

CBNAAT: A new pillar in diagnosis of extra pulmonary tuberculosis

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Background: Worldwide, incidence of extrapulmonary tuberculosis (EPTB) is around 25% of all TB cases which is even more higher in HIV-infected individuals. Till date, there are limited tests for the diagnosis of EPTB with limitations in accuracy and takes longer time to diagnosis with often requiring invasive procedures and expertise. Sensitivity of culture in pleural fluid is 30–50% and of lymph node is around 30–50%. In recent times, new nucleic acid amplification diagnostic technologies have proved to give better result due to their rapidity, sensitivity, and specificity. Our study focuses on the same values of sensitivity, specificity, PPV, and NPV of CBNAAT. **Material and Methods:** Retrospective cross-sectional study done from November 2018 to October 2019 in Rohilkhand Medical College, Bareilly. **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 nine 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 five for lymph node, four in pleural effusion, one in ascitic fluid, and two 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.

KEY WORDS: CBNAAT, EPTB, Diagnosis

INTRODUCTION

With an increase in detection of new cases and deaths every year, tuberculosis (TB) remains a leading public health problem worldwide.^[1] Till date, there are limited tests for the diagnosis of extrapulmonary TB (EPTB) with limitations in accuracy and takes longer time to diagnosis with often requiring invasive procedures and expertise. Sensitivity of culture in pleural fluid is 30–50% and of lymph node is

around 30–50%.^[2-4] 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 TB in India, it can be definitely called world capital for TB.^[5,6]

Diagnosing EPTB is even more challenging because in most of the reports, acid-fast staining was found to be positive in less than 10% of patients, whereas pleural fluid cultures for *Mycobacterium tuberculosis* were positive in ranging from 12% to 70% and pleural biopsies revealed granulomas in 46 to 95% of patients with the diagnosis of tuberculous pleural effusion.^[7] Extrapulmonary infection with members of the *M. tuberculosis* complex remains a diagnosis that is often difficult to establish, since the number of bacilli in extra pulmonary TB is very less as compared to the bacillary load in pulmonary TB. Furthermore, collection of extrapulmonary specimen is very difficult as it

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often requires invasive procedure and it is very difficult to obtain the sample. In recent times, attention has been devoted to new nucleic acid amplification diagnostic technologies, due to their rapidity, sensitivity, and specificity.^[8] The CBNAAT works on real-time PCR to amplify an *M. tuberculosis*-specific sequence of the *rpoB* gene. To determine rifampin resistance, the *rpoB* gene is probed with molecular beacons.^[9,10] Although the test has a limit of detection of 131 colony forming units per mL, 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
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 TB patients.

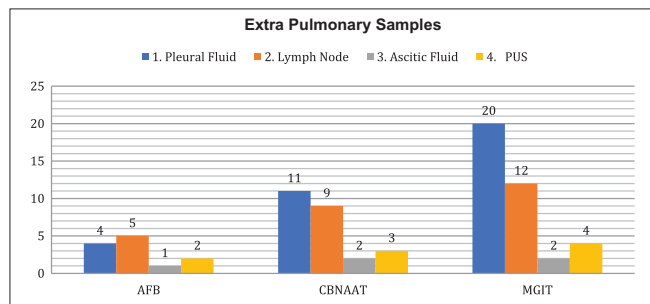


Figure 1: Total number positive extrapulmonary sample

| Table 1: Total distribution of cases | | |
|--------------------------------------|-------------------|-------------|
| Extra pulmonary <i>n</i> =69 | Pleural fluid | 39 (56.52%) |
| | Pus from any site | 5 (3%) |
| | Lymph Node | 21 (15.7%) |
| | Ascitic Fluid | 4 (5.7%) |

| Table 2: How samples tested for various tests | | | | | | | |
|---|------------|------------|-------------------|------------|------------------|--------------|-------------------------|
| 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 number=69 (100%) | 12 (17%) | 25 (36%) | 12 (17.3%) | 38 (55%) | 10 (14.4%) | 24 (34%) | 12 (17.3%) |

MATERIALS AND METHODS

Study Type

This was a retrospective cross-sectional study.

Study Place

This study was conducted at Rohilkhand Medical College and Hospital and Bareilly, U.P.

Period

This study was from November 2018 to October 2019.

Selection Criteria

All patients with clinical suspicion of TB (extra pulmonary) according to RNTCP and the WHO guidelines, will undergo Fluorescent Microscopy, CBNAAT, and BACTEC Culture (MGIT) for *M. tuberculosis* and any of these test comes positive will be included in the study.

Procedure

Collection of samples

All the patients were clearly explained about the procedure and the samples were collected after following universal precautions [Figure 1 and Table 1].

Body fluids

Under strict aseptic precautions around 10–15 ml of body fluids such as pleural, ascitic fluid was collected by aspiration.

Lymph node and pus sample

Under strict aseptic precautions using needle and syringe, as much material as possible of pus was aspirated from the abscess, for examples, like cold abscess, Psoas abscess, and cutaneous abscesses.

All samples were subjected to smear examination for Acid Fast Bacilli (AFB) using fluorescence microscopy technique, CBNAAT under Revised National TB Control Program, and MGIT culture [Table 2].

The reports of the patients were then followed up and entered in the pre-designed and pretested pro forma and final diagnosis was made.

Ethical Approval

The Ethical committee approval from the institution was taken for conducting this study.

Statistical Analysis

The data were coded and entered, its clearing and compiling was done on a Microsoft Excel spreadsheet and then it was imported into Statistical Package for the Social Sciences version 23 for statistical analysis. Data were analyzed by applying frequency, percentage, mean, and standard deviation.

Observations

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 [Table 3].

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

ascitic fluid, and three pus, respectively. In case of microscopy, the results were five for lymph node, four in pleural effusion, one in ascitic fluid, and two in pus, respectively.

Comparing the both tests sensitivity and specificity were as follows. 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 [Table 4].

DISCUSSION

CBNAAT has now become one of the most important diagnostic techniques for TB. 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 extrapulmonary TB. 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 aid the use of GeneXpert MTB/RIF assay in routine EPTB diagnosis. Similar studies were carried out by Lawn *et al.*^[15] who employed the GeneXpert MTB/RIF assay for diagnosis of EPTB.

Limitations of the Study

The most important drawback with CBNAAT is it can give false positive result as demonstrated by some study. It is difficult to generalize result, sample size being. It is a single centered study.

Table 3: Confirmation of cases using MGIT BACTEC

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

Table 4: Sensitivity, positive and negative predictive values of both tests

| 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 | - | - | - | - |

RECOMMENDATIONS AND CONCLUSION

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

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