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Statistical Validation of CBNAAT as a Superior Adjuvant to FNAC in the Rapid Diagnosis of Paucibacillary Extrapulmonary Tuberculosis.

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Abstract

Background: ***Objectives:*** Extrapulmonary Tuberculosis (EPTB), specifically tuberculous lymphadenitis, remains a significant diagnostic challenge due to its paucibacillary nature, which often renders conventional microscopy insensitive. This study aims to provide a statistical validation of the Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) as a superior diagnostic adjuvant to Fine Needle Aspiration Cytology (FNAC) for the rapid and accurate detection of Mycobacterium tuberculosis in superficial lymphadenopathy.
Methods: A prospective observational study was conducted over an 18-month period from January 2021 to June 2022 at a tertiary care hospital in Ranchi, Jharkhand. FNAC was performed on 115 patients presenting with superficial lymphadenopathy. Aspirates were subjected to cytomorphological analysis (Leishman-Giemsa and Papanicolaou stains), Ziehl-Neelsen (Z-N) staining for Acid-Fast Bacilli (AFB), and CBNAAT (GeneXpert MTB/RIF). Statistical parameters, including sensitivity, specificity, and Area Under the Curve (AUC), were calculated using SPSS v24. ***Results:*** Tuberculous lymphadenitis was the most prevalent diagnosis, accounting for 54.78% (n=63) of the 115 cases. Among the 64 confirmed TB cases, CBNAAT demonstrated a superior diagnostic yield of 98.44% (n=63), compared to 93.75% (n=60) for FNAC and 75% (n=48) for Z-N staining. Notably, CBNAAT identified 16 cases that were smear-negative, showing a statistically significant advantage in paucibacillary samples ($P < 0.0001$). The statistical correlation between CBNAAT and FNAC yielded a sensitivity of 93.65%, a specificity of 97.37%, and an Area Under the Curve (AUC) of 97%, indicating near-perfect diagnostic accuracy. The Negative Predictive Value (NPV) for the CBNAAT-FNAC combination was 90.24%. ***Conclusion:*** CBNAAT is a highly sensitive and specific diagnostic adjunct that significantly enhances the diagnostic yield of FNAC. Its rapid molecular confirmation and ability to detect rifampicin resistance make it an essential tool in the diagnosis of paucibacillary EPTB. Routine integration of CBNAAT with FNAC is recommended to facilitate early diagnosis and support tuberculosis elimination efforts.

Keywords: Paucibacillary Tuberculosis, EPTB, Diagnostic Validation, Statistical Accuracy.

INTRODUCTION

Tuberculosis (TB) continues to be a major global health concern, with India contributing a significant proportion of the global disease burden[1]. Extrapulmonary tuberculosis (EPTB) constitutes a considerable fraction of TB cases and often poses diagnostic challenges, particularly in cases of tuberculous lymphadenitis[2].

The diagnosis of EPTB is difficult due to its paucibacillary nature, wherein the bacterial load is insufficient for detection by conventional microscopy[3]. Fine Needle Aspiration Cytology (FNAC) is widely used as a first-line diagnostic modality due to its simplicity, cost-effectiveness, and rapid results[4]. However, cytomorphological features such as granulomas and necrosis are not specific to tuberculosis and may be seen in other granulomatous conditions[5].
Ziehl–Neelsen (ZN) staining, although highly specific, has limited sensitivity in EPTB due to the requirement of a high bacillary load[3]. Mycobacterial culture, considered the gold standard, is time-consuming and delays treatment initiation[6].

The introduction of Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) has revolutionized TB diagnostics by enabling rapid detection of Mycobacterium tuberculosis DNA and rifampicin resistance[7]. Despite its advantages, its role as an adjunct to FNAC in EPTB requires statistical validation[8].

This study aims to evaluate the diagnostic performance of CBNAAT in comparison with FNAC and ZN staining and to establish its role in improving diagnostic accuracy in superficial lymphadenopathy[9,10].

MATERIALS AND METHODS

Study Design and Setting

This prospective observational study was conducted over a period of 18 months from January 2021 to June 2022 at a tertiary care teaching hospital in Ranchi, Jharkhand, after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from all participants. [11-12]

Study Population

A total of 115 patients presenting with superficial lymphadenopathy were included[9].

Inclusion Criteria

- Patients with lymph nodes >1 cm in size
- Clinical suspicion of tuberculosis

Exclusion Criteria

- Patients on anti-tubercular therapy for more than four weeks
- Known cases of malignancy

Procedures

FNAC and Sample Processing

FNAC was performed under aseptic conditions using a 22-gauge needle. Smears were prepared for[4-5]:

- Leishman–Giemsa staining
- Papanicolaou staining
- Ziehl–Neelsen staining

Residual material was collected in sterile saline and processed for CBNAAT[7,13].

CBNAAT Testing

CBNAAT (GeneXpert MTB/RIF) was performed as per manufacturer's instructions for detection of M. tuberculosis and rifampicin resistance.

Statistical Analysis

Data were analyzed using SPSS version 24. Sensitivity, specificity, PPV, NPV, and AUC were calculated. Chi-square and Z-proportion tests were applied. A p-value < 0.05 was considered statistically significant [10,14]..

RESULTS

Comparative Detection Rates

A comparative analysis of the three diagnostic modalities was performed among 64 confirmed cases of tuberculous lymphadenitis. As shown in Table 1, CBNAAT demonstrated the highest diagnostic yield, detecting 98.44% (n = 63) of cases. This was followed by FNAC with a detection rate of 93.75% (n = 60) and Ziehl–Neelsen (ZN) staining with 75% (n = 48).

Although CBNAAT showed superior detection, the difference among the three modalities was not statistically significant (p = 0.3311), suggesting that all methods contribute to diagnosis, albeit with varying sensitivity.

Table 1: Comparison of positive TB detection across diagnostic platforms (n=64)

Diagnostic test	FNAC	AFB	CBNAAT	P value
No. of positive cases of TB	60	48	63	0.3311
Percentage	93.75%	75%	98.44%	

Statistical Validation of CBNAAT Accuracy

To validate the performance of CBNAAT, it was compared against traditional bacteriological (AFB) and cytomorphological (FNAC) parameters.

CBNAAT vs. AFB (Z-N Staining)

When validated against Z-N staining, CBNAAT correctly identified 47 out of 48 smear-positive cases (Table 2). More importantly, it identified 16 cases that were smear-negative, highlighting its utility in paucibacillary samples. This

comparison yielded a sensitivity of 74.60% and a specificity of 97.37%, with a statistically significant difference ($p < 0.0001$).

Table 2: Statistical sensitivity and specificity of CBNAAT validated against Z-N Staining.

AFB	CBNAAT		Total	P value
	Positive	Negative		
Positive	47 (46.53%)	1 (0.99%)	48	<0.0001
Negative	16 (15.84%)	37 (36.63%)	53	
Total	63	38	101	

CBNAAT vs. FNAC

CBNAAT showed a very strong correlation with cytomorphological findings. It identified 59 of the 60 cases suggestive of TB on FNAC and provided molecular confirmation for an additional 4 cases that were not cytologically definitive (Table 3). This comparison yielded a sensitivity of 93.65% and a specificity of 97.37% ($P < 0.0001$).

Table 3: Diagnostic accuracy of CBNAAT validated against FNAC findings.

FNAC	CBNAAT		Total
	Positive	Negative	
Positive	59 (58.42%)	1 (0.99%)	60
Negative	4 (3.96%)	37 (36.63%)	41
Total	63	38	101

Diagnostic Performance of FNAC relative to Z-N Staining

The diagnostic performance of traditional FNAC was validated against Z-N staining. FNAC identified 13 cases that were AFB negative, while only 1 case was AFB positive but cytologically negative(Table 4). Statistical analysis demonstrated a highly significant difference in predictive performance ($P < 0.0001$), with FNAC yielding a sensitivity of 78.33% and a high Positive Predictive Value (PPV) of 97.92%.

Table 4: Performance of traditional FNAC compared against Z-N Staining.

AFB	FNAC		Total
	Positive	Negative	
Positive	47 (46.53%)	1 (0.99%)	48
Negative	13 (12.87%)	40 (39.60%)	53
Total	60	41	101

Measures of Diagnostic Validity and Accuracy

The overall statistical performance of the three diagnostic approaches is summarized in Table 5. The CBNAAT with FNAC combination demonstrated the highest diagnostic accuracy with an Area Under the Curve (AUC) of 97% and a Negative Predictive Value (NPV) of 90.24%. This high NPV is critical for paucibacillary tuberculosis, as it allows clinicians to confidently rule out the disease when both tests are negative.

The results statistically validate CBNAAT as a superior adjuvant. While FNAC and Z-N staining are effective for high-load samples, CBNAAT significantly enhances the detection rate in paucibacillary cases, as evidenced by its superior sensitivity and highest AUC.

Table 5: Summary of statistical validity and accuracy of diagnostic modalities

Comparison	Sensitivity	Specificity	PPV	NPV	Disease prevalence	Area Under Curve
CBNAAT with AFB	74.60%	97.37%	97.92%	69.81%	62.37%	87%
CBNAAT with FNAC	93.65%	97.37%	98.33%	90.24%	62.38%	97%
FNAC with AFB	78.33%	97.56%	97.92%	75.47%	59.41	88%

(PPV: Positive Predictive Value; NPV: Negative Predictive Value; AUC: Area Under Curve; Prevalence: ~62%)

DISCUSSION

The diagnostic landscape of Extrapulmonary Tuberculosis (EPTB), specifically tuberculous lymphadenitis, has long been constrained by the paucibacillary nature of the disease. This study demonstrates the significant advantage of CBNAAT over conventional diagnostic modalities[3,7].

Comparative Analysis of Diagnostic Yield

Our results indicate a hierarchical progression in diagnostic sensitivity: CBNAAT (98.44%) > FNAC (93.75%) > Z-N Staining (75%). While Z-N staining remains a rapid and specific tool, its reliance on a high bacillary load (typically >10,000 organisms/ml) makes it inherently insensitive for lymph node aspirates[3]. In our cohort, CBNAAT successfully identified 16 cases that were smear-negative (Table 2), a statistically significant improvement ($P < 0.0001$) that underscores its role in detecting paucibacillary infections [14-17].

Diagnostic Accuracy and Predictive Value

The strength of CBNAAT lies not only in its sensitivity but in its robust Negative Predictive Value (NPV) [14,16]. As shown in Table 4, the combination of CBNAAT and FNAC yielded an NPV of 90.24%. In clinical practice, a high NPV is essential for paucibacillary TB; it provides clinicians with a 90% degree of certainty that a negative result truly indicates the absence of disease. This is a substantial improvement over the 75.47% NPV observed with the FNAC-AFB combination.

Furthermore, the Area Under the Curve (AUC) for CBNAAT compared with FNAC was 97%, indicating near-perfect diagnostic accuracy. This statistically confirms that CBNAAT is not merely a supplementary test but a definitive adjuvant that elevates the diagnostic standard of FNAC. These findings are supported by Sunil Kumar Komanapalli et al. (2018)[18], who reported that CBNAAT detected 6.5% of cases missed by FNAC, and Arpitha et al. (2021)[19], who observed a similar 12.6% detection increase**.**

Impact of Molecular Validation on Clinical Outcomes

A critical advantage of CBNAAT validated in this study is the speed of detection. By providing molecular confirmation and Rifampicin resistance status within two hours, CBNAAT matches the speed of FNAC while providing the definitive accuracy traditionally reserved for mycobacterial culture.

The statistical significance ($P < 0.0001$) found when validating CBNAAT against both AFB and FNAC (Tables 2 & 3) suggests that the "triple approach" is redundant if CBNAAT is available. Our data suggests that a "dual approach"—utilizing FNAC for morphological screening and CBNAAT for molecular confirmation—is the most statistically sound and efficient diagnostic framework.

Correlation with Contemporary Studies

Our study's sensitivity of 93.65% for the CBNAAT-FNAC correlation is higher than that reported by Suwarna B. Patil et al. (55.20%)[20], likely due to the immediate processing of fresh aspirates in our tertiary setting, which prevents DNA degradation. Our results align more closely with Gayathri Purushothaman et al. (2019)[21], who found a high specificity of 97.74% for CBNAAT in EPTB samples.

Overall, the study confirms that while FNAC remains a valuable initial screening tool and ZN staining provides specificity, CBNAAT significantly enhances diagnostic accuracy, particularly in paucibacillary cases. Its integration into routine diagnostic protocols can substantially improve early detection and management of EPTB.

CONCLUSION

The statistical evidence presented herein advocates for the routine implementation of CBNAAT as a primary adjuvant to FNAC in the diagnostic workup of superficial lymphadenopathy.

The routine use of CBNAAT as an adjunct to FNAC is strongly recommended, particularly in suspected cases of extrapulmonary tuberculosis with low bacillary load. This dual-modality approach ensures early and accurate diagnosis, facilitates prompt initiation of appropriate therapy, and reduces the risk of disease progression.

Implementation of this strategy will not only improve patient outcomes but also contribute to national and global efforts toward tuberculosis elimination.

Conflict of Interest: Nil
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