Panacea Journal of Medical Sciences 2024;14(3):748–752

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Panacea Journal of Medical Sciences

Journal homepage: http://www.pjms.in/

Original Research Article

Precision in practice: Comparative evaluation of cytomorphological findings, acid-fast bacilli staining and cartridge-based nucleic acid amplification test for diagnosing suspected cervical tubercular lymphadenopathy

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ARTICLE INFO

Article history:
Received 05-02-2024
Accepted 28-02-2024
Available online 21-12-2024

Keywords:
Extra Pulmonary Tuberculosis
Granulomatous lymphadenitis
Cervical lymph nodes

ABSTRACT

Introduction: India has the highest burden of tuberculosis with an incidence of 2,590,000 in 2021. Extrapulmonary TB constitutes 15-20% of all TB cases with the lymph node being the most common site. Due to its paucibacillary nature, EPTB is a diagnostic and therapeutic challenge.
Objectives: Comparing FNAC with AFB staining and CB-NAAT for diagnosing cervical tubercular lymphadenopathy.
Materials and Methods: A study was conducted at Dr. B.R. Ambedkar Medical College and Hospital, Bangalore from Oct 2021 to Jan 2023. 102 suspected cases of cervical tubercular lymphadenopathy were included. Smears were made with FNAC aspirates, stained with H&E, PAP, and AFB, and the remaining aspirate was sent for the CB-NAAT test.
Results : In 102 cases, the most affected age group is 21-30 years with more females. Most cases were seen in level V. The 46 cases showed granulomatous lymphadenitis with necrosis. The highest number of CBNAAT and AFB positivity is seen in necrotizing lymphadenitis. CBNAAT showed the most positivity in caseous (87.5%) and the least in blood mixed (35.38%). Overall, CBNAAT showed positivity in 54 cases and AFB showed positivity in 19 cases. CBNAAT demonstrated greater sensitivity (81.97%) than AFB (31.15%) while maintaining high specificity (90.2%) and positive predictive value (92.6%).
Conclusion: CBNAAT is a newer and more sensitive diagnostic test for extrapulmonary tuberculosis as compared to FNAC. It is recommended to use CBNAAT in addition to smear microscopy and cytology in FNAC specimens for early and accurate diagnosis and prompt treatment of EPTB.

1. Introduction

Before the COVID-19 pandemic, Tuberculosis (TB) caused more deaths than any other single infectious agent, including HIV/AIDS.[1] As of 2021, the number of TB patients notified has increased by 19% compared to 2020.[2]

Tuberculosis (TB) is an ancient bacterial disease affecting humans, caused by Mycobacterium tuberculosis. It manifests in two main types: pulmonary TB (PTB) and extrapulmonary TB (EPTB). Extrapulmonary TB occurs when the infection affects organs outside the lungs, with the lymph nodes being the most frequently affected site.[3] EPTB cases constitute approximately 15-20% of all TB cases.[4]

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https://doi.org/10.18231/j.pjms.2024.133

The most common manifestation of mycobacterial infections is cervical lymphadenitis,[5] and TB of the head and neck can be difficult to diagnose due to a large number of smear-negative cases. This often leads to positive cases being missed, which increases the burden of TB.[6]

Conventional methods for diagnosing EPTB include fine-needle aspiration cytology of lymph nodes and demonstrating acid-fast bacilli (AFB) using Ziehl-Neelson (ZN) staining.[7] Fine-needle aspiration cytology of lymph nodes can be presumptively diagnosed with tuberculous lymphadenitis morphologically.[8] The cartridge-based nucleic acid amplification test (CBNAAT) is a real-time polymerase chain reaction (PCR) assay designed to swiftly detect the presence of Mycobacterium tuberculosis and rifampicin resistance in biological samples, providing results within a two-hour timeframe. Acknowledged by the World Health Organization (WHO) in December 2010, this technology stands as a pivotal advancement in global tuberculosis (TB) control efforts.[9]

2. Materials and Methods

The prospective study was done at DR B. R Ambedkar Medical College & Hospital, Bangalore from October 2021 to January 2023. The study consists of clinically suspected cervical tubercular lymphadenopathy patients referred from various departments of this hospital for FNAC.

2.1. Inclusion criteria

Patients aged above 18 years and below 65 years who are clinically suspected with cervical tubercular lymphadenopathy.

2.2. Exclusion criteria

1. Individuals with a confirmed history of tuberculosis.
2. Patients currently undergoing anti-tubercular drug treatment.
3. Cases where aspirates or samples were insufficient for a cytopathological diagnosis.
4. Patients who declined to provide consent for participation.

2.3. Study procedure

Prior to conducting Fine-Needle Aspiration Cytology (FNAC), the patients were provided with an informed consent form in the language of their choice. Their clinical history and relevant investigations were thoroughly reviewed. During the Fine Needle Aspiration Cytology (FNAC) procedure, a 22-gauge needle, coupled with a 10 ml syringe, was employed to ensure the collection of sufficient and representative samples. Adequate smears were made and immediately fixed and prepared for cytomorphological analysis using Hematoxylin and Eosin (H&E) and Papanicolaou stain (PAP). One smear was air-dried and stained for acid-fast bacilli while the remaining aspirate was rinsed into a Falcon tube with 5 ml of normal saline and processed for CB-NAAT testing. The procedures were executed in adherence to the operator manual specifications outlined by the Central TB Division, Government of India, for the Gene Xpert system. The assay sample reagent, comprising a mixture of sodium hydroxide and isopropanol in a 2:1 ratio, was meticulously introduced to the sample. Following a 15-minute incubation period at room temperature with periodic agitation, 3ml of the processed sample was meticulously loaded into the cartridge and seamlessly integrated into the CB-NAAT instrument module. The subsequent assay steps and conclusive results were automatically presented on the Gene Xpert monitor approximately 1 hour and 50 minutes after initiation.[10]

2.4. Ethics

The study was conducted only after receiving approval from the Institutional Ethics Committee (IEC), with reference number 158.

2.5. Statistical analysis

Microsoft Word and Excel generated graphs, tables, etc. Data was coded and entered into a statistical package for social science (SPSS) software version 20.00 and analyzed.

3. Results

A total of 102 cases underwent FNAC based on clinical and radiological findings. Females were the majority (Table 1) and the most common age group was 21-30 [Figure 1].

Table 1: Table showing gender and number of cases.

Gender	Number of cases
Male	42
Female	60

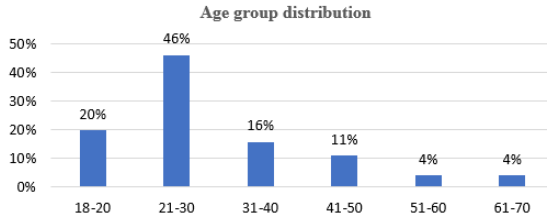

Figure 1: Graph illustrating affected age groups.

Upon examination, all cases showed lymph node enlargement. Of these, 63 cases were discrete, and 39 were matted. Most cases were found in level V, as shown in [Table 2].

Table 2: Distribution of level of involvement of lymph nodes.

Level of lymph node	No. of cases
I	13
II	5
III	14
IV	26
V	44

During aspiration, most cases showed blood mixed with aspirate. The CBNAAT and AFB tests were more positive in the caseous aspirate. [Table 3]

Table 3: Types of aspirates with CBNAAT and AFB positivity.

Types of aspirates	Number	No of CBNAAT positive cases	No AFB-positive cases
Blood mixed	65	23	6
Purulent	29	24	8
Caseous	8	7	5

Among cytomorphological findings of 102 cases [Table 4], 46 cases showed granulomatous lymphadenitis with necrosis [Figure 2],12 cases showed granulomatous lymphadenitis without necrosis [Figure 3] and 3 cases showed necrotizing lymphadenitis [Figure 4].

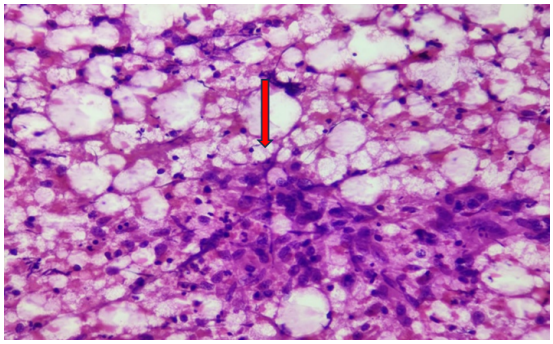

Figure 2: FNAC finding showing granulomatous lymphadenitis with necrosis. (H &E stain 400X)

In this study, among 102 cases 54 cases showed CBNAAT positive, and 19 cases showed AFB positive [Figure 5].

In total, CBNAAT exhibited positivity in 54 cases, while AFB demonstrated positivity in 19 cases. In comparison to FNAC, CBNAAT displayed a sensitivity of 81.97%, a specificity of 90.2%, a positive predictive value of 92.6%, and a negative predictive value of 77.1%. In contrast, AFB exhibited a sensitivity of 31.15%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 49.4%.

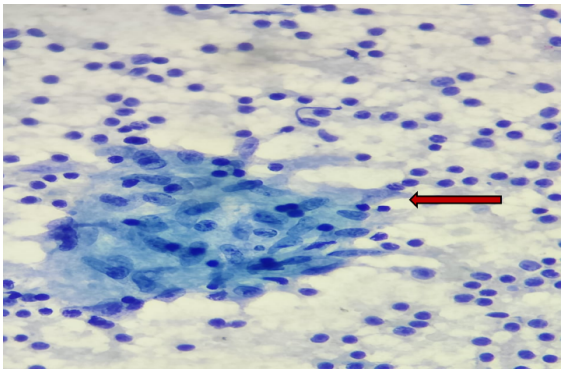

Figure 3: FNAC findings are showing granulomatous lymphadenitis without necrosis. (PAP stain,400X)

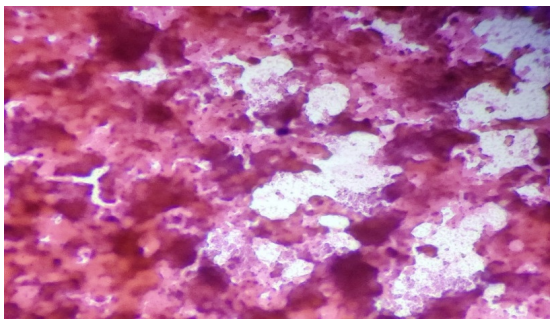

Figure 4: FNAC shows wide areas of necrosis. (PAP stain,100X)

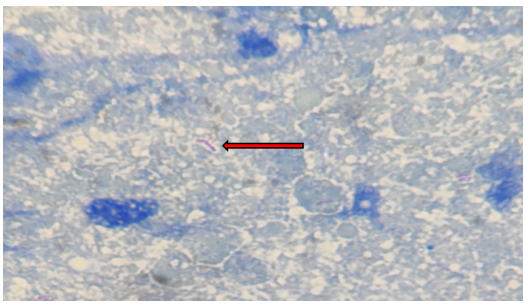

Figure 5: FNAC findings show the presence of AFB against a necrotic background in ZN staining. (Oil immersion,1000X)

4. Discussion

This prospective study, conducted at a tertiary care hospital, aimed to diagnose cervical tubercular lymphadenitis by comparing FNAC with AFB staining and CBNAAT. FNAC, recognized as the primary diagnostic test, holds significance in detecting tubercular lymphadenitis.[17]

In this study, we have included only cervical lymph nodes as it is the most common site of EPTB.[3]

This present study shows the greatest number of cases affected in the 21-30 age group with female preponderance. This is similar to studies conducted by Mulualem et al, Bryan Rock et al and Adhikary et al[11,12,18] [Table 5].

Table 4: Cytomorphological findings with CBNAAT and AFB positivity.

Cytomorphological findings	Number of cases	Percentage	No of CBNAAT positive cases	No AFB P ositive cases
Granulomatous lymphadenitis with necrosis	46	45.09 %	39	14
Granulomatous lymphadenitis without necrosis	12	11.76 %	8	3
Necrotizing lymphadenitis	3	2.94 %	3	2
Reactive lymphadenitis	25	24.5%	2	0
Lymphoproliferative disorder	5	4.90%	0	0
Mets	5	4.90%	0	0
Suppurative inflammatory disease	4	3.92%	2	0
Chronic lymphadenitis	2	1.96%	0	0

Table 5: Compares age group and sex preponderance with the other studies.

Study	Period	Age group	% of cases	Females
Present study	Oct 2021 – Jan 2023	21-30	46%	58.82%
Mulualem et al	May-Sept 2013	16-30	58%	53.1%
Bryan Rock et al [11]	Jan1993-Dec 2003	15-24	43%	54%
Adhikary et al [12]	Mar 2021- Feb 2022	21 - 30	27.85 %	59.49 %

Table 6: Compares the sensitivity and specificity of the present study with the other studies.

Study	Study Period	Sensitivity	Specificity
Present study	October 2021-January 2023	81.97%	90.2%
Arpitha et al [13]	July 2019- June 2020	80%	86%
Suwarna B. Patil et al [14]	January 2019 - December 2019	55.5 %	83.80%
Lavanya et al [15]	April 2018 to March 2019	28.75%	88.7%
Komanapalli SK et al [16]	April 2017- March 2018	84.25 %	86.71%

All 102 cases showed lymph node enlargement and the highest number of patients presented with discrete swelling (61.76 %) and the majority of the lymph nodes were seen in level V (43.14 %). These findings are comparable with the study done by Sreenidhi et al. [19]

While analyzing the types of FNAC aspirates, CBNAAT showed the most (87.5%) positivity in caseous aspirate and the least (35.38%) in hemorrhagic aspirate. This is similar to the study conducted by Tadesse M et al which shows the most (69%) positivity in caseous aspirate and the least (41.7 %) in hemorrhagic aspirate. [18] Hence CBNAAT is less sensitive to blood-mixed samples.

In the study conducted by Khare et al 2014, smears from lesions having only caseous necrosis showed maximum AFB positivity (94.1%) which is comparable with our study which also showed maximum AFB positivity (62.5%) in caseous aspirate. [20]

The presence of clusters of epithelioid cells, with or without giant cells and/ or caseous necrosis is the diagnostic criteria for tuberculosis in FNAC. [21] This study shows 100% CBNAAT positivity in necrotizing lymphadenitis which is similar to the findings in the study done by Kalyani Gouda et al [22] and Anjali R et al. [23]

Out of 102 cases, FNAC is positive in 61 cases and confirmed in 19 cases by AFB and 50 cases by CBNAAT. Among 41 FNAC-negative cases, CBNAAT could detect Tb in 3.9 % of cases. Thus, CBNAAT showed its importance in finding out missed cases among FNAC and AFB negative cases.

This present study showed sensitivity of CBNAAT is 81.97% which is more than that of AFB (31.15%). Similar findings are also noted in studies conducted by Lavanya et al and Suwarna B. Patil et al. [14,15]

This study also showed specificity of CBNAAT is more than that of its sensitivity. These are comparable with the studies conducted by Arpitha K et al, Lavanya et al Suwarna B. Patil et al and Komanapalli SK et al [13–16] [Table 6].

5. Conclusion

According to this study, CBNAAT is more effective than AFB in detecting EPTB in FNAC missed cases. Moreover, CBNAAT is more sensitive than AFB. While culture is considered the gold standard test for EPTB diagnosis, CBNAAT has a faster turnaround time. Therefore, in countries like India, where the burden of TB is high, CBNAAT can be used as a rapid diagnostic test to prevent

underdiagnosis and provide early treatment.

6. Source of Funding

None.

7. Conflict of Interest

None.

8. Acknowledgement

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Cite this article: Anushree C N, Choudhary S, Sanghamitra Maharana S, Nair BS, Krisha M, Raju A. Precision in practice: Comparative evaluation of cytomorphological findings, acid-fast bacilli staining and cartridge-based nucleic acid amplification test for diagnosing suspected cervical tubercular lymphadenopathy. *Panacea J Med Sci* 2024;14(3):748-752.