

Diagnosis of Extrapulmonary Tuberculosis by Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) and Detection of Rifampicin Resistance on Fine-Needle Aspiration Samples: An Institution-based Study

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Abstract

Background: Extrapulmonary tuberculosis (EPTB) is still a major health problem in developing countries. Diagnosis of EPTB is difficult due to paucity of acid-fast bacilli in clinical specimens. **Objectives:** To study the diagnostic role of cartridge-based nucleic acid amplification test (CBNAAT) and rifampicin resistance in EPTB on fine-needle aspiration samples. **Materials and Methods:** It is a prospective study comprising 79 patients of clinically suspected EPTB cases for 1 year duration. All patients underwent fine-needle aspiration cytology (FNAC) with Ziehl–Neelsen (ZN) staining followed by CBNAAT for confirmation of tuberculosis (TB) and to detect rifampicin resistance. Data have been collected and analyzed regarding sensitivity, specificity, positive predictive value, and negative predictive value of FNAC in comparison with CBNAAT. **Results:** Out of 79 patients, 47 (59.49%) patients were male and cervical lymph node was the most common site of aspiration (42, 52.16%). Granuloma with necrosis (38, 48.10%) followed by necrosis only (14, 17.72%) were the common findings on FNAC. Out of 79 suspected EPTB patients, CBNAAT confirmed 62 patients as having TB. Specificity of FNAC with ZN staining was 100.0%; however, sensitivity was low (43.55%) compared to CBNAAT. Only three cases were detected as rifampicin resistant. **Conclusion:** Compared to FNAC, CBNAAT has very good sensitivity and specificity in the diagnosis of EPTB. It has also as added advantage of detecting rifampicin resistance at the same time. CBNAAT should be used for diagnosis of EPTB wherever the facility is available.

Keywords: Cartridge-based nucleic acid amplification test (CBNAAT), extrapulmonary tuberculosis, fine-needle aspiration cytology, rifampicin resistance, Ziehl–Neelsen staining

INTRODUCTION

Tuberculosis (TB) is a leading health problem in developing countries. A patient with *Mycobacterium tuberculosis* (MTB) complex is identified from a clinical specimen, either by culture or by a newer method such as molecular line probe assay. In countries that lack the laboratory capacity to routinely identify MTB, a pulmonary case with one or more initial sputum smear examinations positive for acid-fast bacilli (AFB) is also considered to be a “definite” case, provided that there is a functional external quality assurance system with blind rechecking.^[1] Extrapulmonary tuberculosis (EPTB) accounts for about

25% of TB cases.^[2] EPTB refers to a case of TB involving organs other than the lungs, e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, and meninges.^[1] Diagnosis of EPTB is challenging due to high rates of smear negativity.^[3]

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Fine-needle aspiration cytology (FNAC) with Ziehl–Neelsen (ZN) staining is widely used for the diagnosis of EPTB in under-resource settings like us. Although a presumptive diagnosis can be made easily, but due to low sensitivity, many times definitive diagnosis is not possible. Cartridge-based nucleic acid amplification test (CBNAAT) is a comparatively new, fully automated, real-time heminested polymerase chain reaction (PCR) system that is a most sensitive rapid test for the diagnosis of TB in paucibacillary samples, and World Health Organization (WHO) also recommends this technique.^[4] In 2014, WHO has recommended CBNAAT over conventional tests for the diagnosis of EPTB for specimens from non-pulmonary sites.^[5] In addition to diagnosis, CBNAAT can detect rifampicin resistance at the same time.^[6] CBNAAT is not routinely performed in EPTB as samples are not readily available in the Revised National Tuberculosis Control Programme (RNTCP) center because a patient is unable to give samples by himself or herself.

Considering these facts, this study was undertaken with the following aims and objectives:

- i. To diagnose EPTB and rifampicin resistance using the CBNAAT technique in fine-needle aspiration samples.
- ii. To calculate sensitivity, specificity, and diagnostic accuracy of FNAC with ZN staining, taking CBNAAT as gold standard.

MATERIALS AND METHODS

Study design, subjects, and area

This prospective, cross-sectional, observational study was carried out in a tertiary care medical hospital situated in West Bengal, India. The duration of this study was 1 year from March 2021 to February 2022 comprising 79 cases of suspected EPTB coming to the pathology department of the FNAC clinic.

Inclusion criteria

All clinically suspected EPTB patients of both sexes regardless of age, sent for FNAC from various out-patient departments (OPDs) and wards and had undergone FNAC from accessible sites followed by the CBNAAT test.

Exclusion criteria

- i. Uncooperative patients
- ii. Patients having inaccessible sites, i.e. lesions in deep-seated locations from where FNA could not be done without ultrasonographic or computed tomographic guidance
- iii. Patients who were already diagnosed with EPTB
- iv. Patients on anti-tubercular treatment
- v. FNA samples where the CBNAAT test could not be done due to any reason
- vi. Those who were not willing to give consent.

Ethical consideration

The study was approved by the institutional ethics committee (vide memo no: IEC/2021/02/004, dated February 12, 2021). Informed consent from the participants or from legal guardians and assent was taken from the patients whenever applicable.

Methods

Detailed history, demographic information, clinical findings, and laboratory investigation findings of all suspected patients of EPTB sent for FNAC to the pathology department were collected in pretested proforma.

After that, FNA from the accessible site was taken and smears were stained with Leishman–Giemsa stain for routine cytology and ZN stain for AFB. Rest of the sample with needle and syringe was sent to the RNTCP center for CBNAAT. Repeated aspiration was done when needed for good yield of material. CBNAAT uses heminested real-time reverse transcriptase PCR assay to amplify a specific sequence of the *rpo b* gene to detect TB. Cytological findings and CBNAAT report and rifampicin resistance report were collected. Analysis by CBNAAT was done using Xpert-MTB/RIF manufactured by Cepheid endorsed by WHO. It was performed according to the manufacturer's instructions (CEPHEID, Sunnyvale, California).

Processing the specimen

According to the standard operating procedure, the sampling reagent (containing NaOH and isopropanol) was added at 2:1 ratio to the sample and kept for 15 min at room temperature with intermittent shaking. In all, 3 mL of this treated sample was transferred to the cartridge and the cartridge was inserted in the module of the CBNAAT machine. This was an automatic process, and the remaining assay steps were completed. The results were displayed on the monitor attached to GeneXpert in about 2 h. Detection of mycobacteria and rifampicin resistance was carried out in the same setting.

Statistical analysis

For statistical analysis, data were entered into Microsoft Excel spreadsheet and analyzed by Statistical Package for Social Sciences, version 20.0.1 (IBM Corporation, Armonk, New York). Reports of FNAC with ZN staining and CBNAAT were collected, and the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of ZN staining were calculated taking CBNAAT reports as gold standard.

RESULTS

In our study, majority of patients were females than males (47 females vs 32 males) out of total 79 cases [Table 1]. The

Table 1: Sociodemographic and clinical characteristics of suspected EPTB patients (N = 79)

Variables	Total EPTB patients	Percentage (%)
Sex		
Male	32	40.51
Female	47	59.49
Age group (in years)		
0–10	4	5.06
11–20	10	12.66
21–30	22	27.85
31–40	12	15.19
41–50	16	20.25
51–60	6	7.59
61–70	7	8.86
>70	2	2.54

EPTB: extrapulmonary tuberculosis

Table 2: Sites of fine-needle aspiration

Site	No (%)
Cervical lymph node	42 (53.16)
Supraclavicular lymph node	16 (20.25)
Submandibular lymph node	10 (12.65)
Axillary lymph node	5 (6.33)
Inguinal lymph node	1 (1.27)
Skin nodule	3 (3.80)
Breast lump	1 (1.27)
Leg ulcer	1 (1.27)
Total	79 (100.0)

Table 3: Cytologic findings on FNA smears

Cytologic findings	No of cases (%)
Granuloma with necrosis	38 (48.10)
Granuloma without necrosis	12 (15.20)
Necrosis only	14 (17.72)
Scattered epithelioid cells without necrosis	4 (5.06)
Pus only	11 (13.92)
Total	79 (100.0)

FNA: fine-needle aspiration

most common age group in our study was 21–30 years, comprising 22 cases (27.85%). Among the different sites, cervical lymph node was the commonest site (42, 53.16%) of fine-needle aspiration, followed by supraclavicular lymph node swelling [Table 2]. Inguinal lymph node, breast lump, and leg ulcers were the least common sites of aspiration, with only one patient in each category.

When we examined the cytology slides, granuloma was the most common cytological finding comprising 50 cases; however, 12 cases did not associate with necrosis [Table 3]. Aspiration of only necrotic material and pus were also seen in a significant number of patients.

Table 4: Comparison of ZN staining with CBNAAT findings

ZN stain	CBNAAT		Total
	Positive	Negative	
Positive	27	0	27
Negative	35	17	52
Total	62	17	79

ZN: Ziehl–Neelsen, CBNAAT: cartridge-based nucleic acid amplification test, TP: true positive, TN: true negative, FP: false positive, FN: false negative, PPV: positive predictive value, NPV: negative predictive value

Sensitivity = $TP \times 100 / TP + FN = 27 \times 100 / 62 = 43.55\%$

Specificity = $TN \times 100 / TN + FP = 17 \times 100 / 17 = 100\%$

PPV = $TP \times 100 / TP + FP = 27 \times 100 / 27 = 100\%$

NPV = $TN \times 100 / TN + FN = 17 \times 100 / 52 = 32.69\%$

Diagnostic accuracy = $(TP + TN) \times 100 / TP + TN + FP + FN = 44 \times 100 / 79 = 55.7\%$

Out of 79 samples from suspected EPTB patients, 27 samples showed AFB in ZN stain and 62 samples were positive in CBNAAT [Table 4]. In all, 17 samples of suspected EPTB patients were negative in CBNAAT, and no AFB was detected in these samples. The sensitivity of FNAC with ZN stain was only 43.55%, whereas specificity was 100% [Table 4]. Diagnostic accuracy of ZN stain on the FNA sample was 55.7% in the diagnosis of EPTB in our study.

Out of 62 CBNAAT confirmed cases, only three cases showed rifampicin resistance (4.84%).

DISCUSSION

EPTB is a major burden of mortality and morbidity as there is difficulty in the diagnosis due to paucibacillary nature and subclinical presentation. Owing to varied clinical presentation, clinical suspicion of EPTB may not be in the mind of clinician, which also causes delay in the diagnosis.^[7,8] Conventional microscopy and ZN staining have low sensitivity that causes delay in the diagnosis.

Owing to advances in the field of genetic diagnosis, the nucleic acid amplification method has been developed in recent years for the diagnosis of MTB in both pulmonary and extrapulmonary TB cases.^[8] CBNAAT is being routinely used in RNTCP centers in India for the diagnosis and detection of rifampicin resistance in sputum samples of suspected pulmonary TB patients. However, its application in the diagnosis of EPTB is minimal as patients are unable to give samples for CBNAAT in suspected EPTB cases by their own.

FNAC is a first-line investigation process in the diagnosis of any superficial lesions, which can be performed as an out-patient procedure. Thus, FNAC can be used to collect samples from suspected cases of EPTB who have come to OPD, and samples can be stained for routine cytology and ZN stain to detect AFB as well as samples can be sent for CBNAAT study.

The present study showed that majority of patients are females (59.49%) and the most common age group affected is 21–30 years (22 cases, 27.85%). Majority of previous studies found similar findings with female preponderance.^[9-12] However, few other studies within India suggested that EPTB, especially tubercular lymphadenopathy, is common among males.^[13-15] The most common age group affected in our study was 21–30 years. This finding is also like studies done by Patil *et al.*^[10] and Samaila *et al.*^[16]

In many developing countries including India, the most common form of EPTB is lymph node TB.^[10,16] We have also found lymph node as the most common site of EPTB. Among lymph nodes, cervical lymph node accounts for majority of EPTB in our study accounting for 53.16% of all EPTB cases. This finding corroborates well with various previous studies done in India and abroad.^[10,11,13,16,17] Clinically known as scrofula, most of patients with lymph node TB present with palpable swelling in the cervical area, with or without fistula or sinus formation. Complications may occur such as inflammation of overlying skin and cold abscess formation.

When we stained the slides for cytological examination, granuloma with necrosis (48.10%) was the commonest finding followed by necrosis only (17.72%). A good number of aspirates showed pus only (13.92%). Various previous studies found that in lymph node EPTB, the most common cytologic finding is epithelioid granuloma with necrosis, which corroborates well with our findings.^[18,19] However, we have found only necrosis without epithelioid granuloma as the second most common pattern in cytology, but majority of the previous studies have found epithelioid granuloma without necrosis as the second most common pattern. This variation may be since a good number of our patients reported to us lately due to lack of awareness and ignorance. In absence of systemic symptoms or having mild systemic symptoms, the patients usually ignored their symptoms and presented late.

We did ZN staining of all FNA samples and sent for CBNAAT study. Only 27 samples were positive for AFB in ZN stain and 62 samples (patients) were positive in CBNAAT. As culture of MTB was not available in our setup, we took CBNAAT as gold standard and found that sensitivity of ZN stain on FNA smear was 43.55%, which is very low. However, specificity of FNA ZN stain was 100% in our study. The overall diagnostic accuracy of ZN stain was 55.7% in our study.

Siddegowda *et al.* in their study comprising 100 cases of suspected EPTB found that FNAC has sensitivity 87.7%, specificity 73.8%, PPV 63.8%, NPV 90.5, and overall diagnostic accuracy 78.0% compared to CBNAAT.^[6] Lavanya *et al.* found GeneXpert has sensitivity 83.3% and specificity 84.8% in diagnosing EPTB over ZN staining, and rifampicin resistance was noted in 6.4% cases.^[9] Patil

et al. compared diagnostic performance of CBNAAT versus ZN stain and found sensitivity 84.04% and specificity 80.57%.^[10] Another study done by Tadesse *et al.* calculated diagnostic accuracy of cytology compared to CBNAAT and found sensitivity 80%, specificity 57.8%, PPV 79.1%, and NPV 59.1%.^[12] In another study of 50 cases done by Dronadula *et al.*, it was found that 31 cases were positive in cytology, whereas 29 cases were positive in CBNAAT.^[20] In another study done by Mavenyengwa *et al.*, 32.20% samples were found positive for TB by CBNAAT assay, whereas only 24.05% were found to be positive by microscopy.^[21] Komanapalli *et al.* concluded that CBNAAT has an important role in diagnosing EPTB, and in addition, it also has an added advantage of detecting rifampicin resistance.^[22]

In our study, rifampicin resistance in the EPTB cases was 4.84% (3/62). Various previous studies on EPTB found approximately similar percentage of rifampicin resistance. Fuladi *et al.* and Dronadula *et al.* found 4.6% and 3.45% cases, respectively, to be rifampicin resistant, which is like our study. However, Kumari *et al.* found 19.51% (eight cases out of 41) rifampicin resistant, which is much higher than our study. Another study done by Singh *et al.*, comprising 57 EPTB patients, did not find any rifampicin-resistant cases.^[13] The variation may be due to different socioeconomic statuses and inclusion of treatment-naïve patients in our study.

We have found that although cytology is an easy method, it can just predict the diagnosis of EPTB in suspected patients. ZN staining can give a definitive diagnosis, but the sensitivity is low. There can be various reasons for low positivity of ZN staining, such as poor sample yield, improper staining technique, and observer variation.^[23] Again, searching for AFB in EPTB samples is time consuming as samples are almost always paucibacillary except persons with immunodeficient state. Comparing to that, CBNAAT is a comparatively sensitive method for detecting TB with an added advantage of detecting rifampicin resistance, which aids in patient management.

CBNAAT has few reasons of false-negative results, which decrease its sensitivity. Improper collection and transport of samples is one of the important causes of false negative result of CBNAAT.^[24] Rarely, PCR inhibitors and other endogenous or exogenous host proteins may interfere and lead to false negative results.^[20] Another cause of false negative CBNAAT is solid nature of cheesy material, which may have very low bacillary load compared to liquid caseous material having high bacillary load.^[25] Therefore, CBNAAT should be repeated with fresh sample if there is high clinical suspicion of EPTB to exclude false negative result. Very rarely, contamination and presence of dead MTB bacilli in the samples can give false positive CBNAAT report.^[20] This is not a problem in culture of MTB, which is the reference method.

Our study has many limitations. First, as we do not have the facility for culture of MTB, we took CBNAAT as gold standard. Another important limitation of our study is that we took only the FNA samples of suspected EPTB patients. That is why our sample size is small. Had we taken all samples such as pleural fluid samples, ascitic fluid samples, bone and joint samples, gastrointestinal samples, or so on, our sample size would be bigger, and it will reflect actual burden of EPTB in our nearby area.

CONCLUSION

FNAC is a simple and easy procedure that can help in the diagnosis of EPTB in suspected patients. FNAC along with ZN staining has very good specificity but less sensitivity. In this connection, CBNAAT has a very good sensitivity and specificity in diagnosing EPTB in FNA samples with added advantage of detecting rifampicin resistance. We recommend that CBNAAT should be done in all suspected EPTB samples collected by FNA in any settings where MTB culture is not available.

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Conflicts of interest

There are no conflicts of interest.

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