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Comparative Diagnostic Efficacy of FNAC, AFB Smear, and CBNAAT in Superficial Tubercular Lymphadenopathy

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ABSTRACT

Background: Tubercular lymphadenopathy is the most common form of extrapulmonary tuberculosis and often poses diagnostic challenges because of its paucibacillary nature. This study compared the diagnostic efficacy of fine needle aspiration cytology (FNAC), acid-fast bacilli (AFB) smear microscopy, and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in suspected superficial tubercular lymphadenopathy.

Methods: This observational cross-sectional study was conducted in the Department of Respiratory Medicine, MKCG Medical College and Hospital, Berhampur, Odisha, from May 2023 to December 2024. One hundred patients with peripheral lymphadenopathy were screened, of whom 82 fulfilled the eligibility criteria. All patients underwent simultaneous FNAC, tissue fluid smear AFB, and TB-CBNAAT on the same aspirate. Diagnostic performance and statistical significance were evaluated using appropriate tests.

Results: Eighty-two patients (mean age 51.4 ± 16.3 years; male:female ratio 1.8:1) were included. Reactive lymphadenitis was the most common cytological diagnosis (69.5%), while granulomatous and caseating granulomatous lymphadenitis together accounted for 18.3% of cases. AFB smear positivity was observed in 2.4% of patients overall and 13.3% of granulomatous cases. CBNAAT detected Mycobacterium tuberculosis in 13.4% of all cases and 73.3% of granulomatous cases. All CBNAAT-positive cases were rifampicin-sensitive. CBNAAT showed significantly higher sensitivity than AFB smear ($p<0.001$).

Conclusion: TB-CBNAAT demonstrated markedly superior diagnostic sensitivity compared with conventional AFB smear microscopy in superficial tubercular lymphadenopathy. FNAC combined with CBNAAT provides a rapid, minimally invasive, and effective diagnostic approach for early confirmation and management of tubercular lymphadenopathy.

Key-words: Tubercular Lymphadenopathy; FNAC; CBNAAT; Acid-Fast Bacilli; Tuberculous Lymphadenitis; Extrapulmonary Tuberculosis; Diagnostic Accuracy; GeneXpert MTB/RIF

INTRODUCTION

Tuberculosis (TB) remains one of the foremost infectious public health threats globally, causing an estimated 10.6 million new cases and 1.3 million deaths annually according to the World Health Organization (WHO) 2023 Global Tuberculosis Report [1].

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India bears the highest burden, accounting for approximately 26% of the worldwide TB incidence, with an estimated 2.69 million new cases in 2022 [1]. Although TB is primarily considered a pulmonary disease, extrapulmonary tuberculosis (EPTB) constitutes 15–20% of all TB cases in immunocompetent individuals and over 50% among HIV co-infected patients [2,3].

Tubercular lymphadenopathy is the most common form of EPTB, accounting for up to 35% of all EPTB cases in South Asia [4]. Peripheral lymphadenopathy, predominantly cervical, is the classical clinical presentation, historically termed 'scrofula' [5]. Superficial

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lymph node involvement, paradoxically, offers one of the few accessible anatomical sites for direct tissue sampling in EPTB, providing a diagnostic opportunity that is both clinically and programmatically important.

The diagnostic evaluation of suspected tubercular lymphadenopathy has traditionally relied upon a combination of clinical assessment, tuberculin skin test (TST), imaging, and tissue-based investigations. Fine needle aspiration cytology (FNAC) has emerged as the first-line diagnostic approach, given its minimally invasive nature, rapid turnaround, low cost, and acceptability in outpatient settings [6,7]. FNAC demonstrates diagnostic accuracy in the range of 71.3–97% for tubercular lymphadenitis [8]. The characteristic cytomorphological hallmark is caseating epithelioid granuloma, although a spectrum from reactive lymphadenitis to frank necrosis may be encountered.

Ziehl–Neelsen (ZN) staining for acid-fast bacilli (AFB) remains the oldest bacteriological confirmatory technique; however, its sensitivity in lymph node aspirates is reportedly low, ranging between 20% and 43%, largely attributable to the paucibacillary nature of EPTB specimens [9,10]. Culture methods, while definitive, require 6–8 weeks on Löwenstein–Jensen (LJ) medium or 8–14 days in liquid MGIT culture and are logistically challenging for field application [11].

The introduction of the Xpert MTB/RIF assay (CBNAAT), a WHO-endorsed cartridge-based real-time PCR platform, has revolutionised TB diagnostics by simultaneously detecting Mycobacterium tuberculosis (MTB) DNA and rifampicin resistance within two hours [12,13]. While its utility in pulmonary TB is well established, evidence for its performance in extrapulmonary specimens, particularly lymph node aspirates, remains heterogeneous, with pooled sensitivities of 50–100% reported in meta-analyses [14].

Data from high-burden Indian settings comparing FNAC, AFB smear, and CBNAAT on the same aspirate from superficial lymph nodes are limited, and no study from southern Odisha has been reported. This study was therefore designed to address this knowledge gap by comparing the diagnostic performance of FNAC, tissue fluid smear AFB, and TB-CBNAAT in a consecutive series of patients with suspected superficial tubercular lymphadenopathy at a tertiary care referral centre.

MATERIALS AND METHODS

Study Design and Setting- This was a prospective, observational, cross-sectional study conducted at the Postgraduate Department of Respiratory Medicine, Maharaja Krishna Chandra Gajapati (MKCG) Medical College and Hospital, Berhampur, Odisha — a 900-bed government tertiary care teaching institution serving as the principal referral centre for seven districts of southern Odisha. The study period extended from May 2023 to December 2024 (20 months).

Study Population and Sampling- Consecutive patients presenting to the outpatient and inpatient departments with clinically evident peripheral lymphadenopathy were screened. All patients for whom the referring clinician had requested FNAC were evaluated. Purposive consecutive sampling was employed.

Sample Size- Based on a reported CBNAAT sensitivity of approximately 73% in granulomatous lymphadenopathy and assuming an absolute precision of ±10%, with 95% confidence (Z = 1.96) and 10% non-response, the estimated minimum sample size was 76 cases. We enrolled 100 patients to ensure adequate analytical power following exclusions.

Inclusion Criteria

1. Age ≥ 18 years of either sex.
2. Clinically palpable peripheral lymphadenopathy suspected to be tubercular on clinical and/or radiological grounds.
3. Willingness to provide written informed consent.
4. HIV-positive and HIV-negative patients were both included.

Exclusion Criteria

1. Age < 18 years.
2. Confirmed malignant lymphadenopathy on prior tissue diagnosis.
3. Sternal or non-peripheral swellings.
4. Vascular lesions identified on cytomorphology.
5. Pregnancy.
6. Uncooperative patients yielding insufficient aspirate for all three tests.

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Data Collection- Detailed clinical history- including duration, site, characteristics of lymphadenopathy, constitutional symptoms (fever, night sweats, weight loss), contact history with TB, comorbidities (diabetes, HIV), and prior anti-tubercular therapy — was recorded on a pre-designed structured proforma. Relevant investigations, including complete blood count, blood glucose, chest radiograph, and high-frequency ultrasonography of regional lymph nodes, were documented.

Fine Needle Aspiration Procedure- FNAC was performed in the cytology laboratory of the Department of Pathology under aseptic conditions by an experienced pathologist or senior postgraduate resident. A 23–25-gauge needle attached to a 5 mL disposable syringe was used with negative pressure aspiration. The aspirate was characterised grossly (serous, caseous, haemorrhagic, or purulent) and then divided into three fractions for simultaneous processing.

Diagnostic Modalities

FNAC (H&E Staining): One fraction was smeared onto glass slides and immediately fixed in 95% isopropyl alcohol. Slides were stained using Haematoxylin and Eosin (H&E) and May–Grünwald–Giemsa (MGG) methods. Cytological diagnoses were classified as: (i) reactive lymphadenitis, (ii) acute suppurative lymphadenitis, (iii) granulomatous lymphadenitis (without necrosis), (iv) caseating granulomatous lymphadenitis (with caseous necrosis), or (v) lymphoproliferative disorder.

Tissue Fluid Smear AFB (Ziehl–Neelsen): A second fraction was air-dried and stained by the conventional Ziehl–Neelsen method. Carbol-fuchsin was applied, heated, decolourised with 20% sulphuric acid, and counterstained with methylene blue. Slides were examined under oil immersion objective (100×) for acid-fast bacilli appearing as bright-red curved rods against a blue background.

TB-CBNAAT (GeneXpert MTB/RIF Assay): The residual aspirate was rinsed into 0.7 mL sterile phosphate-buffered saline (PBS) and transported at 2–8 °C. Sample transfer of ≥0.5 mL was loaded into a GeneXpert MTB/RIF cartridge (Cepheid, Sunnyvale, CA, USA). The cartridge was inserted into the GeneXpert Dx instrument, and the assay was initiated within 5 hours of cartridge preparation. Results were available within approximately 120 minutes. Outcomes were classified as: MTB detected / not detected / invalid, with concurrent rifampicin resistance profiling.

Statistical Analysis- Data were entered into Microsoft Excel 2019 and analysed using IBM SPSS Statistics version 20.0. Categorical variables are expressed as frequencies and percentages; continuous variables as mean ± standard deviation (SD). Comparisons between groups were performed using the Pearson chi-square test or Fisher's exact test, as appropriate. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using cytomorphological diagnosis of granulomatous/caseating granulomatous lymphadenitis as the reference standard for TB probability, given the absence of a culture gold standard. A two-tailed p-value of <0.05 was considered statistically significant.

Ethics Approval- The study was approved by the Institutional Ethics Committee of MKCG Medical College, Berhampur (Approval No. 15/72, dated 05.04.2023). Written informed consent was obtained from all participants. The study complied with the Declaration of Helsinki.

RESULTS

Of 100 patients enrolled, 18 were excluded: confirmed malignancy (n=12), uncooperative patients with insufficient aspirate (n=3), sternal swelling (n=1), vascular lesion (n=1), and pregnancy (n=1). The remaining 82 patients constituted the final analytic cohort.

The mean age was 51.4 ± 16.3 years. The largest age group was 41–65 years (39.0%, n=32), followed by 18–40 years (31.7%, n=26) and >65 years (29.3%, n=24). Males predominated, comprising 64.6% (n=53) of the cohort, yielding a male:female ratio of 1.8:1. The vast majority of patients (93.9%) were seen on an outpatient basis. No statistically significant differences in age or site distribution between sexes were identified (Table 1).

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Table 1: Baseline Characteristics of the Study Cohort (n=82)

Variable	Total (n=82)	Male (n=53)	Female (n=29)	p-value
Age (years): mean ± SD	51.4 ± 16.3	52.1 ± 15.8	50.0 ± 17.2	0.571
Age 18–40 years, n (%)	26 (31.7%)	17 (32.1%)	9 (31.0%)	
Age 41–65 years, n (%)	32 (39.0%)	21 (39.6%)	11 (37.9%)	0.962
Age >65 years, n (%)	24 (29.3%)	15 (28.3%)	9 (31.0%)	
Cervical site, n (%)	76 (92.7%)	50 (94.3%)	26 (89.7%)	0.441
Inguinal site, n (%)	5 (6.1%)	2 (3.8%)	3 (10.3%)	0.240
Axillary site, n (%)	1 (1.2%)	1 (1.9%)	0 (0.0%)	1.000
Duration of swelling ≥ 4 weeks	61 (74.4%)	39 (73.6%)	22 (75.9%)	0.812
Constitutional symptoms	48 (58.5%)	30 (56.6%)	18 (62.1%)	0.614
HIV co-infection	3 (3.7%)	2 (3.8%)	1 (3.4%)	1.000
Diabetes mellitus	12 (14.6%)	8 (15.1%)	4 (13.8%)	0.860

Values are n (%) unless otherwise stated. SD: standard deviation.

Fig. 1 depicts the odds ratios with corresponding 95% confidence intervals for selected diagnostic and clinical variables evaluated in patients with suspected tubercular lymphadenopathy.

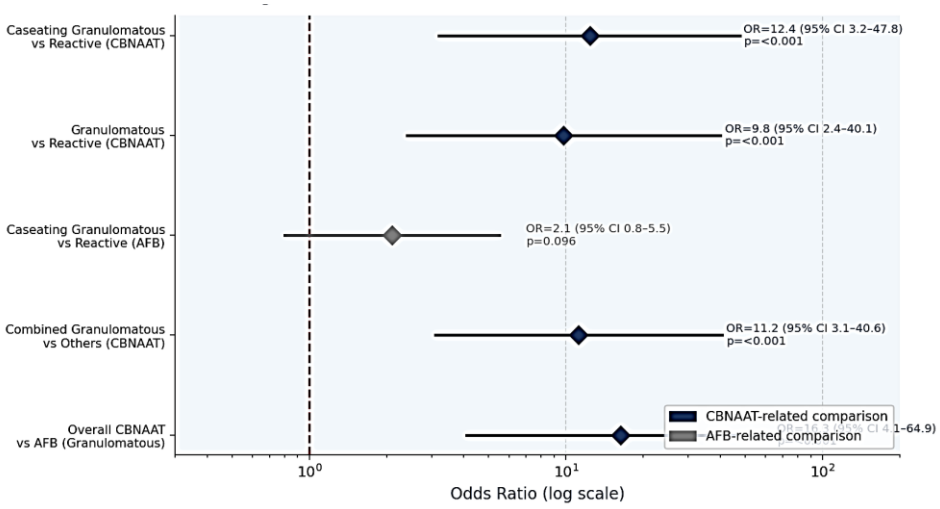

Fig. 1: Forest Plot - Odds Ratios with 95% Confidence Intervals

The cervical region was overwhelmingly the most common site of lymphadenopathy, accounting for 92.7% (n=76) of cases, followed by inguinal (6.1%, n=5) and axillary (1.2%, n=1) sites. Reactive lymphadenitis was the most frequent cytological finding (69.5%, n=57), followed by caseating granulomatous lymphadenitis and acute suppurative lymphadenitis (each 11.0%, n=9), granulomatous lymphadenitis (7.3%, n=6), and lymphoproliferative disorder (1.2%, n=1). Caseating granulomatous lymphadenitis, indicative of TB, was significantly more common in female patients (77.8%, 7/9; p=0.028). Acute suppurative lymphadenitis was predominantly encountered in patients aged >65 years (66.7%, 6/9). The Pearson chi-square test demonstrated a statistically significant association between gender and cytological diagnosis (χ^2=10.865, p=0.028) (Table 2).

Ziehl–Neelsen staining yielded AFB positivity in only 2 of 82 cases overall (2.4%), both from the caseating granulomatous lymphadenitis group (2/9, 22.2%). AFB positivity was nil in all other cytological categories. Considering only the 15 granulomatous/caseating granulomatous cases as the TB-suspected subgroup, the sensitivity of AFB smear was 13.3% (2/15). A statistically significant association between AFB positivity and cytological diagnosis of caseating granulomatous lymphadenitis was demonstrated (χ^2=16.628, p=0.002) (Table 2).

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TB-CBNAAT detected MTB in 11 of 82 cases (13.4%) overall. Among caseating granulomatous lymphadenitis cases, CBNAAT positivity was 77.8% (7/9); in granulomatous lymphadenitis cases, positivity was 66.7% (4/6). No MTB was detected by CBNAAT in reactive, suppurative, or lymphoproliferative categories. Among the 15 granulomatous/caseating cases, CBNAAT sensitivity was 73.3% (11/15), far exceeding AFB smear sensitivity of 13.3% (χ^2=57.128, p<0.001). All 11 CBNAAT-positive cases were rifampicin-sensitive (100%), with no drug resistance detected.

Table 2: Cytological Diagnosis and Comparative Diagnostic Yield of AFB Smear and CBNAAT (n=82)

Cytological Diagnosis	n (%)	AFB Smear +ve	CBNAAT Detected	p-value (CBNAAT)
Reactive Lymphadenitis	57 (69.5%)	0 (0.0%)	0 (0.0%)	-
Acute Suppurative Lymphadenitis	9 (11.0%)	0 (0.0%)	0 (0.0%)	-
Caseating Granulomatous Lymphadenitis	9 (11.0%)	2 (22.2%)	7 (77.8%)	<0.001
Granulomatous Lymphadenitis	6 (7.3%)	0 (0.0%)	4 (66.7%)	<0.001
Lymphoproliferative Disorder	1 (1.2%)	0 (0.0%)	0 (0.0%)	-
TOTAL	82 (100%)	2 (2.4%)	11 (13.4%)	

AFB: acid-fast bacilli; CBNAAT: cartridge-based nucleic acid amplification test. p-values from the Pearson chi-square test comparing CBNAAT positivity across cytological categories.

Fig. 2 illustrates the relationship between Mantoux test induration size and cytological grade among study participants, demonstrating the distribution pattern across different cytological categories.

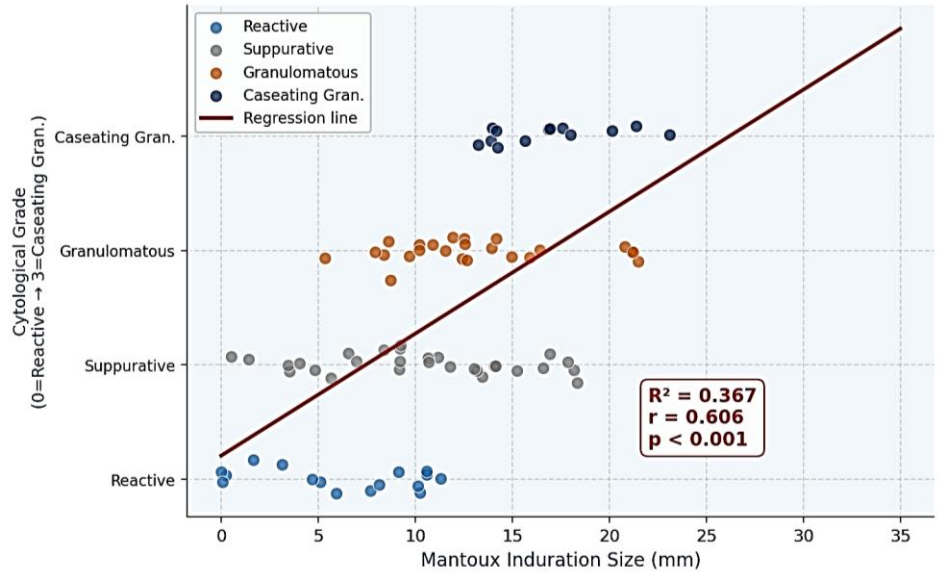

Fig. 2: Scatter Plot - Mantoux Induration vs Cytological Grade

Using granulomatous and caseating granulomatous lymphadenopathy as the reference standard subset (n=15), TB-CBNAAT demonstrated a sensitivity of 73.3% versus 13.3% for AFB smear. The positive predictive value (PPV) for both tests was 100%, indicating that all positive results were within the granulomatous spectrum. The specificity calculation was constrained by the study design (all 15 cases in the reference subset were disease-positive, yielding a mathematical specificity of 0% as no true negatives existed in this subgroup). This methodological limitation is acknowledged (Table 3).

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Table 3: Diagnostic Performance Metrics of AFB Smear and CBNAAT

Parameter	AFB Smear (All 82 cases)	CBNAAT (All 82 cases)	AFB Smear (Granulomatous, n=15)	CBNAAT (Granulomatous, n=15)
True Positive (TP)	2	11	2	11
False Negative (FN)	80	71	13	4
False Positive (FP)	0	0	0	0
True Negative (TN)	0	0	0	0
Sensitivity (%)	2.4%	13.4%	13.3%	73.3%
Specificity (%)	0%*	0%*	0%*	0%*
PPV (%)	100%	100%	100%	100%
NPV (%)	—	—	—	—

** Specificity = 0% because the reference standard subset (n=15) comprised exclusively disease-positive (granulomatous) cases — no true negative was available. PPV=100% as all positive tests occurred within the granulomatous subset. †Among the 15 granulomatous/caseating granulomatous cases.*

DISCUSSION

This study presents a systematic, simultaneous comparison of three diagnostic modalities — FNAC, tissue fluid smear AFB, and CBNAAT — applied to the same aspirate from superficial lymph nodes in 82 patients with suspected tubercular lymphadenopathy, conducted over 20 months at a high-burden tertiary care centre in southern Odisha. The findings demonstrate a compelling and statistically significant superiority of CBNAAT over conventional AFB smear microscopy in the granulomatous lymphadenopathy subgroup, with sensitivities of 73.3% and 13.3%, respectively ($p<0.001$).

The peak incidence of tubercular lymphadenopathy in our cohort was observed in the 41–65-year age group (39%), consistent with a study by Singh *et al.*, who reported a similar peak in the same age band (48.59%) [15]. This predominance may reflect cumulative occupational exposure to infectious TB contacts and potential reactivation of latent infection in middle-aged adults with emerging metabolic comorbidities. Male predominance (64.6%; M:F = 1.8:1) is comparable to Vimal *et al.* (1.07:1) and Moore *et al.* (3:1) [16,17]. The higher male representation likely reflects differential healthcare-seeking behaviour and greater occupational exposure in the largely rural catchment population of MKCG hospital.

The cervical group was involved in 92.7% of cases — higher than figures reported by Shairoly Singh *et al.* (60.6%) and Vimal *et al.* (50.8%) [15,16]. This higher cervical predilection in our cohort may represent a referral bias, as patients with cervical swellings are more likely to seek early evaluation at tertiary facilities. Furthermore, the tonsillar portal of entry and contiguous spread from mediastinal nodes favour early cervical involvement in a population with high pulmonary TB prevalence.

Reactive lymphadenitis comprised 69.5% of the cytological diagnoses, a substantially higher proportion than typically reported in TB-endemic studies. This finding may be interpreted as reflecting either an early-stage immune response in immunocompetent patients or a presentation bias in which patients with early, non-specific lymphadenopathy are referred for FNAC before cytological hallmarks of TB evolve. Granulomatous and caseating granulomatous categories together accounted for 18.3% of cases (n=15), consistent with the recognised paucity of classical cytomorphological features in paucibacillary lymph node TB. A notable and statistically significant gender-based cytological asymmetry was identified: caseating granulomatous lymphadenitis (the TB-suggestive category) was predominantly found in female patients (77.8%; $\chi^2=10.865$, $p=0.028$). This observation, while biologically intriguing, may partly reflect the higher immunological reactivity in females, facilitating more robust granuloma formation and caseous necrosis, or may represent a hormonal modulation of the granulomatous response — a phenomenon warranting further investigation.

The ZN smear positivity rate in our cohort was 2.4% overall (2/82) and 13.3% in the granulomatous subgroup (2/15). This is consistent with the well-documented low sensitivity of ZN microscopy in lymph node EPTB, arising

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from the paucibacillary burden, anaerobic microenvironment of caseous material, and degradation of mycobacterial wall antigens within necrotic tissue. Comparative AFB positivity rates from published studies ranged from 12.5% (Chandrasekar *et al.*) to 33.33% (Annam *et al.*) in granulomatous lymph node aspirates (Table 4) [18–21]. The lower positivity in our cohort may partly reflect the modest volume of aspirate available for ZN smear given the tripartite division of specimen, and the predominantly paucibacillary nature of cases presenting without overt abscess formation.

The 73.3% CBNAAT sensitivity in the granulomatous subgroup is consistent with the pooled sensitivity of 83.1% reported by Denkinger *et al.* in their systematic review and meta-analysis of Xpert MTB/RIF for EPTB [14]. The numerically lower figure in our study may be attributed to the relatively small sample size of the reference subgroup (n=15), the paucibacillary burden of peripheral lymph node specimens, and the potential degradation of mycobacterial DNA in necrotic material. Importantly, CBNAAT detected TB in 4 of 6 granulomatous lymphadenitis cases (66.7%) that lacked frank caseation on FNAC, confirming the pivotal role of molecular testing in cytomorphologically non-diagnostic or pre-caseating-stage disease. The molecular basis for this superior sensitivity over ZN microscopy relates to CBNAAT's ability to amplify mycobacterial IS6110 insertion sequence DNA from as few as 131 bacilli per millilitre, including non-viable and fragmented organisms released from necrotic foci—a scenario where intact, stainable AFB are absent.

All 11 CBNAAT-positive cases were rifampicin-sensitive (100%). This finding is contextually important: rifampicin resistance on Xpert is a reliable proxy for MDR-TB. The absence of resistance in this cohort is clinically reassuring and permits prompt initiation of standard first-line 2HRZE/4HR therapy per NTEP 2024 guidelines, without the need for second-line drug sensitivity testing [22]. The single-step diagnosis and simultaneous resistance profiling within two hours underscore CBNAAT's operational advantage over culture-based methods requiring weeks of incubation.

Table 4. AFB Positivity and CBNAAT/PCR Sensitivity in Tubercular Lymphadenopathy

Author (Year)	Study Design	n	AFB Positivity (%)	CBNAAT/ PCR Sensitivity (%)
Khubnani *et al.*	Prospective	-	21.8%	-
Gangane *et al.*	Prospective	-	27.0%	-
Annam *et al.*	Cross-sectional	-	33.3%	-
Chandrasekar *et al.*	Cross-sectional	-	12.5%	-
Denkinger *et al.*	Meta-analysis	-	-	83.1% (pooled)
Daley *et al.*	Systematic review	-	-	25–100%
Present study	Observational	82	13.3%†	73.3%†

† Among the 15 granulomatous/caseating granulomatous cases. CBNAAT: cartridge-based nucleic acid amplification test; PCR: polymerase chain reaction.

CLINICAL IMPLICATIONS

The combined algorithm of FNAC (for morphological characterisation) plus TB-CBNAAT (for bacteriological confirmation) is superior to FNAC alone or FNAC with AFB smear in diagnosing superficial tubercular lymphadenopathy. We propose the following diagnostic pathway for resource-constrained settings: (1) FNAC should be the first-line investigation; (2) all aspirates demonstrating granulomatous or caseating granulomatous morphology should undergo concurrent CBNAAT on the same aspirate; (3) in cases of reactive or suppurative cytology in high-risk individuals, CBNAAT may also be considered given its high PPV (100%) and near-real-time turnaround; (4) excision biopsy for histopathology and culture should be reserved for FNAC/CBNAAT-negative cases with high clinical suspicion, or when lymphoma cannot be excluded.

STRENGTHS

Strengths include the prospective design, simultaneous application of three modalities on the same aspirate (avoiding sampling variability), and adherence to NTEP programmatic protocols. The study is the first from

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southern Odisha to compare FNAC, AFB smear, and CBNAAT in superficial lymph node TB.

LIMITATIONS

(i) Absence of a definitive gold standard (mycobacterial culture); (ii) relatively small granulomatous subgroup (n=15) limiting power for specificity estimation; (iii) single-centre design limiting generalisability; (iv) absence of clinical follow-up data confirming treatment response; (v) IGRA not performed due to resource constraints. Future multicentre studies incorporating culture, histopathology, and IGRA with follow-up are warranted.

CONCLUSIONS

TB-CBNAAT is a significantly more sensitive diagnostic tool than conventional AFB smear microscopy for confirming Mycobacterium tuberculosis in superficial tubercular lymphadenopathy, particularly in specimens demonstrating granulomatous morphology on FNAC. In this high-burden Indian setting, CBNAAT detected TB in 73.3% of cytologically granulomatous lymph node aspirates compared to only 13.3% by ZN smear — a near sixfold improvement. The simultaneous, automated detection of rifampicin resistance within two hours further enhances its operational utility. FNAC supplemented with TB-CBNAAT on the same aspirate constitutes an optimal, minimally invasive, and rapid diagnostic strategy for superficial tubercular lymphadenopathy. Routine adoption of this algorithm within India's National TB Elimination Programme is recommended to reduce diagnostic delays, prevent inadvertent empirical treatment without bacteriological confirmation, and facilitate early detection of drug resistance.

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Research concept- Swaraj Kumar Dey
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