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Original Research Article

Correlation of Fine-Needle Aspiration Cytology (FNAC) of Granulomatous Lymphadenitis with Ancillary Investigations like Ziehl-Neelsen (ZN) Staining and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) to Confirm Tubercular Etiology

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Conflict of interest: Nil

Abstract:

Introduction: Granulomatous lymphadenitis is a chronic inflammatory condition that can be associated with lymphoproliferative disorders, infectious diseases and autoimmune conditions. Among infectious diseases, tuberculosis is the most common disease caused by Mycobacterium tuberculosis. In developing countries like India, where the disease burden is highest FNAC serves as a minimally invasive, cost-effective primary diagnostic tool for evaluating lymphadenopathy. However, due to its limitation's ancillary techniques like ZN staining and CBNAAT can be employed to confirm tubercular etiology. It is also necessary to rule out other causes of granulomatous lymphadenitis. ZN staining is a simple, rapid and economical method for detecting acid-fast bacilli (AFB). CBNAAT is based on real time polymerase chain reaction (RT PCR) which has significantly higher sensitivity and specificity compared to ZN staining.

Aims: To study various cytomorphological patterns of granulomatous lymphadenitis and correlate with ZN stain and CBNAAT findings.

Materials and Method: This cross-sectional study was carried out for a period of 6 months from November 2024 to April 2025 at Akash Institute of Medical Sciences and Research Centre. 105 cases diagnosed as granulomatous lymphadenitis on FNAC were included in this study. Clinical details, site of aspiration, type of aspirate were noted. Smears prepared from material obtained on fine-needle aspiration were fixed in ethanol and stained with hematoxylin and eosin. Further, ZN stain was performed on air-dried smears. Samples for CBNAAT was collected simultaneously in a falcon tube with normal saline.

Results: A total of 105 cases diagnosed as granulomatous lymphadenitis on FNAC were evaluated and correlated using ZN stain and CBNAAT. Higher incidence was observed in females, accounting for 60%. Majority of patients (33.33%) belonged to 16-30 years age group. Cervical region (71.43%) was the most common site of lymphadenopathy. On FNAC, most common aspirate was blood-mixed aspirates seen in 52.38% of cases. Among the cytomorphological patterns, caseating granulomatous lymphadenitis was the most common observed in 57.14% of cases. Mycobacterium tuberculosis was detected in 84.76% of cases using CBNAAT and 70.48% of cases using ZN staining.

Conclusion: A single time sample collection with FNAC for routine cytology, ZN staining for AFB and CBNAAT for detection of Mycobacterium offers comprehensive and effective approach in rapid diagnosis of tuberculous lymphadenitis. In resource limited settings where advance techniques are not available and in cases of blood-mixed aspirate where CBNAAT sensitivity is lower, FNAC still remains cost-effective initial diagnostic tool in evaluating lymphadenopathy. Diagnostic yield and accuracy of FNAC are significantly enhanced when used in conjunction with ancillary techniques to confirm tubercular lymphadenitis. This study revealed that FNAC findings of granulomatous lymphadenitis cases showed a stronger correlation with CBNAAT compared to conventional ZN stain.

Introduction

Granulomatous lymphadenitis is a chronic inflammatory condition that can be associated with lymphoproliferative disorders, infectious diseases and autoimmune conditions. Among infectious diseases, Tuberculosis is the most common communicable disease caused by Mycobacterium tuberculosis. [1]

In 2020, Tuberculosis affected 10 million individuals globally, during the same period it led to 1.4 million deaths. Currently, India is at the first position among eight countries carrying high burden of Mycobacterium tuberculosis. [2] In developing countries like India, where the disease burden is highest FNAC serves as a minimally invasive, cost-effective primary diagnostic tool for evaluating lymphadenopathy and also an easy way for collecting material for bacteriological examination. [1]

FNAC has several limitations, to overcome these ancillary techniques like ZN stain and CBNAAT can be employed with cytological assessment to improve diagnostic accuracy. [1] ZN stain is simple, rapid microbiological method for detection of acid-fast bacilli (AFB). [2] CBNAAT is fully automated and is based on real time polymerase chain reaction (RT PCR) which has significantly higher sensitivity and specificity compared to ZN staining in detection of Mycobacterium tuberculosis. [1]

Aims: To study various cytomorphological patterns of granulomatous lymphadenitis and correlate with ZN stain and CBNAAT findings.

Materials and Methodology

This cross-sectional study was carried out for a period of 6 months from November 2024 to April 2025 at Central Laboratory of tertiary care center.

A total of 105 cases diagnosed as granulomatous lymphadenitis on FNAC were included in this study. Clinical data such as history of fever, cough, contact with TB patient, past history of TB, site of aspiration, type of aspirate were noted.

Fine-needle aspiration was performed by using 23-24G needle attached to 10ml syringe. A total of 4 smears were prepared, two of these were fixed in alcohol and stained with routine haematoxylin and eosin for cytological evaluation while, other two air-dried smears were stained with ZN stain for detection of AFB. After ZN stain, the stained smears were examined under oil immersion, and atleast 100 fields were scanned to declare as negative. However, presence of red beaded, slightly curved bacilli against blue background was considered positive.

For CBNAAT, the sample was combined with sample reagent in 1:2 ratio and incubated at room temperature for 15 minutes. After 15 minutes using a Pasteur pipette 2ml of the prepared sample mixture was transferred into test cartridge which was loaded into CBNAAT machine.

Inclusion Criteria:

- Patients of all ages, both males and females.
- All lymphadenopathy cases diagnosed as granulomatous lymphadenitis on FNAC.
- All patients with informed consent.

Exclusion Criteria:

- Cases which are already diagnosed, on treatment and recurrent cases of tuberculosis.
- Inadequate aspirate

Statistical Analysis: Statistical analysis was performed using SPSS 28.0 software and descriptive statistics were employed for obtaining frequencies and percentages. Chi-square test was done to understand the significance in qualitative variables. P-value less than 0.05 was considered statistically significant.

Results

A total of 105 cases diagnosed as granulomatous lymphadenitis on FNAC were evaluated and correlated using ZN stain and CBNAAT.

Majority of patients belonged to 16-30 years age group with 35 cases (33.33%) while children below 15 years and elderly above 60 years were least affected. Higher incidence was observed in females, accounting for 63 cases (60%) and male to female ratio is 1:1.5. Cervical region was the most common site of lymphadenopathy with 75 cases (71.43%) followed by axillary region with 20 cases (19.05%).

On FNAC, most common type of material aspirated was blood-mixed aspirate seen in 55 cases (52.38%) followed by cheesy aspirate with 28 cases (26.67%) and least common type of aspirate was pus with 4 cases (3.81%). Further, four different types of cytomorphological patterns were observed in patients with granulomatous lymphadenitis. Among the cytomorphological patterns, caseating granulomatous lymphadenitis was the most common observed in 60 cases (57.14%) while least number was observed in suppurative granulomatous lymphadenitis with 6 cases (5.72%).

Positivity for Mycobacterium tuberculosis was 84.76% by CBNAAT and 70.48% by conventional ZN staining and about 14.28% cases which were negative for AFB on ZN stain were positive for Mycobacterium tuberculosis on CBNAAT.

Chi-square value is 5.37 and p-value is 0.0205 which is statistically significant.

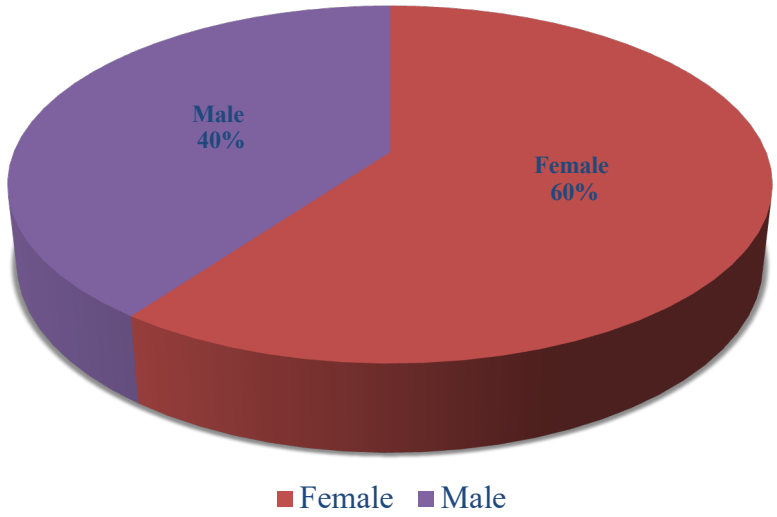


Figure 1: Gender Distribution

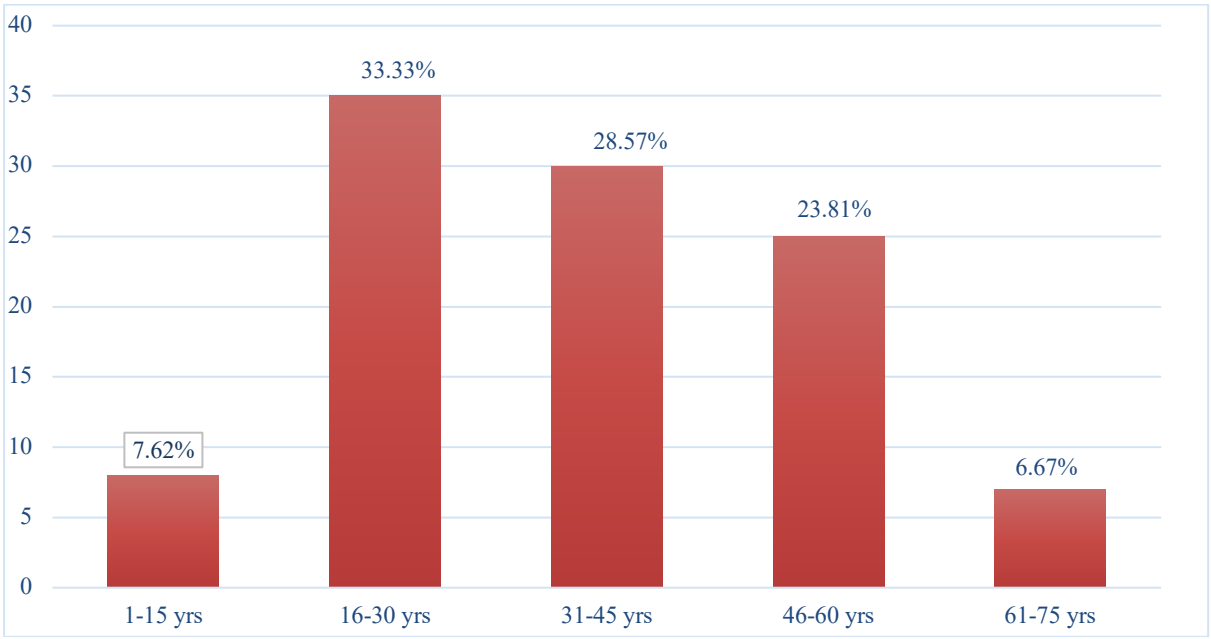


Figure 2: Age Distribution

Table 1: Nature of Material Aspirated on FNAC

Type Of Aspirate	Number of Cases	Percentage
Blood mixed aspirate	55	52.38%
Cheesy	28	26.67%
Pus mixed blood	10	9.52%
Blood mixed cheesy	8	7.62%
Pus	4	3.81%

Table 2: Site of Lymph Node Involved

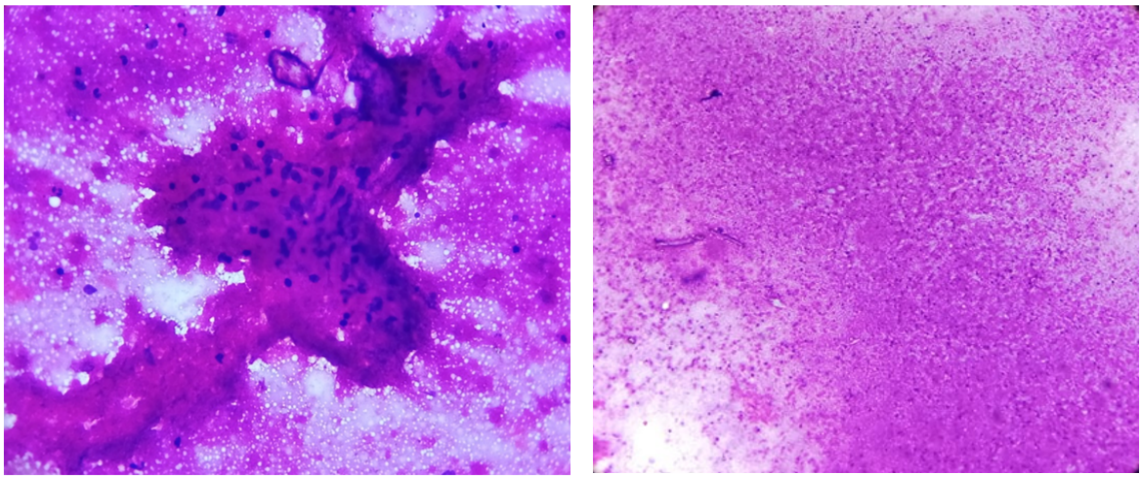
Site	Number of Cases	Percentage
Cervical	75	71.43%
Axillary	20	19.05%
Supraclavicular	7	6.66%
Inguinal	3	2.86%

Table 3: Cytomorphological Patterns on FNAC

Pattern	Number of Cases	Percentage
Caseating granulomatous lymphadenitis	60	57.14%
Non-caseating granulomatous lymphadenitis	30	28.57%
Only caseous necrosis without granuloma	9	8.57%
Suppurative granulomatous lymphadenitis	6	5.72%

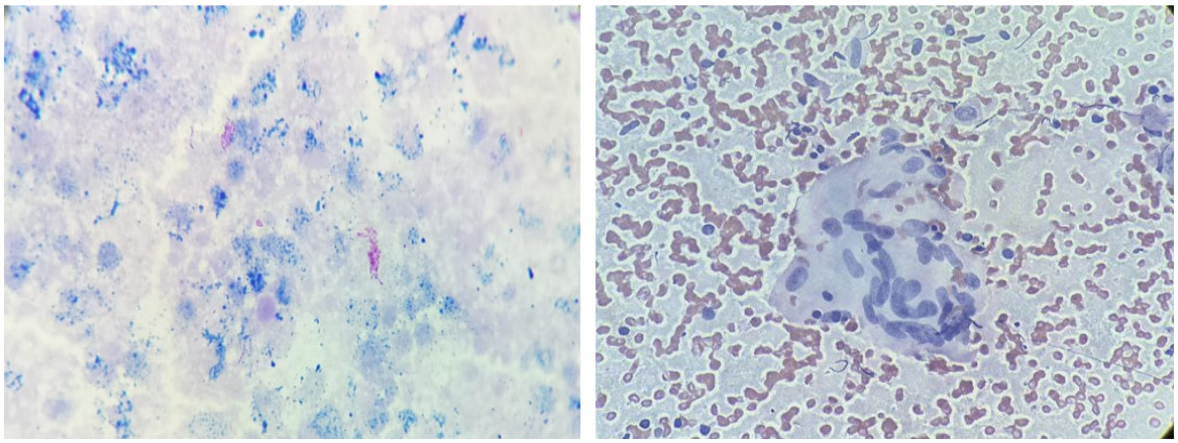
Table 4: Comparison Between ZN STAIN and CBNAAT

FNAC	FNAC Cases	ZN Stain	CBNAAT
Caseating granulomatous lymphadenitis	60 (57.14%)	48 (80%)	55 (91.67%)
Non-caseating granulomatous lymphadenitis	30 (28.57%)	18 (60%)	22 (73.33%)
Only caseous necrosis without granuloma	9 (8.57%)	5 (55.55%)	7 (77.78%)
Suppurative granulomatous lymphadenitis	6 (5.72%)	3 (50%)	5 (83.33%)
Total	105	74(70.48%)	89(84.76%)



Caseating granulomatous lymphadenitis **Caseous necrosis**

Figure 3: (a) Caseating granulomatous lymphadenitis; (b) Caseous necrosis



ZN stain demonstrating AFB **Non-caseating granulomatous lymphadentis**

Figure 4: (a) ZN stain demonstrating AFB; (b) Non-caseating granulomatous lymphadentis

Discussion

Granulomatous lymphadenitis is a chronic inflammatory condition characterized by formation granuloma, which is a collection of epithelioid cells surrounded at the periphery by lymphocytes and may contain multinucleate giant cells. [1]

The present study was an attempt to assess various cytomorphological patterns of granulomatous lymphadenitis and correlate with ZN stain and

CBNAAT findings. In the present study, the most common cytomorphological pattern observed is caseating granulomatous lymphadenitis (57.14%) followed by non-caseating granulomatous lymphadenitis (28.57%) which when compared to other studies, similar distribution pattern is observed though proportions vary.

Parigi M et al reported slightly lower rates of caseating granulomatous lymphadenitis (47.62%) but a higher proportion of non-caseating granulomatous (36.66%), while the percentage of only caseous necrosis remains identical to the present study (8.57%). Siddegowda M et al documented a much lower incidence caseating granulomatous lymphadenitis (13%). Mitra S K et al reported higher incidence of caseating granulomatous lymphadenitis (40%) while the percentage of non-caseating granulomatous lymphadenitis is lower (21%). [1,3,6]

Overall, the comparison highlights considerable variability among studies, with caseating granulomatous lymphadenitis being major pattern, though its frequency differs widely across different research studies.

Total sample positivity for Mycobacterium tuberculosis in the present study is 84.76% by CBNAAT and 70.48% by conventional ZN staining. Similar trends were observed in studies by Parigi M et al, with CBNAAT positivity of 82.85% and ZN stain positivity of 65.71%, Manju MD et al, with CBNAAT positivity of 79.49% and ZN stain positivity of 23.08% and Patil SB et al, with CBNAAT positivity of 55.20% and ZN stain positivity of 46.35%. [1,4,5]

FNAC findings of granulomatous lymphadenitis cases showed a stronger correlation with CBNAAT compared to conventional ZN staining and CBNAAT was found to be more accurate when compared ZN staining.

Table 5: Comparison of ZN STAIN and CBNAAT Positive Results with Other Studies

Sl. No.	STUDY	ZN Stain (%)	CBNAAT (%)
1.	Present study	70.48%	84.76%
2.	Parigi M et al.	65.75%	82.85%
3.	Manju MD et al.	23.08%	79.49%
4.	Shetty D et al.	34%	60%
5.	Patil SB et al.	46.35%	55.20%

Table 6: Comparison of Cytomorphological Features with Other Studies

Sl. No.	Study	Caseating Granulomatous Lymphadenitis	Non-Caseating Granulomatous Lymphadenitis	Only Caseous Necrosis	Suppurative Granulomatous Lymphadenitis
1.	Present study	57.14%	28.57%	8.57%	5.72%
2.	Parigi M. et al.	47.62%	36.66%	8.57%	7.14%
3.	Siddegowda M et al.	13%	22%	12%	21%
4.	Mitra S. K. et al.	40%	29.5%	30.5%	Nil

Conclusion

A single time sample collection with FNAC for routine cytology, ZN staining for AFB and CBNAAT for detection of Mycobacterium offers comprehensive and effective approach in rapid diagnosis of tuberculous lymphadenitis.

Diagnostic yield and accuracy of FNAC are significantly enhanced when used in conjunction with ancillary techniques to confirm tubercular etiology. CBNAAT is a rapid and sensitive test for detection of Mycobacterium tuberculosis even in patients with low bacillary load.

In resource limited settings where advance techniques are not available and in cases of blood-mixed aspirate where CBNAAT sensitivity is lower, FNAC still remains cost-effective initial diagnostic tool in evaluating lymphadenopathy.

Hence cytopathology techniques like FNAC must be coupled with other ancillary investigations like ZN stain and CBNAAT for accurate results.

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