive. All these 16 smear positive samples have 100% sensitivity in CBNAAT. Smear and CBNAAT positive samples (16 and 45 respectively) were sent for culture. In culture out of 16 smear positive samples 15 are culture positive 1 is culture negative, which is false positive in CBNAAT.

In culture out of 45 CBNAAT positive samples 29 culture positive, 16 culture negative. In culture out of 32 positive samples 29 true positive and 3 are false negative in CBNAAT. Sensitivity, specificity, PPV, NPV of smear in sputum samples are 46.88%, 98.5%, 93.75%, 79.76%, while that of CBNAAT are 90.6%, 76.47%, 64.44%, 94.55% respectively.

In the present study sensitivity of sputum smear positive, culture positive samples are 100%, which is very much similar to studies like Bodmer et al.,[13],- 99.8%, Armand et al., [14]- 100%.

Sensitivity of the CBNAAT in smear negative samples may be increased by more than one sample. In the present study CBNAAT Sensitivity of smear positive and smear negative sputum samples are 100% and 82.35% respectively. In the Present study 2.22% rifampicin resistance detected in CBNAAT which is also positive in culture. In a study conducted by Prabhakaran et al.,[15] 1.8% was reported as resistance to Rifampicin, and in Sowjanya et al.,[16] study Rifampicin resistant was 1.9%. Present study comparable with these two studies.

Table 1: Comparison of AFB Smear and CBNAAT with culture results

SPUTUM	AFB smear		CBNAAT	
	Positive	Negative	Positive	Negative
Culture Positive	15	17	29	3
Culture Negative	1	67	16	52

Table 2: CB-Naat versus AFB and culture status in all samples

All Samples	AFB Negative		AFB Positive	
	Culture Positive	Culture Negative	Culture Positive	Culture Negative
CBNAAT	14	16	15	0
MTB Detected	3	51	0	1
MTB Not detected				

Table 3: Comparison of sensitivity, specificity and PPV, NPV of CBNAAT in smear positive, culture positive and smear negative, culture positive sputum samples

	Sputum Samples			
	Sensitivity	Specificity	PPV	NPV
Smear Positive Culture Positive cases	100%	100%	100%	100%
Smear Negative Culture Positive cases	82.35%	76.12%	46.67%	94.40%

Table 4: Comparison of sensitivity, specificity and positive predictive value, negative predictive value of AFB smear and CBNAAT in sputum samples.

	Sputum AFB	Sputum CBNAAT
Sensitivity	46.88%	90.60%
Specificity	98.50%	76.47%
Positive Predictive value	93.75%	64.44%
Negative predictive value	79.76%	94.45%

Conclusions

CBNAAT is a rapid, reliable, attractive tool with least bio-safety concern and requires minimally trained staff, is best alternative to conventional methods of tubercular diagnosis and detects rifampicin resistance simultaneously. The result is available in less than two hours and hence screening capacity of healthcare center can be increased. It also helps to avoid injudicious use of anti-tubercular drugs. Its efficacy is comparable to culture. It is MTB specific, no cross reaction with non-tubercular mycobacteria. It ultimately helps to initiate early treatment, curbing transmission and reduce the mortality.

Limitations of the Study

More than one sample might increase the sensitivity of smear negative samples. This study had no extra pulmonary samples, even though extra pulmonary TB is more common in PLHIV.

Conflicts of Interest: None declared.

Acknowledgements: None.

References

- Sachdeva KS, Kumar A, Dewan P, Kumar A, Satyanarayan S. New Vision for Revised National Tuberculosis Control Programme (RNTCP): Universal access "Reaching the unreached. *Ind J Med Res* 2012;135(5):690-4.
- India, Ministry of Health and Family Welfare, Central TB Division, Directorate General of Health Services, TB INDIA 2014 Revised National TB Control Programme, Annual status report. 2014.
- World Health Organization data: Tuberculosis profile in India. [Internet] Available from: https://extranet.who.int/sree/Reports?op=Replet&name=%2F%20WHO_HQ%20Reports%2F%202%20FPROD%2F%20FEXT%2F%20FTBCountry%20Profile&I%20SO2=IN&LAN=EN&outtype=html. Last accessed 2019 on May 12.
- Urdea M, Penny LA, Olmsted SS, Giovanni MY, Kaspar P, Shepherd A, et al. Requirements for high impact diagnostics in the developing world. *Nature* 2006;444:1:73-9.
- "WHO endorses new rapid tuberculosis test", WHO Media center Press release, Geneva, 8 December 2010. [Internet] Available from: www.who.int/mediacentre/news/releases/2010/.
- World health organization-policy statement: automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system. Geneva: world health organization, 2011.
- Robert B, Elizabeth S, Danica H, JoAnn K, Padmapriya B, Michelle RO, et al. Evaluation of the analytical performance of the Xpert MTB/RIF assay. *J Clin Microbiol* 2010;48(7):495-2501.
- Helb D, Martin J, Elizabeth S, Catharina B, Ellen W, Ken Ho, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of ondemand, near-patient technology. *J Clin Microbiol* 2010;48:229-37.
- Weyer K, Van der Walt M, Brand J, Lancaster J Levin J. Survey of tuberculosis drug resistance in South Africa: final report, 2003 Pretoria Medical Research Council.
- Van Ric A, Page - Shipp L, Scott L. Xpert (B) MTB / RIF for point-of care diagnosis of TB in high -HIV burden, resource-limited countries: hype or hope? *Expert Rev Mol Diagn* 2010;10:937-46.
- Deivanayagam CN, Rajasekaran S, Senthilnathan V, Jasekhar RK, Raja K, Chandrasekar C. Clinico-radiological spectrum of tuberculosis among HIV sero-positives a Tambaram study. *Indian J Tub* 2001;48:3.
- Swaminathan S. Pulmonary tuberculosis in HIV positive individuals: Preliminary report on clinical features and response to Treatment. *Indian J Tub* 2002;49:189.

13. Bodmer T, Strohle A. Diagnosing Pulmonary Tuberculosis with the Xpert MTB/RIF Test. *J Vis Exp* 2012;(62):3547.
14. Armand S. Comparison of the Xpert MTB/RIF Test with an IS6110-TaqMan Real-Time PCR Assay for Direct Detection of Mycobacterium tuberculosis in Respiratory and Nonrespiratory Specimens. *J Clin Microbiol* 2011;1772-6.
15. Rathinam P, Sanakaralingom I. (2017). Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) For Diagnosis of Pulmonary Tuberculosis In HIV: Results From Madurai District, Tamilnadu (*IOSR- JDMS*) 2017;16(8)Ver. VI:66-8.
16. Sowjanya DS, Behera G, Ramana Reddy VV, Praveen JV. CBNAAT: a Novel Diagnostic Tool for Rapid and Specific Detection of Mycobacterium Tuberculosis in Pulmonary Samples. (*IJHRMIMS*), ISSN2394-8612(P), ISSN2394-8620(O),Oct-Dec2014.

How to cite this article: Reddy DVP, Kumari SL, Kumar KR, Rao CH RNB, Sanjana PH. Role of cartridge -based nucleic acid amplification test (CBNAAT) for early diagnosis of pulmonary tuberculosis in HIV. *Indian J Immunol Respir Med* 2019;4(2):114-7.