Section: Pathology

Original Research Article

CORRELATION BETWEEN CYTOMORPHOLOGICAL DIAGNOSIS OF EXTRA-PULMONARY TUBERCULOSIS BY FNAC OF HEAD AND NECK LYMPHNODE WITH CBNAAT RESULTS

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Received *: 23/09/2026*
Received in revised form *: 23/10/2026*
Accepted *: 05/11/2026*

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DOI: 10.70034/ijmedph.2026.4.325

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2026; 16 (4); 2170-2175

ABSTRACT

Background: Tuberculosis (TB) affecting lymph nodes is a prevalent form of extra-pulmonary disease. Conventional diagnosis via fine-needle aspiration cytology (FNAC) has diagnostic limitations due to low sensitivity, necessitating rapid and specific tests like the Cartridge Based Nucleic Acid Amplification Test (CBNAAT).

Materials and Methods: A prospective study of 150 patients presenting with head and neck lymphadenopathy was conducted at Dr. M.K. Shah Medical College and Research Centre starting in July 2022. Aspirates obtained via FNAC were evaluated for cytomorphological patterns and subjected to CBNAAT.

Results: Among 150 cases (61 males, 89 females; mean age 34 years), tuberculous lymphadenitis accounted for 44 cases (29.3%). Cytomorphological patterns in the TB cases included only necrosis (47.7%), granulomatous inflammation without necrosis (36.3%), and granulomatous inflammation with necrosis (15.9%). Overall CBNAAT positivity in cytologically suspected TB cases was 59% (26 out of 44). CBNAAT positivity was highest in cytological patterns showing granuloma with necrosis (85.7%) and necrosis only (80.9%). Conversely, patterns demonstrating granuloma without necrosis yielded a significantly lower CBNAAT positivity rate (18.7%). The CBNAAT assay demonstrated a sensitivity of 59%, a specificity of 94%, a positive predictive value of 81.25%, and a negative predictive value of 84.7%. All CBNAAT positive cases were Rifampicin sensitive.

Conclusion: CBNAAT is a rapid, highly specific adjuvant diagnostic tool for evaluating tuberculous lymphadenopathy. Its ability to simultaneously detect Mycobacterium tuberculosis and screen for Rifampicin resistance directly from FNAC material reduces delays in diagnosis and prevents inappropriate empirical treatments.

Keywords: Tuberculous lymphadenitis, FNAC, CBNAAT, cervical lymphadenopathy, Mycobacterium tuberculosis, Rifampicin resistance.

INTRODUCTION

Tuberculosis is a major cause of ill health and one of the main causes of mortality at the global level by being a communicable disease.[1] Infectious agent for Tuberculosis is bacillus Mycobacterium Tuberculosis, which is spread by coughing through the infected person. Incidence rate of TB in India is 188/10000 population according to WHO TB statistics for 2021. In India, TB kills 480000 persons each year and more 1400 persons each day.[2] Patients with human immunodeficiency virus (HIV) infection extra- pulmonary TB constitutes almost 53-62% of TB.[3] It has affected individuals of all ages and backgrounds, striking both the affluent and the impoverished.[4,5] Certain genetic variations, poverty,

overcrowded living conditions, and inadequate access to healthcare services are strongly associated with increased risk of TB, exposure to environments where TB bacteria thrive can increase the likelihood of infection, age, sex, and comorbidities (HIV infection or diabetes) are biological factors that affect susceptibility to TB. The disease often presents as multifocal lymphadenopathy in HIV-positive patients. The increasingly recognized deadly interaction between diabetes mellitus (DM) and tuberculosis (TB) has prompted the implementation of bi-directional screening for both conditions globally.[6,7] Evidence has become available suggesting that use of immunomodulator drugs has been associated with the development of fatal TB in rheumatoid arthritis.[7,8] Data are also emerging suggesting that tobacco smokers have about three-fold higher risk of TB than non-smokers; even after adjustment for other factors.[9,10]

The disease predominantly, affects the lungs, but can also spread to the extra-pulmonary sites and one of those sites can be Lymph node. Among the extra-pulmonary TB, LNTB is the most common type of EPTB.[11] Tubercular lymphadenitis is seen in nearly 35% of extra pulmonary TB (Extra Pulmonary TBconstitutes15-20%of all cases of TB). Most people (~90%) who develop the disease are adults, with male predominance. The likely route of infection in the case of cervical lymph node is considered to be the tonsil.[12] Similarly, the uvula,[13] the pharynx,[14] and the larynx,[15,16] have been reported to be the sites of primary TB. In these instances, the infection may follow the common mode of entry, that is through inhalation. The infection may then spread via the lymphatics to the nearest cervical lymph nodes. In the early stages, lymph nodes may appear discreet clinically. Periadenitis causes lymphnodes to become matted and fixed. They can merge and form caseous pus, which may break through deep fascia, resulting in a fluctuant swelling known as a collar-stud abscess. The skin above becomes hardened, breaks down, and forms a sinus that, if untreated, can persist for years. Healing can happen in stages, possibly leading to calcification or scarring. Mycobacterial lymphadenitis has shown marked geographical variation. In several studies from India.[17-20] MTB has been the most common pathogen isolated from patients with mycobacterial lymphadenitis accounting for almost all the cases.[17-20] TB is curable and preventable. During the past decade, the clinical pattern and presentation of tuberculosis has changed dramatically. Much of the traditional learning about this disease is no longer true and tuberculosis has become a new entity.[21] About 85% of people who develop TB disease can be successfully treated with a 6 months drug regimen and regimens of 1-6 months can be used to treat TB infection. National Tuberculosis Elimination Programme (NTEP) is a public health initiation of the Government of India that organizes its anti-tuberculosis efforts.

There are different methods for detecting tuberculosis under NTEP like Culture, AFB (ZN) staining, CBNAAT, FNAC. Smears with necrosis and epithelioid cell granulomas have the highest chance of AFB positivity. With the high tuberculosis load in a country like India; ZN stain has some limitations like low sensitivity ranging from 20% to 43%,[22] and specificity is 98.2.23,24 Culture method has limitation of taking weeks because of the slow growth of TB bacilli. For the accurate diagnosis of LNTB; biopsy with histopathological examination and culture are needed, but these are very time-consuming. Impactful control of TB occurs when rapid diagnosis and treatment are given. FNA of Lymphnode and CBNAAT assay are rapid detection methods.[11] FNAB of nodes provides a high level of diagnostic accuracy.[25] FNAC is a routinely done cytological technique in diagnosing tubercular lymphadenitis as it has sensitivity and specificity of 88-96%. Because of early availability of results, simplicity, minimal trauma and complications, the aspiration cytology is now considered as a valuable diagnostic aid.[26] In our population with high tubercular disease burden, presence of epithelioid cell granuloma is considered as an evidence of tubercular lymphadenitis.[21] FNAC has limitations like it requires practice and skills; sample obtained may not be representative of the lesion. CBNAAT is an automated Cartridge Based Nucleic Acid Amplification Test. Cartridge based nucleic acid amplification test (CBNAAT) can be done from FNAC material which act as an adjuvant for the diagnosis and can also detect Rifampicin resistant cases.[27] They provide a rapid and reliable diagnosis as the threshold for detection is low 10–100cfu/ml.[28] CBNAAT test result is available within 2hours.17 Sensitivity and Specificity of CBNAAT is 85.7% and 93.5% respectively. Inclusion of CBNAAT in the initial diagnosis of tubercular lymphadenopathy in addition to the FNAC would decrease the over diagnosis of tuberculosis and in judicious use of anti-tuberculosis treatment (ATT).[29] Currently, CBNAAT is preferred and is first mode of investigation in EPTB and in children.[30]

MATERIALS AND METHODS

The present prospective study was done in the Department of Pathology at Dr.M.K.Shah Medical College and Research Centre, Ahmedabad during period from July, 2022 onwards. A total of 150 samples from the patients who presented with head and neck lymphadenopathy attending outpatient and admitted patients were included into the study after obtaining institutional ethical committee approval.

RESULTS

In this study 150 patients were subjected to FNAC for cervical lymphadenopathy. There were 61 males and 89 females in the study. The male:female ratio in this

study was 0.68:1, with a slight female preponderance. The age at presentation ranged from 1 year to 82 years with mean age 34 years in the middle of third decade. Maximum number of patients were in the 21 – 40 years age group (62 cases, 41%) followed by the age group 41 – 60 years (39 cases, 26%), followed by age group 0-20 years (36 cases, 24%), age group 61– 80 years (12 cases,08%) and the least in the age group 81-100 years (01 case,01%). Majority of them presented with solitary node (91.33%) and followed by bilateral nodes (8.66%). 57% patients (86/150) did not have constitutional symptoms at presentation; however, when these did occur (42.6%) they were most commonly fever and weight loss. Out of 44 cases of Tuberculosis, 27 cases (61.36%) were having symptoms at the time of presentation. 17out of 44 tuberculosis cases (38.6%) were not having any constitutional symptoms. All cases (150) were sent for CBNAAT in which 26 cases out of 44 cytologically diagnosed tuberculosis came positive. Out of 26 majority of cases (21) showed only necrosis pattern on cytology with 17 cases (80.9%) showed CBNAAT positive, followed by 16 cases showed granuloma without necrosis cases showed pattern on cytology with only 3 cases (18.7%) showed CBNAAT positive and 7 cases showed granuloma with necrosis pattern with 6 cases (85.7%) showed CBNAAT positive results. 1 case out of 7 cases of acute reactive lymphadenitis (14.28%) showed CBNAAT positive, 5 cases of chronic non-specific lymphadenitis (5.88%) showed CBNAAT positive. In present study granulomas without necrosis showed 12.5% AFB positive and 18.7% CBNAAT positive whereas in 7 cases of granulomas with necrosis showed 28.5% AFB positive and 85.7% CBNAAT positive. In 21 of only necrosis showed 85.7% AFB positive and 80.9% CBNAAT positive. Smears showing predominantly necrosis has shown more AFB positivity (85.7%) than the other cases. All the cases sent for CBNAAT in our study were Rifampicin sensitive. Chronic non-specific lymphadenitis was noted in 85 patients (56.6%). Among these patients 33 patients (majority) in 21 – 40 age group. Tuberculous lymphadenitis accounted for a total of 44 cases (29.3%). Cold abscess, a delayed presentation of tuberculosis was the dominant sub category in this group accounting for 47.7% (21 cases). Granulomatous inflammation without coexistent caseous necrosis was noted in 16 cases (36.3%). Acute reactive lymphoid hyperplasia was noted in 07 patients (4.6%), among these patients 03 patients were in 0 – 20 age group. Metastatic malignancy was observed in 07 cases (4.6%). Suppurative lymphadenitis was noted in 03 cases.

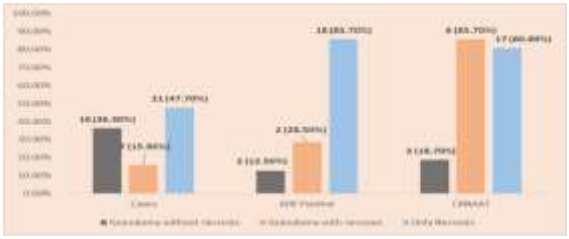


Figure 1: CBNAAT result.

Table 1: Summary of FNAC of cervical Lymphadenopathy

Age (Years)	Sex	Acute Lymphadenitis	Chronic Lymphadenitis	Suppurative lymphadenitis	TB Lymphadenitis	Lympho-proliferative	Metastatic Malignancy
0-20	M	00	13	01	05	00	00
0-20	F	03	10	00	05	00	00
21-40	M	01	09	00	05	00	00
21-40	F	01	24	02	17	01	01
41-60	M	01	07	00	06	02	04
41-60	F	00	14	00	05	00	00
61-80	M	00	02	00	01	01	02
61-80	F	01	04	00	00	00	00
81-100	M	00	01	00	00	00	00
81-100	F	00	00	00	00	00	00
TOTAL		07	85	03	44	04	07

DISCUSSION

It was Dudgeon and Patrick in1927 who first used FNAC in diagnosing TB lymphadenitis followed by Tempka and Kubiczek, Mahanta et al,[31] FNAC is a first line investigation in the diagnosis of lymph node lesions.[27] We noticed a female preponderance of cases in our study. Similar findings have been described by Shaila et al,[21] Hemalatha et al,[3] and Pamra et al,[32] while Subhan Ali et al,[27] and Laishram et al. have described male preponderance of the cases.[33] In present study, maximum number of cases were seen in the age group of 21-40 years which was in concordance with the study by Bhavani et al.[26] For cytodiagnosis of tubercular lymphadenitis, we need to demonstrate granulomas composed of epithelioid cells, Langhans giant cells with or without caseous necrosis along with demonstration of AFB positivity +. Studies by Subhan Ali et al,[27] Gomes et al,[34] and Das et al,[35] also followed the same cytological parameters. In tuberculous lesions the present study showed 36.3% granulomatous without necrosis,15.9% granulomatous with necrosis and 47.7% only necrosis whereas study done by Subhan Ali et al,[27] showed 51.2% granulomatous without necrosis, 34.2% granulomatous with necrosis and 14.6% only necrosis and by Bhavani et al,[26] showed 9.8% granulomatous without necrosis, 27.17%

granulomatous with necrosis and 5.28% only necrosis.

Table 2: The various cytological pattern comparison with other studies as shown

Pattern	Cases	Present Study	Bhavani et al,[26]	Ligthelm et al,[36]	Devi et al,[37]	Naesreen et al,[38]	Subhan Ali et al,[27]
Granulomatous	44	29.3%	42.26%	66.7%	31.2%	17.9%	39.4%
Reactive (Chronic and Acute)	92	61.3%	35.47%	20.8%	55.2%	12.7%	36.5%
Metastatic	07	4.66%	11.32%	8.3%	6.25%	19.7%	14.4
Suppurative	3	2%	9.8%	2.1%	-	-	6.7%
Lymphoproliferative	4	2.66%	1.13%	-	6%	49.7%	2.8%

In cases of extra-pulmonary tuberculosis, clinicians often struggle with low suspicion levels due to the variability in symptoms and presentation. Tuberculous lymphadenitis, for instance, poses a diagnostic challenge as conventional methods exhibit low sensitivity, typically ranging from 0% to 40%. This limitation stems from the difficulty in detecting Mycobacterium tuberculosis bacilli, given their low load, and the routine inability to detect drug resistance. Culture methods, while offering some improvement with yields varying from 30% to 80%, are still not entirely reliable. Furthermore, drug susceptibility testing adds to the complexity and time consumption, typically taking 2 to 8 weeks, thereby delaying treatment decisions.[39] During this diagnostic limbo, patients may receive inappropriate or ineffective treatments, allowing the disease to progress unchecked. This not only increases the likelihood of tuberculosis-related morbidity but also perpetuates the transmission of drug-resistant strains to others, particularly within family circles where transmission risks are heightened. This, in turn, can exacerbate resistance patterns. Consequently, there's a significant delay in achieving a definitive diagnosis.[29] Though cytological diagnosis of tuberculosis, coupled with AFB stain, has been the preferred method for diagnosing extra-pulmonary tuberculosis, it too comes with limitations and pitfalls, as previously discussed. To mitigate these challenges, there arose a pressing need for a simple and rapid diagnostic tool. Enter the CBNAAT, a new diagnostic test developed to address this very need. The CBNAAT method streamlines the diagnostic process by purifying, concentrating, amplifying, and identifying targeted rpoB nucleic acid sequences, all within are mark ably short time frame of approximately 120 minutes. To conduct the CBNAAT examination, clinical specimens are treated with sample reagents at a ratio of 3:1. The closed specimen contain erundergoes manual agitation twice over a 15-minute period at room temperature, after which 2 ml of the inactivated material (equivalent to 0.5 ml of decontaminated pellet) is transferred to the test cartridge.

One of the standout features of CBNAAT is its high specificity, which eliminates the possibility of cross-reactions with other bacterial species. This specificity is achieved through the utilization of three specific primers and five unique molecular probes designed to target the rpoB gene of Mycobacterium tuberculosis.[40] This highly specific approach ensures accurate detection of the tuberculosis-causing pathogen. CBNAAT operates as a rapid and fully automated test, relying on PCR (Polymerase Chain Reaction) technology to detect DNA directly from clinical specimens. Additionally, it offers the crucial benefit of detecting Rifampicin resistance, providing valuable information for treatment decisions. In the present study, out of 16 cases of granulomatous without necrosis showed 12.5% AFB positive and 18.7% CBNAAT positive whereas in 07cases of granulomatous with necrosis showed 28.5% AFB positive and 85.7% CBNAAT positive. In 21 cases of only necrosis showed 85.7% AFB positive and 80.9% CBNAAT positive. The overall AFB showed 50% positivity in suspected cases of tuberculosis which is higher than the study done by Subhan Ali et al,[27] Aggarwal et al,[41] and lower than the study done by Nidhi et al.[42] The CBNAAT results in the present study showed 59%which was in concordance with the study done by Srwar et al,[43] and Moure et al.[44] Various studies showed 36%-96% CBNAAT Positivity. Low CBNAAT positivity may be due to various technical reasons like the second or third pass of FNAC sent for CBNAAT, amount of material sent. Also, saline was used as diluent in our study, while in many other studies N acetyl cysteine was used as the diluent. In our study CBNAAT test showed Sensitivity of 59%, Specificity 94%, Positive predictive value 81.25%, Negative predictive value 84.7%. There were no Rifampicin resistance cases in the present study which was also seen in study by Subhan Ali et al,[27] whereas the studies conducted by Fuladi et al,[45] Gupta et al,[39] Shakeel et al,[46] showed 4.6%, 5% and 6.4% of Rifampicin resistant cases respectively.

Limitations: This study was conducted at a single tertiary-care centre with a relatively small sample size, which may limit the generalizability of the findings. CBNAAT results may have been influenced by the quantity of aspirated material and the use of saline as a diluent. The absence of rifampicin-resistant cases also limits the assessment of its ability to detect drug resistance in this cohort.

CONCLUSION

FNAC combined with CBNAAT is a useful approach for evaluating suspected tuberculous lymphadenitis. CBNAAT demonstrated high specificity (94%) but

moderate sensitivity (59%) in this study, highlighting that a negative result does not exclude tuberculosis. Integrating cytomorphological findings with molecular testing can support timely diagnosis and enable rapid detection of rifampicin resistance when present. Further multicentric studies with appropriate microbiological confirmation are warranted.

Conflict of interest: No

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