CARTRIDGE-BASED NUCLEIC ACID AMPLIFICATION TEST, AN IMPORTANT DIAGNOSTIC TOOL FOR TUBERCULOSIS BOTH PULMONARY AND EXTRA-PULMONARY TUBERCULOSIS

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ABSTRACT

Objective: Tuberculosis (TB) is severe infectious disease caused by *Mycobacterium tuberculosis* and imposes significant morbidity and mortality. Early diagnosis of active TB forms pulmonary and extrapulmonary is very important and the diagnostic methods such as culture test and Ziehl-Neelsen (ZN) stain displayed low diagnostic accuracy. Cartridge-based nucleic acid amplification test (CBNAAT) is widely used for the diagnosis of TB with good accuracy rate. Hence, the current study was commenced to assess the diagnostic usefulness of CBNAAT for the diagnosis of *mycobacterium* TB (MTB) and compared with ZN staining.

Methods: This was a prospective study conducted on 250 sputum samples of pulmonary TB (new cases) and also patients who were receiving the treatment for TB. 5 mL of sputum sample including CSF, pericardial or ascitic fluid, pus, or lymph nodes aspirates were collected and subjected to ZN staining and CBNAAT.

Results: Mean age observed was 48.12±8.76 years. 30 (12%) showed smear positive and 220 (88%) were smear negative for ZN staining. Further, samples have been subjected to CBNAAT showed 60 (24%) MTB positive, and 190 (74%) showed MTB negative. CBNAAT showed sensitivity and specificity of 91.5% and 89%. Out of 60 positives for MTB by CBNAAT, MTB has been identified in 28 patients.

Conclusion: CBNAAT is a great tool in the early diagnosis of pulmonary and extrapulmonary TB.

Keywords: Tuberculosis, Ziehl-Neelsen stain, Cartridge-based nucleic acid amplification test, Pulmonary and extra pulmonary tuberculosis.

INTRODUCTION

Tuberculosis (TB) is a bacterial infection affected by *Mycobacterium tuberculosis* (MTB) and globally one of the important causes for mortality and ranks above HIV/AIDS. Nearly 1/4th of the global population affected by TB and thus they servs as a carrier for the risk of spreading the disease. In 2019, as per the Global TB Report, the mortality rate in people infected with TB is 1.5 million in 2018, and around 10 million of individuals are affected with the disease [1]. Nearly, about 8 countries occupy the two-thirds of the disease, and in this study, India occupies the first position monitored by China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa [1]. Multidrug-resistant TB (MDR-TB) is one of the major public threats, and as per the World Health Organization, data around 484,000 new cases are resistance to rifampicin (RIF), the most widely used first-line agent and mostly 78% of MDR-TB cases are due to RIF. The burden of MDR-TB burden is higher in India, China, and the Russian Federation and around 6.2% of MDR-TB cases are due to extensively drug-resistant TB. Globally, 56% of patients are effectively treated for MDR-TB patients. Globally, the annual incidence rate of TB is decreasing by 2%, and the main milestone of End TB Strategy is to enhance the annual declining rate 4–5% in the coming years [2].

Extrapulmonary TB (EPTB) contributes major issue in emerging nations, and lymphadenopathy is the common demonstration of EPTB [2]. In India as per the 2020 statistics out of 2,404,815, all TB cases nearly 26% (640,399) are affected with EPTB [3]. The most commonly occurred EPTB forms are peripheral lymph node TB and to next the TB pleural effusion. In immunosuppressed and HIV-seropositive cases, the most frequently encountered types are disseminated and military TB [4].

Diagnosis of lymph node TB is not specific, and in some cases, there is lack of active TB forms. Conventional Ziehl-Neelsen (ZN) stain for the recognition of acid-fast bacilli is important for TB diagnosis and management, but its sensitivity is very low and ranges between 20% and 43% [5]. *Mycobacterium* culture technique is the gold standard for the identification TB, but its timeline consuming and requires more laboratory safety measures [5]. A newer molecular method such as PCR gives faster results, but it is costly and cannot be employed in low-resource settings [6]. Recently, there has been lot of attention in novel nucleic acid intensification analytical procedures due to their fast and high-sensitive results. Cartridge-based nucleic acid amplification test (CBNAAT) is used for the early detection of smear-negative pulmonary and EPTB, MDR-TB, and TB-HIV and thus elicits important role for TB control. In this backdrop, we have evaluated the diagnostic usefulness of CBNAAT aimed at the diagnosis of MTB and compared through ZN staining.

METHODS

This was a prospective observational research directed at the Department of Pulmonary medicine, Bhima Bhoi Medical College of Balangir, Odisha from January 2019 to January 2021.

We have subjected samples probable of TB for CBNAAT aimed at the identification of TB and RIF-resistant TB. In this study, 250 sputum samples were collected in patients suggestive of pulmonary TB (new cases) and also patients who were receiving the treatment for TB. The samples were collected in pre-sterilized falcon tubes, and details of the patients were included in the collection tubes. As per the standard procedures, 5 mL of sputum sample or 2 mL of fluids including CSF, pericardial or ascitic fluid, pus, or lymph nodes aspirates were collected and subjected to ZN staining and CBNAAT.

Inclusion criteria
Patients with the suspicion of pulmonary TB including with cough symptoms with or without expectoration for more than 2 weeks, loss of weight and appetite, and hemoptysis were included in the study.

Exclusion criteria

Samples with a lack of information about clinical history and patient with lung malignancies or fungal diseases were not included in the study.

CBNAAT is a PCR technique for the identification of TB and RIF. It is a self-enclosed one-use, cartridge which processes the sample automatically with application and detection facilities. The sample reagent was added to the sample at the ratio of 2:1 ratio for liquefaction process and to deactivate the bacteria and then 2 mL of the sample was added into the cartridge and loaded for the assay procedure. The following interpretation was made, presence of MTB; exposure of MTB with RIF resistance, manifestation of MTB with no RIF resistance; presence of MTB with indeterminate RIF resistance, and no result [7].

Data analysis

Descriptive statistics has been developed to depict the frequency using SPSS statistical software. Chi-square test was applied for the definite variables. The sensitivity, specificity, and positive and negative predictive value were done to assess the diagnostic usefulness of CBNAAT.

RESULTS

In this study, a total of 250 cases suspected of TB constructed on the scientific and radiological have been analyzed. The mean age of the study participants were 48.12 ± 8.76 years. Maximum subjects fit to the age category between 21 and 40 years, 120 (48%) then 41–60 years, 95 (38%) respectively. Out of 250 patients, 140 (56%) were males and 110 (44%) were females.

The sample for analysis included lymph node aspirate in 142 (58%) followed by sputum 75 (30%), CSF 25 (10%), and others included 8 (3.2%), respectively.

In the present observation, 250 samples have been exposed to ZN staining and CBNAAT. Among the 250 samples, 30 (12%) showed smear positive and 220 (88%) were smear negative for ZN staining. Further, all the samples have been exposed to CBNAAT showed 60 (24%) MTB positive and 190 (74%) showed MTB negative.

In the present study, CBNAAT identified MTB in 29 out of 30 ZN smear-positive and 31 out of 220 ZN smear-negative patients. The smear positivity rate for ZN smear was 12% and CBNAAT positivity was 24%. The sensitivity and specificity of CBNAAT were 92% and 88%, and it was significant as compared to ZN staining ($p<0.05$) (Table 1).

The sensitivity and specificity of CBNAAT for pulmonary samples was 99.5% sensitivity and 95% specificity and was significant as compared to ZN staining. Out of 60 positives for MTB by CBNAAT, MTB seems to be identified in 32 patients for pulmonary sample (Table 2).

Likewise, when the CBNAAT results are associated with ZN staining for extrapulmonary samples, CBNAAT exhibited sensitivity also specificity 91.5% and 89%. Out of 60 positives for MTB by CBNAAT, MTB seems to be identified in 28 patients. The results were shown in Table 3.

DISCUSSION

TB is a multifaceted infectious pathology triggered by category of bacteria usually referred as *Mycobacterium* TB complex. The wide range of species which affects the humans are MTB and Mycobacterium africanum also further organism which affects the animals are Mycobacterium bovis, Mycobacterium caprae, Mycobacterium microti, and Mycobacterium pinnipedii [8].

Worldwide, the incidence of pulmonary TB is 85% and the EPTB is 15% [9]. The most affected type of EPTB includes lymphatics, pleural cavity, meningeal, bones, and genitourinary tract. However, the EPTB prevalence varies among the different geographical regions and countries [10].

Table 1: Detection rate of ZN and CBNAAT for both pulmonary and extra pulmonary samples

Test	ZN staining			
CBNAAT	+ve	-ve	Frequency	Percentage
Positive	29	31	60	24
Negative	1	189	190	76
Total	30	220	250	100
%	12	88	100	

ZN: Ziehl-Neelsen, CBNAAT: Cartridge-based nucleic acid amplification test

Table 2: Comparison ZN and CBNAAT for pulmonary TB samples

Variables	ZN staining		
CBNAAT	Positive	Negative	Frequency
Positive	32	28	60
Negative	18	12	30
Total	50	40	90

ZN: Ziehl-Neelsen, CBNAAT: Cartridge-based nucleic acid amplification test, TB: Tuberculosis

Table 3: Comparison ZN and CBNAAT for pulmonary TB samples

Variables	ZN staining		
CBNAAT	Positive	Negative	Frequency
Positive	28	32	60
Negative	12	18	30
Total	40	50	90

ZN: Ziehl–Neelsen, CBNAAT: Cartridge-based nucleic acid amplification test, TB: Tuberculosis

However, most of the global TB control programs has omitted the EPTB, since it is taken as consideration to be non-infectious as a result of global epidemic. However, a recent study from England stated that lively TB disease among the household associates of EPTB showed increased prevalence, with a rate of 400/10,000 screened and thus pointed out that EPTB has substantial risk for the transmission of TB [11]. In addition, slow EPTB decline rate as compared to pulmonary TB might affect the goals set by WHOEND-TB targets [12].

In the current observation, the mean age of the cases seems to be 48.12 ± 8.76 years. In another observation performed by Zeka *et al*. [13], the mean age of the patients seems to be 47.5 ± 22.2 years. In our study, male preponderance was observed with 56% of males. Similarly, in a study done by Mukherjee *et al*. [14], 69.3% were males and the low rate of TB incidence in females might be due to various clinical and demographic factors and the differences in the access to health care between males and females [15]. In addition, the majority of the women showed low disease rate as that of the men during diagnosis and thus implicating earlier diagnosis in females [16].

We have observed ZN smear and CBNAAT positivity rate of 12% and 24%, respectively, and CBNAAT showed sensitivity of 92% and specificity of 88%, respectively, as that of the ZN. Wide range of studies showed CBNAAT sensitivity; in a research performed in Italy, CBNAAT sensitivity also specificity is reported to be 81.3% and 99.8% as compared to culture test [17]. In Zeka *et al*. [13] study, using the EPTB samples, CBNAAT sensitivity also specificity was found to be 52.1% and 100% as compared to culture referencing system. In another study conducted in South Africa by Scott *et al*. [18], the CBNAAT sensitivity was 59% as that of the culture. In a study conducted among the German patients by Hillemann *et al*. [19], the CBNAAT sensitivity was 77.3% as that of the culture. Previous researches directed in India by Vadwai *et al*. [20] showed CBNAAT sensitivity of 81% as compared to culture, and in additional research finished by Suzana *et al*. [21], CBNAAT

sensitivity was 62%. In Krishna *et al.* study, the CBNAAT sensitivity when compared to CRS was 68.5% [22].

CONCLUSION

CBNAAT is a great tool in the early identification of pulmonary and EPTB. Thus, CBNAAT should be recommended for suspected cases of TB even in low-resources settings.

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CONFLICT OF INTEREST

None to declare.

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