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Original Research Article

Diagnosis of pulmonary tuberculosis with cartridge based nucleic acid amplification test and light emitting diode fluorescent microscopy: a comparative study

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ABSTRACT

Background: Due to low sensitivity and inability to detect drug resistance, smear microscopy limits its impact on TB control. Culture methods and drug susceptibility testing is complex, time consuming, and takes around 6-8 weeks. A new diagnostic test, cartridge based nucleic acid amplification test (CBNAAT) was developed based on real-time polymerase chain reaction (RT PCR). Objective of this study to compare the results of CBNAAT for diagnosis of pulmonary tuberculosis with LED fluorescent microscopy and sputum culture.
Methods: A cross-sectional study was conducted in the department of Chest and TB, CIMS, Bilaspur. Each Sputum sample of presumptive TB patients were tested with CBNAAT, sputum smear microscopy by light emitting diode (LED) fluorescent microscopy (FM) and solid and liquid culture for diagnosis of Tuberculosis. Results of CBNAAT, Fluorescent Microscopy and Culture for detection of Mycobacterium Tuberculosis were compared.
Results: The sensitivity and specificity for CBNAAT were 97% and 100% respectively; while that for Fluorescent microscopy were 70% and 100% respectively. The positive and negative predictive value for CBNAAT was 100% and 96% respectively. The positive and negative predictive value for Fluorescent microscopy was 100% and 73% respectively.
Conclusion: CBNAAT is having high sensitivity and specificity for diagnosis of pulmonary tuberculosis. It should be routinely used under national health programme to detect a tuberculosis case efficiently.

Keywords: Cartridge based nucleic acid amplification test, Culture, Fluorescent, Light emitting diode, Microscopy, Sputum

INTRODUCTION

Tuberculosis (TB) is caused by bacteria M. tuberculosis (MTB). Worldwide, TB is one of the leading causes of death from a single infectious agent (above HIV/AIDS).

According to WHO global TB report, In 2017, TB caused an estimated 1.3 million deaths (range, 1.2-1.4 million) among HIV-negative people and there were an additional 300 000 deaths from TB (range, 266 000–335 000) among HIV-positive people.[1] Globally, the best estimate is that 10.0 million people (range, 9.0-11.1 million) developed TB disease in 2017: 5.8 million men, 3.2 million women and 1.0 million children. There were cases in all countries and age groups, and two thirds were in eight countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%). These and 22

other countries in WHO's list of 30 high TB burden countries accounted for 87% of the world's cases.

Drug-resistant TB continues to be a public health crisis. The best estimate is that, worldwide in 2017, 558 000 people (range, 483 000–639 000) developed TB that was resistant to rifampicin (RR-TB), the most effective first-line drug, and of these, 82% had multidrug-resistant TB (MDR-TB). Three countries accounted for almost half of the world's cases of MDR/RR-TB: India (24%), China (13%) and the Russian Federation (10%).

Globally, 3.5% of new TB cases and 18% of previously treated cases had MDR/RR-TB. Among cases of MDR-TB in 2017, 8.5% were estimated to have extensively drug-resistant TB (XDR-TB). India has the highest number of Tuberculosis (TB) cases in the world, with over 2 million TB cases every year. Annually, one fourth of the global incident TB cases occur in India. Early and accurate diagnosis is the first critical step in controlling TB.

Light-emitting diode fluorescent microscopy (LED-FM) which uses auramine-stained smear techniques is simpler, cheaper and having a longer lifespan. It has on average 10%more sensitive than conventional ZN-LM.[2] Additional advantage of this technology is that lower magnifications can be used, enabling rapid screening over a wider area of the smear to be seen and resulting in up to 4 times faster examination of smear. This technology is believed to be more beneficial in TB high-burden and resource-limited settings. Due to low sensitivity and inability to detect drug resistance, smear microscopy limits its impact on TB control.

Culture methods and drug susceptibility testing is complex, time consuming, and takes around 6-8 weeks. While patients await diagnosis, they are likely to receive inappropriate or in effective treatment and consequently disease may progress. This results in an increased chance of morbidity from tuberculosis. To address this issue specially for high-burden countries and a new diagnostic test, cartridge based nucleic acid amplification test (CBNAAT) was developed which was rapid, fully automated and was based on real-time polymerase chain reaction (RT PCR) that detects deoxyribonucleic acid (DNA) directly from the clinical specimens and also detects rifampicin resistance and delivered the results within hours.[3] In this study we compared the results of CBNAAT, LED Fluorescent microscopy and sputum Culture for diagnosis of pulmonary Tuberculosis. The study generated new data about the diagnostic performance of CBNAAT and LED Fluorescent microscopy in presumptive TB patients at tertiary care center in Chhattisgarh.

METHODS

A cross-sectional study was conducted in the department of Chest and TB, CIMS, Bilaspur from January 2017 to March 2019. Presumptive TB patients both new and those with previously treated for TB, and multi-drug resistant TB (MDR-TB) suspected patients including PLHIV and non-HIV cases were included in the study. Patients who were on anti-TB medication (follow-up patients), were excluded.

Each Sputum sample were tested with CBNAAT, sputum smear microscopy by light emitting diode (LED) fluorescent microscopy (FM) and solid and liquid culture for diagnosis of Tuberculosis.

Samples were processed and smears were prepared by the auramine-rhodamine acid-fast staining method.

For CBNAAT examination the sample reagent were added at a 2:1 ratio to sputum samples. The closed specimen container was manually agitated twice during a 15 minute period at room temperature, before 2 ml of the inactivated material was transferred to the test cartridge for the analysis. Sputum samples were transported at appropriate temperature to IRL for culture.

Results of CBNAAT, Fluorescent Microscopy and Culture for detection of Mycobacterium Tuberculosis were compared. Data processing and statistical analysis were performed using the GraphPad instat (Windows version 3.10).

RESULTS

There were 548 sputum specimens collected during the study period of which 340(62%) samples were of males and 39(7%) sample were of PLHIV. Among the samples provided 298(54%) samples were culture positive, 290(53%) CBNAAT samples had MTB detected while only 210(38%) FM smear was positive. Out of 290 CBNAAT positive Samples, 49(16.8%) samples had Rifampicin resistance, 31(10.6%) samples were of PLHIV of which 2(6.4%) samples had Rifampicin resistance, which were confirmed with culture. The comparison of CBNAAT and FM smear against sputum culture are shown in Table 1 and 2.

The sensitivity and specificity for CBNAAT were 97% and 100% respectively; while that for Fluorescent microscopy were 70% and 100% respectively. The positive and negative predictive value for CBNAAT was 100% and 96% respectively. The positive and negative predictive value for Fluorescent microscopy was 100% and 73% respectively. The performance of CBNAAT and FM smear against sputum culture are shown in Table 3 and 4.

DISCUSSION

Current recommendations for the control of tuberculosis emphasize early case detection so as to allow treatment of patients and there by limit the transmission of the bacilli. The main stay for its control is the rapid and accurate

identification of the infected individuals. The laboratory plays a critical role in diagnosis of pulmonary tuberculosis. The simplest rapid method is the detection of acid-fast bacilli by smear microscopy but has less sensitivity. Sputum culture is widely regarded to be the most sensitive and specific test for the detection of pulmonary TB, but its routine use in resource- limited settings is hampered by excessive cost, slow turnout, and the need for adequate laboratory infrastructure.4 CBNAAT is a molecular method of rapid diagnosis of tuberculosis.

Table 1: Comparison of results of Fluorescent Microscopy (FM) with Culture (N= 548).

Type of test	Culture (Positive)	Culture (Negative)	Total
FM (Positive)	210 (38%) (True positive)	0 (0%) (False positive)	210 (38%) (Total FM positive)
FM (Negative)	88 (16%) (False negative)	250 (46%) (True negative)	338 (62%) (Total FM negative)
Total	298 (54%) (Total culture positive)	250 (46%) (Total culture negative)	548 (100%) (Total Samples; N)

Table 2: Comparison of results of CBNAAT with Culture (N= 548).

Type of test	Culture (positive)	Culture (Negative)	Total
CBNAAT (Positive)	290 (53%) (True positive)	0 (0%) (False positive)	290 (53%) (Total CBNAAT positive)
CBNAAT (Negative)	08 (1%) (False negative)	250 (46%) (True negative)	258 (47%) (Total CBNAAT negative)
Total	298 (54%) (Total culture positive)	250 (46%) (Total culture negative)	548 (100%) (Total Samples; N)

Table 3: Performance of FM for detection of TB against Culture.

Characteristics	Values (%)	95% CI
Sensitivity	70	64.8 to 75.6
Specificity	100	98.5 to 100
PPV	100	98.2 to 100
NPV	73	69.0 to 78.5

PPV positive predictive value, NPV negative predictive value, CI confidence interval

Table 4: Performance of CBNAAT for detection of TB against culture.

Characteristics	Values (%)	95% CI
Sensitivity	97	94.7 to 98.8
Specificity	100	98.5 to 100
PPV	100	98.7 to 100
NPV	96	93.9 to 98.6

PPV positive predictive value, NPV negative predictive value, CI confidence interval

In our study, only 548 sputum specimens were included. The sensitivity of CBNAAT for pulmonary samples was 97% when compared to Fluorescent Microscopy (FM) which was 70%. In this study findings suggest that CBNAAT has higher sensitivity for MTB detection than Fluorescent microscopy in Pulmonary Tuberculosis. The WHO has also recommended the CBNAAT for routine use under programmatic conditions.[5]

In a study done by Panayotis et al, 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 the sensitivity of CBNAAT in 60 pulmonary samples which included sputum, BAL, bronchial aspirate and gastric aspirate was 79%. In a study done Kandi et al the sensitivity of CBNAAT for pulmonary samples was 79% when compared to sputum smear which was 42%.[6-8]

Various studies conducted across India has suggested the usage of CBNAAT up-fornt for people living with HIV (PLHIV).[9]

The limitation of this study is that only sputum samples are examined with Fluorescent microscopy, CBNAAT and Culture. Other pulmonary (e.g. BAL) and extrapulmonary samples could be examined with CBNAAT and various studies has shown very good results with CBNAAT for MTB detection in extrapulmonary specimens.

CONCLUSION

CBNAAT has shown good results and is nearly matching the results of Culture. CBNAAT is having high sensitivity and specificity for diagnosis of pulmonary tuberculosis. It should be routinely used under national health programme to detect a tuberculosis case efficiently.

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