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ORIGINAL ARTICLE

Comparative Study of Truenat with Cytomorphology and AFB Positivity for Diagnosis of TB Lymphadentitis in Tertiary Care Hospital

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Abstract

Introduction: Lymph node TB (LNTB) is the most common form of Extra Pulmonary TB (EPTB) in India. Fine needle aspiration cytology (FNAC) is a simple diagnostic technique but cytomorphological features of LNTB can overlap with lesions other than TB.Conventional smear microscopy with the Ziehl-Neelsen (ZN) stain is aneasy method for detecting acid-fast bacilli (AFB). Now a days, Nucleic acid amplification tests (NAAT) is the only rapid test currently recommended by the WHO for TB diagnosis. **Aim and Objectives:** To compare the TRUENAT with FNAC finding and AFB positivity by ZN staining and to study the association of TRUENAT and AFB positivity with cytomorphological patterns. **Study Design:** Aobservational cross-sectional hospital based study. **Material and Mathods:** One hundred patients were subjected to FNAC and TRUENAT was performed.Result of TRUENAT was compared with cytological findings and AFB positivity. **Results:** Out of 100 patients 55% were TRUENAT positive for TB while ZN staining for AFB was positive in only 42% cases indicating that more cases were diagnosed by TRUENAT. **Conclusion:** TRUENAT on FNA samples is more efficient diagnostic test in suspected TB Lymphadenitis. However, it can't replace the FNAC for diagnosis of TB Lymphadenitis.

Key Words

Cytology, Lymph Node, TRUENAT, ZN staining

Introduction

Tuberculosis (TB) is an old disease.[1] World Health Organization (WHO) declared TB as the leading cause of death from a single infectious agent and one of the top ten causes of death worldwide.[2]As per the Global TB report 2024, the WHO African and Southeast Asia regions accounted for 81% of the combined total number of deaths caused by TB among people with and without HIV; India accounted for 26% of such deaths.[3]

Lymph node TB is the most common form of Extra Pulmonary TB (35%). To reduce the morbidity and mortality caused by tuberculosis,it is essential to early diagnose the cases so that timely treatment can be started.Fine needle aspiration cytology (FNAC) is a simple and rapid diagnostic technique for diagnosing TB lymphadenitis.[4] In low-resource environments, cytology and standard smear microscopy for acid-fast bacilli (AFB) serve as the primary diagnostic tools for LNTB.

But the cytology and AFB have their own limitations as they can't detect the drug resistance also culture takes larger time.

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CBNAAT /TRUENAT is an automated, heminested real-time polymerase chain reaction (PCR) that simultaneously detects Mycobacterium tuberculosis and rifampicin resistance.This technique is specially helpful for paucibacillary TB.[5]

Primary outcome of thestudy was aimedto compare TRUENAT with cytomorphology and AFB positivity in patients of tuberculosis.

Secondary outcome:

1. To study the association of TRUENAT positivity with the cytomorphological patterns.
2. To study the association of AFB positivity with the cytomorphological patterns.

Material and Methods

1. Study design: Hospital-based cross sectional study.

2. Study population: Data were collected from 100 cases at a tertiary referral centrein Department of Pathology, Muzaffarnagar Medical College, Muzaffarnagar (U.P.) over 12 months, from June 2023 to May 2024 after the approval from Institutional Ethical Committeeletter no MMC/IEC/2023/241 dated 20/3/2023.

Inclusion criteria:

All palpable and enlarged lymph nodes clinically suspected to have TB and patients willing to participate were included.

Exclusion Criteria:

1. FNAC samples that were not suspected TB cases.
2. Cases where enough material was unavailable for both ZN staining and NAAT.
3. Neoplastic lesions.
4. Non-cooperative patients

3. Sampling procedure: Purposive sampling.

4. Statistical analysis and software: A suitable statistical significance test was used for statistical analysis along with SPSS17/20 statcal2 software. The p-value of less than 0.05 was considered significant for statistical analysis.

5. Sample size: 100 cases (hospital based study, according to last three year average)

Approval of the study protocols was received from the Institutional Ethics Committee of the Muzaffarnagar Medical College, Muzaffarnagar (U.P.) (IEC No. MMC/IEC/2023/241) dated 20/3/2023

6. Procedure: Afterwritten informed risk consentunder aseptic precautions, the FNAC specimen was done using a 22- or 23-gauge needle. The Giemsa stained slides were examined under microscope with 10X & 40X lens while the ZN-stained slide was examined with oil immersion lens.

Smears having following Cytomorphological patterns were taken as positive for tuberculosis.

The TRUENAT was performed using **Xpert (Molbio Diagnostics private limited VERNA GOA) machine**.

7. Data Analysis Procedure: Data was analysedusing SPSS17/20 statcal2 software.

Result

The age group of patients ranged from 6 to 73 years, and mean age was 33.3 years. Female male ratio was 1.77:1.

In our study, 45 (45.0%) patients presented with fever followed by cough (17%) and weight loss (16.0%). Single lymph node enlargement was seen among 71 (71.0%). Cervical lymph nodes was most commonly enlarged lymph node (77.0%).

Cases were divided into 4 groups on microscopic examination. Necrotizing granulomatous lymphadenitis was present among 44.0% followed by Granulomatous lymphadenitis (32.0%), Only necrotizing lymphadenitis (12%) and Necrotizing lymphadenitis 12%.

In present study, AFB was seen in among 42.0%. The highest AFB positivity was observed in **Only necrotizing lymphadenitis** (67%).Chi square was calculated, p value for this was 0.034 which was less than 0.05, indicating that the result of AFB positivity is significant according to cytological diagnosis which is one of the secondary outcome of this study (Table 2).

In our study, TRUENAT positivity was seen among 55.0%. Maximum TRUENAT positivity (75%) was seen in Only necrotizing lymphadenitis. P value was 0.042, indicating that the association between TRUENAT positivity and cytological diagnosis was statistically significant, depicting the other secondary outcome. (Table 3)

Overall, AFB was positive in 42 cases while TRUENAT was positive in 55 cases. Out of these 42 AFB positive cases, TRUENAT came positive in 41 cases, TRUENAT detected 14 cases which were AFB negative, signifying the detection of more TB cases by TRUENAT than ZN staining. Significant association was found between AFB and TRUENAT (p value < 0.05). (Table 4) So, the primary outcomes in this study shows TRUENAT is able to diagnose more TB cases than AFB and various cytomorphological patterns.

Discussion

TB affects both extra pulmonary and respiratory tract locations. Fine needle aspiration cytology (FNAC) now a days being recognized as a first line diagnostic technique. Standard diagnostic algorithm for TBLN in India

Table 1: Distribution of Demographic and Clinical Characteristic.

Age group (in years)	No. of Cases (N)	Percentage (%)
Less than 40	69	69.0
40–60	21	21.0
More than 60	10	10.0
Gender		
Male	36	36.0
Female	64	64.0
Anatomical site		
Cervical	77	77.0
Axillary	15	15.0
Supraclavicular	7	7.0
Inguinal	1	1.0
Aspirate appearance		
Blood mixed	69	60.0
Pus	29	29.0
Thick Material	11	11.0

Table 2 : Distribution of AFB positivity according to different Cytomorphological pattern.

Cytomorphological Pattern	Number of cases (N)	AFB POSITIVITY	
		Number of cases (N)	Percentage (%)
Granulomatous lymphadenitis	32	8	25%
NGL-Likely to be tubercular	44	22	50%
Only necrotizing lymphadenitis	12	8	67%
Necrotizing lymphadenitis with suppuration	12	4	33%
Total	100	42	100%
Chi square	5.86		
p value	0.034		

NGL- Necrotizing Granulomatous lymphadenitis, AFB-Acid fast bacillus TRUENAT-True Nuclear Amplification Technology

recommends FNAC along with Ziehl–Neelsen (ZN) staining for acid fast bacilli (AFB), Mycobacterial culture is a gold standard method, useful as a definitive diagnosis but due to lengthy culture period, causes delay in the treatment.[6]

The Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) assay is a real-time polymerase chain reaction (PCR) cartridge-based assay for the simultaneous detection of Mycobacterium tuberculosis complex and rifampicin resistance within two hours. The World Health Organization (WHO) endorsed this technology in December 2010.[7]

LNTB can present at any age. In our study, mean age of patient was 33.38±18.95 yrs. This was in concordance with Dagar *et al*.[8] and Chand *et al*.[9]An individual is energetically most active during age of 20-40 years and therefore chances of exposure are more.

In present study, female predominance seen. This is similar to Shetty and Vyas[10] and Fazal *et al*[11] The increased occurrence among females can be related to poor nutritional condition and poorer overall level of living in emerging nations.

In present study, 45.0% patients presented with fever. Studies by Parigi *et al*.[12] and Kumar *et al*.[13] was in concordance with our study.

Single lymph node enlargement was seen among 71%

Table 3: Distribution of TRUENAT positivity according to different Cytomorphological pattern.

Pattern	No. of cases (N)	TRUENAT Positivity	
		Number of cases (N)	Percentage (%)
Granulomatous lymphadenitis	32	15	46.8
NGL-Likely to be tubercular	44	25	56.8
Only necrotizing lymphadenitis	12	9	75
Necrotizing lymphadenitis with suppuration	12	6	50
Total	100	55	100
Chi square Test	4.92		
P value	0.042		

Table 4: Association between AFB and TRUENAT.

		TRUENAT		Total
		Positive	Negative	
AFB (ZN staining)	Positive	41	1	42
	Negative	14	44	58
	Total	55	45	100
	Chi square Test	7.11		
	p value	0.013		

AFB-Acid fast bacillus

cases. Nidhi *et al*.[14], Shetty and Vyas *et al*.[10] and Kumar *et al*.[13] observed similar findings.

In this study, cervical lymph nodes were predominantly involved (77%), it was in concordance with Garima *et al* (69%)[15] and Masilamani *et al* (83.5%).[16] Aspirate in our study was blood mixed (60%), pus (29%) and thick cheesy material (11%) which was in concordance with Kumar *et al*[13], Masilamani *et al*.[16], Arpitha *et al*.[17] and Hemalatha *et al*.[18].

In present study, Necrotizing granulomatous lymphadenitis was present among 44.0% Parigi *et al*[12], Hemlatha *et al*.[18] and Narang *et al*.[19] also observed similar findings.

In our study, AFB was seen in ZN staining among 42.0%. Different AFB positivity was found in different studies like Dagar *et al* (36.5%)[8] and Masilamani (55.6%) *et al*.[16]

TRUENAT positivity was seen among 55 cases (55.0%) which was in concordance with the study done by Moure *et al*.[20]and Srwar *et al*.[21]

Overall TRUENAT was positive in 55%. TRUENAT was positive 75%in 'Only necrotizing lymphadenitis' followed by 56.8% in 'Necrotizing granulomatous lymphadenitis' and it was 50 % in 'Necrotizing lymphadenitis suppuration' and least 46.8% in

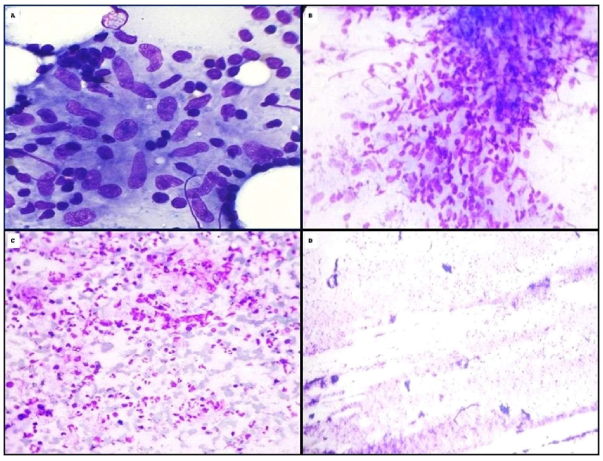

FIGURE Photomicrograph Giemsastain, 400X)
A-Granulomatous lymphadenitis showing epithelioid granuloma
B- Epithelioid cell granuloma with necrosis
C- Necrosis with suppuration
D-Only Necrotising lymphadenitis

'Granulomatous lymphadenitis'. Parigi *et al* observed TRUENAT positivity 90% in Caseating granulomatous lymphadenitis.[12] In developing country such as India, early diagnosis of TB along with accurate detection of drug resistance are critical for prevention of TB transmission and development of resistant cases. In

resource-limited settings, adoption of newer techniques remains challenging due to the involved start-up and training costs.

Conclusion

Early diagnosis and timely treatment is necessary for the prevention of TB. FNAC and ZN staining was the primary diagnostic methods employed for the detection of TB lymphadenitis.TRUENAT is the latest less time-consuming technique and had an advantage of detecting FNAC missed cases, especially suppurative abscess and can detect TB even if bacilli load is less.FNAC combined with TRUENAT emerged as a reliable diagnostic approach due to its rapid turnaround time and high sensitivity, facilitating early diagnosis and management of tubercular lymphadenopathy.

Recommendations

We recommend that when the smear contains only necrotic material with or without inflammatory cells and AFB is negative, it is advisable to exclude TB by mycobacterium culture and even by a therapeutic trial, if needed.

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Conflict of Interest: Nil

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