EFFICACY OF CB-NAAT IN DETECTING SPUTUM NEGATIVE PULMONARY TUBERCULOSIS

K. N. Mohan Rao[1], Kolla Vinod[2], Pradeep Ishwarappagol[3], Vivek Gundappa[4], Mohammed Parveez[5], Niaz Farhat[6]

[1]*Professor and HOD, Department of Pulmonary Medicine, Rajarajeswari Medical College and Hospital, Bengaluru.*
[2]*Assistant Professor, Department of Pulmonary Medicine, Rajarajeswari Medical College and Hospital, Bengaluru.*
[3]*Junior Resident, Department of Pulmonary Medicine, Rajarajeswari Medical College and Hospital, Bengaluru.*
[4]*Junior Resident, Department of Pulmonary Medicine, Rajarajeswari Medical College and Hospital, Bengaluru.*
[5]*Junior Resident, Department of Pulmonary Medicine, Rajarajeswari Medical College and Hospital, Bengaluru.*
[6]*Junior Resident, Department of Pulmonary Medicine, Rajarajeswari Medical College and Hospital, Bengaluru.*

ABSTRACT

BACKGROUND
Tuberculosis (TB) is a major health problem in India. The World Health Organization has recently in 2010 endorsed the GeneXpert MTB/RIF assay for rapid detection of smear negative and multidrug-resistant tuberculosis.

The aim of the study is to evaluate the role of Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in sputum AFB negative Pulmonary Tuberculosis (PTB).

MATERIALS AND METHODS
This is an observational study conducted in the Department of Pulmonary Medicine, RRMCH, Bengaluru, between August 2016 to June 2017. Patients who presented with symptoms and signs suggestive of pulmonary tuberculosis, chest x-ray showing features of pulmonary tuberculosis were included in the study whose sputum for AFB was negative. Sputum/BAL samples from these patients were sent for CBNAAT.

RESULTS
CBNAAT was done for 80 samples of the patients who were having history and CXR suggestive of pulmonary tuberculosis with sputum AFB negative. Out of these 80 sputum-AFB negative samples, 29 (36%) were CBNAAT positive. Most of the cases were men aged >50 yrs. Most diabetics (78%) were CBNAAT positive. Most diabetics had HbA1C >10. Most diabetics had lower zone infiltrates on CXR.

CONCLUSION
Because of its simplicity, rapidity and sensitivity, this seems to be a very novel tool for diagnosis of extrapulmonary tuberculosis from clinical samples and that it should be researched more thoroughly.

KEYWORDS
Pulmonary Tuberculosis, Mycobacterium Tuberculosis, CBNAAT.

BACKGROUND
Tuberculosis (TB) is a major health problem in India. India continues to be the highest TB burden country in the world in terms of the absolute numbers of incidence cases each year.[1] Mortality due to TB is the third leading cause of Years of Life Lost (YLL) in the country. The estimated incidence (new TB cases per year) is 2.8 million cases in 2015 (CI- 112 to 355) per 1,00,000 population) with a confidence interval of 1.47 to 4.65 million. The estimated mortality due to TB is 4,80,000 (CI- 3,80,000-5,90,000). Approximately, 5% of the incident TB cases have comorbidity with HIV, though this proportion varies depending on the HIV prevalence of the population.[2]

There are certain risk groups that are more susceptible to getting infected including- young adults (more commonly males), those in developing countries, healthcare workers who are around the disease frequently and those whose immune systems are weak as in those who have HIV or smoke.[3,4] In fact, TB is the leading cause of death in those infected with HIV and HIV-TB comorbidity has been widely studied.[4,5]

Early case detection is vital to interrupt the transmission of TB disease. Smear microscopy is the cornerstone for the diagnosis of TB in resource-limited settings, but it has only modest (35-80%) sensitivity and a poor Positive Predictive Value (PPV).[6] Culture is the "gold standard" for final determination and also permits drug susceptibility testing. However, it remains largely inaccessible in resource limited settings as a result of infrastructure and financial limitations. Even where accessible, culture results are typically not available for 2-6 weeks.[7]

RNTCP screens around 20 million TB symptomatic by microscopy and initiates around 1.5 million cases of TB on treatment annually since 2007-08. Rapid molecular diagnostics introduced since 2009 and scaled up from 2012 onwards has ensured that line probe assay and CBNAAT testing is available throughout the country. In 2016, 5,20,000 patients have been tested and 35,000 rifampicin resistant/MDR-TB patients have been diagnosed. Second line DST using liquid culture systems are in place and are being scaled up to cover the entire country by December 2017.[2]

CBNAAT marks an important development in the field of rapid molecular TB diagnostics. This assay was rapidly endorsed by the WHO (World Health Organization) in December 2010 as a replacement for sputum smear microscopy, particularly in settings with high rates of HIV-associated TB and multidrug-resistant TB developed for testing sputum samples.

This multifunctional diagnostic platform is an automated closed system that performs real-time PCR and can be used by operators with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 hours. The test detects DNA specific for Mycobacterium tuberculosis by polymerase chain reaction. More recently, however, evaluations of the assay have extended to a variety of non-respiratory clinical samples from patients with extrapulmonary tuberculosis. A definitive diagnosis can be made by detection of M. tuberculosis with the improvement of Nucleic Acid Amplification Techniques (NAAT) in TB detection, sensitivity of tests for TB detection has been rising.

GeneXpert test is a semi-quantitative nested real-time PCR in-vitro diagnostic test with two uses-

a. The detection of Mycobacterium tuberculosis complex DNA in sputum samples or concentrated sediments prepared from induced or expectorated sputum that are either Acid-Fast Bacilli (AFB) smear positive or negative.

b. The detection of rifampicin resistance associated mutations of the rpoB gene in samples from patients of rifampicin resistance.[7,8]

This study aims to assess the performance of CBNAAT test for diagnosis of TB in immunocompromised patients (diabetes mellitus).

MATERIALS AND METHODS

This study is conducted to evaluate the clinical value of CBNAAT (cartridge-based nucleic acid amplification test) MTB/RIF assay in patients with suspected pulmonary tuberculosis by comparing with ZN staining.

We also evaluated for the rifampicin resistance if present from the same test.

This is an observational study conducted in the Department of Pulmonary Medicine, Rajarajeswari Medical College and Hospital, Bengaluru, between August 2016 to June 2017. Patients who presented with symptoms and signs suggestive of pulmonary tuberculosis, chest x-ray showing features of pulmonary tuberculosis were included in the study whose sputum AFB was negative. BAL was done to the patients who were unable to produce sputum, even after 3% hypertonic normal saline nebulisation. Sputum/BAL samples from these patients were sent for CBNAAT.

Inclusion Criteria
Age >15 yrs.
All suspected cases of tuberculosis.
Defaulter, relapse, reactivation and treatment failure case of tuberculosis.

Exclusion Criteria
Known MDR tuberculosis.
All extrapulmonary tuberculosis cases.

Methods- For the test procedure, the sample is poured into a single-use disposable cartridge that is placed in the Xpert module with the results produced in less than 2 hours. The system automatically interprets all results from measured fluorescent signals with embedded calculation algorithms into the following categories invalid, if PCR inhibitors are detected with amplification failure; negative or positive. If positive, the strain was categorised as susceptible or resistant to rifampicin.

Standard Assay Procedure of CBNAAT- The assay utilises single-use plastic cartridges with multiple chambers that are preloaded with liquid buffers and lyophilised reagent beads necessary for sample processing, DNA extraction and heminested rt-PCR.[9,10]

Clinical sputum samples (or decontaminated sputum pellets) are treated with sodium hydroxide and isopropanol-containing Sample Reagent (SR). The SR is added to the sample (currently recommended at a 3:1 ratio for sputum pellets and a 2:1 ratio for unprocessed sputum samples) and incubated at room temperature for 15 mins. The treated sample is then manually transferred to the cartridge, which is loaded into the GeneXpert instrument. Subsequent processing is fully automated. The cartridge incorporates a syringe drive, a rotary drive and a filter upon which M. tuberculosis bacilli are deposited after being liberated from the clinical material. The test platform employs a sonic horn that inserts into the cartridge base to cause ultrasonic lysis of the bacilli and release of the genetic material. The assay then amplifies a 192 bp segment of the rpoB gene using a heminested RT-PCR reaction. Mycobacterium tuberculosis is detected by the five overlapping molecular probes (probes A-E) that collectively are complementary to the entire 81-bp rpoB core region.[10,11]

M. tuberculosis is identified when at least two of the five probes give positive signals with a Cycle Threshold (CT) of d"38 cycles and that differ by no more than a prespecified number of cycles. The basis for detection of rifampicin resistance is the difference between the first (early CT) and the last (late CT) M. tuberculosis-specific beacon (ACT). The system was originally configured such that resistance was reported when ACT was >3.5 cycles and sensitive if d"3.5 cycles.[7]

RESULTS

CBNAAT was done for 80 samples of the patients who were having history and CXR suggestive of pulmonary tuberculosis with sputum AFB negative. Out of these 80 sputum AFB-negative samples, 29 (36%) were CBNAAT positive and rest were negative. Most of the cases were men aged >50 yrs. Most diabetics (78%) were CBNAAT positive. Diabetics had lower zone infiltrates on CXR.

Age	Male	Female	CBNAAT +	CBNAAT -
<25 yrs.	6	5	3	8
25-50 yrs.	18	10	6	22
>50 yrs.	28	13	20	21
Total	**52**	**28**	**29**	**51**
Table 1. Demographic Data				

In our study, patients aged less than 25 years were 11, age between 25 to 50 years were 28 and remaining 41 were aged above 50 years. Most of the patients were aged above 50 years.

	CBNAAT +	CBNAAT -	Total
Male	22	30	**52**
Female	7	21	**28**
Total	**29**	**51**	**80**
Table 2. CBNAAT Results			

Out of 52 males suspected, tuberculosis patients 22 (42%) were CBNAAT positive and among 28 females suspected tuberculosis patients 7 (25%) were CBNAAT positive. Most of the male patients turned out to be positive for CBNAAT.

Diabetics	CBNAAT +	CBNAAT -	Total	% CBNAAT +
Men	15	3	18	83.3%
Women	3	2	5	60%
Table 3. Diabetes Mellitus Status				

In our study, there were total 23 diabetics, in which 18 were males and remaining 5 were female patients. Among 18 males 15 (83.3%) were CBNAAT positive and among 5 females 3 (60%) were CBNAAT positive.

| Diabetics CBNAAT Positive	HbA1C <10	HbA1C >10
18	6	12
Table 4. Diabetes Control Status		

In this study, out of 18 diabetic CBNAAT positive patients, most of them- 12 (66.6%) had HbA1C more than 10 indicating most uncontrolled diabetics (immunocompromised) had tuberculosis.

Diabetics	CXR Lower Zones Involved	CXR Other Zones Involved
Men (18)	15 (83.3%)	3 (16.9%)
Women (5)	3 (60%)	2 (40%)
Table 5. Chest X-Ray Involvement		

In this study, 15 (83.3%) male patients out of 18 diabetics had lower zone involvement on CXR and 3 (60%) female patients out of 5 had lower zone involvement on CXR. This indicates most diabetics who were CBNAAT positive had lower zone involved.

SUMMARY
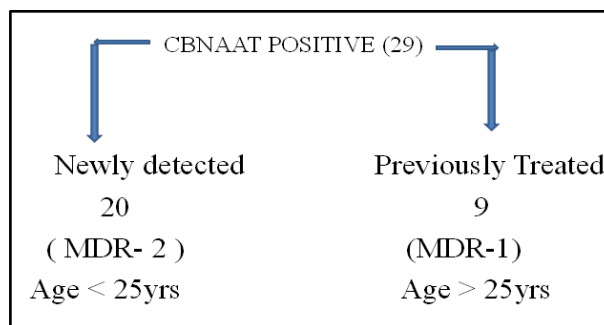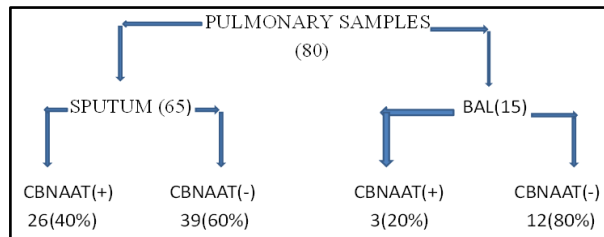
Out of total 29 CBNAAT positive cases, 20 cases were newly detected among which 2 were primary rifampicin resistance cases and both were females aged below 25 years and 9 cases were previously treated cases among which 1 was rifampicin resistance, which was aged above 25 years.

DISCUSSION

In a study conducted by Mohanty T et al[12] shows out of 71 patients who were smear AFB-negative cases, sputum for CBNAAT was positive in 23 (32%) patients, which correlates with our study, out of 80 sputum AFB-negative samples 29 (36%) were CBNAAT positive and rest were negative. Incidence was more in diabetics, i.e. out of 23 diabetics who were suspects of tuberculosis 18 were positive for CBNAAT (78%) and most of the cases had HbA1C >10 (12 cases out of 18, which is 66.6%) and most diabetics had lower zone involvement on chest x-rays (78%).

CONCLUSION

Many of AFB negative samples came to be positive with CBNAAT indicating CBNAAT assay is highly sensitive and specific technique. We also found, out of 29 samples, which came positive with CBNAAT, 3 were resistant to rifampicin, which we would have missed with AFB staining or by other conventional methods. The result of the study revealed a maximum positivity rate by CBNAAT, which indicated that it is a more sensitive technique as compared to conventional methods.

REFERENCES

[1] World Health Organization. Global tuberculosis report 2014. Geneva: WHO 2014.
     http://apps.who.int/iris/bitstream/10665/137094/1/9789241564809_eng.pdf?ua=1.

[2] RNTCP Guidelines 2017.

[3] World Health Organization. c1948. Tuberculosis fact sheet. Geneva (Switzerland): WHO global TB programme 2014.

[4] Goldman L, Schafer AI. Tuberculosis: disease overview. In: Goldman L, Schafer AI, eds. Goldman's cecil medicine: expert consult premium edition. 24th edn. St. Louis (MO): Saunders Elsevier 2011.

[5] Cruz-Knight W, Blake-Gumbs L. Tuberculosis: an overview. Prim Care 2013;40(3):743-756.

[6] Mathew P, Kuo YH, Vazirani B, et al. Are three sputum acid-fast bacillus smears necessary for discontinuing tuberculosis isolation? J Clin Microbiol 2002;40(9): 3482-3484.

[7] Sowjanya DS, Behera G, Reddy VVR, et al. CBNAAT: a novel diagnostic tool for rapid and specific detection of mycobacterium tuberculosis in pulmonary samples. IJHRMIMS 2014:28-31.

[8] Rattan A, Kalia A, Ahmad A. Multidrug-resistant mycobacterium tuberculosis: molecular perspectives. Emerg Infect Dis 1998;4(2):195-209.

[9] Boehme CC, Nicol MP, Nabeta P et al. Feasibility, diagnostic accuracy, and effectiveness of decentralized use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 2011;377(9776):1495-1505.

[10] Helb D, Jones M, Story E, et al. Rapid detection of mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 2010;48(1):229-237.

[11] Blakemore R, Story E, Helb D, et al. Evaluation of the analytical performance of the Xpert MTB/RIF assay. J Clin Microbiol 2010;48(7):2495-2501.

[12] Mohanty T, Panigrahi SK, Pattnaik M, et al. Study on diagnostic modalities in smear negative pulmonary tuberculosis with special reference to sputum induction (SI CBNAAT), bronchoscopy (BAL CBNAAT and BAL culture). J Evid Based Med Healthc 2017;4(47):2858-2862.