

Analysis of the Diagnostic Yield of Nucleic Acid Amplification Tests in Immunocompetent Patients with Tuberculous Cervical Lymphadenitis

Dr. Rajesh Sharma¹, Dr. Richa Sharma², Dr. Kotha Poorna Sai³

^{1,2,3}Department of General Medicine, Saraswathi Institute of Medical Sciences, Hapur

Abstract: ***Background:** Tuberculous lymphadenitis is the most prevalent form of extrapulmonary tuberculosis (TB), accounting for approximately 30–40% of such cases. Nucleic acid amplification tests (NAATs), such as TrueNAT, offer a highly sensitive and rapid diagnostic method for tuberculous cervical lymphadenopathy, including the detection of rifampicin resistance in positive cases. **Objectives:** 1) To assess the diagnostic effectiveness of TrueNAT in identifying Mycobacterium tuberculosis in fine - needle aspirate (FNA) samples from cervical lymph nodes, compared to Ziehl - Neelsen (ZN) staining. 2) To evaluate the diagnostic yield of TrueNAT by comparing it with cytological findings from FNA samples of cervical lymph nodes and with necrotic findings in these nodes observed via ultrasonography (USG) of the neck. **Materials and Methods:** This prospective study included 50 patients from July to December 2023. Demographic data and clinical histories were collected. FNA samples underwent TrueNAT testing, cytological analysis, and ZN staining. USG of the neck was performed to detect necrosis in the cervical lymph nodes. **Results:** The TrueNAT test showed a positivity rate of 70%. Its sensitivity and specificity were evaluated against cytology results, acid - fast bacilli (AFB) detection on ZN staining, and necrotic findings in cervical lymph nodes via USG. The sensitivity and specificity of TrueNAT were 80.49% and 77.78%, respectively, when compared with FNA cytology showing necrosis; 17.14% and 93.33%, respectively, with AFB positivity on ZN staining; and 74.29% and 33.33%, respectively, with lymph node necrosis observed on USG. **Conclusion:** The TrueNAT assay is recommended as an adjunctive test to conventional cytological examination of FNA samples from lymph nodes for the rapid diagnosis of tuberculosis, along with simultaneous detection of rifampicin resistance.*

Keywords: Cervical Lymphadenitis, TrueNAT assay, Tuberculosis, Ziehl - Neelsen staining

1. Introduction

Tuberculosis (TB) is a chronic, infectious disease that poses significant risks of morbidity and mortality if not diagnosed and treated promptly. In 2021, approximately 10.6 million people contracted TB, with 1.6 million deaths reported among TB patients. Extrapulmonary TB constitutes around 15–20% of all TB cases. In India and other developing nations, tuberculous lymphadenitis is the most prevalent form of extrapulmonary TB, accounting for 30–40% of cases. In contrast, nontuberculous mycobacteria (NTM) are the leading cause of lymphadenopathy in developed countries.

Given the high burden of TB in India, there is a significant likelihood of initial drug resistance in both pulmonary and extrapulmonary forms of the disease. Early detection of drug resistance is crucial for initiating appropriate treatment promptly. The treatment of lymph node TB often begins based on histopathological evidence of granulomas found in excision biopsy or fine - needle aspirate (FNA) specimens, even in the absence of definitive microbiological proof of TB. Traditionally, the diagnosis of lymph node TB relies on histopathological examination and corroborative evidence, such as a positive Mantoux test and elevated erythrocyte sedimentation rate.

A definitive diagnosis of tuberculous lymphadenitis necessitates the detection of Mycobacterium tuberculosis in FNA or biopsy specimens or the growth of the organism in a culture sample. Diagnosing lymph node TB is challenging due to its paucibacillary nature. The sensitivity of smear examination for acid - fast bacilli (AFB) in FNA samples is relatively low, ranging from 27 to 50%. Cytological diagnosis

of lymph node TB involves identifying AFB and granulomatous inflammation, often with caseous necrosis (necrotizing granulomas). However, various factors, including sample quality and quantity, interobserver variability, and shared microarchitectural patterns with other diseases, complicate the cytological diagnosis of tuberculous lymphadenitis.

Culture has reduced sensitivity in paucibacillary TB, and also it takes several weeks for results and requires a highly equipped laboratory. Due to the abovementioned factors, the recent focus has been shifted to molecular diagnosis or nucleic acid amplification (NAA) methods, which detect the mycobacterial DNA in the samples and also detect rifampicin resistance. There are two types of NAAT available in the market—one is CBNAAT/Gene Xpert, and the other one is TrueNAT. Both tests are objective tests with little or no variations in the result. CBNAAT/Gene Xpert detects M. tuberculosis bacteria and simultaneously detects rifampicin resistance within 2 hours from biological samples, but it requires continuous electricity and an advanced laboratory setup. In contrast, TrueNAT can run without electricity and doesn't require an advanced laboratory setup. Regarding culture, the pooled sensitivity and specificity of the NAAT method for diagnosing TB in patients with cervical lymphadenopathy were 83.1 and 93.6%, respectively. NAAT can help to confirm a diagnosis in patients suspected of lymph node tuberculosis (LNTB) when considered alongside the results of Fine needle aspiration cytology (FNAC), noting that a negative NAAT test does not rule out LNTB.³ In the current study, we evaluated the diagnostic yield of TrueNAT for the early diagnosis of tuberculous cervical lymphadenitis.

2. Materials and Methods

Study Design

After obtaining Institutional Ethics Committee clearance a prospective study was conducted to evaluate the diagnostic yield of NAAT among tuberculous cervical lymphadenitis patients who consulted the Department of General Medicine at Saraswathi medical college, Hapur between July and December 2023.

Objectives

- To evaluate the diagnostic yield of TrueNAT for detecting M. Tuberculosis bacteria in the fine needle aspirated samples suggestive of tuberculous cervical lymphadenitis compared with Ziehl–Neelsen (ZN) staining.
- To evaluate the diagnostic yield of TrueNAT for the diagnosis of TB in comparison with the cytology report of FNA samples of cases of tuberculous cervical lymphadenitis.
- To evaluate the diagnostic yield of TrueNAT for the diagnosis of TB in necrotic cervical lymph nodes on ultrasonography (USG) neck.

Inclusion Criteria

- Age >12 years.
- Immunocompetent patients with palpable or radiologically detected cervical lymphadenopathy.
- Lymph node FNA cytology is suggestive of granulomatous inflammation with or without necrosis, or lymph node FNA showing AFB on ZN staining.

Exclusion Criteria

- Age <12 years.
- Patients who did not give consent for this study.
- Patients who were taking anti - TB treatment.
- Patients with a history of human immunodeficiency virus and on immunosuppressants.

3. Methodology

Demographic data and clinical data, including clinical symptoms and signs related to cervical lymphadenopathy, were noted for all enrolled patients at the time of consultation. The previous history of sputum - positive pulmonary TB and contact history with sputum - positive pulmonary TB patients were recorded. USG neck was performed to assess features of necrotic lymph nodes. USG - guided lymph node aspiration was conducted using the FNA method in all patients, and aspirated samples were sent for (1) the cytological examination, (2) ZN staining for AFB, and (3) TrueNAT assay to detect AFB and rifampicin - resistance. Patients with cervical lymphadenitis whose FNA cytology report suggested granulomatous inflammation with or without necrosis or demonstrated AFB on ZN staining were enrolled in our study. TrueNAT, among the NAAT tests, was considered for the study of the diagnostic yield of NAAT. TrueNAT is one type of NAAT that can be conducted without electricity and without advanced laboratory setup. It can detect M. Tuberculosis Bacilli from the biological sample within 2 hours.

Statistical Analysis

Data were preprocessed using Microsoft Excel to calculate mean, median, and standard deviation. The t - test was used for parametric variables to compare groups of subjects. Quantitative data are expressed as mean \pm standard deviation or median (interquartile range), while qualitative variables are expressed as numbers and percentages. The Chi - squared test was used to check the association between two categorical data. P - values < 0.01 were considered significant. The data were captured and analyzed using the software package Statistical Package for the Social Sciences (SPSS) version 25.

4. Results

The demographic, clinical, radiological, and microbiological characteristics of the enrolled patients in the study are described in Table 1.

Table 1: Demographic, clinical, and radiological variable

Characteristics	Sample size (n = 50)
Demographic characteristics	
Age (years)	33.28 ± 13.26
Sex (M:F)	2:3
BMI	18.31 ± 3.92 kg/m ² In TrueNAT positive: 17.78 ± 3.85 kg/m ² In TrueNAT negative: 19.66 ± 4.03 kg/m ²
Symptoms and signs	
Swelling over the neck	50 (100%)
Pain over neck swelling	25 (50%)
Anorexia	33 (66%)
Weight loss	33 (66%)
Low-grade fever	24 (48%)
Night sweats	23 (46%)
Cough	12 (24%)
TrueNAT result	
Positive	35 (70%)
Negative	15 (30%)
Site of cervical lymphadenopathy on USG neck	
Left side only	28 (56%)
Right side only	16 (32%)
Bilateral	6 (12%)
Comorbidities	
Diabetes mellitus	7 (14%)
Hypertension	5 (10%)
Hypothyroidism	4 (8%)
ischemic heart disease	1 (2%)
History of pulmonary TB (PTB)	
Previous history of sputum-positive PTB	3 (6%)
Contact history of sputum-positive PTB	9 (18%)
AFB positivity	
ZN stain positive	7 (14%)
TrueNAT positive	
Occupation	35 (70%)
Semi-skilled worker*	22 (44%)
Housewife	16 (32%)
Student	7 (14%)
Skilled worker**	5 (10%)

5. Discussion

In our study, 50 patients with cervical lymphadenopathy and FNA cytology reports suggestive of TB were enrolled. The TrueNAT result of the cervical lymph node aspirate was compared with the cytology result, ZN staining for AFB, and necrotic findings in the cervical lymph node on the USG neck. Among the 50 patients enrolled, 20 (40%) patients were male, of which 16 (32%) were TrueNAT positive, and 30 (60%) patients were female, of which 19 (38%) were TrueNAT positive on cervical lymph node fine needle aspirate (FNA). The M: F ratio of 2: 3. The mean age of the patients was 33.28 ± 13.26 years. Most patients, 28 (56%), were 21–40 years. The median age of the patients was 28 years, with an interquartile range of 21–39 years. Purohit et al., 4 Kamal et al., 5 Mukhida et al., 6 Tanwir, 7 and Goyal⁸ observed female predominance with a comparable peak age onset in their studies. Female preponderance may be due to the country's social dynamics. Most females stay or work inside their houses in a closed environment. So, less air ventilation tends to increase the overall risk of developing infectious diseases. There was no significant statistical correlation of age and

gender with the TrueNAT result of the patients with cervical lymphadenopathy (Chi - square = 1.823; p = 0.1770). The mean body mass index (BMI) of the patients with a TrueNAT positive result was 17.78 ± 3.85 kg/m², and the mean BMI of the patients with a TrueNAT negative result was 19.66 ± 4.03 kg/m². Overall, the mean BMI of the patients was 18.31 ± 3.92 kg/m². There was no statistical association between the BMI of the patients and the TrueNAT result (unpaired t - test = 1.53; p = 0.13).

In our study, 22 (44%) patients were semi - skilled workers, followed by 16 housewives (32%), 7 students (14%), and 4 skilled workers (8%). Among all the patients, 18 semi - skilled workers (36%) were positive for TrueNAT results, followed by 10 housewives (20%) and 6 students (12%). Kamal et al.⁵ had 37 housewives (56.8%), followed by 16 students (24.6%), 11 semiskilled workers (16.9%), and 6 skilled workers (9.2%) in their study. No significant statistical correlation existed between occupation and TrueNAT results of the patients with cervical lymphadenopathy. In our study, palpable cervical swelling (100%) was the most common presenting feature, followed by anorexia in 33 (66%) patients, weight loss in 33

(66%) patients, pain over the neck swelling in 25 (50%) patients, fever in 24 (48%) patients, night sweats in 23 (46%) patients, and cough in 11 (22%) patients. The frequency of localized and systemic symptoms described by Purohit et al., 4 Kamal et al., 5 and Tanwir⁷ are comparable with our study.

In our study, nine (18%) patients had a contact history of sputum - positive pulmonary TB patients, of which five were positive on TrueNAT. There is no statistically significant association between the contact history of pulmonary TB and the TrueNAT result of the patients with cervical lymphadenitis (Chi - square = 0.0107, p = 0.9177, and not significant at p < 0.01). Purohit et al., 4 Kamal et al., 5 and

Nur⁹ observed a history of contact with sputumpositive pulmonary TB patients in 10.13, 26.2, and 18.3% of patients, respectively. In our study, 41 (82%) patients showed necrotic granuloma on the cytology report. The sensitivity and specificity of the TrueNAT method for diagnosing TB in patients with cervical lymphadenitis were 80.49% and 77.78%, respectively, compared with the necrosis finding on the cytology report of cervical lymph node FNA (Table 2). The positivity rate for TrueNAT was 70%. Studies done by Purohit et al., 1 Tanwir, 7 Nur, 9 Haridas et al., 10 reported necrotic granuloma in 42.86, 62.28, 58.4, 60.89, 63.8, 80, and 91.7%, respectively.

Table 2: Comparison and statistical association between TruNAT results and necrosis on cytology reports

TrueNAT results	Cytology reports s/o		Total		
	Granulomatous inflammation with records	Granulomatous inflammation without records			
Positive	33 (66 %)	2 (4 %)	35 (70 %)	Chi Square= 9.317 p= 0.0022 Not significant at p <0.01	Sensitivity= 80.49% Specificity= 77.78%
Negative	8 (16 %)	7 (14 %)	15 (30 %)		
Total	41 (82 %)	9 (18 %)	50 (100 %)		

Table 3: Comparison and statistical association between TruNAT results and granulomatous inflammation on cytology reports

TrueNAT results	Cytopathology results showing granulomatous inflammation		Total		
	Yes	No			
Positive	33 (66 %)	2 (4 %)	35 (70 %)	Chi Square= 0.2702 p= 0.6032 Not significant at p <0.01	Sensitivity= 70.21% Specificity= 33.33%
Negative	14 (28 %)	1 (2 %)	15 (30 %)		
Total	47 (94 %)	3 (30 %)	50 (100 %)		

Our study showed a statistically significant association between the cytology report showing necrosis and TrueNAT results in diagnosing TB in patients with cervical lymphadenitis. (Chi - square = 9.317, p = 0.0022 and significant at p < 0.01). Goyal⁸ showed a sensitivity of 77.27% and specificity of 57.1% compared to the cytology of cervical lymph node aspirated samples, and the positivity rate for cartridge - based nucleic acid amplification test (CBNAAT) was 72.5%.

Another study by Denkinger et al. showed a sensitivity of 83.1% and specificity of 93.6% compared to the cytology of lymph node aspirated samples for Xpert M. tuberculosis/ rifampicin resistance assay (MTB/RIF). Mukhida et al.⁶ showed a sensitivity of 85.71% and specificity of 48.54% compared to cytology for cervical lymph node aspirated samples, and the positivity rate for Xpert MTB/RIF was 61.37%. Habous et al.¹⁵ observed a sensitivity of 76.92% and specificity of 100% compared to the cytology of lymph node aspirated samples for GeneXpert. Bankar et al.¹⁶ observed a sensitivity of 100% and specificity of 64.06%, compared to the cytology of lymph node aspirated samples. The positivity rate was 41.43% for Xpert MTB/RIF. There was no statistically significant association when granulomatous inflammation, irrespective of necrosis in the cytology report,

was compared with the TrueNAT result (Chi - square = 0.2702, p = 0.6032, and not significant at p < 0.01) (Table 3). This signifies that whenever there is necrosis in the FNAC report, the sensitivity and specificity of the TrueNAT method to detect M. tuberculosis increase.

In our study, 7 (14%) patients showed AFB positivity on ZN staining of FNA samples of cervical lymph nodes, of which 6 (85.71%) patients were TrueNAT positive. Among 29 patients (58%) with AFB negative on ZN staining, 17 (58.62%) were TrueNAT positive. There was no statistically significant association between TrueNAT results and AFB results in diagnosing TB in patients with cervical lymphadenitis (Chi - square = 0.2848, p = 0.593595, not significant at p < 0.01). The sensitivity and specificity of the TrueNAT assay for detecting AFB on the FNA sample compared to the ZN staining method for AFB were 17.14 and 93.33%, respectively (Table 4). The smear quality, staining method, technician expertise, and scanty bacilli in the FNA samples are responsible for the relatively lower sensitivity and higher specificity rates in ZN staining, which ultimately help to decide to start the anti - TB treatment. In the study by Goyal, 8 AFB by ZN staining of the cervical lymph node aspirate samples showed a sensitivity and specificity of 45.45 and 100%, respectively, with an AFB positivity rate of 39.2%.

Table 4: Comparison and statistical association between FNA and AFB positivity by ZN staining and TruNAT results

TrueNAT results	FNA/ AFB result		Total		
	Yes	No			
Positive	6 (12 %)	29 (58 %)	35 (70 %)	Chi Square= 0.2848 p= 0.593595 Not significant at p <0.01	Sensitivity= 17.14% Specificity= 93.33%
Negative	1 (2 %)	14 (28 %)	15 (30 %)		
Total	7 (14 %)	43 (86 %)	50 (100 %)		

Table 5: Comparison and statistical association between TrueNAT results and necrotic lymph node on USG neck

TrueNAT results	USG neck showing necrotic lymph node		Total	Chi Square= 0.043 <i>p</i> = 0.8366 Not significant at <i>p</i> <0.01	Sensitivity= 74.29% Specificity= 33.33%
	Yes	No			
Positive	26 (52 %)	10 (20 %)	36 (72 %)		
Negative	9 (18 %)	5 (10 %)	14 (28 %)		
Total	35 (70 %)	15 (30 %)	50 (100 %)		

A total of 35 (70%) patients had necrosis of the lymph node on the USG of the neck, of which 26 (74.29%) were TrueNAT positive and 9 (25.71%) were TrueNAT negative. The sensitivity and specificity of TrueNAT compared with necrosis in cervical lymph nodes on the USG of the neck were 74.29 and 33.33%, respectively, but no statistically significant association was observed (Chisquare = 0.043, *p* = 0.8366, and not significant at *p* < 0.01) (Table 5). In our study, six (12%) had lung lesions on chest X - ray, of which three patients were female and had a history of previous pulmonary TB. Studies done by Kamal et al., 5 Mukhida et al., 6 observed lung lesions on chest X - ray in 3.1, 7.5, and 16% of patients, respectively. In our study, the TrueNAT assay identified all specimens as rifampicin - sensitive. However, the sensitivity and specificity of the TrueNAT assay in detecting rifampicin resistance were not attempted because it requires comparison with the liquid culture method, a gold - standard method, for diagnosing TB. A study conducted by Haridas et al.10 did not detect any rifampicin resistant case in this study on Xpert MTB/ RIF assay.

6. Conclusion

The present study supports the TrueNAT assay as a reliable reverse transcription polymerase chain reaction - based test for the rapid diagnosis of lymph node TB. It can be used as an adjunctive test to conventional cytological examination, smear microscopy, and ultrasonic evaluation of necrotic cervical lymphadenopathy for the diagnosis of lymph node TB. The sensitivity and specificity of NAAT for detecting M. Tuberculosis increase whenever there is granulomatous necrosis on cytology of the lymph node and USG neck showing findings of necrotic lymph nodes.

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