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Original Research Article

Utility of CBNAAT in Diagnosis of Pulmonary and Extrapulmonary Tuberculosis in (GMC Doda) India

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Abstract

Background: Tuberculosis is the ninth leading cause of death worldwide. India contributes to about one fifth of global TB burden. It is very important to diagnose early and treat Tuberculosis to cut down transmission of Tuberculosis. The sensitivity of smear microscopy and its inability to detect drug resistance limits its impact on TB control. We compared the cartridge-based nucleic acid amplification test (CBNAAT) results for diagnosis of pulmonary and extra pulmonary tuberculosis with the conventional methods like sputum smear.

Material and Methods: We conducted a retrospective study in department of Microbiology to analyze the utility and yield of CBNAAT from Jan 2017 to December 2017. We included all patients who were subjected to CBNAAT in the study period. Data was collected from DOTS centre and CBNAAT centre. We collected total number of samples tested for CBNAAT, indication for CBNAAT, result of smear microscopy for AFB and CBNAAT. The study population included all the pulmonary and extra pulmonary presumptive TB cases who were subjected for further investigations.

Conclusion: CBNAAT is one of the rapid diagnostic tests available in the country and it should be routinely used under the public and private health sector effectively to detect a tuberculosis case.

Keywords: Cartridge-based nucleic acid amplification test (CBNAAT); Tuberculosis; Sputum smear; AFB; Sensitivity; Specificity.

Abbreviations: TB: Tuberculosis; CBNAAT: Cartridge-Based Nucleic Acid Amplification Test; FNAC: Fine Needle Aspiration Cytology; ATT: Anti-Tuberculosis Treatment; RNTCP: Revised National TB control Programme; BAL: Bronchoalveolar Lavage; MDR-TB: Multi-drug resistant TB; MTB: Mycobacterium Tuberculosis.

Introduction

Tuberculosis is a major communicable disease causing significant mortality and morbidity worldwide especially in India. TB is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS.[1] In 2016, the incidence of Tuberculosis in India was 2.79 million with mortality rate of 32/ lakh population.[2] India constitutes 24% of the total TB burden.[2] Early detection of TB cases is the key to successful treatment and reduction of disease transmission and most deaths from TB could be prevented with early diagnosis and appropriate treatment. Since past many years, smear microscopy and conventional cultures have been used as diagnostic modality for pulmonary tuberculosis. Smear microscopy has variable sensitivity

(45–80%) mainly in patient with smear negative TB, extra pulmonary TB and there are issues related to quality control[3,4] and conventional solid culture techniques take long turn around time of 2–6 weeks and is costly.[5] For faster diagnosis, liquid culture (Mycobacterium Growth Indicator Tube) techniques were developed but the mean turnaround time is still long of 21 days.[6] Such delays in diagnosis increase morbidity and mortality that predispose to secondary resistance and cause transmission of resistant strains. Recently nucleic acid amplification tests (NAAT) were developed for rapid detection of TB and identification of drug resistance. However, conventional NAATs require well-trained technical staff and sophisticated equipments.[7] WHO recommended use of a Cartridge Based Nucleic Acid Amplification test (CB-NAAT), for diagnosis of TB in December 2010. In 2013, WHO endorsed conditional recommendation for Xpert MTB/RIF as the initial diagnostic test in all adults with presumptive tuberculosis and MDR TB.[8] Xpert MTB/RIF is an automated, seminested real-time PCR that detects MTB and tests every positive sample for rifampicin sensitivity using molecular beacons.[9] Thus, results for both, presence of MTB and rifampicin resistance, are available within 2 hours with good sensitivity and specificity. It is a cartridge based nucleic acid amplification test (CBNAAT) that does not have any specific pre-requites for its set-up and does not require much technical training. Further, as the reagent used for processing is bactericidal and tubercle bacilli are inactivated in vitro, biosafety risks are eliminated, thus enabling its use as a rapid point-of-care diagnostic test.

As this Study is conducted in the Rural mountainous region of J&K India; The CBNAAT was First time introduced in this area in 2016.

To address this issue there was a need for a simple and rapid diagnostic tool at least for high-burden countries and a new diagnostic test known as cartridge based nucleic acid amplification test (CBNAAT) was developed which was rapid, fully automated and was based on polymerase chain reaction (PCR) that detects deoxyribonucleic acid (DNA) directly from the clinical specimens and also detects rifampicin resistance.[2] This diagnostic test was designed to purify, concentrate, amplify and identify targeted rpoB nucleic acid sequences, and delivered the results in about 120 minutes.

In this study, we compared the CBNAAT results for diagnosis of pulmonary and extrapulmonary tuberculosis with the conventional methods like sputum smear.

Methodology

We conducted a retrospective study in the department of Microbiology to analyze the utility and yield of CBNAAT from January 2017 to December 2017. We included all patients who were subjected to CBNAAT in the study period. Data was collected from DOTS centre and CBNAAT centre. We collected total number of samples tested for CBNAAT, indication for CBNAAT , result of smear microscopy for AFB and CBNAAT Specimen subjected to CBNAAT was either sputum, gastric lavage, BAL or extrapulmonary fluid sample (Pleural fluid, pus, synovial fluid, ascitic fluid). Tissues were not subjected to CBNAAT due to non-availability of homonizer at our institute. A minimum of 2.5 ml of sample was considered adequate for analysis and bloody specimen was rejected. Specimen was collected in Falcon tubes and analysis was done on the same day and results were given within a day.

For CBNAAT examination the sample reagent were added at a 3:1 ratio to clinical specimens. The closed specimen container was manually agitated twice during a 15 minute period at room temperature, before 2 ml of the inactivated material (equivalent to 0.5 ml of decontaminated pellet) was transferred to the test cartridge. All the specimens that is pulmonary and extrapulmonry suspected of Tuberculosis were included in the study and AFB as well as CBNAAT of all samples was done simultaneously. Cases other than suspected were excluded from the study.

Results

In the present study, Out of 290 clinically suspected cases of pulmonary and extrapulmonary Tuberculosis, 220 cases were Pulmonary samples and 70 cases were extrapulmonary samples.

Pulmonary samples were in the form of Sputum samples in majority of the cases and Gastric lavage samples.

Extra-pulmonary samples were in the form of Pleural fluid, Ascitic fluid, Synovial fluid, Pus and lymph node samples taken with the help of Fine Needle Aspiration Cytology (FNAC) department of pathology.

Out of 220 pulmonary samples, CBNAAT was positive in 180 (82%) samples and negative in 40 (18%) samples, and out of 70 extra-pulmonary samples, CBNAAT was positive in 60 (86%)

samples and negative in 10 (14%) samples.

Out of 220 cases of Pulmonary samples, 92 cases were correctly diagnosed by AFB stain as Tuberculosis and were included in True positive cases, 8 cases as scanty/suspicious which later on CBNAAT were proved to be Negative and were included in the false positive cases. Total number of negative cases diagnosed on AFB stain were 125 out of which true negative were 37 and false negative were 88.

In our study on pulmonary samples by AFB stain, Sensitivity was found to be 51%, specificity was 92.5%, positive predictive value 96.84% and Negative predictive value 29.6%.

Table: Correlation of AFB stain with CBNAAT in diagnosing Pulmonary Tuberculosis.

Diagnostic accuracy of ZN Stain in diagnosing Mycobacterium Tuberculosis in Pulmonary (Sputum samples). (n=220)			
	Gold Standard CBNAAT		
AFB Stain	**Disease Present**	**Disease not Present**	**Total**
Test positive	True positive 92	False positive 3	95
Test negative	False negative 88	True negative 37	125

Table: Pulmonary sample parameters.

Parameter	Percentage
Sensitivity	51%
Specificity	92.5%
Positive predictive value	96.84%
Negative predictive value	29.6%

In the present study of 290 clinically suspected cases, 70 were extrapulmonary samples.

Out of 70 cases of extra-pulmonary samples, 35 cases were correctly diagnosed by AFB stain as Tuberculosis and were included in True positive cases. 2 cases as scanty/suspicious which later on CBNAAT were proved to be Negative and were included in the false positive cases. Total number of negative cases diagnosed on AFB stain were 33 out of which true negative were 8 and false negative were 25.

In our study on extrapulmonary samples by AFB stain, Sensitivity was found to be 58.33 %, specificity was 80%, positive predictive value 95% and Negative predictive value was 24.24% respectively.

Table: Correlation of AFB stain with CBNAAT in diagnosing Extrapulmonary Tuberculosis.

Diagnostic accuracy of ZN stain in diagnosing Mycobacterium Tuberculosis in Extrapulmonary samples (n=70)			
	Gold Standard CBNAAT		
AFB Stain	**Disease Present**	**Disease not Present**	**Total**
Test Positive	True positive 35	False Positive 2	37
Test Negative	False Negative 25	True Negative 8	33

Table: Extra- Pulmonary samples parameters.

Parameter	Percentage
Sensitivity	58.33%
Specificity	80%
Positive Predictive value	95%
Negative Predictive value	24.24%

Discussion

In Our study on overall 290 samples, CBNAAT has higher sensitivity for detection of pulmonary and extrapulmonary tuberculosis cases. The WHO 2012 has also recommended the CBNAAT for routine use under programmatic conditions.[10] But in our Predominantly Rural mountainous region of Jammu and Kashmir India (GMC DODA) This procedure was introduced in year 2016. This procedure was done FREE OF COST.

In the present study, only 290 specimens were included; among them 220 were pulmonary and 70 were extrapulmonary. Among the 220 pulmonary presumptive TB cases, 4 were unable to produce adequate amount of quality sputum and hence gastric lavage was performed for collection of the specimen. The sensitivity of CBNAAT for pulmonary samples was 82% as compared to sputum smear which was 51%. Sensitivity of smear negative pulmonary samples can be increased by including more than one sample for diagnosis. Out of 70 extra-pulmonary samples, the sensitivity of CBNAAT was (86%) as compared to AFB stain which was 58.33%.

The false positive cases could be because of misinterpretation of smears. The false negative cases could be because of technical error in the form of poor slide preparation,poor staining technique, observational error etc. In case of false positive /Suspicious cases, repeat sample is advised or CBNAAT is to be performed to confirm the diagnosis in order to avoid un-necessary treatment, side effects of drugs and Stress to the patient and family members.

In a study done by Panayotis et al,[11] the sensitivity and specificity of CBNAAT in 80 pulmonary samples were 90.6% and 94.3% respectively. In a study done by Armand et al[12] the sensitivity of CBNAAT in 60 pulmonary samples which included sputum, BAL, bronchial aspirate and gastric aspirate was 79%. Inclusion of CBNAAT in the initial diagnosis of tubercular lymphadenopathy in addition to the FNAC would decrease the over

diagnosis of tuberculosis and injudicious use of anti-tuberculosis treatment (ATT).

The operational feasibility studies conducted under the Revised National TB Control Programme (RNTCP) have demonstrated the feasibility of the machine to efficiently work under Indian settings.[13]

Conclusion

Thus in the present study yield of diagnosis was highest with CBNAAT and out of 220 pulmonary samples CBNAAT was positive in 180 (82%) and out of 70 extra-pulmonary samples, CBNAAT was positive in 60 (86%) samples.

Sensitivity of AFB stain in pulmonary samples in our study was 51% and specificity was 92.5%. Sensitivity of AFB stain in extra pulmonary samples was 58.33% and specificity was 80.0%. Though AFB stain was inferior to CBNAAT in diagnosing Mycobacterium tuberculosis but this is effective in diagnosing Tubercular cases in those areas where this CBNAAT procedure is unavailable and in those cases which don't afford to do this expensive procedure.

To conclude, CBNAAT is one of the rapid diagnostic tests available in the country and it should be routinely used under the public and private health sectors efficiently to detect a tuberculosis case.

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