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Original Article

USE OF CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST FOR RAPID DETECTION OF MYCOBACTERIUM TUBERCULOSIS IN PULMONARY AND EXTRAPULMONARY TUBERCULOSIS

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ABSTRACT

Objective: Tuberculosis is an airborne infection caused by *Mycobacterium tuberculosis*. Timely diagnosis and treatment are important to prevent the spread of infection. Cartridge-based nucleic acid amplification test (CBNAAT) provides a valuable tool in the early detection of TB. This study is undertaken to evaluate the utility of CBNAAT for the detection of MTB. Comparison of cartridge-based nucleic acid amplification testing with ZN staining.

Methods: This prospective observational study was carried out in the Department of Microbiology, BLDEDU's Shri B. M. Patil Medical College, Hospital and RC and Dr. Karigoudar Diagnostic Laboratory, Vijayapur. A total of 129 samples from patients with the presumptive diagnosis of TB based on history, clinical presentation, and radiological findings were included in the study. All samples were subjected to ZN staining, and Cartridge-based nucleic acid amplification test and data were analyzed.

Results: The present study showed ZN smear positivity of 7.75% and CBNAAT positivity of 19.38%. CBNAAT sensitivity and specificity were 90% and 86.55, respectively, compared with ZN staining with a significant P value of <0.001.

Conclusion: CBNAAT helps diagnose TB and detect rifampicin resistance within 2-3 h with high sensitivity and specificity. Rifampicin resistance detection is of great concern, which otherwise leads to treatment failure and on time spread of multidrug resistance TB, leading to increased morbidity and mortality.

Keywords: Tuberculosis, CBNAAT, Rifampicin resistance

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INTRODUCTION

Tuberculosis (TB) is a communicable disease caused by the bacillus *Mycobacterium tuberculosis* (MTB), an airborne infection. It causes pulmonary TB (PTB) involving lungs in about 85-90% of cases and extrapulmonary TB (EPTB) involving pleura, the lymphatic system, the central nervous system, the bones and joints, and the genitourinary system accounting for about 15-20% of TB cases. TB is a major global health problem in developing countries. Globally around 10 million people suffer from TB infection every year, accounting for one-third of the world's population. The increased occurrence of TB in underdeveloped countries is due to poor hygienic conditions, overcrowding, and the increased occurrence of HIV infection. India contributes to one-fourth of the world's TB burden. According to WHO 2018 report, India was having the world's largest number of TB patients. As of 2019, India reported the largest number of individuals suffering from drug-resistant TB. As of 2019 report, 21.5 lakh cases were notified as TB compared to 18.3 lakh cases of TB in 2018. This is mainly due to the rapid cartridge-based nucleic acid amplification test (CBNAAT) method for the early TB diagnosis. With timely detection and treatment, most TB cases can be cured, and the forward spread of infection can be reduced [1]. Prompt and accurate detection of TB is needed for early and effective treatment, thereby reducing the burden of TB [2]. The overall priority for TB care and control is to improve early case-detection. Hidden undiagnosed cases of TB are of major global concern. The innovation of CBNAAT is an important tool to fight against TB. CBNAAT provides a valuable tool in the early detection of smear-negative PTB, EPTB, TB-HIV, and Multidrug-resistant tuberculosis (MDR-TB). Its high sensitivity, specificity, and less turnaround time for timely diagnosis of pulmonary and extrapulmonary cases and detection of resistance towards rifampicin provide a potential role for controlling TB infection [3].

The CBNAAT has advantages over conventional methods for diagnosing tuberculosis (TB) and detecting rifampicin-resistance. It is very costly. So in a low-resource hospital setup, CBNAAT must be used in a manner that will have the peak impact on patient care. In this context, the present study was undertaken to evaluate the utility of CBNAAT for the detection of MTB and to compare CBNAAT with ZN staining.

MATERIALS AND METHODS

This Prospective observational study was carried out in the Department of Microbiology, BLDEDU's Shri B. M. Patil Medical College, Vijayapur and Dr. Karigoudar Diagnostic Laboratory, Vijayapur. The study duration was from March 2018 to August 2019. Patients with the presumptive diagnosis of TB based on history, clinical presentation, and radiological findings were included in the study. A total of 129 samples collected from suspected patients were included in the present study. The patients on treatment with TB drugs in the last 2 mo were excluded from this study. From each patient, either a minimum of 5.0 ml of sputum sample or 2 ml of fluid (CSF, pus, ascitic fluid, and pericardial fluid) and aspirate from lymph nodes were collected according to standard protocol. All samples were subjected to Ziehl–Neelsen staining and CBNAAT, and data were analyzed.

CBNAAT is a polymerase chain reaction (PCR) based method for the detection of TB and resistance to Rifampicin. CBNAAT device is a disposable, single-use, self-enclosed cartridge with automated sample processing, amplification, and detection facility. The sample reagent will be added to the sample in a 2:1 ratio to liquefy and inactivate the bacteria in the sample, 2 ml sampled into the cartridge, and loaded into the assay procedure device. All further steps are automated. The test results are categorized into the following result patterns: No-MTB detected; MTB detected-

Rifampicin resistance detected; MTB detected no rifampicin resistance detected; MTB detected rifampicin resistance indeterminate, and an invalid result [3].

Statistical analysis

Statistical analyses were performed using SPSS version 20.0 software for MS-Windows. P-value was calculated using the Chi-Square test (P-value of <0.05 = significant).

RESULTS

A total of 129 samples collected from the patients suspected of tuberculosis based on clinical and radiological findings were analyzed. The most common age group suspected of TB was from 21-40 y (41.08%), followed by 41-60 y (24.03%). The mean age of the subject was found to be 33.55±19. In that, 59.56 (43.41%) were males, and 73 (56.59%) were females with a ratio of 1:1.3 (M: F). Samples included lymph node aspirate 42 (32.56%), sputum 25 (19.38%), CSF 17 (13.18%), endometrial tissue10 (7.75%), synovial fluid 3 (2.33%) and others like Urine, Stool, Peritoneal fluid 32 (24.81%) among 129 samples. All these 129 samples were subjected to Ziehl Neelsen staining and CBNAAT. Out of these 129 samples subjected to Ziehl Neelsen staining, 10 (7.75%) samples were smear-positive, and 119 (92.25%) were smear-negative for acid-fast bacilli. All these samples were also tested by CBNAAT, which showed 25 (19.38%) MTB positive, and 104 (80.62%) MTB negative. The CBNAAT detected MTB in 09 out of 10 ZN smear-positive and 15 out of 115 ZN smear-negative cases. The present study showed ZN smear positivity (7.75%) and CBNAAT positivity (19.38%) with CBNAAT sensitivity and specificity of 90% and 86.55% when compared with ZN staining with a significant P value of <0.0001. CBNAAT showed 100% sensitivity and 100% specificity when compared to ZN staining for pulmonary samples. In the same way, when the CBNAAT results are compared with ZN staining for extra pulmonary samples, CBNAAT showed 87.5% sensitivity and 81.18% specificity. Out of 25 positives for MTB by CBNAAT, MTB was detected in 15 cases. Among the 15 cases, females (08) and males (07) were 21-40 y old. In the age group 41-60 y, MTB was detected in 5 cases, male (04) and female (01). In the age group <20 y, 4 cases showed MTB positive results, and all were females. In >60 y, 1 MTB was detected in male patient. Overall, MTB was detected in high number among males (21.43%) than females (17.81%) by CBNAAT. Age, sex, and sample-wise distribution of MTB detection by assay have been shown in fig. 1 and 2. Out of 25 detected for MTB by CBNAAT, 10 were tested for rifampicin resistance; in that 10, 2 (20%) were rifampicin resistance, and the remaining 08 (80%) were sensitive to rifampicin. While 15 were not tested due to low bacillary load.

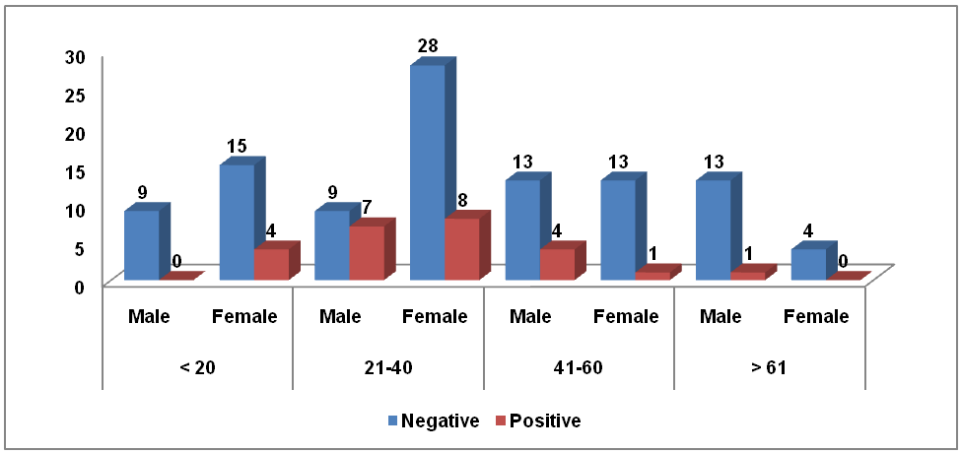

Fig. 1: Age and sex-wise detection of MTB by CBNAAT

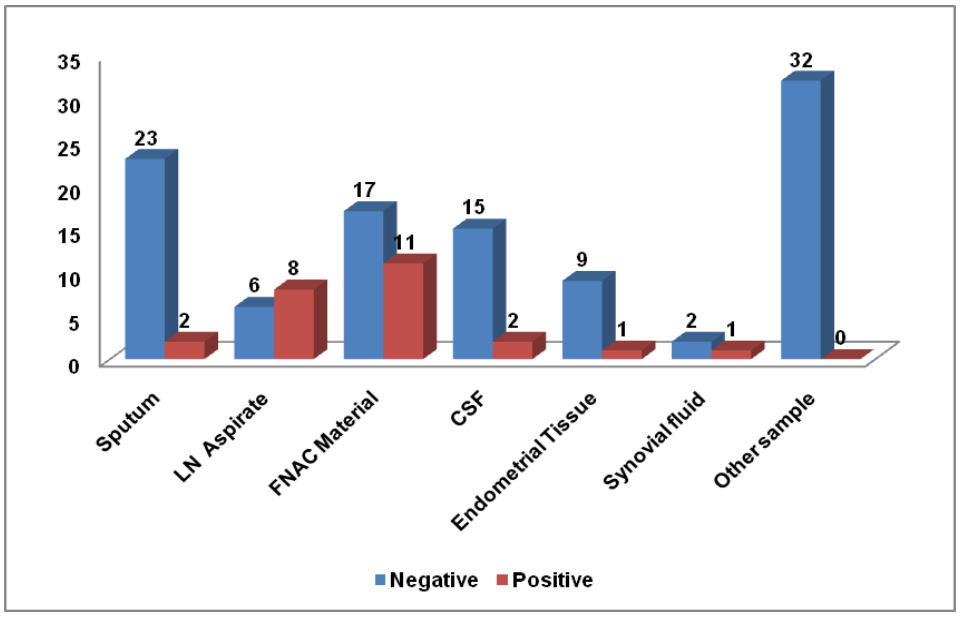

Fig. 2: Sample wise detection of MTB by CBNAAT

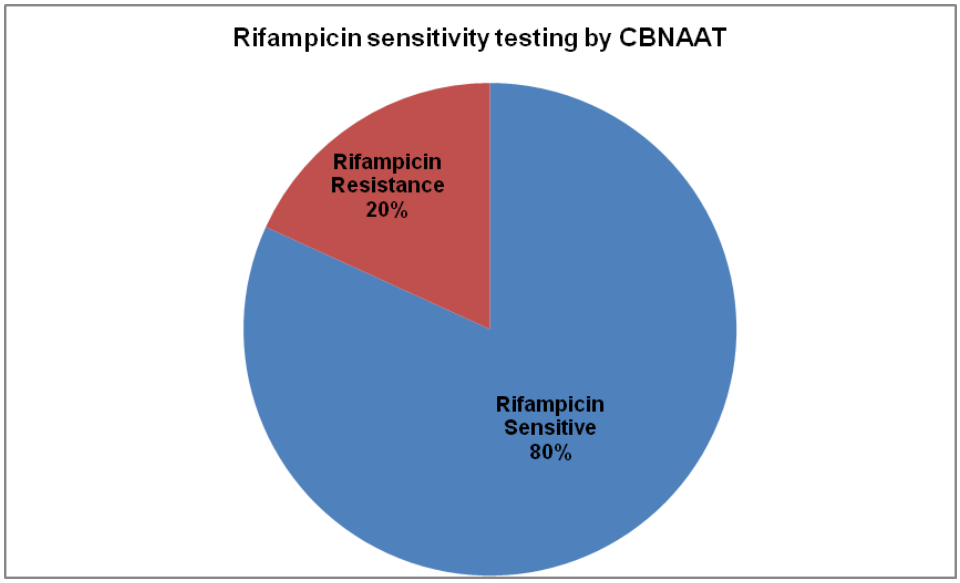

Fig. 3: Rifampicin resistance testing by CBNAAT

Table 1: Comparison of CBNAAT with ZN stain (pulmonary and extrapulmonary samples)

Test	ZN stain			
CBNAAT	**Positive**	**Negative**	**Total**	**%**
Positive	9	16	25	19.38
Negative	01	103	104	80.62
Total	10	119	129	100
%	7.75	92.25	100	

χ^2= 29876, P<0.0001, Sensitivity = 90%, Specificity = 86.55%, Positive predictive value =36%, Negative predictive value =99.04%

Table 2: Comparison of CBNAAT with ZN stain (Pulmonary Sample)

Test	ZN stain			
CBNAAT	**Positive**	**Negative**	**Total**	**%**
Positive	2	0	2	6
Negative	0	34	34	94
Total	2	34	36	100
%	6%	94%	100	

χ^2= 19.434, P<0.0001, Sensitivity = 100%, Specificity = 100%, Positive predictive value =100%, Negative predictive value =100%

Table 3: Comparison with CBNAAT ZN stain (Extra Pulmonary Sample)

Test	ZN stain			
CBNAAT	Positive	Negative	Total	%
Positive	7	16	23	25
Negative	1	69	70	75
Total	8	85	93	100
%	9%	91%	100	

χ^2= 15.02, P = 0.0001, Sensitivity = 87.5%, Specificity = 81.18%, Positive predictive value =30.43%, Negative predictive value =98.57%

DISCUSSION

India has more than a million cases each year that are not notified and remain undiagnosed [4]. According to the World Health Organization, in 2019, India continued at the top list, with tuberculosis (TB) having the majority number of MDR-TB cases. Early and effective treatment depends on the timely diagnosis of TB. The overall main concern for TB care and control is to improve early case-detection, mainly in ZN smear-negative PTB and EPTB. EPTB diagnosis is considered a major concern because clinical samples obtained from inaccessible sites may have a low bacillary load, thus decreasing diagnostic test's sensitivity. As a result, an enormous number of undiagnosed cases of TB will be missed out, leading to a serious global health issue [5].

TB affects all ages. In India, the infection rate is 20% at the age of 15–24 y age and 2% in 0–14 y of age group. In a developed country, the disease is more common in the older age group and more prevalent in males than females [6]. The present study analyzed 129 samples obtained from patients suspected of TB based on clinical and radiological findings. The most common age group suspected of TB was from 21-40 y (41.08%), followed by 41-60 y (24.03%). The mean age of the subjects was found to be 33.55±19.59. Males were 56 (43.41%), and females were 73 (56.59%) with a 1: 1.3 (M: F)

ratio. In another study, 194 (48.1%) were females, and 209 (51.9%) were males with M: F ratio of 1: 1.08. The mean age of the subjects found to be 35.3±15.9. Most subjects (40.9%) were in 21-35 y of age [7]. In another study, the mean age of the subjects was 33±17.13 y [8]. These findings were in concordance with our findings of the present study.

The highest number of samples were from Lymph node aspirate 42 (32.56%), sputum 25 (19.38%), followed by CSF 17 (13.18%), endometrial tissue 10 (7.75%), synovial fluid 3 (2.33%), and others like urine, stool, peritoneal fluid 32 (24.81%). In contrast to our study, the other study included 387 (96.0%) sputum and 7 (1.7%) bronchial wash, extrapulmonary samples 9 (2.3%) [6]. Most of the clinicians focus on ZN smears positive patients who are highly infectious. Patients with ZN smear-negative are also accountable for about 17% of transmission of TB infection, which could not be ignored and has a high impact on public health [9].

The present study showed ZN smear positivity (7.75%) and CBNAAT positivity (19.38%) with CBNAAT sensitivity and specificity of 90% and 86.55% when compared with ZN staining with a significant P value of <0.0001. In a study conducted at Mayo Hospital Lahore, CBNAAT reported 90.1% and 98.3% of sensitivity and specificity for MTB detection compared to ZN smear, 77.7% and 91.4% sensitivity specificity, respectively. Among 130 ZN smear-negative cases, 52 (40.0%) were MTB positive by CBNAAT. Of the 13 ZN smear-positive, CBNAAT detected MTB in 9 (69.2%) while 4 (30.8%) were of *non-tubercle mycobacteria* based on Para-nitro-benzoic acid test. Delay in the diagnosis of ZN Smear negative TB possesses difficulties in the treatment aspect. In such cases, early diagnosis and timely treatment are most important. If we go for the culture, it takes around 6-8 w for MTB detection. Newer diagnostic technique such as CBNAAT helps in increased TB detection rate rapidly. We can treat the patient at the earliest and can prevent the patient from becoming infectious for others [7]. A recently published study by Buchelli Rmirez HL *et al.* showed lower ZN smear positivity of 53% and higher MTB positivity of 82% by CBNAAT [10]. In the present study, the CBNAAT showed 100% sensitivity and 100% specificity for pulmonary samples than ZN staining. In the same way, when CBNAAT results are compared with ZN staining for extrapulmonary samples, CBNAAT showed 87.5% sensitivity and 81.18% specificity. This may be because of low bacillary load in extrapulmonary samples, with smear-negative and CBNAAT positive results. Based on the above facts, the CBNAAT assay forms an important diagnostic tool for diagnosing extrapulmonary tuberculosis for early treatment and preventing disease spread.

Bajrami *et al.* reported 24.1% MTB positive by culture, while 29.3% MTB positive by CBNAAT. Compared with culture, the CBNAAT assay achieved 82.3% sensitivity and specificity [11]. In the present study, CBNAAT detected MTB with 87.5% sensitivity and 81.18% specificity among extrapulmonary samples compared with ZN staining. In our study, one sample was not detected for *mycobacterium tuberculosis* by CBNAAT but was positive by ZN staining. This may be due to non-mycobacterium tuberculosis found to ZN staining positive and CBNAAT result negative. The present study showed that ZN staining and CBNAAT for MTB detection improve the case detection rate. In a study conducted at Hyderabad, CBNAAT for pulmonary samples showed the sensitivity and specificity of 79.2% and 89.5%, respectively, and for extrapulmonary samples, the sensitivity and specificity of 85.7% and 93.5%, respectively. The above study suggested that CBNAAT has higher sensitivity for detecting pulmonary and extrapulmonary tuberculosis cases [12].

Panayotis *et al.* observed CBNAAT sensitivity of 90.6% and specificity of 94.3% among the 80 pulmonary samples [13]. Similarly, in another study, the sensitivity of CBNAAT was 79% among all pulmonary samples. The CBNAAT showed a higher sensitivity for lymph node aspiration (94.74%) than ZN smear positivity (73.68%). Similarly, the present study also showed a higher sensitivity for lymph node aspirate (96.43%) by CBNAAT than ZN staining. Clearly, it indicates CBNAAT would increase the early detection of lymph node tuberculosis compared to and in addition to the ZN staining on FNAC aspirate material [14]. Steingart *et al.*, as part of a WHO, developed updated guidelines and observed higher sensitivity and specificity in TB suspected patients, with or without HIV co-infection on the utility of the CBNAAT method [15]. A study from South Africa also certified that ZN stained microscopy of sputum smears combined with detection of MTB by CBNAAT is the most cost-effective strategy for diagnosis of ZN smear-negative TB [16].

Studies done from various places of different countries by the CBNAAT method were showed variations in their sensitivity and specificity. These variations in sensitivity and specificity by CBNAAT assay can be explained by differences in their inclusion and exclusion criteria. Detection of MTB by conventional methods has lower sensitivity and time consuming [13]. Molecular methods like the CBNAAT technique have transformed TB detection with rapid diagnosis, high sensitivity, and specificity.

The emergence of MDR-TB and extensively drug-resistant tuberculosis (XDR-TB) makes the treatment and control of tuberculosis difficult. Rapid detection of drug-resistant strains is important for the successful treatment of drug-resistant tuberculosis [17]. CBNAAT allows early and rapid detection of rifampicin resistance, facilitating early drug resistance detection [15]. The specificity of CBNAAT in detecting rifampicin resistance was very high (98%) [18]. In our present study, out of 25 detected for MTB by CBNAAT, 15 were not tested due to low bacillary load, 10 were tested for Rifampicin resistance; of these 10, 2 (20%) were rifampicin resistance, and the remaining 8 (80%) were sensitive to rifampicin. In a study where 1201 sputum samples were analyzed, MTB has detected in 268 (22.31%) samples, and rifampicin resistance was detected in 30 (2.49%) samples. 2 (33.33%) rifampicin-resistant samples were from treatment failures cased [19]. In another study, Rifampicin resistance was detected with 100% sensitivity and specificity [8]. A higher rate of rifampicin resistance was found in our study compared to the above study. The WHO in 2010 permitted the use of the Xpert MTB/RIF (CBNAAT) technique for the early and rapid detection of MTB and MDR-TB [20].

CONCLUSION

The development of CBNAAT is an important rapid tool to battle against TB. CBNAAT testing on EPTB samples showed encouraging results. CBNAAT also provides an available part in the early diagnosis of smear-negative PTB and EPTB cases. CBNAAT helps in the timely diagnosis of TB and detects rifampicin resistance cases within 2-3 h. The present study showed that ZN staining along with CBNAAT (True NAAT) improves the case detection rate in both pulmonary and extrapulmonary TB cases and also showed a promising result in the diagnosis of TB in extrapulmonary samples compared to pulmonary samples; otherwise, if the ZN results are negative, samples should be subjected to the culture, which takes months together to detect TB and another couple of months to detect drug resistance.

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AUTHORS CONTRIBUTIONS

All authors designed the experiments. Rashmi Karigoudar, Mahesh Karigoudar, Sanjay Wavare performed the experiments. Rashmi Karigoudar, Sanjay Wavare, Smita Bagali, and Lakshmi Kakhandki analyzed the data. Rashmi Karigoudar and Mahesh Karigoudar wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest

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