

EVALUATION OF CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST IN DIAGNOSIS OF PULMONARY TUBERCULOSIS

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ABSTRACT

BACKGROUND

Tuberculosis still continues to be one of the commonest cause of infectious disease related morbidity in the developing world. Diagnosis of pulmonary tuberculosis is mostly relied on sputum smear microscopy and sputum for mycobacterial culture. In recent years, cartridge based nucleic acid amplification test (CBNAAT) has been recommended by World Health Organization as a rapid diagnostic test for detection of tuberculosis and rifampicin resistance.

The aim of this study is to determine the diagnostic yield of CBNAAT in pulmonary tuberculosis (PTB) and to compare its efficacy between sputum smear-positive and sputum smear-negative pulmonary tuberculosis.

MATERIALS AND METHODS

A prospective observational study was conducted in the Department of Respiratory Medicine in a teaching hospital in eastern India for a period of one year. All re-treatment cases of PTB, newly detected smear-negative PTB and all human immunodeficiency virus-PTB co-infected cases were recruited for the study and sputum for CBNAAT was performed in all of them. Results were analysed in light of diagnostic yield of CBNAAT with special emphasis on comparing CBNAAT results between different subgroups- sputum smear-positive, sputum smear-negative PTB and immunocompromised patients.

RESULTS

Out of 228 cases of PTB, 190 were sputum smear negative and 38 cases were sputum smear positive. Mean age of the study population was 33 ± 17.13 years. Sensitivity of sputum smear for AFB was 16.67% (CI-12.07%-22.15%) with a negative predictive value (NPV) of 20.83% (CI- 19.89%-21.81%). Sputum smear negativity was found to be more common in females. CBNAAT was positive in 111 cases. Overall, sensitivity of CBNAAT was 48.68% (95% CI- 42.03%-55.37%). Sensitivity of CBNAAT varied significantly between sputum smear-positive PTB (100%, CI- 92.89%-100%) and sputum smear-negative PTB (38.42%; CI- 31.47%-45.74%) (p- <0.0001). Sensitivity of CBNAAT was 33.33% (CI-17.96%-51.83%) in PTB-HIV co-infected patients. Rifampicin resistance was detected in five (2.2%) patients with 100% sensitivity and specificity.

CONCLUSION

CBNAAT adds significantly to the diagnostic yield of PTB in comparison to sputum smear microscopy, but its sensitivity is lower in sputum smear-negative cases. It has additional advantage of identifying rifampicin resistance with high sensitivity and specificity.

KEYWORDS

Pulmonary Tuberculosis, Nucleic Acid Amplification Test, Smear Positive, Smear Negative, Human Immunodeficiency Virus.

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BACKGROUND

Pulmonary tuberculosis is still one of the commonest cause of infectious disease related morbidity and mortality in the developing countries.^[1] Diagnosis of pulmonary tuberculosis (PTB) mostly relies on identification of acid-fast bacilli (AFB) in sputum smear, but its limitation is low sensitivity.^[2,3] Conventional mycobacterial cultures (Solid culture in Lowenstein-Jensen medium) takes about 6-8 weeks' time;

newer liquid culture methods like BACTEC or Mycobacterial growth indicator tube (MGIT) gives relatively rapid results but is costly.^[4,5] Cartridge based nucleic acid amplification test (CBNAAT) is a nested polymerase chain reaction (PCR) technique that identifies small quantities of genetic elements of Mycobacterium tuberculosis from clinical specimens and it can identify resistance to rifampicin, the surrogate marker of multi-drug resistant (MDR) tuberculosis, at the same time. CBNAAT is completely automated, has minimal biosafety hazard and can give result within two hours. World Health Organization has endorsed the use of this rapid molecular diagnostic test for diagnosis of tuberculosis with special emphasis on drug-resistant tuberculosis, human immunodeficiency virus (HIV) and TB co-infection, paediatric tuberculosis, extrapulmonary tuberculosis and smear-negative pulmonary tuberculosis.^[6,7] There has been paucity of data from eastern India regarding diagnostic role of CBNAAT specially in diagnosis of sputum smear-negative PTB

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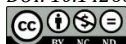
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cases. In this background, the present study was carried out to determine the diagnostic role of CBNAAT in PTB except in new microbiologically confirmed cases without HIV co-infection.

MATERIALS AND METHODS

A prospective, observational study of all adult cases (above 12 years of age), of new sputum smear negative, clinically diagnosed PT, all TB-HIV co-infected patients and all re-treatment cases of pulmonary tuberculosis, admitted or attending outpatients department in the Department of Respiratory Medicine of a teaching Hospital in Kolkata was carried out over a period of one year (July 2016 – June 2017).

Clinically Diagnosed TB Case [8]

Presumptive Pulmonary TB: refers to a person with any of the symptoms and signs suggestive of PTB including cough > 2 weeks, fever > 2 weeks, significant weight loss, haemoptysis, abnormality in chest radiograph.

Microbiologically confirmed Pulmonary tuberculosis: refers to a presumptive pulmonary TB patient with sputum positive for acid-fast bacilli or positive for Mycobacterium tuberculosis on culture, or positive for tuberculosis through quality assured rapid molecular diagnostic test.

Clinically Diagnosed TB Case

Refers to a presumptive pulmonary TB patient who is not microbiologically confirmed, but has been diagnosed with active TB by a clinician on the basis of clinical findings and having radiological lesions consistent with active parenchymal tuberculosis on chest x-ray/CT scan of the thorax (nodular consolidation with or without cavity in apex, tree in bud appearance).

Written informed consent was taken from all patients and the study was cleared by the institute's ethics committee.

Inclusion Criteria

1. All cases of clinically diagnosed sputum smear-negative pulmonary tuberculosis.
2. All re-treatment cases of microbiologically confirmed pulmonary tuberculosis (recurrent, failure, treatment after loss to follow-up).
3. All presumptive pulmonary tuberculosis cases with HIV co-infection.

Exclusion Criteria

1. Age less than 12 years;
2. New microbiologically confirmed PTB patients without HIV co-infection;
3. Patients not giving consent for the study.

Study Protocol

All patients who fulfilled the case definition and inclusion criteria and who consented for the study were considered for subsequent investigation and analysis. Patients were evaluated for clinico-demographical parameters such as age, sex, symptoms with duration, comorbidities, sputum smear status for acid-fast bacilli (AFB). Two samples of sputum were collected from each patient in Falcon tubes designated for sample collection for CBNAAT and were sent for CBNAAT at B.N. Bose Hospital, Kolkata as per Revised National Tuberculosis Control Programme (RNTCP) protocol for

testing for CBNAAT for Mycobacterium tuberculosis (Cepheid, GX-IV Processing Unit: 11.00" w x 12.00" h x 11.70" d, GXIV-4-D) [6, 8, 9]. Sputum samples for CBNAAT was also sent in 40 cases of community-acquired pneumonia and 10 cases of suspected lung nodule/mass lesion to look for false positive results but CBNAAT result was negative in all 50 cases. Chest X-ray posteroanterior (PA) view was done in all patients of microbiologically confirmed and clinically diagnosed PTB cases. High-resolution Computed Tomography (HRCT) scan of thorax with contrast was done additionally in clinically diagnosed PTB cases for obtaining some greater anatomical details regarding radiological evidence of active pulmonary tuberculosis (Nodular consolidation with or without cavity in apex, tree in bud appearance, bilateral involvement). Blood were sent for testing for HIV infection at the integrated counselling and testing centre (ICTC) of our hospital. Relevant haematological investigations like fasting blood sugar, complete haemogram, urea, creatinine and baseline liver function test were also done in all patients.

SA composite diagnostic index (Comprising of sputum AFB smear and/or radiology, and/or clinical judgement, and/or response to antitubercular drugs) was considered as the reference standard for diagnosis of pulmonary tuberculosis in this study and result of CBNAAT was compared with that composite diagnostic index.

Statistical Analysis

Statistical analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL) software for MS-Windows. Descriptive frequencies were expressed using mean and standard deviation. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and negative likelihood ratio were calculated with 95% confidence interval (CI) where relevant. P value was calculated using Fisher's exact test and Chi Square test as applicable and P value of less than 0.05 was considered significant.

RESULTS

Categorisation of Pulmonary TB Cases

A total of 228 cases of microbiologically confirmed or clinically diagnosed PTB were encountered during the study period, of which 179 cases were new cases of clinically diagnosed PTB with negative sputum smear and 49 were re-treatment PTB cases. Twenty of the re-treatment cases were recurrent PTB, 11 were treatment after loss to follow-up, seven were treatment after failure and 11 belonged to other previously treated category. So, overall 190 cases had sputum smear for AFB negative and 38 cases were tested positive for AFB in sputum.

Among 190 cases of sputum smear negative clinically diagnosed PTB cases, 33 were PTB with HIV co-infection, 11 were other previously treated cases and rest 146 were immunocompetent new sputum smear-negative PTB.

Demographic Profile

Overall mean age of PTB patients in the study population was 33 years \pm 17.13 years (mean \pm SD). 59.2% (135 out of 228) patients were male. Mean age in the female population was slightly lower (28.23 \pm 15.63 years) compared to the mean age in the male group (36.35 \pm 17.3 years). There was no significant difference in mean age between sputum smear-positive PTB and sputum smear-negative PTB population, but

sputum smear-negative PTB was significantly more prevalent in females in the study population (p<0.04) [Table 1]. Out of 190 cases of sputum smear-negative PTB cases, 82 (43.16%) were females, in contrast to 11 (28.95%) females out of 38 sputum smear-positive PTB. Diabetes mellitus was the commonest comorbidity, being found in 15 (7.89%) cases.

Microbiologic Diagnostic Spectrum

Sputum smear for AFB was positive in 16.67% (38 out of 228 cases). Sputum for CBNAAT detected Mycobacterium tuberculosis in 111 patients. CBNAAT was found to be positive in all 38 (100%) cases of sputum smear-positive PTB, but in 73 out of 190 (38.42%) cases of sputum smear-negative PTB (p<0.0001) [Table 2]. Thus, sputum for CBNAAT testing resulted in a relative increase in detection rate of microbiologically confirmed tuberculosis cases by 32.02% compared to sputum smear microscopy.

Overall, in this study population, sensitivity of sputum smear for AFB was only 16.67% (95% CI- 12.07%-22.15%) with specificity of 100%, negative predictive value (NPV) of 20.83% (95% CI- 19.89%-21.81%) and negative likelihood ratio of 0.83 (95% CI- 0.79-0.88). On the other hand, against a composite diagnostic index of PTB (comprising of sputum AFB smear, and/or chest radiograph and/or HRCT Thorax, and/or clinical judgement, and/or response to antitubercular drugs), overall sensitivity of sputum CBNAAT was 48.68% (95% CI- 42.03%-55.37%), with specificity of 100% (95% CI-

92.89%-100%). Sensitivity of CBNAAT was found to be 100% (95% CI -90.75%-100%) for microbiologically confirmed PTB, but sensitivity came down to 38.42% (95% CI- 31.47%-45.74%) for sputum smear-negative PTB (p<0.0001) [Table 3]. Specificity were 100% (95% CI- 92.89%-100%) in both the groups. Sputum for CBNAAT was found positive in 93.88% (46 out of 49) of re-treatment cases of PTB, on the contrary, it was positive in 36.31% (65 of 179) of newly diagnosed sputum smear-negative PTB (p<0.0001).

On detailed analysis of sputum smear-negative PTB group, CBNAAT showed detection of Mycobacterium tuberculosis in 36.99% (54 of 146) of immunocompetent newly smear negative cases, 33.33% (11 of 33) of HIV infected smear negative patients and 72.72% (8 of 11) of other previously treated cases [Table 4]. There was no significant difference in yield of sputum CBNAAT between immunocompetent and immunocompromised patients (p=0.56) but sputum microscopy yield was significantly lower in immunocompromised group (p=0.01).

Sputum for CBNAAT detected "Rifampicin resistance" in five patients-two of them were in the re-treatment group (treatment after lost to follow-up), two were new case of PTB with HIV co-infection and one was new sputum smear-negative PTB without HIV infection. All these cases of rifampicin resistance were also confirmed to be MDR cases by Line probe assay (LPA).

	Sputum smear-positive PTB (n=38)	Sputum smear-negative PTB (n=190)
Age (mean±SD) in years	32.97±13.64	33.005±17.71
Male: Female ratio	2.45:1	1.30:1
Diabetes mellitus	7	8
HIV infection	0	33

Table 1. Demographic Characters

	Sputum AFB Smear positive (n=38)	Sputum AFB Smear Negative (n=190)
Sputum CBNAAT positive (n=111)	38	73
Sputum CBNAAT negative (n=117)	0	117

Table 2. Diagnostic Yield of CBNAAT versus Sputum Smear Microscopy

	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (PPV)	Negative Predictive value (NPV)	Negative Likelihood Ratio
Overall PTB	48.68% (42.03%-55.37%)	100% (92.89%-100%)	100%	29.94% (27.36%-32.66%)	0.51 (0.45-0.58)
Sputum smear-positive PTB	100%	100% (92.89%-100%)	100%	100%	
Sputum smear-negative PTB	38.42% (31.47%-45.74%)	100% (92.89%-100%)	100%	29.94%	0.62 (0.55-0.69)

Table 3. Diagnostic Sensitivity and Specificity of Sputum CBNAAT in PTB

	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	Negative Likelihood Ratio
Smear negative PTB immunocompetent (n=157)	39.49% (31.79%-47.59%)	100% (92.89%-100%)	100%	34.48% (31.69%- 37.39%)	0.61 (0.53-0.69)
Smear negative PTB immunocompromised	33.33% (17.96%-51.83%)	100% (92.89%-100%)	100%	69.44% (64.10%-74.31%)	0.67 (0.52-0.85)

Table 4. Diagnostic Yield of CBNAAT in Smear-negative PTB- Immunocompetent Versus Immunocompromised Patients

Name of the Study	Overall PTB		Smear-Positive PTB		Smear-negative PTB	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Dewan R et al	40.00%	100%	100%	100%	32.58%	100%
Theron G et al	78.7%	94.4%	94.7%	95%	46.8%	94.4 %
Geleta et al	65.5 %	96.3 %	95.2 %	96.3 %	48.6 %	96.3 %
Agarwal M et al	86.8%	93.1%	100%	90%	79.1%	93.1%
Sharma SK et al	95.7%	99.6%	99.2%	99.6%	77.7%	99.6%
Sowjanya DS et al	70.24%	100%	99.08%	100%	37.50%	100%
Boehme CC et al	92.2%	99.2%	98.2%	99.2%	72.5%	99.2%

Table 5. Sensitivity and Specificity of CBNAAT-Results of Different Studies

DISCUSSION

India accounts for around one-fourth of the global tuberculosis cases.^[10] Detection of AFB in sputum smear is a simple, rapid, inexpensive and very specific for diagnosis for PTB, its limitation is its low sensitivity.^[2,3] Sputum culture for Mycobacterium tuberculosis is more sensitive and specific, but it takes 2-8 weeks' time depending on the method used and is costly.^[4,5] Chest x-ray is neither sensitive nor specific for diagnosis of PTB.^[11] So, there was a long felt need for a newer rapid diagnostic test for PTB with improved sensitivity and specificity. WHO has endorsed the use of CBNAAT as a rapid diagnostic test for diagnosis of tuberculosis and prioritised areas like drug-resistant tuberculosis, paediatric tuberculosis, TB-HIV co-infection, extrapulmonary tuberculosis and sputum smear-negative PTB for use of CBNAAT.^[6]

In this study, mean age of PTB patients was 33 years \pm 17.13 years (mean \pm SD) with slight male preponderance (59.2%). Dewan et al^[12] have also reported that mean age of patients in their study was 35 \pm 9 years, 69% of their patients were in 20-40 years age group and 76% were male. Sputum smear-negative PTB was found to be more common in females. Diabetes mellitus (n=15) was a common comorbidity in our study population. Increased prevalence of diabetes mellitus and tuberculosis is well documented in the published literature.^[13,14] HIV infection was seen in 33 cases (14.47%) with male predominance (57.6%). Dewan et al^[12] and Theron G et al^[15] have reported a higher value of TB-HIV co-infection of 40% and 27.08% respectively in their studies, though both the studies were conducted in high HIV prevalence setting.

Sensitivity of sputum smear for AFB was very low (16.67%) with a low NPV (20.83%) in excluding PTB in this study. Geleta DA et al^[16] have also found a very low sensitivity (9.3%) of conventional sputum smear microscopy by Ziehl-Neelsen staining. CBNAAT showed an advantage of increase in diagnosis of microbiologically confirmed PTB cases by 32% over and above the cases diagnosed by sputum smear for AFB. Dewan R et al^[12] and Geleta DA et al^[16] have also mentioned an increase in microbiologically confirmed PTB diagnosis by 29% and 31% respectively by CBNAAT compared to sputum smear microscopy.

Overall, CBNAAT was positive in 48.68% (95% CI- 42.03%-55.37%) PTB cases in this study, but its result varied significantly between 100% (95% CI -90.75%-100%) in sputum smear-positive PTB and 38.42% (95% CI- 31.47%-45.74%) in sputum smear-negative PTB. Similar results of very high sensitivity of CBNAAT in smear positive cases have been reported by most of the studies, but sensitivity of CBNAAT in smear negative cases varied between 35%-79.1% among several studies [Table 5].^[12,15,16-20] Specificity of CBNAAT was 100% (95% CI- 92.89%-100%) in this study in

both smear positive and smear negative group and is in line with other studies which have also demonstrated a very high specificity of CBNAAT in diagnosis of pulmonary tuberculosis [Table 5].^[12,15,16-20]

Sensitivity of sputum smear microscopy was even lower in this study in patients infected with HIV, all of the TB-HIV co-infected patients were sputum smear negative in comparison to 38 out of 195 immunocompetent patients being sputum smear-positive on microscopy (p-0.01). However, there was no statistically significant difference in sensitivity of CBNAAT between sputum smear-negative PTB cases with or without HIV co-infection (p-0.56). These findings were similar to the observations of Van Rle A et al^[21] and Carriquiry G^[22] et al in their respective studies. In this study, sputum for CBNAAT resulted in an increase in microbiologically confirmed TB cases by 33.3% in HIV infected patients. Dewan R et al^[12] have also reported a 29% increase in diagnosis of PTB by application of sputum CBNAAT over sputum smear microscopy in HIV infected patients. Among all smear negative cases, sensitivity of CBNAAT was found to be significantly higher (72.73%; 95% CI -39.03%-93.98%; p value- 0.023) with NPV of 94.34% and negative likelihood ratio of 0.27 in cases of sputum smear-negative tuberculosis with previous history of antitubercular drug intake.

Rifampicin resistance was detected in five (2.2%) cases of PTB. Two of these five cases were sputum smear-positive with previous history of antitubercular treatment default; two were sputum smear-negative PTB with HIV co-infection and one patient was a new case of sputum smear-negative PTB. Sensitivity (100%; 95% CI-47.82%-100%), specificity (100%; 95% CI- 98.36%-100%), PPV (100%) and NPV (100%) of CBNAAT for identifying rifampicin resistance were very high in this study. All of the five cases of rifampicin-resistant PTB in this study were confirmed as MDR-TB cases by LPA, so sensitivity and specificity of CBNAAT as a surrogate marker of MDR TB was also 100%. This finding has been supported by the study of Sharma SK et al,^[18] where sensitivity and specificity of CBNAAT was found to be 94.5%-99% and 97.7%-99.3% respectively. Theron G et al^[15] have also shown that with the use of second generation software for identifying rifampicin resistance, specificity and NPV for rifampicin resistance were 100% by CBNAAT. Rifampicin resistance on CBNAAT has been found to be a good surrogate marker for MDR-TB in their study, too, as 90% of rifampicin-resistant cases were confirmed as MDR by subsequent analysis. Although, sputum for CBNAAT is very good in rapidly identifying Rifampicin resistance in PTB patients, a study from Mumbai have reported there are good number of Isoniazid resistant but Rifampicin sensitive cases, where only relying on CBNAAT result may lead to missing out of drug-resistant PTB cases.^[23]

Limitation of this study was that we did not compare CBNAAT with sputum for mycobacterial culture, the current gold standard for diagnosis of PTB. Future studies in this field comparing result of CBNAAT with mycobacterial cultures are needed.

CONCLUSION

CBNAAT is a very useful and rapid test for diagnosis of PTB, but its limitation is that its sensitivity is modest in smear-negative PTB with or without HIV co-infection in comparison to very high sensitivity in smear-positive PTB. In spite of its modest sensitivity in smear-negative PTB and TB-HIV co-infection, in terms of absolute numbers, CBNAAT adds significantly to the number of microbiologically confirmed PTB in these patients. Main advantage of CBNAAT lies in its ability in rapid diagnosis and early detection of rifampicin resistance. Sputum for CBNAAT should be sent in all cases of TB-HIV co-infection, sputum smear-negative PTB and all retreatment cases of PTB for an early and confident diagnosis of PTB and to look for rifampicin resistance.

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