A study on the role of Cartridge Based Nucleic Acid Amplification Test (CBNAAT) in the Diagnosis of Sputum Negative Pulmonary Tuberculosis

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Abstract

Background: Tuberculosis(TB) is among the top threats to public health worldwide; with increasing incidence of drug resistance and smear-negative cases. Smear-negative TB has been an area of diagnostic dilemma until the advent the Xpert MTB/RIF assay. The Xpert MTB/RIF assay also known as CBNAAT (Cartridge Based Nucleic Acid Amplification Test) is a novel integrated molecular diagnostic test for rapid detection of Mycobacterium tuberculosis as well as rifampicin (RIF) resistance.

Objective: To evaluate the role of CBNAAT in the diagnosis of sputum negative pulmonary tuberculosis.

Methods: A prospective cross-sectional study was conducted in a tertiary healthcare centre, among new sputum negative pulmonary TB cases. Patients with negative sputum samples showing signs and symptoms or chest X-Ray suggestive of TB were included in the study. These cases were sent for CBNAAT testing.

Results: A total of 75 sputum-AFB negative samples were tested by CBNAAT. Out of these, 54 were detected positive for MTB and 21 were negative. Rifampicin resistance was seen in 4 samples. This study shows 72% detection rate by CBNAAT.

Conclusion: CBNAAT is a breakthrough in the field of TB diagnosis because of its simplicity, rapidity and diagnostic accuracy particularly in cases of sputum negative TB.

Keywords: Pulmonary tuberculosis, Mycobacterium tuberculosis, CBNAAT, Rifampicin.

Introduction

Tuberculosis (TB) is one among the leading causes of death globally. In 2019, 10 million people fell ill with TB, and an estimated 1.2 million died from the disease[1]. Tuberculosis is a chronic bacterial infection caused by Mycobacterium tuberculosis (MTB), characterized pathologically by the formation of granulomas[2]. As per WHO Global Tuberculosis Report 2020, India accounts for a quarter of global tuberculosis burden, with 2.2 million incident cases annually and the largest share of drug-resistant tuberculosis globally (27%)[1].

Detection of smear-negative and drug-resistant tuberculosis has been an area of diagnostic dilemma in recent times. The primary diagnostic tool, Sputum smear microscopy for Acid-Fast bacilli (AFB) has low sensitivity and more false negative results are reported particularly in smear-negative TB due to low bacillary load. Microscopy detects only half the number of TB cases[3] and cannot detect drug resistance. Chest X-Ray (CXR) as a diagnostic tool is more sensitive but less specific[4]. Moreover, there is significant intra- and inter observer variation in the readings of CXRs[4]. Over-reliance of chest X-Rays can lead to misdiagnosis and hence should be used as an adjunct alongside other diagnostic tools. Mycobacterial culture is a standard and most sensitive test for detection of TB, however its use in clinical practice is limited due to slow turnaround time (6-8 weeks), strict biosafety requirements and costly infrastructure[5].

Diagnostic delays leads to continued transmission and worse clinical outcomes. As reported from O. Opota et al, 55% of smear-negative TB patients had clinical presentation suggestive of high transmission potential[6]. Another study reported transmission in contacts of smear negative cases to be as high as 17%[7]. To overcome this problem, more sensitive and rapid diagnostic methods for smear-negative TB are paramount.

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The advent of Xpert MTB/RIF assay (Cepheid, Sunnyvale, USA), also known as CBNAAT (Cartridge Based Nucleic Acid Amplification Test) led to a paradigm shift in the diagnosis of TB. It is a novel integrated molecular diagnostic test for rapid detection of Mycobacterium tuberculosis as well as rifampicin resistance. It was endorsed by WHO in 2010 and is now a mainstay in diagnosis of TB as the most sensitive rapid test for TB diagnosis in paucibacillary respiratory samples[8]. Several studies evaluating CBNAAT performance in smear-negative pulmonary TB revealed sensitivity ranging from 47% to 87%[9].

CBNAAT is positioned as the initial diagnostic test for all people suspected of having TB in India's national algorithm for TB diagnosis. Also, CBNAAT is the follow-on test for sputum-negative presumptive TB[10]. This study was conducted at a tertiary health care centre, Davangere district, central part of Karnataka, India. No such studies have been conducted in the specified study area, hence we aim to assess the performance of CBNAAT in diagnosis of new sputum negative Pulmonary tuberculosis cases.

Materials and Methods:

This was a prospective cross sectional study done over a period of three months from May 2019- July 2019. We included 75 outpatients of both genders, aged above 18 years showing signs and symptoms or chest X-Ray suggestive of tuberculosis, and initial two sputum samples negative for smear microscopy. Clinical features of tuberculosis include fever, cough or night sweats for more than 2 weeks, hemoptysis, pyrexia of unknown origin, malaise, fatigue and/or weight loss for more than 1 month. Patients with past history of tuberculosis, co-infection with HIV/AIDS, or patients with extra-pulmonary TB were excluded. Those who had taken anti-tubercular treatment previously were also excluded from the study.

Routine diagnostic procedures were followed as per protocol. Smear microscopy (Fluorochrome staining) was performed. The smear slides were flooded with Auramine O (for 15 min) and decolourized using acid alcohol (2 min). After rinsing with distilled water, smear was flooded with potassium permanganate (2 min). It is again rinsed with distilled water, air dried and focused under Fluorescent Microscope (FM). Smear negative samples were sent for CBNAAT testing.

Ethical approval was obtained from the Institutional Ethics Committee. Approval of the District Tuberculosis Officer (DTO) was obtained for conducting the study. Objectives of the study were explained in local language and a written informed consent was taken from all the study participants prior to data collection.

Data collection was conducted in two phases; Phase 1: A questionnaire was used to collect basic information of the study participants, their socio demographic details and associated co morbidities. The Socio-economic class was determined by Below poverty line/ Above poverty line ration card held by the study participants; followed by Phase 2: The individuals were subjected for CBNAAT testing at the tertiary health care centre, Davangere.

CBNAAT (Cartridge Based Nucleic Acid Amplification Test) also known as Xpert MTB/RIF assay (developed by Cepheid, Sunnyvale, USA) is the WHO-recommended rapid diagnostic test for detection of TB and rifampicin resistance. The system consists of the device, a computer, a barcode scanner, and software; used in conducting the tests and viewing the results[11]. The CBNAAT instrument is a fully automated, closed (and therefore safe) real-time PCR based assay. It employs single-use disposable cartridges that hold PCR reagents and host the PCR process[12] This greatly simplifies testing as all the key steps of sample processing, nucleic acid amplification and detection of target sequences are integrated and automated within the cartridge. The cartridges are self-contained and cross-contamination between samples is eliminated[13].

The cartridges were labelled with corresponding sample IDs of the patients (barcode). Clinical sputum samples were collected in conical, screw-capped falcon tubes. The Xpert MTB/RIF sample reagent (SR) was directly added to these sputum samples in the ratio 2:1. After placing the cap, the tube was shaken vigorously 10-20 times and incubated at room temperature. After 10 min, the tube is shaken again for 10-20 times and kept aside for 5 min. Final samples should be liquefied with no visible clumps of sputum. 2ml of this sample is transferred to the Xpert MTB/RIF cartridge using a sterile pipette. The cartridge lid is closed. Create test was opted in the software and cartridge barcode is scanned for patient sample ID. The cartridge is inserted in the modules of CBNAAT instrument. The module door is closed and program initiated. After the program completed, the cartridge was removed and disposed off in the Biohazard Waste disposal bin and the results were recorded electronically[12].

The system automatically interprets all results using fluorescent signals. The assay amplifies a 192-bp segment of rpoB gene of M. tuberculosis using a heminested real-time PCR reaction[13]. MTB is detected by five unique probes (A-E) called molecular beacons that detect the rpoB gene. More than 95% of mutations associated with rifampicin resistance occur in 81-bp core region of rpoB gene. These five molecular beacons, each labelled with coloured fluorophore

are complimentary to entire 81-bp core region and respond to specific target sequence within the gene. The generation of all five fluorescent colours during PCR amplification indicates rifampicin susceptible MTB result, while any mutation within the core region prevents the binding of respective molecular beacon, resulting in absence of colour which indicates rifampicin resistance. CBNAAT provided results of both MTB and rifampicin resistance in two hours[14].

The data was entered in Microsoft Excel and analyzed using SPSS (Statistical Package for the Social Sciences) software. Descriptive analyses done by summarizing continuous and categorical variables; Continuous variables were summarized using mean (SD), Categorical variables using proportion. Data is presented in percentage, proportions and ratio. To study the association of categorical variables chi-squire test is used. Statistical significance set at 0.05% level of significance ($P < 0.05$).

Results:

A total of 75 patients with clinical features of tuberculosis and among whom the initial two sputum samples are negative for Acid-Fast Bacilli microscopy participated in this study.

The age of the participant ranged from 18 to 72 years, with mean age and standard deviation 43.01 +/- 15.21 yrs. Among these participants 58 (77.3%) were male and 17 (22.7%) were female. Majority of them were Hindu 62 (82.7%) based on religion and 46 (62.6%) of them were employed. Among the participants 33 (44%) of them belonged to below poverty line families 34 (45.3%) of the participants were from rural background.

Table 1: Socio demographic details

Sl	Variable		Number(%)
1	Age	<30 yrs	19 (25.3%)
		30 -50yrs	31 (41.3%)
		>50 yrs	25 (33.3%)
2	Gender	Male	58 (77.3%)
		Female	17 (22.7%)
3.	Residency	Urban	41(54.7%)
		Rural	34 (45.3%)
4.	SES	Below Poverty Line	42 (56.0%)
		Above Poverty Line	33 (44.0%)
5	Occupation	Employed	46 (62.2%)
		Unemployed	29 (37.8%)

Among the participants 9 (12%) were hypertensive and 10 (13.33%) were diabetic; among whom 6 (8%) were both diabetic and hypertensive. The duration of co morbidities varied from 2- 15 yrs.

Among the study participants with sputum AFB-negative samples, 54 (72%) were CBNAAT positive and 21 (28%) were negative; 42 (56.0%) were Chest X ray positive, 18 (24%) were negative and 15 (20%) were in error/inconclusive.

Among the study participant's majority (72%) of them were observed to be CBNAAT positive.

Table 2: Percentage of CBNAAT positive cases

Sl	CBNAAT results	No of samples	Percentage
1	CBNAAT positive	54	72%
2	CBNAAT negative	21	28%
Total		75	100

Among the 54 samples detected positive by CBNAAT, 50 (92.5%) were identified as sensitive to Rifampicin and 4 (7.4%) were identified as Rifampicin resistant.

Table 3: Percentage of Rifampicin resistance detected by CBNAAT (n=54)

Sl	Drug Resistance Pattern	No of samples	Percentage
1	Rifampicin Sensitive (RS)	50	92.5%
2	Rifampicin Resistant (RR)	4	7.4%
Total		54	100

There was no statistical significant association observed for CBNAAT results along with socio demographic data and co morbidities.

Discussion:

In this study, we evaluated the role of CBNAAT in the diagnosis of sputum-negative pulmonary TB cases. Out of 75 sputum-negative samples, 54 were detected positive by CBNAAT, which was missed by smear microscopy. CBNAAT shows an overall 72% detection rate.

Our study shows 72% detection rate of MTB by CBNAAT which is comparable to the results of the study conducted by Sowjanya DS et al[15]. The study compared the performance of CBNAAT with smear microscopy (ZN staining). CBNAAT detected MTB in 144 out of 205 samples showing 70.24% detection rate, thereby higher compared to 52.68% detection rate shown by microscopy. Among these 144 samples detected MTB positive by CBNAAT, 36 were sputum smear negative samples. This shows CBNAAT has better detection rate and is more efficient in diagnosing sputum negative TB than microscopy.

A study reported 72% sensitivity of CBNAAT in smear-negative patients, and a specificity of 100%[16]. Another study conducted by Boehme CC, reported sensitivity 72.5% for sputum negative cases with a single MTB/RIF test, 85.1% for two MTB/RIF tests, and 90.2% for three MTB/RIF tests[17].

A multicenter study conducted by Boheme CC et al reported 76.9% sensitivity of CBNAAT in detection of smear-negative, culture-positive patients. The study found 99.0% specificity of CBNAAT for detection of MTB. The authors also found the use of CBNAAT reduced median time to treatment for smear-negative TB from 56 days (39-81) to 5 days (2-8). Thus concluding that CBNAAT is a rapid point-of-care test, hence reduces diagnostic delays, associated transmission of disease and mistreatment[18].

Our findings, 72% detection rate of MTB by CBNAAT is comparatively more than recent studies by Anand MK et al (42.1%) and Rao KM et al (36%)[19,20]. Also, among the samples detected positive for MTB, 7.4% were Rifampicin resistant, which is more than the study (2.78%) at Vizianagram and Bengaluru (5.4%), and less compared to study (15.1%) reported from Belgaum and Dharwad[15,19,20].

Rifampicin is associated with resistance to other anti-tubercular drugs, mainly Isoniazid (93% of cases), which makes rifampicin resistance a surrogate marker for detecting cases of multi-drug resistant TB (MDR-TB)[19]. A study conducted by Boheme CC et al reported CBNAAT sensitivity and specificity for rifampicin resistance 94.4% and 98.3% respectively[18]. This data suggests that CBNAAT detects rifampicin resistance with high efficiency and can be used for screening of MDR-TB.

Our study posed several limitations. Firstly, the study was conducted for a short duration and the relatively small sample size limited the study power. Secondly, we could not carry out a direct comparison between CBNAAT results and microscopy as we included only smear negative samples. Hence, more measures of diagnostic accuracy like specificity, predictive values or error rates could not be assessed.

Finally, the variation in results from other studies may be resulted from the difference in inclusion criteria, as we excluded pediatric, HIV/TB co-infected and extra-pulmonary TB cases. The low bacillary load from these sub-groups of patients could result in higher negative results from CBNAAT.

Nevertheless, CBNAAT is a novel diagnostic technique with several advantages over conventional methods and has greatly improved TB diagnosis over the years. The most distinguishable asset of CBNAAT is the simultaneous detection of TB and rifampicin resistance with a rapid turnaround time of 2 hours. CBNAAT has high sensitivity, specificity and can be easily done by routine staff with minimal training, requires minimum hands-on time and negligible safety concerns for persons handling the samples[16,17,18]. Furthermore, CBNAAT was shown to be cost effective compared to microscopy, making its implementation feasible in low and middle income settings.

Conclusion

In this study we observed a substantial number of Acid Fast Bacilli negative samples came to be positive with CBNAAT indicating CBNAAT assay is more sensitive and specific technique. 72% of the sample came to be CBNAAT positive among which 7.4% were rifampicin resistant. The results in this study revealed maximum positivity rate by CBNAAT, than compared to other conventional methods. With increasing coverage of CBNAAT services, it will help in early diagnosis, treatment, reduction in drug resistance Tuberculosis.

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