VOLUME - 9, ISSUE - 8, August - 2020 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Microbiology

COMPARATIVE PERFORMANCE ANALYSIS OF DIFFERENT METHODS OF DIAGNOSIS IN PATIENTS WITH SUSPECTED PULMONARY TUBERCULOSIS

Dr. Aparna Aparajita*	Senior Resident, Department of Microbiology, RIMS, Ranchi. *Corresponding author
Dr. Manoj Kumar	Prof. & H.O.D., Department of Microbiology, RIMS, Ranchi.
Dr. Ashok Kumar Sharma	Associate Professor, Department of Microbiology, RIMS, Ranchi.

ABSTRACT

Background: Tuberculosis occurs in every part of world and India accounts for two thirds of new TB cases. A number of tests are available to diagnose TB, ranging from conventional microscopy to nucleic acid tests which are new tools for rapid and early diagnosis. The conventional microscopy has low sensitivity and culture is considered as gold standard but takes ample time for positivity. The newer outlook with nucleic acid amplification tests provide rapid and early detection of tuberculosis with rifampicin resistance especially in patients with high clinical suspicion of infection with Mycobacterium tuberculosis as well as decrease the transmission of disease. The study was carried out to compare such rapid tests with liquid culture and microscopy using ZN stain and AO stain.

Aim: To evaluate the sensitivity, specificity, positive predictive value, negative predictive value of cartridge based nucleic acid amplification test (CBNAAT) using sputum samples in patients suspected of pulmonary tuberculosis and compare with AFB smear microscopy using ZN stain, AO stain and acid fast bacilli culture (AFB liquid culture).

Materials And Methods: The study was a cross sectional study conducted from April 2018 to October 2018 in Department of Microbiology, RIMS, Ranchi. A total of 157 sputum samples were tested. The sensitivity, specificity, positive predictive value, negative predictive value of CBNAAT, ZN stain, AO stain were calculated using Liquid culture of Mycobacterium tuberculosis by MGIT 960 as reference standard.

Results: A total of 157 samples were evaluated . Out of these 28 were positive by all the four methods. The sensitivity, specificity, PPV & NPV of CBNAAT were 89.6%, 82.8%, 75.3% and 93.1% . The sensitivity, specificity, PPV & NPV of AFB smear microscopy using ZN stain were 48.3%, 100%, 100% and 76.7% and that of fluorescent microscopy with AO stain were 75.8%, 91.9%, 84.6% & 86.7% respectively.

Conclusion: CBNAAT has higher sensitivity than AFB smear microscopy in sputum samples. Fluorescent microscopy has higher sensitivity than conventional microscopy and can help detect the bacteria in samples with less bacterial load in lesser time. Culture is the gold standard and so results that are culture negative but CBNAAT positive should be correlated with history and clinical presentation.

KEYWORDS :

INTRODUCTION

Tuberculosis is one of the major air borne infectious bacterial disease caused by *Mycobacterium tuberculosis*. It ranks as the second leading cause of death from an infectious disease worldwide after HIV. As per WHO Global TB report 2019, every year 10 million people fall ill with TB, of which 1.5 million people die from TB each year making it the world's top infectious killer. About 58 million lives were saved through diagnosis and treatment between 2000 and 2018.(1) Therefore, early diagnosis and prompt treatment of TB are crucial to reduce morbidity and mortality, secondary drug resistance, and transmission of TB. Tuberculosis can involve any organ system in the body. Pulmonary TB is the most common presentation of TB affecting lungs. Microscopy detects only half the number of TB cases and cannot detect drug resistance.(1)

ZN smear microscopy is a rapid and cheap method to detect acid fast bacilli, but has lesser sensitivity.(2) Fluorescent microscopy greatly improves the diagnostic yield of sputum smear especially in patients with low density of bacilli that are likely to be missed on Ziehl Neelsen stained smears. The method is economical in both time and expense and recommended for laboratories handling very large number of samples.

Culture is a slow method despite being the gold standard and takes 2-6 weeks time to yield a final result requiring technical expertise and proper infrastructure.(1,3,4) Nucleic Acid Amplification Tests(NAAT) have been developed that can detect even low density organisms rapidly. CBNAAT utilizes a DNA-PCR technique for simultaneous detection of *Mycobacterium tuberculosis* and Rifampicin resistance related mutations giving result in 2hrs,so recommended by WHO as initial diagnostic test in all persons with signs and symptoms of TB. It has high diagnostic accuracy for pulmonary tuberculosis and is highly useful in patients with HIV and in paediatric age group(5,6)

AIM

The study was aimed at evaluating sensitivity, specificity, positive predictive value, negative predictive value of CBNAAT assay in patients suspected of pulmonary tuberculosis and compared with AFB smear microscopy using ZN stain, AO stain and liquid AFB culture.

MATERIALS AND METHODS

The study was a cross-sectional study carried out in the Department of Microbiology, RIMS, Ranchi, from April 2018 to October 2018. A total of 157 sputum samples were received and tested for the presence of *Mycobacterium tuberculosis*. Patients suspected of pulmonary tuberculosis including symptoms of cough with or without expectoration for > 2weeks, weight loss, fatigue, haemoptysis and loss of appetite were included in the study. The samples without a clinical history, without request for all the four tests and patients with history of lung malignancy of fungal infections were excluded from the study.

Two sputum samples were received from each patient in sterile containers. Each sputum sample was divided into four parts, one part for CBNAAT testing, 2nd part for ZN smear microscopy, 3rd part for AO staining and fluorescent microscopy and 4th part for MGIT 960 liquid culture. No less than 2ml of sputum sample

was used for MGIT 960 testing. For AFB smear and culture, the sample were homogenized and concentrated using N-acetyl L cysteine- sodium hydroxide(NALC- NaOH) method.

CBNAAT testing was performed according to the manufacturer's instructions.(7) Sample reagent was added to the untreated sputum at a ratio of 2:1, manually agitated and kept for 10mins at room temperature, shaken and kept for 5 mins; 2ml of inactivated material was transferred to the test cartridge and inserted into the test platform. Only electronic results were used for comparison. ZN staining was performed with the 2nd part and microscopy done to see the presence of acid fast bacilli. Red coloured acid fast bacilli were considered positive. 3rd part of the sputum was stained by auramine which is a fluorescent dye. Using fluorescent microscopy, the tubercle bacilli when examined under ultra violet illumination, appeared as bright rods against a dark background. 4th part was processed using N- acetyl-L cysteine – sodium hydroxide method(NALC-NaOH), cultured on MGIT 960 , a liquid culture system. Sodium hydroxide is a decontaminating agent and also a mucolytic and homogenizer. It also reduces the concentration of NaOH required. When tubes were flagged positive by the system, ZN staining and culture on 5% sheep blood agar were performed from the tube directly to look for any contamination. The tubes were checked for positivity till 42 days. Differentiation between Mycobacteria other than tuberculosis(MOTT) and *Mycobacterium tuberculosis* were done using immunochromatographic test kit with MPT 64 antigen.

The sensitivity, specificity, positive and negative predictive value for diagnosis of pulmonary tuberculosis were calculated for AFB microscopy and CBNAAT using culture as the gold standard. Samples that were positive and negative in culture were taken as true positive and true negative. Culture negative but CBNAAT positive samples were considered false positive samples. CBNAAT negative and culture positive samples were considered false negative.

RESULTS

A total of 157 samples from suspected cases of pulmonary tuberculosis were received with request for all the four methods of diagnosis and were tested accordingly. 28 samples were positive by all the four methods. Of all the samples 69 samples were positive by CBNAAT and 58 were flagged positive by MGIT 960, 28 were positive by ZN stain and 52 were positive by Auramine O stain. Overall sensitivity, specificity, PPV and NPV of AFB smear microscopy when liquid culture was taken as the reference was calculated.(Table-1) The sensitivity, specificity, PPV & NPV of CBNAAT were 89.6%, 82.8%, 75.3% and 93.1% .(Table-1,2) The sensitivity, specificity, PPV & NPV of AFB smear microscopy using ZN stain and light microscopy were 48.3%, 100%, 100% and 76.7%.(Table-3) The sensitivity, specificity, PPV & NPV of AFB using fluorescent microscopy using AO stain were 75.8%, 91.9%, 84.6% and 86.7% respectively.(Table- 4)

Table 1: Sensitivity, Specificity, Positive And Negative Predictive Value Of Sputum Samples With Culture As Reference Standard.

	SENSITIVITY	SPECIFICITY	PPV	NPV
CBNAAT	89.6%	82.8%	75.3%	93.1%
ZN STAIN	48.3%	100%	100%	76.7%
AO STAIN	75.8%	91.9%	84.6%	86.7%

Table-2: Sensitivity, Specificity, Positive And Negative Predictive Value Of CBNAAT In Sputum Samples With Culture As Reference Standard.

	CBNAAT	CULTURE	
	No. of samples	POSITIVE	NEGATIVE
POSITIVE	69	52	17
NEGATIVE	88	6	82
Sensitivity	89.6%(CI-78.8%-96.1%)		
Specificity	82.8%(CI-73.9%-89.6%)		
PPV	75.36%(66.3%-82.6%)		
NPV	93.1%(86.4%-96.7%)		

Table-3: Sensitivity, Specificity, Positive And Negative Predictive Value Of Light Microscopy Using ZN Stain In Sputum Samples With Culture As Reference Standard.

	ZN Stain	CULTURE	
	No. of samples	POSITIVE	NEGATIVE
POSITIVE	28	28	0
NEGATIVE	129	30	99
Sensitivity	48.28%(CI-34.9% - 61.7%)		
Specificity	100%(CI-96.3%-100%)		
PPV	100%(96.3%-100%)		
NPV	76.74%(72-80.8%)		

Table-4: Sensitivity, Specificity, PPV & NPV of Fluorescence Microscopy Using AO Stain In Sputum Samples With Culture As Reference Standard.

	AO Stain	CULTURE	
	No. of sample	POSITIVE	NEGATIVE
POSITIVE	52	44	0
NEGATIVE	105	14	91
Sensitivity	75.8%(CI-62.8%-86.1%)		
Specificity	91.9%(CI- 84.7%-96.4%)		
PPV	84.6%(CI-73.6%-91.6%)		
NPV	86.7%(CI-80.4%-91.1%)		

Table-5: Studies Showing Overall Sensitivity And Specificity Of CBNAAT In Suspected Pulmonary Tuberculosis Cases.

S.No	STUDY	SENSITIVITY	SPECIFICITY
1.	Dewan R et al.	40%	100%
2.	Theron G et al.	78.7%	94.4%
3.	Geleta et al.	65.5%	96.3%
4.	Agrawal M et al.	86.8%	93.1%
5.	Sharma SK et al.	95.7%	99.6%
6.	Sowjanya DS et al.	70.2%	100%
7.	Boehme CC et al.	92.2%	99.2%

DISCUSSION

Our study compares various modalities of diagnosis right from the conventional methods to the new rapid methods for diagnosing TB. Rapid diagnosis of TB is a necessity in our country because of the high patient load as well as the risk of ongoing transmission of infection. CBNAAT is a simple diagnostic assay that requires minimal technical expertise and also provides results within 2 hours. Our study thereby shows that CBNAAT is a very good tool to detect positive tuberculosis patients in those with high suspicion of pulmonary tuberculosis. It has higher sensitivity for detection in smear positive patients. It is valuable as an add on test following smear microscopy in patients previously found to be smear negative. Major drawback of direct smear examination using ZN staining for diagnosing TB, although cheap and easy to use is that it requires good and experienced microscopy and technical expertise. It is also a problem with paucibacillary samples. Liquid culture by MGIT960 is rapid as compared to the solid culture on LJ media but has high contamination rates and requires good technical expertise. In study done by Agrawal M et al the sensitivity and specificity, PPV and NPV were 86.8% , 93.1%, 78.5% and 96% in suspected pulmonary TB patients.(8) In study done by Sharma SK et al the sensitivity, specificity were 95.7% & and 99.6%. (9) Studies done by various authors have shown an overall sensitivity and specificity of CBNAAT to be 40%-92% and 93%-100%.(Table-5)(8,9,10,11,12,13,14)

CONCLUSION

CBNAAT has greater sensitivity and specificity followed by AO stain and ZN stain in smear microscopy. Culture though

considered as a reference takes time to become positive, cannot give simultaneous rifampicin resistance status and has higher contamination rates as well. CBNAAT is a very good choice in our setting for rapid diagnosis of suspected pulmonary tuberculosis patients and it can also provide with the rifampicin resistance status of the patient. Also, Auramine O staining can be a good option in screening patients suspected of pulmonary tuberculosis where there is high case load as compared to ZN staining. The fluorescent stains can be more efficient as it is less time consuming compared to ZN method for detecting Tubercle bacilli in sputum samples.

REFERENCES

1. World Health Organization. Global tuberculosis report 2019. Geneva: WHO; 2019.
2. Fitzgeraid DW, Sterling TR, Hass DW,Tuberculosis . In: Mandell GL, Benett JE, Dolin R editors. Mandell,Douglas , and Benett's Principles and Practice of infectious diseases. 7th ed:Churchill Livingstone/Elsevier;2010.
3. Evans CA. GenXpert – a game changer for tuberculosis control? PLOS MED. 2011;8:e1001064.
4. Centers for Disease Control and Prevention (CDC). Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. MMWR Morb Mortal Wkly Rep. 2009;58:7-10.
5. Shah I, Gupta Y. Role of molecular tests for diagnosis of tuberculosis in children. Pediatric Oncall Journal. 2015;12(1):www.pediatriconcall.com/Journal/Article/
6. World Health Organization: Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert mtb/rif system. Policy statement 2011. http://whqlibdoc.who. int/publications/2011/9789241501545_eng.pdf.
7. Xpert MTB RIF kit insert http://www.cepheid.com/manageddownloads/xpertmtb-rif-english-package-insert-301-1404-rev-b-february-2015.pdf [12] Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology, 12th edition. The C.V Mosby Co. St.Louis. 2007, chapter 45 Pg: 478-509.
8. Agrawal M, Bajaj A, Bhatia V, et al. Comparative study of gene Xpert with ZN stain and culture in samples of suspected pulmonary tuberculosis. Journal of Clinical and Diagnostic Research 2016;10(5):DC09-DC12.
9. Sharma SK, Kohli M, Yadav RN, et al. Evaluating the diagnostic accuracy of Xpert MTB/RIF assay in pulmonary tuberculosis. PLoS One 2015;10(10): e0141011.
10. Dewan R, Anuradha S, Khanna A, et al. Role of cartridge-based nucleic acid amplification test (CBNAAT) for early diagnosis of pulmonary tuberculosis in HIV. JIACM 2015;16(2):114-7.
11. Theron G, Peter J, van Zyl-Smit RV, et al. Evaluation of the Xpert MTB/RIF Assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. Am J Respir Crit Care Med 2011;184(1):13240.
12. Geleta DA, Megerssa YC, Gudeta AN, et al. Xpert MTB/RIF assay for diagnosis of pulmonary tuberculosis in sputum specimens in remote health care facility. BMC Microbiology 2015;15:220.
13. Sowjanya DS, Behera G, Reddy VVR, et al. CBNAAT: a novel diagnostic tool for rapid and specific detection of Mycobacterium tuberculosis in pulmonary samples. International Journal of Health Research in Modern Integrated Medical Sciences 2014;1:28-31.
14. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 2010;363(11):1005-15.