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Cross-sectional study on evaluation of peripheral tubercular lymphadenopathy with special emphasis on smear AFB microscopy, AFB liquid culture and Xpert MTB/RIF from fine needle aspiration samples in Presumptive, Partial responder and Presumptive relapsed cases

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Abstract

Background: Peripheral tubercular lymphadenopathy is the most common form of extrapulmonary tuberculosis. Patients are categorised into presumptive, partial responder, and presumptive relapsed cases, where early and accurate diagnosis followed by proper treatment decisions significantly impact outcomes. Conventional smear microscopy has limited sensitivity, while culture and molecular methods such as Xpert MTB/RIF improve diagnostic yield and detect drug resistance. This study aimed to evaluate and compare the diagnostic yields of smear AFB microscopy, AFB BACTEC culture and Xpert MTB/RIF using FNA (Fine Needle Aspiration) samples in different clinical categories of peripheral tubercular lymphadenopathy. ***Methods:*** This cross-sectional study included 103 clinically suspected cases of peripheral lymph node tuberculosis, categorized into presumptive (n=40), partial responders (n=23), and presumptive relapsed cases (n=31). Fine needle aspiration was performed, and samples were subjected to Ziehl–Neelsen smear microscopy, AFB BACTEC culture, and Xpert MTB/RIF testing. Clinical data, including age, sex, BMI and symptoms were recorded and analysed. ***Results:*** Out of 103 cases, 94 were diagnosed as TBLN. Distribution included 43% presumptive, 22% partial responders and 35% presumptive relapsed cases. Xpert MTB/RIF demonstrated the highest detection rate across all categories (85% in presumptive, 86.9% in partial responders, and 83.8% in relapsed cases) with no statistically significant discordance [p = 0.0998]. Unlike Presumptive category, there were statistically significant discordance noted between the diagnostic yields of Xpert MTB/RIF and that of AFB BACTEC culture in partial responders (p = 0.00028) and presumptive relapsed cases (p = 0.00026). MDR/RR-TB prevalence was 5.3% overall. Most patients were underweight (BMI <18.5), and fever was the most common presenting symptom (77%). Cervical Level 2 and Level 5 lymph nodes were most frequently involved. ***Conclusion:*** Xpert MTB/RIF showed superior diagnostic yield over smear microscopy and culture in peripheral TBLN and effectively detected rifampicin resistance. Incorporating molecular diagnostics into routine evaluation of lymph node TB can enhance early detection and appropriate management in treatment naïve presumptive peripheral TBLN cases. But in partial responders and presumptive relapsed cases [patients already exposed to anti-tubercular drug therapy (ATT)], AFB-culture positivity might be the considered as the most reliable indicator for ATT re-initiation.

Keywords: Peripheral Tubercular Lymphadenopathy, Fine Needle Aspiration, Smear Microscopy, AFB BACTEC Culture, Xpert MTB/RIF, MDR-TB.

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INTRODUCTION

Tuberculosis continues to be a major global public health problem and remains one of the leading causes of morbidity and mortality worldwide.[1] Although pulmonary tuberculosis is the most common form, EPTB (Extrapulmonary Tuberculosis) constitutes a significant proportion of total TB cases.[2] Among the various forms of EPTB, TBLN (Tubercular Lymphadenopathy) is the most frequent presentation, particularly involving the cervical lymph nodes.[3] Peripheral lymph node tuberculosis is commonly seen in developing countries

and contributes substantially to the overall disease burden.[4]

Clinically, patients with peripheral TBLN present with lymph node enlargement, which may or may not be associated with constitutional symptoms such as fever, weight loss, night sweats, and cough.[5] Diagnosis of peripheral lymphadenopathy is often challenging due to overlapping features with reactive lymphadenitis, malignancy, and other granulomatous diseases.[6] FNA (Fine Needle Aspiration) cytology is a simple, minimally invasive, and cost-effective procedure widely used for evaluating lymph node swellings.[7]

Conventional ZN (Ziehl–Neelsen) smear microscopy for AFB (Acid-Fast Bacilli) is rapid and inexpensive but has limited sensitivity, particularly in paucibacillary specimens.[8] Mycobacterial culture improves diagnostic accuracy but requires longer turnaround time and specialized laboratory facilities.[9] The advent of molecular techniques such as Xpert MTB/RIF has significantly improved rapid detection of Mycobacterium tuberculosis and simultaneous identification of rifampicin resistance directly from clinical samples.[9]

Early and accurate diagnosis is important in presumptive, partial responder, and relapsed cases, where delayed detection may lead to persistent disease and drug resistance. Therefore, this study aims to evaluate peripheral tubercular lymphadenopathy using smear AFB microscopy, AFB BACTEC culture, and Xpert MTB/RIF from FNA samples to determine their diagnostic utility in different clinical categories.

AIMS AND OBJECTIVES

The study aimed to evaluate peripheral tubercular lymphadenopathy using fine needle aspiration samples with special emphasis on smear AFB microscopy, AFB culture, and Xpert MTB/RIF assay in different clinical categories, including presumptive, partial responder, and presumptive relapsed cases. The objectives were to compare the diagnostic performance of smear AFB microscopy, AFB BACTEC culture, and Xpert MTB/RIF in detecting Mycobacterium tuberculosis from lymph node aspirates, to assess the utility of molecular methods in identifying rifampicin resistance, and to determine the distribution and clinical characteristics of peripheral tubercular Lymphadenopathy across the study groups.

MATERIALS AND METHODS

Study Design

This was a cross-sectional observational study conducted to evaluate peripheral tubercular lymphadenopathy using fine needle aspiration samples. Patients presenting with enlarged peripheral lymph nodes were categorized into three groups: presumptive cases with no prior ATT (Anti-Tubercular Therapy), partial responders with residual lymph nodes after completion of ATT, and presumptive relapsed cases with recurrence or new lymph node enlargement after successful treatment completion. The study aimed to compare the diagnostic utility of smear AFB microscopy, AFB BACTEC culture, and Xpert MTB/RIF assay in these clinical categories, considering CRS (Composite reference standard) positivity as "Gold Standard" for diagnosis of peripheral tubercular lymphadenopathy.

Criteria for CRS positivity

BOX: A (Microbiologically confirmed)

1) AFB AFB BACTEC CULTURE: MTB growth detected
2) Xpert MTB/RIF: MTB DNA detected
3) AFB: Seen on smear microscopy

…**One or more positive**

Or

BOX: B (Clinically diagnosed)

1) Cytology: Granulomatous inflammation &/or caseation necrosis
2) Clinical symptoms present

… **Both positive**

Inclusion and Exclusion Criteria

The study included patients presenting with enlarged peripheral lymph nodes (greater than 1 cm in diameter) involving cervical, axillary, or inguinal regions. Participants were categorized as presumptive cases without prior ATT, partial responders with persistent lymphadenopathy after treatment, and presumptive relapsed cases with recurrence following completion of therapy. Patients who did not meet these clinical criteria or had inadequate samples for laboratory evaluation were excluded from the study.

Data Collection Procedure

After clinical evaluation and categorization, FNA was performed under aseptic precautions from the affected lymph nodes. Aspirated material was divided for cytological examination and microbiological analysis. Smears were prepared and stained using the Ziehl–Neelsen technique for acid-fast bacilli detection. Additional samples were processed for mycobacterial culture using the AFB BACTEC system and molecular testing with Xpert MTB/RIF for rapid detection of Mycobacterium tuberculosis and rifampicin resistance. Clinical details, demographic characteristics, lymph node levels involved, BMI, and associated symptoms were recorded systematically for analysis.

RESULTS

Category	Number of Cases	Percentage (%)
Presumptive	40	43%
Partial Responder	23	22%
Relapsed	31	35%
Total TBLN Diagnosed	**94**	—

Table 1: Distribution of Study Categories (n = 103)

Table 1 illustrates the distribution of cases across the three study categories. Presumptive cases formed the largest group (43%), followed by presumptive relapsed (35%) and partial responders (22%).

Level	Right	Left
Level 1	8	2
Level 2	12	9
Level 3	4	6
Level 4	6	5
Level 5	24	15
Level 6	3	0

Table 2: Level-Wise Involvement of Cervical Lymph Nodes

Table 2 observes that Level 5 cervical lymph nodes were most frequently involved, followed by Level 2. Right-sided involvement was slightly more common overall.

Category	Smear AFB	Xpert MTB/RIF	AFB BACTEC Culture
Presumptive (n=40)	14 (35%)	34 (85%)	29 (72.5%)
Partial Responder (n=23)	9	20 (86.9%)	8 (34.7%)
Relapsed (n=31)	12	26 (83.8%)	12 (38.7%)

Table 3: Diagnostic Detection Rates

Table 3 demonstrates that Xpert MTB/RIF had the highest diagnostic yield across all categories compared to smear microscopy and culture.

Category	RR/MDR-TB Cases	Percentage (%)
Presumptive	3	7.5%
Partial Responder	0	0%
Relapsed	2	6.45%
Total	**5**	**5.3%**

Table 4: Prevalence of RR/MDR-TB (n=94)

Table 4 illustrates that RR/MDR-TB prevalence was 5.3% overall, with higher proportions in presumptive and relapsed groups.

Age Group (in years)	Male	Female
12–18	4	20
19–25	12	20
26–32	10	12
33–39	1	1
40–46	3	6
47–53	1	0
54–60	0	1

Table 5: Age Distribution of Diagnosed TBLN Cases

Table 5 shows that the majority of cases occurred in younger age groups, with female predominance in most age brackets.

BMI Category	TBLN Positive	TBLN Negative
<18.5	65	3
≥18.5	29	6

Table 6: BMI Distribution

Table 6 observes that most TBLN patients were underweight (BMI <18.5), indicating a strong association between malnutrition and TB.

Symptom	Presumptive	Partial Responder	Relapsed
Fever	19	5	13
Cough	2	1	1
Night Sweat	0	0	1
Weight Loss	4	0	2

Table 7: Symptom Distribution among TBLN Cases

Table 7 illustrates that fever was the most common presenting symptom (77%), followed by weight loss, while cough and night sweats were less frequent.

DISCUSSION

Our study population consisted of 103 patients included as per our inclusion criteria. Among them, 94 cases were diagnosed to have tubercular peripheral lymphadenopathy as per CRS (Composite reference standard) positivity. One patient had lymphoproliferative disorder, three had metastatic lymph nodes and 5 cases remained undiagnosed with the diagnostic modalities used in our study.

Female predominance (67%) was noted among the diagnosed cases of TBLN (n=94) based on CRS positivity, with a female-to-male ratio of 2.03:1. Purohit MR et al., also reported a female-to-male ratio of 2.1:1.[10] In the same study, they also reported that 75% of patients were aged between 14 and 35 years. Our study finding did not differ much - we found 82.9% of the affected population were aged between 12 and 32 years.

We found under-nutrition to be an important risk factor for tubercular peripheral lymphadenopathy (Odds ratio = 4.48). About 69% of the TBLN cases had a BMI < 18.5, which is concordant with most of the existing literature. [11,12] Evaluation regarding the presence of diabetes mellitus and HIV co-infection revealed the prevalence of HIV to be 2.12% and that of DM to be 3.2%. We found no co-existence of HIV and DM. Sridhar CB and co-workers in a Bangalore-based study reported 42% prevalence of HIV and 13% prevalence of DM in tubercular lymphadenopathy.[13] Daniela E. Kirwan reported similar data in a study conducted in Lima, Peru.[14] The probable reasons behind the comparatively low prevalence of HIV and DM in our study were: (1) It was a small population-based study, and (2) the majority of our study population were young females not having a sedentary lifestyle, which is a major risk factor for developing diabetes mellitus.[15]

All 40 TBLN patients in the presumptive group came up with Mantoux test (5 PPD) positivity, with a mean induration diameter of 22 mm, SD = 4.82 (95% CI ± 6.8%). The reported reactivity of the tuberculin test in the literature has ranged from none to very strong. [16] Furcolow[17] and Johnston[18] showed 99% positive test results, while Smith reported 20–30% negative reactions.[19] A large number of factors have been reported to cause such variability in tuberculin reactivity.[20]

Lymph node measurements were taken by USG-neck. The largest diameter of the largest affected lymph nodes was considered. Mean diameter was 2.85 cm, SD = 0.66 (95% CI ± 4.70%). A similar finding was reported by Ahmad Z and co-workers, who reported a statistically significant association of >1 cm diameter lymph nodes with tuberculosis. The cervical group of lymph nodes was most commonly affected (98%). Among them, 61.9% involved the right side of the neck. Level V (posterior triangle) lymph nodes were the maximally affected group (42.4%), bilaterally. The presence of multiple and/or matted lymph nodes was significantly noted in tubercular lymphadenopathy. Similar data was reported by Kamal SM et al., who reported the most commonly involved lymph node group as level V (59.4%), followed by level II (42.2%), and a predominance of multiple and matted lymph nodes.[21] About 51% of TBLN patients had systemic symptoms, and the commonest of all was low-grade fever (77%),

followed by weight loss (13%). Nearly similar findings were reported by Kamal SM et al.[21]

We found the prevalence of RR/MDR to be 5.3%, which is higher than that reported by WHO in 2013.[22] The increasing burden of RR/MDR-TB strains in the population along with improper use of ATDs (Anti-Tubercular Drugs) are the most probable causative factors.

In our resource-poor, tuberculosis-endemic country, patients usually get treated with anti-tubercular therapy based on CRS positivity, in concordance with RNTCP 2016 guidelines.[23] Smear cytology and AFB screening from FNA samples have a poor diagnostic yield. Granulomatous inflammation with or without necrosis is not an absolute indicator of tubercular lymphadenopathy; it can be present in various other conditions. On the other hand, tubercular lymphadenopathy can present with only reactive changes of the lymph node. It is a paucibacillary disease, and AFB is not always detected by smear microscopy. [24] If detected at all, it does not essentially always indicate "active" tubercular lymphadenopathy - it can represent dead tubercular bacilli or NTM (Non-Tubercular Mycobacterium). Xpert MTB/RIF is a good rapid diagnostic modality for detecting tubercular mycobacteria and its molecular resistance to rifampicin, and NTM diagnosis can be readily excluded. However, Xpert MTB/RIF amplifies tubercular mycobacterial DNA, whether from live or dead bacilli, thus failing to confirm the diagnosis of "active" tubercular lymphadenopathy.

AFB BACTEC Culture is a liquid culture method that detects the growth of viable mycobacterium. Hence, not only can it detect the presence of live Mycobacterium tuberculosis bacilli, but detection of NTM infection with proper species identification is also possible. If mycobacterial tuberculosis growth is detected, drug susceptibility testing can be performed subsequently.[9] Culture from lymph node excision biopsy is considered the gold standard for detection of active tubercular lymphadenopathy. However, in a recent meta-analysis, Guocan Yu et al. reported that there is no significant difference in diagnostic efficiency for specimens obtained via well-performed FNA versus excision biopsy.[25]

In this study, we found the diagnostic yield of Xpert MTB/RIF to be 85% in CRS+ cases, the highest among all the diagnostic modalities used for detecting tubercular peripheral lymphadenopathy. There was no statistically significant variation of this diagnostic yield among the presumptive, partial responder, and presumptive relapsed categories [p = 0.0998]. In the CRS+ presumptive group of patients, AFB BACTEC culture also yielded a good diagnostic result of 72.5%, without any statistically significant difference from that of Xpert MTB/RIF [p = 0.170]. However, there were statistically significant differences between the diagnostic yield of Xpert MTB/RIF and AFB BACTEC culture in the CRS+ partial responder [p = 0.00028] and relapsed categories [p = 0.00026]. We did not find similar comparative data regarding evaluation of tubercular peripheral lymphadenopathy in the existing literature.

Why is this significant difference observed?

The answer probably lies within the basic principles of Xpert MTB/RIF and AFB BACTEC culture. Xpert MTB/RIF amplifies and detects tubercular mycobacterial DNA, whether from live or dead bacilli.[26] On the other hand, AFB BACTEC culture detects only the growth of viable mycobacterium.[27] A possible and minor drawback of the automated AFB BACTEC culture system is that there could be very few "false negative" cases due to the "low fitness" and "very slow" growth rate of some strains of tubercular bacilli, some of which could also carry resistant mutations.[28,29,30] Considering these facts, we hypothesize that, unlike presumptive cases, in partial responder and presumptive relapsed cases of tubercular peripheral lymphadenopathy, the lymph node lesion is not always due to live tuberculosis bacilli. There could be a considerable proportion of patients in the partial responder and presumptive relapsed categories who have peripheral lymphadenopathy only due to a hypersensitivity reaction to dead tubercular DNA particles – hence, they are detected by Xpert MTB/RIF but not by AFB BACTEC culture. To confirm this hypothesis, a study comprising a larger number of cases along with follow-up is required, which was beyond the scope of this study. We also found no statistically significant correlation between AFB BACTEC positivity and the duration elapsed since last ATD intake [p = 0.34], a finding consistent with a study conducted by Theron and co-workers.[26]

CONCLUSION

This study highlights the significant burden of peripheral tubercular lymphadenopathy and underscores the importance of accurate and timely diagnosis and proper treatment initiation. Fine needle aspiration remains a valuable rapid initial diagnostic tool; however, smear AFB microscopy alone demonstrates limited sensitivity. The incorporation of culture methods improves detection but is time-consuming, whereas Xpert MTB/RIF offers superior diagnostic yield with rapid detection of Mycobacterium tuberculosis and simultaneous identification of rifampicin resistance. The presence of RR/MDR-TB among study cases further emphasizes the need for early molecular testing to guide appropriate treatment strategies.

Every coin has two sides. Having the highest diagnostic yield, Xpert MTB/RIF positivity undoubtedly is the strongest indicator for ATT initiation in treatment naïve

presumptive peripheral TBLN cases. But, in partial responders and presumptive relapsed cases [patients already exposed to anti-tubercular drug therapy (ATT)], AFB-culture positivity might be the considered as the most reliable indicator for ATT re-initiation. This would decrease the unnecessary use of ATDs, so that the drug related adverse effects and emergence of drug resistance as well.

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