ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 8, Issue – 5, September – 2023 , Page No. : 183 – 189

Role of Cartridge Based Nucleic Acid Amplification Test a diagnostic modality in Extra-pulmonary Tuberculosis.

[1]Adil Jokhi, Department of Respiratory Medicine, SGT University, Gurugram, Haryana, India.
[2]Ayush Pandey, Department of Respiratory Medicine, SGT University, Gurugram, Haryana, India.
[3]Mohit Bhardwaj, Department of Respiratory Medicine, SGT University, Gurugram, Haryana, India.
[4]DPS Sudan, Department of Respiratory Medicine, SGT University, Gurugram, Haryana, India.
Corresponding Author: Adil Jokhi, Department of Respiratory Medicine, SGT University, Gurugram, Haryana, India.

Citation this Article: Adil Jokhi, Ayush Pandey, Mohit Bhardwaj, DPS Sudan, "Role of Cartridge Based Nucleic Acid Amplification Test a diagnostic modality in Extra-pulmonary Tuberculosis", IJMSIR- September - 2023, Vol – 8, Issue - 5, P. No. 183 – 189.
Type of Publication: Original Research Article
Conflicts of Interest: Nil

Abstract

Introduction: About. 10 to 15% of total TB cases are of extra pulmonary tuberculosis involving the pleura, lymph nodes, gastrointestinal tract etc and diagnosing such cases is very challenging because of its paucibacillary nature, lack of specific sign and symptoms and often negative acid-fast bacilli smears. The present study was designed to study the role of Cartridge Based Nucleic Acid Amplification Test a diagnostic modality in Extra-pulmonary Tuberculosis.

Material and methods: This Prospective comparative study carried out over 2 years included 100 patients who are visited NTEP clinic in SGT medical college, Haryana.

A detailed history and examination were done for all the suspected patients based on clinic-radiological findings and specimen (pus, pleural fluid, CSF, lymph node) were collected under aseptic precautions and were subjected to CBNAAT and Ziehl-Neelsen staining.

Results: The total number of cases with presumptive extra pulmonary Tb were 100. The most common type of sample was Pleural fluid (44%) and pus aspirate was the second most common type of sample taken (13%). Out of all EPTB samples ZN stain came out to be positive in 19% of the samples while CBNAAT detected presence of MTB in 59% with additional Rifampicin resistance in 30.5% patients.

Conclusion: Though ZN staining is the cheapest and easiest diagnostic modality, but in patients with suspected EPTB CBNAAT is an important diagnostic tool to detect MTB more precisely and with additional advantage of Rifampicin resistance.

Keywords: Extrapulmonary tuberculosis, CBNAAT, MTB, ZN stain

Introduction

Tuberculosis (TB) is one of the deadliest disease and global health problem. It is caused by slow-growing bacteria Mycobacterium tuberculosis (MTB). According to global tuberculosis report 2020 of World Health Organization worldwide 10 million people infected with TB killing 1.5 million people. It is a 2nd major leading infectious cause of death resulting from single infectious agent after HIV. Being single infectious agent, Mycobacterium tuberculosis is the tenth most common

reason for mortality on the planet. The individual who are in close contact with TB patient are likely at higher rate of becoming infected, with an expected 22% infection rate [1]. Owing to it heightened rate of transmission one smear positive case of pulmonary tuberculosis can infects 7-10 persons in a year [2,3].

India represents one fourth of the worldwide TB burden. In spite of common involvement of lungs, TB can infect every organ of the body except hair and nail. Extra pulmonary tuberculosis defined by WHO is M. tuberculosis affecting tissues and organs other than lung parenchyma, most commonly site like Lymph nodes, Pleura followed by Bone and Joins, then it called as extra pulmonary tuberculosis (EPTB) [4, 5].

About 10 to 15% of total TB cases are of extra pulmonary tuberculosis involving the pleura, lymph nodes, gastrointestinal tract etc and diagnosing such cases is very challenging because of its paucibacillary nature, lack of specific sign and difficult to access for sample collection. CBNAAT is able to diagnose 131 bacilli/ml in pulmonary sample leading to additional yield in diagnosis. However, regarding its role in extrapulmonary tuberculosis has limited data available so; the present study was designed to study the role of Cartridge Based Nucleic Acid Amplification Test a diagnostic modality in Extra-pulmonary Tuberculosis.

Material and methods

This Prospective comparative study carried out over 2 years included 100 patients who visited the clinical departments in SGT medical college a tertiary care center Haryana.

Sample processing

Staining method

All respiratory and non-respiratory samples are subjected to Ziehl-neelsen staining procedure. Steps of Ziehl-Neelsen stain described below

1. A new slide is selected and the slide is labeled with the diamond pencil.
2. A tongue shape smear is made from sample portion of the sputum using a broom stick. A good smear is spread evenly, 2 cms x 3 cms in size and is neither too thick nor too thin. Smear preparation heated with a flame. Flame is considered as a sterile zone which coagulates the aerosol raised during smear preparation.
3. Air dry the slide for 15-30 minutes
4. Cover the entire slide with 1% filtered carbol fuchsin stain.
5. The slide is gently heated with flames, until vapors rise.
6. The slide is gently washed with normal temperature tap water until excess carbol fuchsin stain is washed away. At this point, slide looks red in color.
7. 25% sulphuric acid is poured onto the slide and allowed to stand for 2–4 minutes. Sulphuric acid work as a decolourisation. Decolourised slide appears light pink in color.
8. 0.1% methylene blue is poured onto the slide and left for 30 seconds. Then the slide is rinsed gently with tap water and allowed to dry.
9. The slide is checked under the microscope using x40 lens. The 40x objective was used for confirmation of AFB
10. Suitable area examined under x100 oil immersion lens using a drop of immersion oil.
11. All positive and negative slides are stored serially in the same slide-box until instructed by the supervisor.
12. Grading of smear according to field examination and number of bacilli present in the smear.

According to NTEP guidelines, below mentioned table depicts information on grading and number of fields examined.

Table 1: Smear grading for AFB

Examination finding	No. of fields examined	Grading	Result
No AFB in 100 oil immersion fields	100	0	Neg
1-9 AFB per 100 oil immersion fields	100	Scanty*	Pos
10-99 AFB per 100 oil immersion fields	100	1+	Pos
1-10 AFB per oil immersion field	50	2+	Pos
More than 10 AFB per oil immersionfield	20	3+	Pos

With ZN staining, the bacilli appear as red in color, slender rods, standing out clearly against a dark blue background and sputum samples were graded according to the number of bacilli as per WHO guidelines. Where in case of extra-pulmonary samples, the numbers of acid-fast bacilli seen were noted.

CB-NAAT

Cartridge Based Nucleic Acid Amplification Test also known as Xpert MTB/RIF assay was performed on all specimens irrespective of AFB smear positivity to detect tuberculosis and to determine rifampicin sensitivity.

Sample processing

Extra pulmonary samples majorly divided into two main groups.

1. Specimens collected with aseptic precaution, nearly sterile fluids like pleural, pericardial, CSF, ascetic, lymph node.
2. Specimens got contaminated by normal flora or specimens not collected aseptically e.g. urine, pus.

Collection of extrapulmonary samples

Lymph node aspirate: - Lymph nodes samples withdraw by FNAC (Fine needle aspiration cytology) Routine FNAC will be performed by the attending pathologist. For FNAC procedure 10cc syringe and 23G needle needed. Selection of largest and superficial most lymph node to be done by ultrasound neck. Hold the lymph node between index finger and thumb of left hand. Insert the needle in anti-gravity method; aspirate the material by with/without vacuum. The aseptically collected tissues are placed by the physician in sterile containers preferably without fixatives or preservatives.

Pleural fluid: After doing Ultrasound chest to quantify fluid, under guidance of ultrasound 18/20G needle inserted in the intercostals space upper border of the lower rib. This procedure also known as Thoracocentesis. Thoracocentesis has two types: 1. Diagnostic 2. Therapeutic.

Ascitic fluid: Considered a suboptimal specimen as tubercle bacilli are not within the fluid. The minimum volume for ascitic fluid required for processing for Xpert is 2 to5 ml. The fluid is collected using Ascitic tap or paracentesis.

CSF: After taking aseptic precaution, ask patient to lay in semi-recumbent position and at site of 5^{th} lumbar vertebral space insert lumbar puncture needle.

For culture method specimen should not be collected in formalin solution. If histo pathological examination is required, two samples should be collected.

Transportation of samples

Collected aseptic extra pulmonary specimens will need to be transported in cool boxes which maintain temperatures below 20^{o}C (2-8^{o}C) for specimens to be compatible for molecular methods. For transportation and prevention of biohazards triple packing system should be utilized. For sending material across international or state boundaries this container may have to be packed in the same way with an additional outer container. Collected sample should be labeled properly with patient name, address, contact number, collection date and collected sample on container not on cap of the bottle.

Procedure

For lymph odes and other tissues

1. Using sterile pair of forceps and scissors, the tissue specimens were cut into small pieces in a sterile tissue grinder.

2. Approximately 2 ml of sterile phosphate buffer (PBS) was added. PBS P_H is approx. 7.2.
3. The tissue and PBS keep on adding and grinding using a tissue grinder until a homo-generous suspension was obtained.
4. Using a pipette approximately 1 ml of the homogenized suspension specimen was trans-ferred to a sterile, conical screw-capped tube.
5. Xpert MTB assay sample reagent was added at a 2:1 ratio to the re suspended sample and mixed well
6. Vigorously the tube was shaken 10 to 20 times or vortex for atleast 10 seconds.
7. It was incubated for 10 minutes at room temperature, and then the specimen was shaken vigorously again for another 10–20 times or vortexed for atleast 10 seconds.
8. The sample will be incubated additionally for more 5 minutes. This step is designed to reduce the viability of M. tuberculosis in sample by 10^6 folds. It reduces the biohazard risk.
9. The cartridge lid will be opened first and then we will open sample container. Using the provide pipette the liquefied sample will be aspirated to the line on the pipette
10. 2 ml of sample will be transferred into sample chamber of the Xpert MTB. This sample should be dispensed slowly to prevent aerosol formation.
11. Then, we will close the lid firmly and cartridge will be loaded into Gene Xpert MTB.

CSF

For GeneXpert of CSF volume of CSF matters, blood-stained CSF gives false negative result.

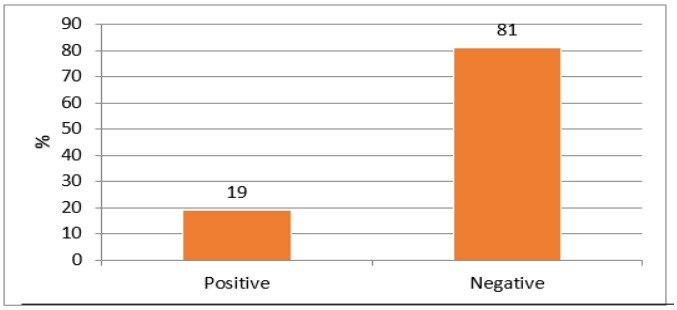
If CSF more than 5ml

1. Transfer entire specimen into the centrifugation tube, and concentrated up to 3000G for 15 minutes.
2. Superficial liquid lying above solid material remove carefully and poured off using a funnel into a discard can which containing 5% phenol.
3. The deposit was re-suspended to a final volume of 2 ml by adding the Xpert MTB/RIF sample reagent
4. Give unique identity number to Xpert/MTB/RIF cartridge.
5. Using a fresh transfer pipette, 2 ml of the concentrated CSF specimen was transferred to the Xpert MTB/RIF cartridge.
6. Load cartridge within the Xpert MTB/RIF following guidelines.
7. Within 2 hours instrument will give us result as MTB bacilli detected or not and Rifampicin status Sensitive/Resistant.

If CSF volume between 1-5 ml

1. Take an equal volume of sample reagent and added into the given volume of CSF.
2. Labelled cartridge with unique identity number, took 2 ml of the sample mixture was directly added to the Expert MTB/RIF cartridge.
3. The cartridge was loaded into the GeneXpert instrument following the manufacturer's instructions.

If CSF volume 0.1-1ml

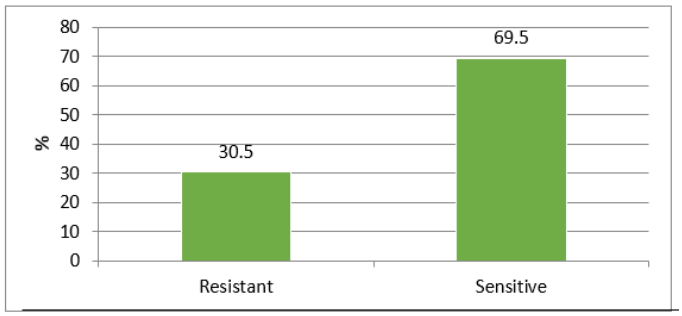


Graph 1

While, CBNAAT detected presence of MTB in 59% (Table 1) with additional Rifampicin resistance in 30.5% patients. (Graph 2)

CBNAAT	No. (n=100)	%
Detected	59	59.0
Not detected	41	41.0

Table 1



Graph 2

Graph 3 shows the predictive values of CBNAAT for the diagnosis of extrapulmonary tuberculosis compared to ZN staining for Acid Fast Bacilli. CBNAAT correctly detected extrapulmonary tuberculosis in 15% patients with sensitivity and specificity of 78.9% and 45.7% respectively.

Discussion

Extrapulmonary tuberculosis is a significant weight of mortality and dreariness because of its paucibacillary nature, subclinical symptoms, and atypical presentation. The routine microscopy, ZN staining and culture have low sensitivity and it is very tedious bringing delay in treatment of these cases. CBNAAT furnishes early diagnosis alongside status of rifampicin sensitivity which is exceptionally helpful for patient future treatment part. It gives the results within 2 hours and plays an important role in diagnosis of extra pulmonary tuberculosis.

India represents around one-fourth of the worldwide tuberculosis cases. Detection of AFB in sputum smear is basically simple, quick, economical and very specific for diagnosis for EPTB; but this technique is limited by it has low sensitivity [6].

Sputum culture for Mycobacterium tuberculosis is precisely sensitive and specific, but it requires approx 2-8 weeks' time depends upon what type of culture method is used and it is costly [7].

So, there was a large gap for a newer rapid diagnostic test for EPTB with improved sensitivity and specificity. WHO has endorsed the use of CBNAAT as a rapid diagnostic test for diagnosis of tuberculosis and promote its used in endemic areas specifically in which prevalence of drug-resistant tuberculosis is more, TB-HIV co-infection, extrapulmonary tuberculosis and sputum smear- negative PTB for use of CBNAAT [8].

In this study, the most common type of sample was Pleural fluid (44%) and pus fluid was the second most common type of sample taken (13%). In the study by Singh et al (2020), of the total 914 clinically suspected tuberculosis specimens ,were collected, in which 683(75%) were pulmonary which included sputum (594/ 87%), bronchoalveolar lavage (27/4%), and gastric fluid specimens (62 /10%) and 231(25%) were presumptive extra pulmonary tuberculosis received from different anatomical sites were: tissue biopsies or fine- needle aspirates (65 /28%), pus (56/24.2%), pleural fluid (55/24%, other body fluids (peritoneal, synovial and pericardial: 24/10.4%) CSF (23/11.3%), endometrial biopsy (4/1.7%) [9].

This study showed that ZN AFB was positive in 19% patients. CBNAAT detected among 59% patients in this study. Kumari et al (2020) found that the positivity for Mycobacterium tuberculosis was 41% by CBNAAT and 24% by conventional ZN staining method. 17% cases were detected with tuberculosis with the help of CBNAAT only and were reported as negative on ZN smears [10].

Rifampicin was resistant in 30.5% patients in the present study. Rifampicin resistance in the study by Kumari et al (2020) was 19.51% (8 cases out of 41). A study by Rao et al (2012) found 13.55% Rifampicin resistance [11] and

Dewan et al (2015) found 25% of rifampicin resistance which was nearby similar to our findings [16]. Whereas Tripathi et al (2016) found a very high (53%) rifampicin resistance in their study [12]. However, Sanjay M et al (2017) found a very low rifampicin resistance of 6.38%. This difference can be due to demographical variation of the populations included in the various studies [13].

This study suggests that CBNAAT is a sensitive tool to detect extra pulmonary tuberculosis, with rifampicin resistance. However, there are some limitations of this study. This study had small sample size and included only patients of extrapulmonary. The studies with larger sample size and including pulmonary and of extrapulmonary samples are required to have more robust findings.

Conclusion

Though ZN staining is the cheapest and simple diagnostic modality, but in patients with suspected EPTB CBNAAT is an important diagnostic tool to detect MTB more precisely. CBNAAT is a very sensitive, critical, successful and used to diagnose for extrapulmonary tuberculosis as compared Z-N staining. This can analyse a greater number of smears negative cases as well as rifampicin resistant tuberculosis very early. This provides an additional edge for management of TB in world where most of cases went undiagnosed. This should be used as an initial diagnostic tool for these cases. Keeping in mind its rapidity in diagnosis as well as identification of rifampicin resistance, CBNAAT should routinely be tested in all forms of EPTB.

References

1. Ahmed N, Hasnain SE. Molecular epidemiology of tuberculosis in India: moving forward with asystems biology approach. Tuberculosis 2011;91:407-13.
2. Kasat S, Biradar M, Deshmukh A, Jadghav S, Deshmukh H. Effectiveness of CBNAAT in the diagnosis of extrapulmonary tuberculosis.Int J Res Med Sci 2018;6:3925-8.
3. Steinbrook R. Tuberculosis and HIV in India. N Engl J Med2007;356:1198-9.
4. Komanapalli S K, Prasad U, Atla B, Vasundhara N, Yendluri D. Role of CB-NAAT in diagnosing extra pulmonary tuberculosis in correlation with FNA in a tertiary care center. Int J Res Med Sci. 2018;6(12):4039-45.
5. Ramirez-Lapausa M, Menendez-Saldana A, Noguerado-Asensio A. Extrapulmonary tuberculosis: an overview. Rev Esp Sanid Penit. 2015;17:3-11
6. World Health Organization. Global Tuberculosis Report-2017. WHOGeneva, Switzerland. 2017
7. Sharma SK, Kohli M, Chaubey J, Yadav RN, Sharma R, Singh BK, et al. Evaluation of Xpert MTB/RIF assay performance in diagnosing extrapulmonary tuberculosis among adults in a tertiary care centre in India. EurRespir J. 2014;44(4):1090-3.
8. World Health Organization. Rapid implementation of the Xpert MTB/RIF diagnostic test. Technical and operational “How to” practical considerations. World Heal Organ Doc. 2011
9. Singh P, Ranjan PK, Ratnesh, S.N Singh. A comparative study of the diagnostic efficacy of CB-NAAT and ZN staining. International Journal of Health and Clinical Research 2020;3(12):153-159
10. Kumari M, Khambra P, Panwar K et.al. Rapid diagnosis of tubercular lymphadenopathy by cartridge-based nucleic acid amplification test

(CBNAAT) and its correlation with Ziehl-Neelsen staining on fine needle aspiration cytology. Int J Health Sci Res. 2020; 10(7):17-21.

11. Rao DP, Sowjanya KL. Role of CBNAAT in rapid detection of Mycobacterium Tuberculosis in PLHIV in a highly prevalent state. J Evid Based Med Healthc. 2012; 3(38):1896–1898.
12. Tripathi R, Sinha P, Kumari R, Chaubey P, Pandey A, et al. Detection of rifampicin resistance in tuberculosis by molecular methods: A report from Eastern Uttar Pradesh, India. Indian J Med Microbiol. 2016;34(1):92–94.
13. Sanjay MG, Radha PM, Babu NP, Sushant M, Jitesh A. Genotypic diagnosis of extrapulmonary tuberculosis- CBNAAT a novel tool. Med Pulse Int J Med. 2017; 4(2);79-82