CBNAAT: A Better Tool for Diagnosis of Tuberculosis

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ABSTRACT

Introduction: With an estimated 9 million new cases and 2 million deaths every year, tuberculosis (TB) remains a leading public health problem worldwide. Current study was done at comparative analysis of cartridge based nucleic acid amplification test with microscopy and mycobacteria growth indicator tube in Extra Pulmonary samples of suspected Tuberculosis Patients.

Material and methods: Rohilkhand medical college and hospital Bareilly, U.P. Retrospective cross-sectional Study**.** From November 2018 – October 2019. Ziehl Neelsen staining was done for all samples. Smears were made from all pulmonary and extrapulmonary samples, heat fixed and stained with strong carbol fuschin.

Results: In total 69 patients we found that there were 21 specimens of patients having suspicion of tubercular lymphadenopathy, 39 of tubercular pleural effusion and 4 of tubercular ascites and 5 pus respectively. Among of which 9 came positive with CBNAAT for lymph node samples, 11 for pleural fluid, 2 for ascitic fluid and 3 pus respectively. In case of microscopy the results were 5 for lymph node, 4 in pleural effusion, 1 in ascitic fluid and 2 in pus respectively.

Conclusion: Comparing the both tests sensitivity and specificity. It was found as CBNAAT was having better sensitivity and specificity compared to microscopy overall.

Keywords: CBNAAT, A Better Tool, Diagnosis of Tuberculosis

INTRODUCTION

With an estimated 9 million new cases and 2 million deaths every year, tuberculosis (TB) remains a leading public health problem worldwide[1]. Worldwide, extrapulmonary tuberculosis (EPTB) accounts for, 25% of all TB cases, and even higher percentages in HIV-infected individuals and children [2-4]. Existing tests for the diagnosis of EPTB are limited in accuracy and time to diagnosis, and often require invasive procedures and special expertise. For pleural TB, culture of pleural fluid has low sensitivity (on average, 30–50%). For lymph node TB, culture of an aspirate has a sensitivity of 60–70%.[5] tuberculosis is associated with high mortality and morbidity in developing country such as India[6]. Tubercular pleural effusion is common in India compared to western countries whereas the commoner cause in later is malignancy. With such increase in number of patients of tuberculosis in India, It can be definitely called world capital for tuberculosis

Diagnosing extrapulmonary tuberculosis is even more challenging because Acid-fast staining was positive in fewer than 10% of patients in most reports, whereas pleural fluid cultures for *M. tuberculosis* were positive in up to 12 to 70% of cases and pleural biopsies revealed granulomas in 50 to 97% of patients with tuberculous pleural effusion[7]. Extrapulmonary infection with members of the Mycobacterium tuberculosis complex (MTBC) remains a diagnosis that is often difficult to establish, since the number of bacteria in

extrapulmonary specimens is often lower than the number in pulmonary specimens. Furthermore, collection of extrapulmonary material often requires invasive procedures, and it is not easy to obtain additional samples. In recent times, attention has been devoted to new nucleic acid amplification diagnostic technologies, owing to their rapidity, sensitivity, and specificity.[8] One of the latest systems, the CBNAAT, was evaluated only recently in a large study with pulmonary specimens. The CBNAAT uses heminested real-time PCR to amplify an *M. tuberculosis*-specific sequence of the *rpoB* gene. To determine rifampin (RMP) resistance, the rifampin resistance-determining region of the *rpoB* gene is probed with molecular beacons[9]. The assay can be carried out in a nearly fully automated manner, including bacterial lysis, nucleic acid extraction and amplification, and amplicon detection. The test runs on the GeneXpert platform (Cepheid, Sunnyvale, CA) using a disposable plastic cartridge with all required reagents[10]. Although the test has a limit of detection of 131 colony forming units per mL, it has been shown to perform less well in EPTB paucibacillary disease, members of the INDEX-TB TAC subcommittees recognized the need for the use of Xpert MTB/RIF for the diagnosis of EPTB in India, because of

1. The widely availability of the test
2. Faster Results
3. Accuracy of the test

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4. Prevention of patients from the possible harms from misdiagnosis

Aim

Comparative study of cartridge based nucleic acid amplification test with microscopy and mycobacteria growth indicator tube in Extra Pulmonary samples Of suspected Tuberculosis Patients.

MATERIAL AND METHODS

Place of study: Retrospective cross-sectional Study was done in Rohilkhand medical college and hospital, Bareilly, U.P from November 2018 – October 2019.

Inclusion criteria

All Patients with clinical suspicion of tuberculosis (extra pulmonary) According to RNTCP and WHO guidelines will undergo Fluorescent Microscopy, CBNAAT and BACTEC Culture (MGIT) for Mycobacterium Tuberculosis and any of these test comes positive will be included in the study

Sample collection and processing

Microscopy

Ziehl Neelsen staining was done for all samples. Smears were made from all pulmonary and extrapulmonary samples, heat fixed and stained with strong carbol fuschin. Intermittent heating of slides was done for 5 minutes and the carbol fuschin was washed off with distilled water. 20% sulphuric acid was used to decolourize the slide till carbol fuschin was extracted from the smear and methylene blue was used to counterstain the smear. The slides were observed under 100X oil immersion and grading of positive sputum smears was done according to RNTCP guidelines. Concentration of remaining samples was done by Petroff's method and auramine O staining was performed on the concentrated sample. The slides were flooded with Auramine O for 15 minutes and decolorized using acid alcohol. Potassium permanganate was used to flood the slide for 1 minute and the smears were dried and observed under fluorescent microscope and graded according to guidelines issued by WHO and followed by Central TB division, India.

Liquid Culture

Liquid culture of all samples was done in Mycobacterial Growth Indicator Tube (BBL MGIT) which contains Middlebrook 7H9 liquid media and an oxygen-quenched fluorochrome, embedded in silicone at the bottom of the tube. During bacterial growth within the tube, the free oxygen is utilized and is replaced with carbon dioxide. With depletion of free oxygen, the fluorochrome is no longer inhibited, resulting in fluorescence within the MGIT tube when visualized under UV light.15 Samples for liquid culture were decontaminated using NALC-NaOH method as recommended by CDC.16 The decontaminated samples (0.5ml) were added to labelled MGIT tubes with 0.8ml of antimicrobial PANTA solution and left at room temperature for 30 minutes after inoculation. They were incubated in the MGIT960 machine till the instrument flagged it positive. Positive tubes were confirmed for Mycobacterium tuberculosis by performing AFB smear and MPT 64 rapid card test (SDbioline).

GeneXpert

Samples were processed according to standardized protocol recommended by WHO.17 The sample was mixed with double volume of Xpert MTB/ Rif sample reagent and vortexed for 10 seconds followed by 5 minutes of incubation at room temperature. 2ml of sample was transferred to Xpert MTB/Rif cartridge and loaded into the machine. The sample combines with the sample processing control (SPC) in the cartridge. A filter captures the sample and SPC. Ultrasonically lysed cells release the DNA from bacterial cells if present. Eluted DNA combines with dried down bead reagents in the cartridge. PCR and detection occurs in the real time and results are ready to be viewed and printed in 1hour 52 minutes on an average.

RESULTS

In total of 69 patients who were taken for purpose of study underwent routine clinical investigations and also had been examined for CBNAAT and microscopy of the samples taken from various samples (lymph node, pleural fluid, ascitic fluid and pus). Both were compared with culture in MGIT BACTEC media for confirmation. Patients were informed and their written consent was also taken

In total 69 patients we found that there were 21 specimens of patients having suspicion of tubercular lymphadenopathy, 39 of tubercular pleural effusion and 4 of tubercular ascites and 5 pus respectively. Among of which 9 came positive with CBNAAT for lymph node samples, 11 for pleural fluid, 2 for

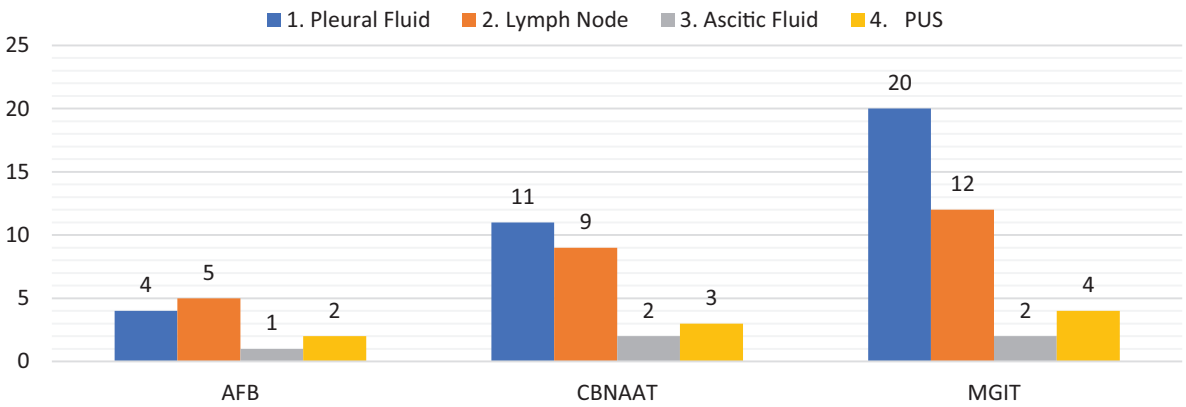

Figure-1: Total no. positive extrapulmonary sample

Extra Pulmonary N= 69	Pleural fluid	39(56.52%)
	Pus from any site	5 (3.%)
	Lymph Node	21(15.7%)
	Ascitic Fluid	4 (5.7%)

Table-1: Showing total distribution of cases

Samples	Microscopy	CBNAAT	Microscopy + CBNAAT	MGIT	Microscopy+ MGIT	CBNAAT + MGIT	Microscopy + CBNAAT + MGIT
Pleural fluid n- 39(56.5%)	4(10%)	11(28.2%)	4(10%)	20(51.2%)	4(10%)	11(28.2%)	4(10%)
Lymph node n-21(30.4%)	5(23.8%)	9(42.8%)	5(23.8%)	12(57%)	3(14.2%)	9(42.8%)	5(23.8%)
Ascitic fluid n-4 (5.7%)	1(25%)	2(50%)	1(25%)	2(50%)	1(25%)	2(50%)	1(25%)
PUS N-5(7.2%)	2(40%)	3(60%)	2(40%)	4(80%)	2(40%)	2(40%)	2(40%)
Total no. =69(100%)	12(17%)	25(36%)	12(17.3%)	38(55%)	10(14.4%)	24(34%)	12(17.3%)

Table-2: Showing how samples tested for various tests

MGIT BACTEC	Number of patients	Percentage
Positive	38	60.9
Negative	31	39.1
Total	69	100

Table-3: Confirmation of cases using MGIT BACTEC

Sample	Type of test	Positive	Negative	Sensitivity (in %)	Specificity (in %)	Positive predictive value (in %)	Negative predictive value (in %)
LYMPH node (n=21)	Microscopy	5	17	41.7	100	100	56.3
	CBNAAT	9	12	75	100	100	75
	MGIT BACTEC	12	9	-	-	-	-
PUS (n=5)	Microscopy	2	3	50	100	100	33.3
	CBNAAT	3	2	50	100	66.7	
	MGIT BACTEC	4	1	-	-	-	-
Pleural fluid (n=39)	Microscopy	4	35	20	100	100	54.3
	CBNAAT	11	28	55	100	100	75
	MGIT BACTEC	20	19	-	-	-	-
Ascitic fluid (n=4)	Microscopy	1	3	50	100	100	66.7
	CBNAAT	2	2	100	100	100	100
	MGIT BACTEC	2	2	-	-	-	-

Table-4: Sensitivity and specificity

ascitic fluid and 3 pus respectively. In case of microscopy the results were 5 for lymph node, 4 in pleural effusion, 1 in ascitic fluid and 2 in pus respectively.

Comparing the both tests sensitivity and specificity. It was found as CBNAAT was having better sensitivity and specificity compared to microscopy overall. Whereas comparing individual samples the sensitivity and specificity were as follows

DISCUSSION

CBNAAT has now became one of the most important diagnostic technique for tuberculosis. CBNAAT basically uses detection of nucleic acid; which can even detect in almost any sample except serum and bony tissue. Microscopy though specific but has very low sensitivity in detection of extra pulmonary tuberculosis Several studies have pointed out the intermediate level sensitivity of GeneXpert, better than smear microscopy but less than broth culture,[11-14]

As per this study GeneXpert also proved helpful in detecting more extrapulmonary cases than the histopathology and AFB stain. The GeneXpert MTB/RIF assay has a better diagnostic potential for specimens, such as pus and aspirates, which is not easily diagnosed by other laboratory techniques. Findings of the study aids the use of GeneXpert MTB/RIF assay in routine EPTB diagnosis. Similar studies were carried out by Lawn and Zumla[15] who employed the GeneXpert MTB/RIF assay for diagnosis of EPTB

Limitations

The most important drawback with CBNAAT is it can give false positive result as demonstrated by some study

Sample size being low it is difficult to generalise result

Single centred study

CONCLUSION

Our study concludes that if patients are suspected for tuberculosis patient must undergo CBNAAT before ruling out possibility of tuberculosis.

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