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An examination of the cytomorphological trends in tuberculous lymphadenitis at a tertiary care hospital of southern Assam

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Abstract

Introduction: Tuberculous lymphadenitis is a leading cause of lymph node enlargement accounting for 195 per 1,00,000 population in India. Fine needle aspiration cytology (FNAC) is rapid and economical compared to other tests and thus plays a crucial role in diagnosing this condition. It prevents unnecessary biopsy of lymph nodes and it can be used for collection of material for cytomorphological and bacteriological examination. This study aimed to assess the cytomorphological patterns of tuberculous lymphadenitis and correlate them with Ziehl-Neelsen (ZN) staining. Primary aim is to assess the cytomorphological patterns of tubercular lymphadenitis and to correlate with the bacteriological results using ZN staining.

Materials and methods: The FNAC results of 100 cases diagnosed with tuberculous lymphadenitis over a period of one year were analyzed from the cytopathology section of Silchar Medical College and Hospital. The findings were classified into three patterns: pattern A - epithelioid granuloma in absence of caseous necrosis, pattern B - epithelioid granuloma with caseous necrosis, and pattern C - caseous necrosis in absence of epithelioid granuloma. The cytomorphological patterns were then correlated with acid-fast bacilli (AFB) positivity.

Results: In individuals between the ages of 21 and 30, tuberculous lymphadenitis was predominantly observed. The cervical lymph node (92%) was the most frequently affected area. Among the different patterns of the condition, Pattern B, which is characterized by the presence of epithelioid granuloma along with caseous necrosis, was found to be the most common (53%). In contrast, Pattern C, which is marked by caseous necrosis without the presence of epithelioid granuloma, exhibited the highest positivity for acid-fast bacilli (80%). The difference in AFB positivity among the patterns was statistically significant (P-value= 0.0003).

Conclusion: FNAC is an effective and economical diagnostic tool for tuberculous lymphadenitis, particularly in resource-limited settings. The study found that Pattern B (epithelioid granuloma with caseous necrosis) was the most common, while Pattern C (caseous necrosis without epithelioid granuloma) exhibited the highest AFB positivity. FNAC, combined with ZN staining, enhances the accuracy of tuberculosis diagnosis, minimizing the need for invasive biopsies. Given the high prevalence of tuberculosis, FNAC should be the first-line investigation for patients presenting with superficial lymphadenopathy, ensuring timely diagnosis and treatment.

Keywords: Cytomorphological patterns, Tuberculous lymphadenitis, Ziehl-Neelsen (ZN) staining, Acid-fast bacilli

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Introduction

Tuberculosis is very common in India accounting for 195 per 1,00,000 population (1). Lymphadenitis is the most common clinical presentation of extrapulomonary tuberculosis with tubercular lymphadenitis accounting for 43% cases in developing countries like India. Diagnosis of tuberculosis can be done by various diagnostic methods as fine needle aspiration cytology, biopsy, Acid fast bacilli culture and polymerase chain reaction .(1) FNAC is a widely used cytological technique for diagnosing tubercular lymphadenitis offering a sensitivity and specificity of 88-96% and is most cost effective and rapid compared to other tests like AFB culture and PCR (2). FNAC can detect tubercular lymphadenitis even in cases where the bacterial load is low or when the disease is in its early stages.

The sensitivity increases significantly when FNAC is combined with Ziehl-Neelsen staining for acid-fast bacilli .The presence of epithelioid cell granulomas, caseous necrosis, and Langhans giant cells on cytology strongly points to a tubercular etiology, leading to accurate diagnosis and exclusion of other causes of lymphadenopathy (e.g., lymphoma, metastatic carcinoma).

It also minimises unnecessary lymph node biopsies and providing material for cytopathological and bacteriological analysis (3).

Its ability to provide rapid cytological and bacteriological insights makes it invaluable in early diagnosis and management of TB, especially in high-burden and resource-limited settings. Combining FNAC with other tests like ZN staining further enhances its diagnostic yield and clinical utility.

To diagnose tuberculosis cytologically, the identification of epithelioid cell granuloma with or without necrosis is essential (4-7). A confirmed diagnosis is achieved by identifying the presence of acid-fast bacilli by doing ZN staining.

This study undertakes a comprehensive examination of the cytomorphological patterns of tubercular lymphadenitis, with a specific focus on correlating FNAC findings with bacteriological confirmation via ZN staining. This study aims to thoroughly analyze the cytomorphological patterns observed in tubercular lymphadenitis using FNAC. The goal is to identify and describe the specific cellular changes and features such as granulomas, caseous necrosis, and giant cells seen in FNAC smears from patients with tubercular lymphadenitis.

Additionally, the study seeks to establish a correlation between the FNAC findings and bacteriological confirmation of tuberculosis through ZN staining. A positive correlation would mean that the cytological features identified in FNAC are consistent with the presence of AFB as confirmed by ZN staining, thereby strengthening the diagnostic value of FNAC for tubercular lymphadenitis.

Materials and methods

The present study was undertaken to assess the cytomorphological patterns of tubercular lymphadenitis and to correlate with the bacteriological results using Ziehl-Neelsen (ZN) staining.

This study was conducted in the Department of Pathology, Silchar Medical College and Hospital, Silchar, Assam, India. The Institutional Ethics Committee approved the study (No.SMCH/ETHICS/M2/2024/46) on 30/08 /2024. The study is compliant with the Helsinki Declaration's ethical guidelines.

Study period

1 year period: From August 2023 to July 2024

Source of data and sample size

100 patients were diagnosed with tuberculous lymphadenitis based on FNAC of peripheral lymphadenopathy and the data were collected from the registers of the cytopathology section of the Pathology department of Silchar Medical College and Hospital. The FNAC slides stained with MGG and ZN stains were reviewed.

Inclusion criteria

Clinically suspected cases of tubercular lymphadenitis and those not on anti tuberculosis treatment (ATT)

Exclusion criteria

- On anti-tubercular treatment.

- FNAC samples yielding inadequate or non-diagnostic material.

Parameters studied

- Detailed clinical history and other investigations of the patients were recorded after taking patient consent.
- Hospital records of the patients.
- Microscopic examination of the FNAC slides.

This study, conducted from 2023 to 2024 at Silchar Medical College and Hospital, Assam, involved 100 patients diagnosed with tuberculous lymphadenitis through FNAC of peripheral lymphadenopathy. Data were collected from cytopathology registers, and FNAC slides stained with MGG and ZN stains were reviewed. Characteristic cytomorphological features, including epithelioid granuloma with or without caseous necrosis and ZN staining, confirmed the diagnosis.

The following techniques were performed in the preparation of FNAC (Fine Needle Aspiration Cytology) slides:

Fine needle aspiration cytology (FNAC) procedure

1. Preparation for the procedure

1.1 Materials required

- **Syringe:** Disposable plastic syringe (10-20 ml capacity)
- **Needle:** 20-22 gauge needle (length varies based on tumor site)
- **Antiseptic solution:** For cleaning the puncture site
- **Glass slides:** For smear preparation
- **Staining reagents:** May-Grunwald Giemsa (MGG) and Ziehl-Neelsen (ZN) stains

1.2 Patient Preparation

- The patient is **positioned comfortably** and reassured to reduce anxiety.
- No local anesthesia is required as FNAC is a minimally invasive procedure.
- The target mass is localized by palpation, and a suitable puncture site is chosen.

2. Aspiration procedure

2.1 Needle insertion and aspiration

1. The skin around the target site is thoroughly cleaned with an antiseptic solution.
2. The lesion is held firmly in place with one hand while the needle is inserted at the predetermined site.
3. Once the needle enters the lesion, the syringe plunger is retracted to create negative pressure for aspiration.
4. The needle is moved within the mass (3-4 oscillations in different directions) to obtain an adequate sample.

2.2 Withdrawal and sample handling

5. Before withdrawing the needle, the plunger is released to equalize pressure, preventing blood contamination.
6. The needle is then removed, and detached from the syringe, and air is drawn into the syringe to facilitate sample expulsion.
7. The needle is reattached, and the aspirated material is expelled onto a clean glass slide.

3. Preparation of Smears

3.1 Spreading the aspirate

- The aspirate is examined macroscopically.
- Semi-solid aspirates are spread using a thick Burker-type cover slip.
- Tissue fragments are gently crushed for uniform distribution.

4. Staining Procedure

4.1 May-Grunwald Giemsa (MGG) Staining

1. The **air**-dried smear is fixed in methanol.
2. May-Grunwald stain is applied for 5-7 minutes, followed by Giemsa stain for 10-15 minutes.

3. The slide is rinsed with buffered water until the excess stain is removed.
4. The slide is then air-dried and ready for microscopic examination.

4.2 Ziehl-Neelsen (ZN) Staining for Acid-Fast Bacilli (AFB)

4.2.1 Primary staining

1. The air-dried and heat-fixed smear is flooded with carbol fuchsin stain.
2. The slide is gently heated until steam appears (without boiling) to ensure dye penetration.
3. It is left to stand for 5-10 minutes to allow effective staining.

4.2.2 Decolorization

1. The slide is washed with water.
2. It is decolorized using acid-alcohol (20% sulfuric acid or 3% hydrochloric acid in ethanol) until the red stain fades.
3. The slide is rinsed again with water.

4.2.3 Counterstaining

1. Methylene blue or malachite green is applied for 1-2 minutes to provide a contrasting background.
2. The slide is washed, air-dried, and ready for microscopic evaluation.

5. Slide Interpretation and reporting

5.1 Cytomorphological classification

The FNAC results are classified into three patterns:

- **Pattern A:** Epithelioid granuloma without necrosis
- **Pattern B:** Epithelioid granuloma with necrosis
- **Pattern C:** Necrosis without epithelioid granuloma, with neutrophilic infiltration

5.2 Statistical Analysis

- **Software Used:** SPSS (Version 21)
- **Test performed:** Chi-square test to correlate cytomorphological patterns with AFB positivity.
- **Significance level:** A **p-value < 0.05** was considered statistically significant.

Results

Out of the 100 patients studied, 42 (42%) were male and 58 (58%) were female, indicating a slight female predominance, with a male-to-female ratio of 1:1.4. The ages of the patients ranged from 1 to 71 years, with an average age of 29.28 years. The disease was most prevalent in the age group of 21 to 30 years, followed by the 11 to 20 years age group (Table 1). Cervical lymph nodes were the most commonly affected, involved in 92% of cases, while inguinal lymph nodes were affected in 8% of cases.

Table 1. The patient information.

		Total (%)
Gender	Male	42
	Female	58
Age group	01-10 years	8
	11-20 years	16
	21-30 years	38
	31-40 years	13
	41-50 years	12
	51-60 years	5
	61-70 years	6
	71-80 years	2

Three distinct cytopathological patterns were identified: Pattern A, which consisted of epithelioid granuloma in the absence of caseous necrosis; Pattern B, which included epithelioid granuloma with necrosis; and Pattern C, characterized by caseous necrosis without epithelioid granuloma (refer to Table 2 and Figures 1 A, B, and C).

Among these patterns, Pattern B was the most prevalent, observed in 53 cases out of 100, representing a partially effective immune response where granulomas are present, but necrosis indicates tissue destruction and active bacterial replication.

Pattern A was the second most prevalent, observed in 42 cases, thus representing a contained immune

response where macrophages are effectively forming granulomas to control bacterial proliferation.

The least prevalent was pattern C, observed in 5 cases, reflecting a defective immune response where the absence of granuloma formation allows bacterial proliferation and leads to extensive tissue damage.

A Ziehl-Neelsen stain for acid-fast bacilli, indicative of tuberculosis, revealed AFB positivity in 47 cases. The distribution of AFB positivity across the three cytopathological patterns was as follows: Pattern B had 33 positive cases out of 53 (62.3%), Pattern A had 10 positive cases out of 42 (23.8%), and Pattern C had 4 positive cases out of 5 (80%). Thus the highest percentage of AFB positivity was seen in Pattern C, where there was no epitheloid granuloma formation indicating a ineffective immune response and thus increased bacillary load.

The differences in AFB positivity among the patterns were statistically significant, with a P value of 0.0003 and a Chi-square value of 16.211.

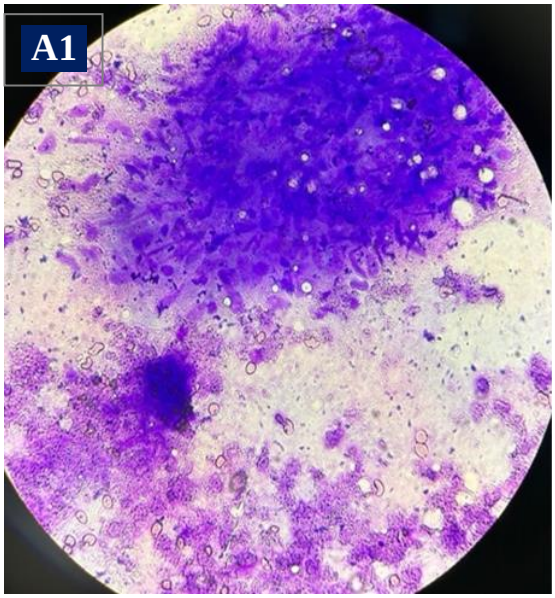
The statistically significant differences confirm that Pattern C is strongly associated with high bacillary load and poor immune control. The lower AFB positivity in Pattern A reflects an effective immune response, while the higher AFB positivity in Pattern B and Pattern C reflects progressive or poorly contained infection.

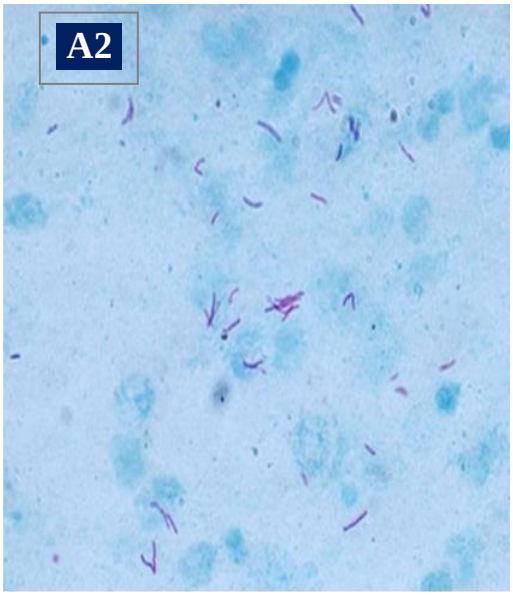
Pattern B's predominance suggests that most cases of tubercular lymphadenitis involve an active immune response with ongoing tissue destruction.

The high AFB positivity in Pattern C highlights the need for aggressive antitubercular therapy in cases where granuloma formation is absent, as these cases are likely to have higher bacterial loads.

The low AFB positivity in Pattern A underscores the protective role of granuloma formation in containing TB infection.

This analysis supports the use of FNAC not only for diagnosis but also for predicting disease severity and guiding treatment strategies.





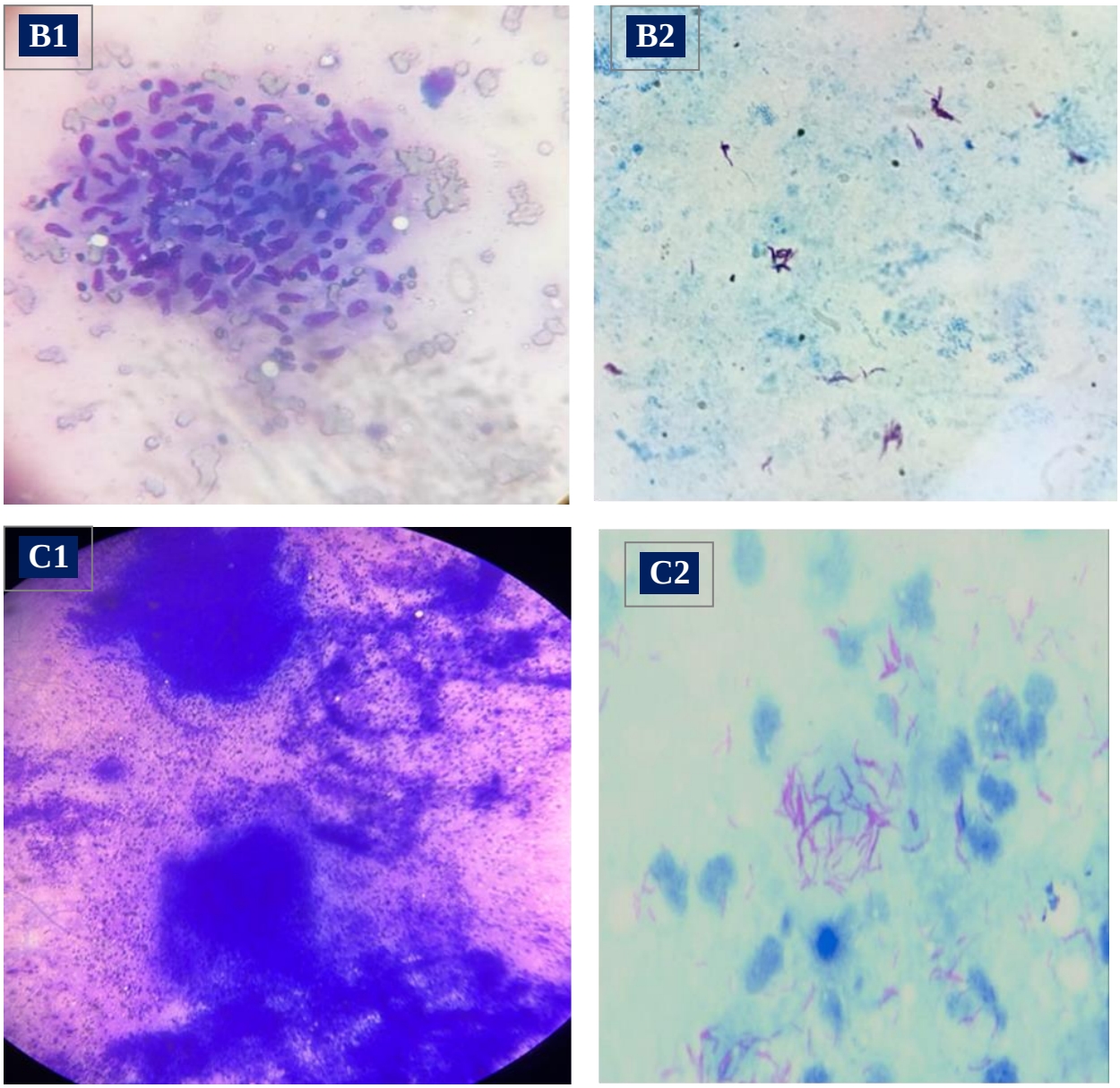


Figure 1. Cytomorphological pattern of tubercular lymphadenitis. A1 and A2: PATTERN B- Epithelioid granuloma with necrosis and its AFB positivity. B1 and B2: PATTERN A- Epithelioid granuloma in absence of necrosis and its AFB positivity. C1 and C2: PATTERN C- Caseous necrosis in the absence of epitheloid granuloma and its AFB positivity.

Table 2. Cytomorphological patterns of tubercular lymphadenitis.

Cytopathological pattern	Total no. of cases	AFB positive cases	AFB negative cases	AFB positivity (%)
Pattern B	53	33	20	62.3%
Pattern A	42	10	32	23.8%
Pattern C	5	4	1	80%
Total	100	47	53	
		P value= 0.0003		

Discussion

FNAC is the primary method for reliably diagnosing tubercular lymphadenitis in patients who present with superficial lymphadenopathy, as it is simple, safe, and cost-effective (8). In our study, the mean age of presentation and the age group exhibiting the highest incidence of tubercular lymphadenitis is consistent with studies conducted by Bhatta S et al. and Hemalatha A et al (9,10) , where the most frequent age group effected was 21-30 years .There was a slight

female predominance(Male: Female-1.4:1) , which aligns with results from Purohit MR, et al (4) (Male: Female- 1.5:1) and Polesky et al. (female to male ratio of 1.9:1) (16).

The most commonly involved lymph node in our study was the cervical lymph node(92%) which corresponds with the study by Bezabith M et al., which reported a 74.2% involvement of lymph nodes, and the research by Paliwal N et al., which indicated a 90% involvement rate (8,13).

The most commonly observed pattern was Pattern B(53%), epithelioid granuloma with caseous necrosis, which was also the most prevalent in studies by Bhatta S et al (9) (53.17%) and Khanna A et al (14) (50.5%). The least common pattern in our study was caseous necrosis without epithelioid granuloma (5%), observed.

The overall positivity for Acid-Fast Bacilli in our study was 47%. An inverse relationship was found between AFB positivity and the presence of granuloma: the highest AFB positivity was seen in smears containing only necrosis and neutrophilic infiltrates, while the lowest was found in smears with only epithelioid granulomas. The patient's cell-mediated immunity triggers a granulomatous response against tubercle bacilli, resulting in lower AFB positivity in smears showing epithelioid granuloma without necrosis. In contrast, smears containing only necrotic material demonstrated higher AFB positivity due to a compromised immune response and the absence of a granulomatous reaction. Paliwal N et al. and Bezabith M et al. reported overall AFB positivity rates of 71% and 59.5%, respectively (9,14,15). The relatively low AFB positivity in our study may be attributed to the predominance of epithelioid granuloma with or without necrosis. Repeating Fine Needle Aspiration Cytology may improve AFB positivity rates. The association between AFB positivity and the three cytomorphological patterns in our study was statistically significant ($p = 0.0003$).

The clinical outcomes can vary based on the patterns and their findings as discussed below:

1. Granuloma with Caseous Necrosis: This pattern indicates an active immune response to the infection. It generally has a favorable prognosis, as it responds well to anti-tuberculosis therapy (ATT). The presence of granulomas and caseous necrosis suggests that the body is successfully containing the infection, leading to a complete resolution with treatment. Relapse rates tend to be low if the treatment is completed properly.

2. Granuloma without Caseous Necrosis: This may indicate an early or less severe form of the infection, where the immune system is managing to contain the bacteria before extensive tissue damage occurs. The prognosis is still good because these cases usually respond well to ATT, and there is often less tissue damage to repair. However, close follow-up is essential to ensure that the infection does not progress to the caseous necrosis stage.

3. Necrosis without Granuloma: This pattern may suggest a compromised or ineffective immune response, as the typical granuloma, which encloses the infection, is absent. This scenario may arise in cases with a high bacillary load or in immunocompromised patients. The prognosis for these cases is generally poorer compared to the other patterns, as there is a higher risk of infection spread and complications. Treatment response can be slower, and these cases require more intensive monitoring and longer follow-up to ensure complete resolution of the infection.

The following limitations must be acknowledged in this study:

1. **Restricted Study Population** – The study only included cases from a single institution (Silchar Medical College and Hospital), limiting its generalizability to a broader population. Cases from private hospitals and rural healthcare centers were not considered.
2. **Exclusion of Certain Diagnostic Methods** – Patients who underwent additional investigations such as CBNAAT could not be included due to a lack of traceability, which may have impacted the study's ability to compare different diagnostic techniques.

These limitations suggest that while the study provides valuable insights, a more comprehensive, multi-center approach could enhance its applicability and accuracy.

Conclusion

This study highlights the diagnostic utility of Fine Needle Aspiration Cytology (FNAC) in identifying cytomorphological patterns of tuberculous lymphadenitis and correlating them with bacteriological confirmation via Ziehl-Neelsen (ZN) staining. The key findings include:

1. **Prevalence and Demographics**: Tuberculous lymphadenitis predominantly affects individuals between 21 and 30 years of age, with a slight female predominance. The cervical lymph nodes are the most commonly involved (92%).

2. **Cytomorphological Patterns**: The most prevalent pattern was **Pattern B** (epithelioid granuloma with caseous necrosis, 53%), indicating an active immune response with ongoing tissue destruction. **Pattern A** (epithelioid granuloma without necrosis, 42%) reflects a well-contained immune response, whereas **Pattern C** (caseous necrosis without granuloma, 5%) suggests a defective immune response and higher bacillary load.

3. **AFB Positivity**: The highest AFB positivity (80%) was observed in **Pattern C**, indicating poor immune containment, followed by **Pattern B (62.3%)** and **Pattern A (23.8%)**. The association between AFB positivity and cytomorphological patterns was statistically significant ($p = 0.0003$).

4. **Clinical Implications**:

   - **Pattern B** cases respond well to anti-tuberculosis therapy (ATT) with a favorable prognosis.
   - **Pattern A** cases require close monitoring to prevent progression to necrosis.
   - **Pattern C** cases, with high bacterial loads, necessitate aggressive ATT and close follow-up due to the risk of complications.

Implications for Diagnosis and Treatment

- FNAC should be the first-line diagnostic approach for superficial lymphadenopathy, particularly in resource-limited settings, as it is minimally invasive, cost-effective, and provides rapid results.
- ZN staining enhances diagnostic accuracy, helping distinguish between different immune responses to tuberculosis.
- Treatment strategies should be tailored based on cytomorphological patterns to ensure better disease management and patient outcomes.
- Further research is needed to validate these findings across multiple healthcare centers and integrate molecular diagnostic tools like CBNAAT for enhanced sensitivity.

This study reinforces FNAC's role in diagnosing tuberculous lymphadenitis and guiding treatment, ultimately contributing to better tuberculosis control strategies.

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Author contribution

All the authors of this research paper have directly participated in the planning, execution, or analysis of the study. **SCh** and **PH** conceived the idea, designed the study, **SCh** and **NCh** collected the data, **SCh** and **PH** performed the statistical analysis and wrote the paper. **ShASh** guided the research project and reviewed the slides and the literature. All the authors of this paper have read and approved the final version submitted.

Conflict of interest

The authors declare that they have no competing interests.

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