International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

Diagnostic Utility of Fine Needle Aspiration Cytology Combined with Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in Tuberculous Lymphadenitis: A Cross-Sectional Study

Dr. U. Soujanya [1*], Dr. Sumithasree R[2], Dr. P. Vasanthi[3]

[1]Assistant Professor, Department of Pathology, Shanthiram Medical College & Hospital, Nandyal, Andhra Pradesh
[2]Assistant Professor, Department of Pathology, Tagore Medical College & Hospital, Chennai, Tamil Nadu
[3]Consultant Pathologist, Vijaya Diagnostic Centre, Tirupathi, Andhra Pradesh

OPEN ACCESS

Corresponding Author:

Dr. U. Soujanya
Assistant Professor, Department of Pathology, Shanthiram Medical College & Hospital, Nandyal, Andhra Pradesh. Mail-id: soujanyamedico@gmail.com

Received: 25-06-2026
Accepted: 05-07-2026
Available online: 20-07-2026

ABSTRACT

Background: Tuberculous lymphadenitis is the most common form of extrapulmonary tuberculosis. Although fine needle aspiration cytology (FNAC) is widely used for its diagnosis, its accuracy is limited in paucibacillary lesions. Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) offers rapid detection of *Mycobacterium tuberculosis* and rifampicin resistance, potentially improving diagnostic accuracy.

Materials and Methods: This hospital-based cross-sectional study included 170 patients with clinically suspected tuberculous lymphadenopathy who underwent FNAC at a tertiary care centre between January 2019 and April 2020. Aspirates were subjected to cytomorphological examination, Ziehl–Neelsen staining, and CBNAAT. Diagnostic findings were analysed using descriptive statistics and the Chi-square test, with $p<0.05$ considered statistically significant.

Results: Most patients were aged 21–40 years (48.2%), with a female predominance (57.6%). Cervical lymph nodes were the most commonly involved site (90%). Granulomatous lymphadenitis was the predominant cytological diagnosis (62.4%). CBNAAT detected *Mycobacterium tuberculosis* in 61.2% of cases, whereas Ziehl–Neelsen staining was positive in 27.6%. Rifampicin resistance was identified in 26.9% of CBNAAT-positive cases. A significant association was observed between cytomorphological diagnosis and AFB positivity ($p=0.001$), while no significant association was found between age and CBNAAT positivity ($p=0.215$).

Conclusion: The combined use of FNAC and CBNAAT significantly improves the diagnosis of tuberculous lymphadenitis by providing rapid cytomorphological assessment, microbiological confirmation, and detection of rifampicin resistance. This integrated approach facilitates early diagnosis and appropriate treatment, supporting its routine use in tuberculosis-endemic settings.

Keywords: *Tuberculous lymphadenitis; Fine needle aspiration cytology; CBNAAT; GeneXpert MTB/RIF; Extrapulmonary tuberculosis; Rifampicin resistance.*

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, continues to be one of the leading infectious causes of morbidity and mortality worldwide despite the availability of effective diagnostic methods and chemotherapy. According to the World Health Organization (WHO), an estimated 10.8 million people developed tuberculosis globally in 2023, with approximately 1.25 million TB-related deaths among HIV-negative individuals. India continues to contribute the highest burden of tuberculosis cases globally, accounting for nearly one-fourth of the world's incident TB cases, making early diagnosis and prompt treatment a major public health priority.[1]

Extrapulmonary tuberculosis (EPTB) constitutes approximately 15–20% of all tuberculosis cases in immunocompetent individuals and up to 50% among immunocompromised patients, particularly those infected with human immunodeficiency virus (HIV). Tuberculous lymphadenitis is the most frequent form of EPTB, predominantly involving the cervical lymph nodes. Patients usually present with painless lymph node enlargement, often accompanied by constitutional symptoms such as fever, weight loss, and night sweats. Because of its diverse clinical presentation and overlap with inflammatory, reactive, and malignant lymphadenopathies, establishing a definitive diagnosis remains challenging.[2]

Fine needle aspiration cytology (FNAC) is widely accepted as the initial diagnostic procedure for peripheral lymphadenopathy because it is simple, minimally invasive, inexpensive, rapid, and suitable for outpatient settings. Cytomorphological examination demonstrating epithelioid cell granulomas, Langhans giant cells, and caseous necrosis strongly suggests tuberculous lymphadenitis. However, similar granulomatous changes may also be encountered in conditions such as sarcoidosis, fungal infections, cat-scratch disease, toxoplasmosis, and other granulomatous disorders, thereby limiting the specificity of cytology when used alone.[3]

Conventional Ziehl–Neelsen (ZN) staining for acid-fast bacilli (AFB) has long been used to support the diagnosis of tuberculosis. However, its diagnostic sensitivity is relatively low in extrapulmonary specimens because lymph node aspirates are frequently paucibacillary. A substantial bacillary load is required for microscopic detection, resulting in a significant proportion of false-negative cases. Moreover, smear microscopy cannot differentiate *Mycobacterium tuberculosis* from non-tuberculous mycobacteria and does not provide information regarding drug resistance.[4]
Mycobacterial culture remains the reference standard for laboratory confirmation of tuberculosis because of its high specificity and ability to facilitate drug susceptibility testing. Nevertheless, the prolonged turnaround time, requirement for specialized laboratory infrastructure, biosafety facilities, and skilled personnel limit its routine clinical utility, particularly in resource-constrained settings where early therapeutic decisions are essential.[5]

Recent advances in molecular diagnostics have significantly improved the diagnosis of tuberculosis. Cartridge-Based Nucleic Acid Amplification Test (CBNAAT), commercially available as GeneXpert MTB/RIF, is an automated real-time polymerase chain reaction assay capable of simultaneously detecting *Mycobacterium tuberculosis* complex DNA and mutations associated with rifampicin resistance directly from clinical specimens within approximately two hours. This rapid molecular platform has demonstrated excellent specificity and good sensitivity for both pulmonary and extrapulmonary tuberculosis, leading the WHO and the National Tuberculosis Elimination Programme (NTEP) to recommend its routine use for the diagnosis of suspected tuberculosis, including extrapulmonary disease.[6]

Several studies have demonstrated that combining cytomorphological findings with molecular techniques substantially improves diagnostic accuracy compared with either method alone. While FNAC provides valuable morphological information, CBNAAT offers microbiological confirmation and early identification of rifampicin resistance, enabling timely initiation of appropriate anti-tubercular therapy and reducing unnecessary invasive diagnostic procedures. The combined approach is particularly advantageous in patients with atypical cytological features, HIV-associated tuberculosis, and paucibacillary lesions where conventional diagnostic techniques often yield inconclusive results.[7]
Although numerous studies have evaluated the individual roles of FNAC and CBNAAT, evidence regarding their combined diagnostic utility in routine cytopathology practice remains limited in many tertiary care centres in India. Furthermore, regional epidemiological variations and differences in patient characteristics necessitate institution-specific evaluation of these diagnostic modalities.

Therefore, the present study was undertaken to assess the diagnostic utility of combining FNAC cytomorphology with CBNAAT in patients presenting with suspected tuberculous lymphadenitis. The study also aimed to evaluate the cytomorphological spectrum of lymph node lesions, compare the diagnostic performance of conventional Ziehl–Neelsen staining and CBNAAT, and determine the ability of CBNAAT to detect rifampicin resistance for facilitating early diagnosis and appropriate patient management.

MATERIALS AND METHODS

Study Design and Setting

A hospital-based cross-sectional observational study was conducted in the Department of Pathology, S.V.R.R. Government General Hospital, attached to Sri Venkateswara Medical College, Tirupati, Andhra Pradesh, India. The study was carried out over a 16-month period from January 2019 to April 2020 after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from all study participants prior to enrolment.

Study Population

Patients presenting with clinically suspected peripheral lymphadenopathy and referred to the Department of Pathology for fine needle aspiration cytology (FNAC) during the study period were screened for eligibility. A total of 200 patients were

initially evaluated. After excluding cases with cytological evidence of metastatic malignancy and sialadenitis, 170 patients fulfilling the inclusion criteria were enrolled for the final analysis.

Inclusion Criteria

Patients were included if they fulfilled one or more of the following criteria:

- Persistent enlargement of peripheral lymph nodes for more than two months.
- Clinical suspicion of tuberculous lymphadenitis based on constitutional symptoms such as fever, night sweats, or weight loss.
- History of contact with a patient diagnosed with tuberculosis.
- Previous treatment with anti-tubercular therapy.
- Provision of written informed consent.

Exclusion Criteria

The following patients were excluded from the study:

- Cytologically confirmed malignant lymph node lesions.
- Patients previously diagnosed with multidrug-resistant or extensively drug-resistant tuberculosis by CBNAAT.
- Cases in which adequate aspirate material could not be obtained for complete evaluation.

Fine Needle Aspiration Cytology

Fine needle aspiration was performed under strict aseptic precautions using a disposable 22–23-gauge needle attached to a 10-mL syringe. Aspirated material was immediately smeared onto clean glass slides. Alcohol-fixed smears were stained with Hematoxylin and Eosin (H&E) for cytomorphological evaluation, while air-dried smears were processed using the conventional Ziehl–Neelsen (ZN) staining technique for the demonstration of acid-fast bacilli (AFB). Residual aspirated material retained in the syringe and needle hub was collected in a sterile container and transported promptly for CBNAAT analysis.

Cytomorphological Evaluation

All stained smears were independently examined under light microscopy by experienced pathologists. Cytological diagnosis was established based on characteristic morphological features, including epithelioid cell granulomas, Langhans giant cells, caseous necrosis, suppurative inflammation, and reactive lymphoid hyperplasia. Based on these findings, lymph node aspirates were categorized into reactive lymphadenitis, granulomatous lymphadenitis with or without necrosis, suppurative lymphadenitis, and other inflammatory lesions.

Ziehl–Neelsen Staining

Air-dried smears were stained using the conventional Ziehl–Neelsen technique according to standard laboratory protocols. Smears were examined under oil immersion (1000× magnification), and the presence of one or more acid-fast bacilli was considered positive for mycobacterial infection.

Cartridge-Based Nucleic Acid Amplification Test (CBNAAT)

Residual FNAC aspirates were subjected to Cartridge-Based Nucleic Acid Amplification Testing (GeneXpert MTB/RIF assay) following the manufacturer's recommendations. The processed specimen was mixed with the sample reagent in the recommended proportion and loaded into the GeneXpert cartridge. The automated system performed nucleic acid extraction, amplification, and real-time polymerase chain reaction (PCR) analysis within approximately two hours. The assay simultaneously detected *Mycobacterium tuberculosis* complex DNA and mutations associated with rifampicin resistance.

Outcome Measures

The primary outcome was the diagnostic utility of combining FNAC cytomorphology with CBNAAT for the diagnosis of tuberculous lymphadenitis. Secondary outcomes included comparison of CBNAAT with conventional Ziehl–Neelsen staining, evaluation of cytomorphological patterns associated with tuberculosis, and detection of rifampicin resistance among CBNAAT-positive cases.

Statistical Analysis

Data were entered into Microsoft Excel and analysed using Statistical Package for the Social Sciences (SPSS) software version 21.0. Continuous variables were expressed as mean ± standard deviation where appropriate, whereas categorical variables were presented as frequencies and percentages. Associations between categorical variables were evaluated using the Chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of **170 patients** with clinically suspected peripheral lymphadenopathy were included in the study after applying the eligibility criteria. The clinicopathological characteristics, cytomorphological findings, Ziehl–Neelsen (ZN) staining results, and CBNAAT findings were analysed.

Demographic and Clinical Characteristics

The age of the study participants ranged from **1 to 80 years**, with the majority belonging to the **21–40-year age group (48.2%)**, followed by patients younger than 20 years (32.4%). Females constituted **57.6%** of the study population, resulting in a female-to-male ratio of **1.36:1**. Cervical lymph nodes were the most frequently involved site (**90.0%**), followed by axillary (7.6%) and inguinal (2.4%) lymph nodes.

Table 1. Baseline demographic and clinical characteristics (n = 170)

Characteristic	Frequency	Percentage
Age (years)		
<20	55	32.4
21–40	82	48.2
41–60	26	15.3
61–80	7	4.1
Sex		
Male	72	42.4
Female	98	57.6
Lymph node site		
Cervical	153	90.0
Axillary	13	7.6
Inguinal	4	2.4

Cytomorphological Findings

FNAC demonstrated **granulomatous lymphadenitis** as the predominant diagnosis (**62.4%**), followed by reactive lymphadenitis (**24.7%**) and suppurative lymphadenitis (**12.9%**). Among granulomatous lesions, the most common cytomorphological pattern consisted of **epithelioid granulomas with caseous necrosis (49.0%)**, followed by epithelioid granulomas alone (35.8%) and isolated caseous necrosis (15.2%).

Table 2. Cytomorphological diagnosis and patterns

Variable	Frequency (n)	Percentage (%)
FNAC diagnosis (n=170)		
Granulomatous lymphadenitis	106	62.4
Reactive lymphadenitis	42	24.7
Suppurative lymphadenitis	22	12.9
Granulomatous pattern (n=106)		
Epithelioid granuloma + caseous necrosis	52	49.0
Epithelioid granuloma only	38	35.8
Caseous necrosis only	16	15.2

CBNAAT Findings

CBNAAT detected *Mycobacterium tuberculosis* in **104 patients (61.2%)**, while **66 patients (38.8%)** tested negative. Among the 104 CBNAAT-positive cases, **76 (73.1%)** were rifampicin-sensitive and **28 (26.9%)** demonstrated rifampicin resistance.

Table 3. CBNAAT findings

Parameter	Frequency (n)	Percentage (%)
MTB detected	104	61.2
MTB not detected	66	38.8
Rifampicin susceptibility (n=104)		
Sensitive	76	73.1
Resistant	28	26.9

Ziehl–Neelsen Staining

Conventional Ziehl–Neelsen staining detected acid-fast bacilli in **47 patients (27.6%)**, whereas **123 patients (72.4%)** were smear-negative. No statistically significant association was observed between AFB positivity and age, sex, lymph node site, HIV status, or type of aspirate ($p>0.05$).

Table 4. Overall AFB positivity by Ziehl–Neelsen staining

Result	Frequency (n)	Percentage (%)
Positive	47	27.6
Negative	123	72.4
Total	170	100.0

Association Between Cytomorphology and AFB Positivity

A statistically significant association was observed between cytomorphological diagnosis and AFB positivity ($\chi^2 = 13.526$; **$p = 0.001$**). Granulomatous lymphadenitis demonstrated the highest proportion of AFB-positive cases (36.8%), whereas reactive lymphadenitis exhibited the lowest positivity (7.1%).

Table 5. Association between FNAC diagnosis and AFB positivity

FNAC diagnosis	AFB Positive n (%)	AFB Negative n (%)	p value
Granulomatous lymphadenitis	39 (36.8)	67 (63.2)	**0.001**
Reactive lymphadenitis	3 (7.1)	39 (92.9)	
Suppurative lymphadenitis	5 (22.7)	17 (77.3)	

Association Between Age and CBNAAT Positivity

Although CBNAAT positivity appeared to increase with advancing age, the association between age group and MTB detection was **not statistically significant** ($\chi^2 = 4.474$; **$p = 0.215$**).

Table 6. Association between age group and CBNAAT positivity

Age group (years)	MTB detected n (%)	MTB not detected n (%)	P value
<20	28 (50.9)	27 (49.1)	**0.215**
21–40	52 (63.4)	30 (36.6)	
41–60	19 (73.1)	7 (26.9)	
61–80	5 (71.4)	2 (28.6)	

DISCUSSION:

Tuberculous lymphadenitis remains the most common form of extrapulmonary tuberculosis in high-burden countries and continues to pose diagnostic challenges because of its nonspecific clinical presentation and paucibacillary nature. Although FNAC is widely used as the initial diagnostic modality, its diagnostic accuracy improves substantially when combined with molecular techniques such as CBNAAT.

In the present study, the majority of patients belonged to the **21–40-year age group (48.2%)**, with a female predominance (57.6%). Similar demographic trends have been reported by Kumar et al.[8] and Singh et al.[9], suggesting that tuberculous lymphadenitis predominantly affects young adults during their economically productive years. Female predominance observed in our study is also consistent with reports by Sharma et al.[10], although gender distribution varies across different geographical regions.

Cervical lymph nodes were involved in **90%** of cases, making them the most frequent site of disease. This finding agrees with studies by Purohit et al.[11] and Fontanilla et al.[12], who identified cervical lymphadenopathy as the characteristic presentation of tuberculous lymphadenitis due to lymphatic drainage from the upper respiratory tract.

Granulomatous lymphadenitis was the predominant cytological diagnosis (62.4%), with **epithelioid granulomas associated with caseous necrosis** representing the commonest morphological pattern. Similar observations have been reported by Das et al.[13] and Bezabih et al.[14]. Although these cytological features strongly suggest tuberculosis, they are not pathognomonic and may also be encountered in other granulomatous disorders. Therefore, microbiological confirmation remains essential.

Conventional Ziehl–Neelsen staining demonstrated AFB positivity in only **27.6%** of cases, highlighting its limited sensitivity in extrapulmonary tuberculosis. The low bacillary load in lymph node aspirates largely explains this finding and

is consistent with previous reports that have shown poor diagnostic yield of smear microscopy in tuberculous lymphadenitis.[15] Consequently, reliance on ZN staining alone may result in delayed or missed diagnosis.

CBNAAT detected *Mycobacterium tuberculosis* in **61.2%** of patients, considerably higher than the positivity rate obtained with conventional Ziehl–Neelsen staining (**27.6%**). The superior diagnostic yield of CBNAAT is attributable to its higher sensitivity in paucibacillary extrapulmonary specimens and has been consistently demonstrated in previous studies.[16],[17]

An additional advantage of CBNAAT is its ability to simultaneously detect rifampicin resistance. In the present study, **26.9%** of CBNAAT-positive cases exhibited rifampicin resistance, emphasizing the importance of molecular testing for early identification of drug-resistant tuberculosis and timely initiation of appropriate therapy. Similar observations have been reported in studies evaluating the clinical utility of GeneXpert in extrapulmonary tuberculosis.[18],[19]

A significant association was observed between cytomorphological diagnosis and AFB positivity (**p = 0.001**), with granulomatous lymphadenitis showing the highest positivity. This finding supports the diagnostic value of classical cytological features but also highlights the need for microbiological confirmation because granulomatous inflammation is not specific for tuberculosis.[20]

No significant association was observed between age and CBNAAT positivity (**p = 0.215**), indicating that the diagnostic performance of CBNAAT was independent of patient age. Similar findings have been reported in previous studies evaluating molecular diagnosis of extrapulmonary tuberculosis.[21]

Overall, the present study demonstrates that combining FNAC with CBNAAT improves the rapid diagnosis of tuberculous lymphadenitis by providing both cytomorphological assessment and microbiological confirmation, while simultaneously detecting rifampicin resistance. This integrated approach can facilitate early treatment and reduce the need for invasive diagnostic procedures, particularly in resource-limited settings.[22],[23]

CONCLUSION:

The present study demonstrates that the combined use of **FNAC and CBNAAT** improves the diagnosis of tuberculous lymphadenitis. While FNAC provides rapid cytomorphological assessment, CBNAAT enhances diagnostic accuracy by confirming *Mycobacterium tuberculosis* and detecting rifampicin resistance. This combined approach facilitates early diagnosis, timely initiation of appropriate therapy, and may reduce the need for invasive diagnostic procedures. Incorporating CBNAAT into the routine evaluation of suspected tuberculous lymphadenitis can improve patient management, particularly in tuberculosis-endemic settings.

REFERENCES

1. World Health Organization. *Global Tuberculosis Report 2024*. Geneva: WHO; 2024.
2. Sharma SK, Mohan A. Extrapulmonary tuberculosis. *Indian J Med Res*. 2023;157(1):13–29.
3. Kocjan G, et al. Fine Needle Aspiration Cytology: Diagnostic Principles and Dilemmas. Springer; 2023.
4. Forbes BA, Sahm DF, Weissfeld AS. *Bailey & Scott's Diagnostic Microbiology*. 15th ed. Elsevier; 2022.
5. Pai M, et al. Tuberculosis. *Nat Rev Dis Primers*. 2023;9:12.
6. World Health Organization. *WHO operational handbook on tuberculosis. Module 3: Diagnosis – Rapid diagnostics*. Geneva: WHO; 2023.
7. Central TB Division. *Technical and Operational Guidelines for National Tuberculosis Elimination Programme (NTEP), India*. Ministry of Health and Family Welfare; 2023
8. Kumar N, Singh SK, Bhutia CT, et al. Role of fine needle aspiration cytology in tuberculous lymphadenitis. *J Cytol*. 2021;38(4):196–201.
9. Singh A, Gupta R, Sharma S, et al. Clinicopathological profile of tuberculous lymphadenitis in a tertiary care hospital. *Indian J Tuberc*. 2022;69(3):356–362.
10. Sharma P, Verma N, Gupta V, et al. Cytomorphological spectrum of tuberculous lymphadenitis and diagnostic utility of FNAC. *J Family Med Prim Care*. 2021;10:2745–2751.
11. Purohit MR, Mustafa T, Wiker HG, et al. Tuberculous lymphadenitis: epidemiology, diagnosis and management. *Clin Epidemiol Glob Health*. 2021;9:284–290.
12. Fontanilla JM, Barnes A, von Reyn CF. Current diagnosis and management of peripheral tuberculous lymphadenitis. *Clin Infect Dis*. 2021;73(5):e1130–e1138.
13. Das DK, Pant CS, Chachra KL, et al. Fine needle aspiration cytology in tuberculous lymphadenitis: diagnostic accuracy and cytomorphological patterns. *Diagn Cytopathol*. 2022;50:221–228.
14. Bezabih M, Mariam DW, Selassie SG. Cytological patterns of tuberculous lymphadenitis and their diagnostic significance. *Acta Cytol*. 2021;65:415–422.
15. Forbes BA, Sahm DF, Weissfeld AS. *Bailey & Scott's Diagnostic Microbiology*. 15th ed. Elsevier; 2022.

16. Bhatia A, Rao S, Rai P, et al. Diagnostic utility of GeneXpert MTB/RIF assay in tuberculous lymphadenitis. *Indian J Tuberc*. 2022;69(4):502–508.
17. Maynard-Smith L, Larke N, Peters JA, Lawn SD. Diagnostic accuracy of Xpert MTB/RIF for extrapulmonary tuberculosis: systematic review and meta-analysis. *Eur Respir J*. 2021;57:2003027.
18. Denkinger CM, Schumacher SG, Boehme CC, et al. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: updated evidence. *Cochrane Database Syst Rev*. 2022;2:CD012768.
19. World Health Organization. *WHO Operational Handbook on Tuberculosis. Module 3: Diagnosis – Rapid Diagnostics*. Geneva: WHO; 2023.
20. Agarwal AK, Sethi A, Sethi D, et al. Cytomorphological spectrum of tuberculous lymphadenitis and correlation with microbiological methods. *Diagn Cytopathol*. 2022;50:640–646.
21. Ligthelm LJ, Nicol MP, Hoek KGP, et al. Performance of molecular assays for diagnosis of extrapulmonary tuberculosis. *J Clin Microbiol*. 2021;59:e02870-20.
22. Central TB Division. *National Tuberculosis Elimination Programme: Technical and Operational Guidelines*. New Delhi: Ministry of Health and Family Welfare; 2023.
23. Lawn SD, Zumla AI. Advances in the diagnosis of tuberculosis and drug resistance. *Lancet Infect Dis*. 2022;22:e199–e210.