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Cartridge Based Nucleic Acid Amplification Test, an Important Diagnostic Tool for Tuberculosis both Pulmonary and Extra-Pulmonary Tuberculosis

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Introduction

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis (MTB), one of the top 10 causes of death worldwide (ranking above HIV/AIDS). About a quarter of the world's population is infected with TB and thus at risk of developing TB disease. The Global TB Report 2019 states that in 2018 a total of 1.5 million people died from TB, an estimated 10 million (range, 9.0–11.1 million) people fell ill worldwide.(1) Eight countries accounted for two-thirds of the total, with India leading the count, followed by China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa.(1) Multidrug-resistant TB (MDR-TB) remains a public health crisis and a health security threat. The WHO estimates that there were 484 000 new cases with resistance to rifampicin (RIF) – the most effective first-line drug, which accounts for 78% MDR-TB cases. The MDR-TB burden largely falls on three countries – India, China, and the Russian Federation. About 6.2% of MDR-TB cases had extensively drug-resistant TB (XDR-TB) in 2018. Worldwide, only 56% of MDR-TB patients are currently successfully treated. Globally, TB incidence is falling at about 2% per year. This needs to accelerate to a 4%–5% annual decline to reach the 2020 milestones of the End TB Strategy.(1)

Extrapulmonary tuberculosis (EPTB) continues to be one of the leading health problem in developing countries. Lymphadenopathy is the commonest form of EPTB.(2) As per the TB India Report 2020 (3), EPTB accounted for 640,399 of the 2,404,815 (26.6%) of all notified cases of TB. The notified EPTB cases in India have varied widely across states and regions. Among various forms of EPTB, two commonly encountered forms are peripheral lymph node TB followed by TB pleural effusion. Disseminated TB and miliary TB are more frequently seen in immunosuppressed and HIV-seropositive individuals.(4)

The investigative parameters for the diagnosis of TB in lymph nodes are neither specific nor does their absence exclude TB involvement. Conventional ZN method for AFB detection plays a key role in diagnosis and also monitoring treatment of TB but has low sensitivity ranging from 20% to 43%.(5) Mycobacterium culture is the gold standard method, but it is time consuming and requires specialized safety precautions in laboratories.(5) Serological techniques lack sensitivity and specificity. Newer molecular techniques such as PCR, although rapid, are costly to be routinely used in developing countries.(6) In recent times, attention has been devoted to new nucleic acid amplification diagnostic technologies, owing to their rapidity, sensitivity, and specificity. Cartridge-based nucleic acid

amplification test (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. So the present study was carried out 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 Pulmonary medicine, Bhima Bhoi Medical College of Balangir, Odisha from January .2019 to January 2021.

In the present study, samples presumptive of TB were subjected to CBNAAT for the diagnosis of TB and rifampicin resistant TB. Total of 250 sputum samples of the patients with symptoms suggestive of pulmonary TB including both new cases and on treatment were received. All specimens were collected in pre-sterilized falcon tubes with three layer packing system, samples along with patient details such as name, address, age, and sex. 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) or aspirate from lymph nodes were collected according to standard protocol. All samples were subjected to Ziehl–Neelsen staining and CBNAAT, and data were analyzed.

Inclusion Criteria

Patients with clinical suspicion of pulmonary TB including symptoms of cough with or without expectoration for >2 weeks, weight loss, fatigue, hemoptysis, and loss of appetite and radio diagnosis were included in the study.

Exclusion Criteria

Samples received without clinical history and patient with history of lung malignancies or fungal infections were excluded from the study.

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.(7)

Statistical analysis

Descriptive statistics were used to depict the frequency using SPSS statistical software. Chi-Square test of independence was used to determine if there was a significant relationship between two nominal (categorical) variables.

The efficacy of CBNAAT was done by calculating the sensitivity, specificity, positive and negative predictive value.

Results

In this study, a total of 250 patients suspected of TB based on the clinical and radiological were analysed. The mean age of the study participants were 48.12± 8.76 years. Majority of the patients were in the age group between 21-40 years, 120 (48%), followed by 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 all the 250 samples were subjected to Ziehl Neelsen (Acid fast) staining and CBNAAT. Among the 250 samples, 30 (12%) showed smear positive and 220 (88%) were smear negative for acid fast staining. Further, all the samples were subjected to CBNAAT, which showed 60 (24%) MTB positive, and 190 (74%) showed MTB negative.

The CBNAAT detected MTB in 29 out of 30 ZN smear-positive and 31 out of 220 ZN smear-negative cases. The present study showed ZN smear positivity 12% and CBNAAT positivity (24%) with CBNAAT sensitivity and specificity of 92% and 88% when compared with ZN staining and found to be significant ($p<0.05$). The results were shown in table 1.

When compared to ZN staining for pulmonary samples, CBNAAT showed 99.5% sensitivity and 95% specificity (Table 2).

Likewise, when the CBNAAT results are compared with ZN staining for extra pulmonary samples, CBNAAT showed 89.7% sensitivity and 82.76% specificity. Out of 60 positives for MTB by CBNAAT, MTB was detected in 28 cases. The results were shown in table 3.

Discussion

TB is one of the primeval chronic and complex infectious diseases which is caused by a group of bacteria belonging to the MTBC. The complex includes the human adapted species of *M. tuberculosis and M. africanum*, and zoonotic pathogens- *M. bovis*, *M. caprae*, *M. microti* and *M. pinnipedii* which affect cattle, goats/sheep, voles and seals/lions, respectively.(8)

Globally, pulmonary TB accounted for 85% whereas EPTB accounted for the remaining 15%.(9)The most common types of EPTB include TB of the lymphatics (TBLN), pleura, bone, meninges, genitourinary tract and peritoneal TB. However, the prevalence of EPTB and its predominant forms varies from country to country.(10)

Global TB control efforts have largely ignored EPTB. This is because EPTB is generally considered non-infectious and as such inconsequential to the global epidemic. However, recent evidence from northwest England has demonstrated that the prevalence of active TB disease among household contacts of EPTB was high (440 per 100 000 contacts screened), indicating that EPTB cases might have substantial impact on TB transmission.(11) Moreover, it is conceivable that the slower annual decline rate of EPTB compared to PTB could retard the progress towards the END-TB targets set by WHO.(12)

In this study, the mean age of the study participants were 48.12± 8.76 years. In a study done by Zeka et al. (13) the mean age of the study population was found to be 47.5 ± 22.2 years. In our male

preponderance was observed with 56% of males. Similarly in a study done by Mukherjee et al. (14) 69.3% were males and the reason might be due to low notification rate for TB in females was more due to epidemiological factors than a differential access of the health care.(15) A higher proportion of women had minimal disease compared to men at the time of diagnosis, suggesting an earlier diagnosis among females.(16)

The present study showed ZN smear positivity 12% and CBNAAT positivity (24%) with CBNAAT sensitivity and specificity of 92% and 88% when compared with ZN. There are studies from different parts of the world with varying sensitivity of CBNAAT, as shown by Tortoli et al., where they conducted a retrospective study in Italy (n = 1476) and found the sensitivity and specificity of CBNAAT to 81.3% and 99.8%, respectively, when compared to CRS.(17) Another study from Turkey conducted by Zeka et al.(13) (n = 48) showed the sensitivity and specificity of CBNAAT against CRS to 52.1% and 100%, respectively, among EPTB samples. Scott et al.(18) from South Africa (n = 1045) found the sensitivity of CBNAAT against culture as 59%. Another study by Hillemann et al.(19) from Germany (n = 521) found the sensitivity of CBNAAT to be 77.3% when compared against culture.Studies from India have also shown varying sensitivity for CBNAAT. Vadwai et al.(20) (n = 283) observed sensitivity of CBNAAT against CRS as 81%, whereas a study by S Suzana et al.(21) involving 494 samples found that sensitivity of CBNAAT was 62%. Another study by Krishna V et al. had observed the sensitivity of CBNAAT against CRS to be 68.5%.(22)

Conclusion

CBNAAT is a great tool in early diagnosis of pulmonary and extra pulmonary tuberculosis. Thus, CBNAAT should be a gold standard investigation, should be done in all cases of suspected TB.

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