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Comparative Study of CBNAAT with Sputum Microscopy by Led Microscopy & Liquid Culture in Sputum Samples of Presumptive Pulmonary Tuberculosis

Thanushree Kurdula[1*], Sravan Kumar M[2], Sumalatha C[3], Phani Kumar B[4], Sunitha P[5]

[1]Junior Resident, Department of Pulmonology, Kakatiya Medical College, Government Chest Disease and Tb Hospital, Warangal, Telangana, India
[2]HOD & Professor, Department of Pulmonology, Kakatiya Medical College, Government Chest Disease and TB Hospital, Warangal, Telangana, India
[3]State TB Epidemiologist, State TB Training & Demonstration Centre, Hyderabad, India
[4]Associate Professor, Department of Pulmonology, Kakatiya Medical College, Government Chest Disease and TB Hospital, Warangal, Telangana, India
[5]Assistant Professor Department of Pulmonology, Kakatiya Medical College, Government Chest Disease and TB Hospital, Warangal, Telangana, India

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***Corresponding author:** Thanushree Kurdula
Junior Resident, Department of Pulmonology, Kakatiya Medical College, Government Chest Disease and Tb Hospital, Warangal, Telangana, India

Abstract **Original Research Article**

Tuberculosis is a communicable disease & a leading cause of death from a single infectious agent (ranking above HIV /AIDS). Early diagnosis is important for early patient management and successful treatment. The present study was a prospective observational study done on 84 suspected Pulmonary Tuberculosis patients visiting Government Chest disease and TB hospital Warangal during the time period of December 2017 to July 2019 & were evaluated with different microbiological tests for further confirmation of pulmonary tuberculosis .Considering liquid culture as the gold standard tool for diagnosis of Tuberculosis, sensitivity , specificity, PPV, NPV of CBNAAT were 78.4%, 60.6%, 75%, 65% respectively and sensitivity, specificity, PPV, NPV of AFB smear were 80%, 47.5%, 39%, 85% respectively in the present study.

Keywords: Cartridge based nucleic acid amplification test, Positive predictive value, Negative predictive value.

INTRODUCTION

Tuberculosis (TB) remains one of the world's deadliest communicable diseases that is caused by Mycobacterium tuberculosis. India is the country with one-fourth global TB burden of TB. Globally, an estimated 10.0 million people fell ill with TB in 2018. There were an estimated 1.2 million TB deaths among HIV negative people in 2018 and an additional 251000 deaths among HIV positive people.

Drug resistant TB continues to be a public health threat. In 2018, there were about half a million new cases of Rifampicin resistant TB. Globally, 3.4 % of new TB cases and 18 % of previously treated cases had multi drug resistant TB or Rifampicin resistant TB.

Early diagnosis is imperative for early patient management, and successful patient outcomes. False negative results and misdiagnosis of TB are common in developing nations, as most TB Control programmes at periphery use sputum microscopy, which has poor sensitivity, and multiple visits are required that leads to higher default. Mycobacterial culture, although considered as the gold standard but is slow and usually takes time to yield a final result and requires proper laboratory infrastructure & technical expertise.

There are Nucleic Acid Amplification Tests which are already deployed in field for all vulnerable population. Many states in India have started Universal DST for which CBNAAT is point of test.

CBNAAT not only provide the advantage of rapidity of diagnosis but also detects even low MTB genomic copies in various biological specimens.

Rationale of Study

There are few clinical experiences where discordances in Sputum Microscopy and CBNAAT are noted and there a very need to understand the

discordance by comparing with Liquid Culture DST with first line Drugs which is gold standard. The rationale of the present study was to compare a genotypic test

CBNAAT which yields results rapidly to culture which is the gold standard for diagnosis of TB with DST (Drug susceptibility testing), which usually takes a long time , which might affect the clinical management .CBNAAT and Microscopy are tests which can also detect dead bacilli which will put the clinician in Dilemma for further course of Action. The rationale of the study was to refrain clinician from all dubious issues in clinical experiences & reveal the most sensitive and specific tool.

AIMS AND OBJECTIVES

To evaluate the sensitivity, specificity, positive predictive value and negative predictive value of Nucleic acid amplification assay (GeneXpert/ CBNAAT)using sputum samples in patients with presumptive pulmonary tuberculosis & compare with LED microscopy for AFB and liquid culture with drug susceptibility testing using first line Anti tubercular drugs.

PATIENTS AND METHODS

The present study was a prospective observational study done on patients visiting Government Chest disease and TB hospital Warangal during the time period of December 2017 to July 2019. Institutional Ethical Clearance certificate was obtained. A total of 84 suspected pulmonary tuberculosis patients are evaluated with different microbiological tests for further confirmation of pulmonary tuberculosis.

Inclusion Criteria

A total of 84 suspected pulmonary tuberculosis patients with

- Age 15 years and above
- Clinical suspicion of pulmonary tuberculosis including symptoms of cough greater than 2 weeks and weight loss / no weight gain, fatigue, haemoptysis of any duration and loss of appetite, fever etc.
- Who are treatment naïve were included in the study and investigated.

Exclusion Criteria

1) Samples received without clinical history.
2) Samples received without request of all three tests.
3) Patient with history of lung malignancies or fungal infections.
4) Age < 15 years of age.

All the procedures like collection of samples and different tests performed were done according to standard protocols after taking informed consent of the patient and permission from the ethical committee of the college.

Tools

Patient was instructed to collect early morning sputum samples upon awakening by coughing as hard as possible after deep breaths and the sputum was collected is a wide mouthed container for sputum microscopy & in Falcons tube for CBNAAT and Culture with DST at same instant. The microscopic examination of the sputum was done by LED microscope, and the falcons tube with sputum was transported to CBNAAT & Intermediate Reference laboratory for liquid culture. The data was tabulated in Microsoft excel spread sheet in a master chart and studied for correlation. Statistical analysis of the data was conducted & the sensitivity, specificity PPV, NPV was calculated for AFB smear and CBNAAT using liquid culture as the gold standard.

OBSERVATIONS AND RESULTS

Out of the 84 cases

- 18 sputum samples were positive by all 3 methods ie , sputum microscopy , CBNAAT & Liquid culture
- 21 samples were negative by sputum microscopy , positive with CBNAAT and Culture
- 19 sputum samples were negative by all three methods
- 3 MOTT sputum samples were identified by culture who were positive by microscopy but negative by CBNAAT
- 5 sputum samples were positive by means of liquid culture only & negative by other two methods
- 4 sputum samples were were both CBNAAT & microscopy positive but culture negative
- 13 samples were positive by CBNAAT only but negative by other two methods.
- 1 sample was positive by direct microscopy alone

The results of Rifampicin sensitivity in CBNAAT & Liquid culture with DST were the same in all cases except one case where RIF sensitivity was seen in CBNAAT, but culture with DST showed RIF resistance.

Table-1: Comparision Table of CBNAAT & Microscopy

CBNAAT	Microscopy			
	Positive		Negative	
	Count	%	Count	%
Detected	21	84.0%	32	54.2%
Not detected	4	16.0%	27	45.8%
Total	25	100.0%	59	100.0%

Microscopy vs CBNAAT	
Sensitivity	84
Specificity	45.8
PPV	40%
NPV	87%

Table-2: Comparision of CBNAAT and Culture

CBNAAT	Culture			
	Positive		Negative	
	Count	%	Count	%
Detected	40	78.4%	13	39.4%
Not detected	11	21.6%	20	60.6%
Total	51	100.0%	33	100.0%

Culture vs CBNAAT	
Sensitivity	**78.4%**
Specificity	**60.6%**
PPV	75%
NPV	65%

Table-3: Comparision of Culture and Microscopy

Culture	Microscopy			
	Positive		Negative	
	Count	%	Count	%
Positive	20	80.0%	31	52.5%
Negative	5	20.0%	28	47.5%
Total	25	100.0%	59	100.0%

Microscopy vs Culture	
Sensitivity	**80.0%**
Specificity	**47.5%**
PPV	39%
NPV	85%

Table-4: Diagnostic accuracy of CBNAAT & AFB smear using sputum samples with AFB liquid culture as the gold standard

Variables	Sensitivity	Specificity	PPV	NPV
CBNAAT	78.4 %	60.6%	75 %	65 %
AFB SMEAR	80%	47.5 %	39%	85 %

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 bacilli. This study was undertaken to assess the utility of various diagnostic modalities for early diagnosis of tuberculosis. Also an attempt was made to compare the sensitivity of CBNAAT with the conventional diagnostic techniques.

For the purpose of the study only new clinically suspected pulmonary tubercular cases were selected. Any case already on anti-tubercular treatment or confirmed to have tuberculosis by any of the microbiological techniques was excluded from the study. This helped us in two ways: we could be sure that all samples which did not show any growth on culture were not due to previous anti-tubercular therapy, and secondly there was no bias regarding the results while processing the sample.

Patient Characteristics

AGE

The youngest patients in our study were 17 year old, both females while the oldest patient was 80 years old. Maximum number of patients with presumptive symptoms were in the age groups of 40-60 years of age.

Tuberculosis mostly affects adults in their most productive years. However, all age groups are at risk.

SEX

In our study, out of 84 patients 52.4 % were males & 47.6 % were females. Many other investigations have also noted male preponderance in their studies. Peter *et al*., [1] and Fandinho FCO *et al*., [2] have reported male to female ratio of 1.8:1 and 1.6:1 respectively. Narang P *et al*., [3] have reported that 61.03% of their subjects were male while 38.97% were females. Our study is comparable to all these studies.

Likely reasons of male preponderance are as follows:

a) In a male dominated society, usually he is the earning member. As he goes out for work, he is more likely to come in contact with an active TB case.
b) Men are more likely to acquire habits like smoking and alcoholism which predispose to TB.

SYMPTOMATOLOGY

Cough with expectoration was the most common symptom in the patients studied, 95.2 % of the cases had cough with expectoration of greater than 2 weeks duration. Fever was the second most common symptom, 94.0 % of the patients studied had fever. 70.2 % of the patients had shortness of breath. 56.0 % of the patients had loss of weight.

67.9 % of the patients had loss of appetite. 36.9 % had chest pain. 15 .5 % of the patients presented with coughing of blood. Out of the total 84 cases, 94.1 % of the cases were Non reactive to HIV anti bodies and 15 % of the cases suffered from Diabetes mellitus.

Thus the most common symptom the patient had was cough with expectoration and the least common common presentation was coughing of blood among the patients included in the present study.

MICROSCOPY

Smear examination following staining is the most effective method for early detection of acid fast bacilli even with development of advanced technology.

Since cultivation of mycobacteria is a time consuming procedure, initial management of individuals, suspected of having tuberculosis is based on results of microscopic examination of the clinical specimens.

Though various methods have been developed for demonstration of Mycobacteria, standard Ziehl Neelson method and Auramine O staining method are the widely used procedures.

In our study, out of 84 cases AFB was demonstrated in 25 cases by Fluorescent microscopy using Auramine O staining.

CDC has recommended the use of fluorescent microscopy for demonstration of Mycobacteria. The overall positivity of Fluorescent microscopy ranges from 30% to 80% in various studies. According to WHO in 2010 accuracy of LED in comparison with reference standards [4].

LED microscopy showed 84% sensitivity (95% confidence interval [CI], 76–89%) and 98% specificity (95% CI, 85–97%) against culture as the reference standard.

The present study the sensitivity of LED microscopy showed 80 % sensitivity Genexpert.

The overall positivity of Genexpert in the diagnosis of tuberculosis reported in various studies.

Sl. No	Authors	Genexpert sensitivity %	Reference no.
1	Geleta *et al*.,	65.5%	[5]
2	Bajrami *et al*.,	82.3%	[6]
3	M Agrawal *et al*.,	86.8%	[7]
4	SK Sharma	95.7%	[8]
5	S Narute *et al*.,	96.9%	[9]
6	Armand S *et al*.,	79%	[10]
7	HS Moussa *et al*.,	93%	[11]
8	Shao Y *et al*.,	94.64%	[12]
9	Arzu N *et al*.,	100%	[13]
10	Present study	78.4 %	

The difference in sensitivity of the Xpert assay on detection of *M. tuberculosis* among studies could be explained by differences in inclusion criteria and techniques used to obtain sputum specimens. Our study

and other studies all confirm that positive acid-fast smear correlated well with Xpert assay and TB culture. Inspite of being the gold standard Culture has its own advantages and disadvantages.

The total cost per test for liquid culture is marginally higher than that of solid culture. For the protection of the staff working in the laboratory, aerosols produced during processing & inoculation need to be minimized .Hence processing should be done in Biosafety Cabins. In case of power failure, infectious particles may no longer be trapped in HEPA filters and may flow back to the open front . Hence the Biosafety Cabins should be connected to a suitable UPS system.

Liquid culture automated readers need continuous power supply. In all settings the instrument should be connected to a suitable UPS, to avoid loss of culture viability. To maintain a liquid culture with DST system, it is very much important to have a ample supply of reagents and consumables within their expiry date, to avoid unnecessary interruptions. It is also important to note that liquid culture is not compatible with cetyl pyridium chloride (CPC). Rather than relying on CPC to control contamination, its best to send the specimens to the laboratory within 4 days of collection. If transport is prolonged specimensmust be maintained at 2-6 degrees Centigrade with the use of Cold boxes & resusable ice packs.

Limitations of the Study

- Sample size of the patients included in the present study was low.
- Follow up of the patients at the end of treatment was not done to look for sputum / culture conversion.

Future Aspects

RNTCP introduced LED-FM at all the DMC's & also there is a rapid rollover of CBNAAT by RNTCP.

Government should be encouraged to procure more of CBNAAT machines to help in earlier & prompt start of treatment to improve TB outcome in high burden TB –HIV co infection settings. Further studies focusing on false positives of CBNAAT will be valuable.

CONCLUSION

Although culture is the gold standard, it takes days to come positive and simultaneous detection of Rifampicin resistance is not possible with it & it detects the Mycobacterium complex and further tests are required to differentiate between the MTB & MOTT. Treatment of the symptomatic patients in a TB burden country should be prompt hence Genotypic methods like CBNAAT, who have a less turn over time with simultaneous detection of Rifampicin resistance can be utilised. Further more of results CBNAAT must be well correlated with clinical and treatment history and subjected to Culture for further confirmation.

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