

Original Research Article

Correlation between cytomorphology and acid fast bacilli positivity in tubercular lymphadenopathy in far Western Nepal: a hospital based study

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ABSTRACT

Background: Tuberculous lymphadenitis is the most common manifestation of extrapulmonary tuberculosis. Fine needle aspiration cytology (FNAC) is a valuable tool in diagnosis of tubercular lymphadenitis. The present study aims to determine the prevalence of different cytomorphological patterns in fine needle aspiration cytology of tubercular lymph nodes and their correlation with Acid Fast Bacilli (AFB) positivity on Ziehi-Neelsen (ZN) Staining.

Methods: Fine needle aspiration cytology of 274 cases diagnosed as tubercular lymphadenitis over a period of a year were reviewed. Cytomorphological patterns were categorized into three patterns. Pattern I: Epithelioid granuloma without necrosis, Pattern II: Epithelioid granuloma with caseous necrosis and Pattern III: Caseous necrosis only. Fischer Exact test was applied to correlate cytomorphological pattern and AFB positivity.

Results: Tuberculous lymphadenitis was most frequent in age group 21-30 years (24.81%). Cervical lymph nodes were the most frequent lymph nodes involved (84.67%). Pattern I was the most common cytomorphological pattern observed (49.3%). Overall AFB positivity was 28.10%. Maximum AFB positivity was seen in pattern III (73.3%).

Conclusions: FNAC is the simple, cost effective and minimally invasive tool to diagnose tuberculous lymphadenitis. Study of both cytomorphological pattern and ZN staining for AFB can improve the diagnostic accuracy.

Keywords: Acid fast bacilli, Cytomorphological pattern, Tuberculous lymphadenitis

INTRODUCTION

Tuberculosis is the leading cause of worldwide morbidity and mortality. According to World Health Organization, it accounts for 6000-7000 people mortality per year in Nepal.¹ Tubercular lymphadenitis is seen in 20-40% of extrapulmonary tuberculosis, being the commonest form of extrapulmonary tuberculosis.²

Tuberculous lymphadenitis can be diagnosed by variety of diagnostic tools such as fine needle aspiration cytology (FNAC), biopsy(histopathology), Ziehi-Neelsen (ZN)

stain for Acid Fast Bacilli (AFB), culture, imaging and molecular tests. Although culture is considered gold standard for diagnosis of tuberculosis, it is not routinely performed as Mycobacteria are slow growing.³ Culture facility is not easily available in all laboratories in our country. FNAC is the first line investigation for the diagnosis of tubercular lymphadenitis as it is simple, cost effective, less invasive and less time consuming.⁴

It has been reported in various studies that FNAC has sensitivity of 97% to 100% and specificity of 88% to 100% for the diagnosis of tubercular lymphadenitis in

endemic areas where as sensitivity and specificity are low in non endemic areas.⁵⁻⁷

Cytological diagnosis of tubercular lymphadenopathy is based on demonstration of different cytomorphological tissue reaction pattern on smear. Spectrum of morphological changes depends upon the stage of disease itself and also the immune status of the patient.^{8,9} Cytomorphological pattern may be broadly classified as:¹⁰

- Pattern I: Epithelioid granuloma without necrosis
- Pattern II: Epithelioid granuloma with caseous necrosis
- Pattern III: Caseous necrosis only.

Definitive diagnosis of tubercular lymphadenitis depends on demonstration of Acid Fast Bacilli (AFB) in cytological smear stained with Ziehl Neelsen (ZN) stain. In developing countries where diagnostic facilities are limited and disease burden is high, presence of epithelioid granuloma is considered as an evidence of tubercular lymphadenitis.¹¹ The present study aims to determine the prevalence of different cytomorphological patterns in fine needle aspiration cytology of tubercular lymph nodes and their correlation with AFB positivity on Ziehl-Neelsen (ZN) staining in far western part of Nepal in hospital setting.

METHODS

This is a retrospective descriptive study conducted in Seti Provincial Hospital and Maya Metro Hospital, Dhangadhi, Kailali, Nepal between January 2018 to December 2018. Total 628 lymph node aspirations were performed during this period out of which 274 cases which were cytologically proven as granulomatous lymphadenitis and tubercular lymphadenitis were studied.

Inclusion criteria: FNAC diagnosis of tubercular and granulomatous lymphadenitis including all age group, gender and site of lymph node involved.

Exclusion criteria: FNAC diagnosis of reactive hyperplasia, suppurative lymphadenopathy, metastatic or hematolymphoid malignancy were excluded from the study.

All FNAC were carried out, examined and reported by coauthor, a senior consultant pathologist. Aspiration was done using 22 gauge needle with standard aseptic precaution after informed consent. The aspirate were smeared on five slides.

One slide was air dried and ZN stain was done, two slides were air dried and stained with May Grunwald Giemsa (MGG) stain and remaining two slides were fixed in 95% ethanol and Papanicolau (PAP) stain was done. The ZN

stained smears were examined under oil immersion for the presence of acid fast bacillus.

Definitive diagnosis of tubercular lymph node was made when ZN stained smear showed presence of AFB. Presumptive diagnosis of tubercular lymphadenopathy was made when smears showed presence of epithelioid granuloma with caseous necrosis.

Smears showing only epithelioid granuloma without caseous necrosis were grouped as granulomatous lymphadenitis (Figure 1).

The latter group were considered suggestive of tubercular lymphadenitis in correlation with clinical and radiological parameters.

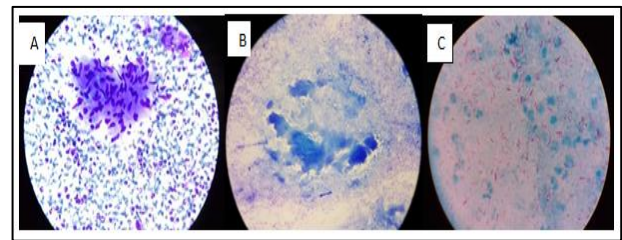


Figure 1: (A) Epithelioid granuloma without necrosis (x400 MGG Stain), (B) Extensive areas of caseous necrosis (x400 MGG stain), (C) Mycobacterium seen in ZN stain(x1000).

Data analysis was done using SPSS version 21 and Fischer Exact test was done to correlate the cytomorphological pattern of tubercular lymph nodes with AFB positivity in ZN staining. A p-value of <0.05 was considered as statistically significant.

RESULTS

Total sample size was 274 patients. Maximum number of patients were in third decade of life and 18.98 % were below 10 years of age. Study involved 47.45% male an 52.55% female (Table 1). Most common site of involvement was cervical lymph nodes (84.67%) (Figure 2).

Table 1: Age and gender distribution.

Age group	Male	Female	Total no. (%)
0-10yr	35	17	52 (18.98)
11-20yr	28	28	56 (20.44)
21-30yr	29	39	68 (24.81)
31-40yr	21	31	52 (18.98)
41-50yr	7	13	20 (7.30)
51-60yr	6	8	14 (5.11)
61-70yr	1	6	7 (2.55)
>70yr	3	2	5 (1.82)
Total	130 (47.45)	144 (52.55)	274 (100)

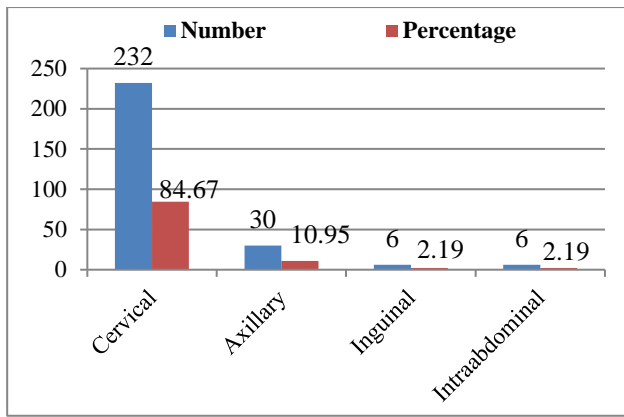


Figure 2: Site of LN involved.

Cytomorphological study showed epithelioid granuloma without necrosis (pattern I) in 135 (49.3%), caseous necrosis with granuloma (pattern II) in 94 (34.3%) and caseous necrosis only (pattern III) in 45 (16.4%) cases.

Total AFB positivity in our study was 28.10 % (77 out of 274 smears). Maximum AFB positivity was seen in necrosis only group (73.3%).

AFB was seen in 45.7% cases in caseous necrosis with granuloma group and epithelioid granuloma without necrosis group showed AFB in only 1 smear (0.74%) (Table 2).

Table 2: Cytomorphology and AFB positivity.

Cytomorphological pattern	No. of cases (%)	AFB +ve cases (%)	AFB -ve cases (%)
Epithelioid granuloma without necrosis (pattern I)	135 (49.3)	1 (0.74)	134 (99.26)
Caseous necrosis with epithelioid granuloma (Pattern II)	94 (34.3)	43 (45.74)	51 (54.26)
Caseous necrosis only, no granuloma (Pattern III)	45 (16.4)	33 (73.33)	12 (26.67)
Total	274 (100)	77 (28.10)	197 (71.90)

Table 3: Statistical analysis cytomorphology and AFB positivity.

Pattern	Cytomorphological feature	AFB +ve	AFB -ve	p value (Fischer exact test)
II	Caseous necrosis with epithelioid granuloma	43	51	p<0.01 (significant)
III	Caseous necrosis only , no granuloma	33	12	
I	Epithelioid granuloma, no caseous necrosis	1	134	p<0.001 (significant)
II	Caseous necrosis with epithelioid granuloma	43	51	
I	Epithelioid granuloma, no caseous necrosis	1	134	p<0.001 (significant)
III	Caseous necrosis only , no granuloma	33	12	

Inter group comparison of cytomorphology with AFB positivity showed significant difference between groups (P<0.01) (Table 3). AFB positivity in relation to site of lymphadenopathy showed no significant difference (P>0.05).

DISCUSSION

FNAC is widely used as the first line investigation of tubercular lymphadenitis in paediatric as well as adults. In our study , most common age group involved was 21-30 years followed by 11-20 years which is similar to the study by Bhatta S et al and Hemlatha A et al.^{12,13} Cervical lymph nodes were the most commonly involved lymph nodes in our study with 84.67% cases. This is similar to study by Patel et al and Bezabih et al which showed cervical lymph node involvement in 83% and 74.2% respectively.^{14,15} Cervical lymph nodes were most commonly involved because organism gains access to the

cervical lymph nodes through the tonsillar lymphoid tissue.¹⁶

In this study, cytomorphological findings were broadly divided into three categories: Epithelioid granuloma without necrosis (pattern I), caseous necrosis with epithelioid granuloma (pattern II) and caseous necrosis only without granuloma (pattern III).^{8,10} Most common cytomorphological pattern observed was pattern I (49.3%). This is similar to study by Vimal S et al, Jagtap S et al., Narayanamurthy et al, where pattern I was most common finding in 42.59%, 48.07% & 37.61% cases respectively.¹⁷⁻¹⁹ However, study by Bhatta S et al, Hemlatha A et al, Masilamani et al revealed pattern II as the most common pattern with 53.17%, 56% and 48.1% cases respectively.^{12,13,16} Pattern III was the most common finding in a study by Paliwal N et al.²⁰ In this study pattern II and III were seen in 34.3% and 16.4% cases respectively.

The broad range of cytomorphological pattern observed in tuberculosis is the result of continuous interaction between bacterial virulence and individual hypersensitivity and immunity to infection.^{8,9} In this study, epithelioid granuloma without necrosis was the most common cytomorphological finding. Although tuberculosis is the commonest etiology of epithelioid granuloma in endemic areas, it can be caused by wide variety of other causes like leprosy, sarcoidosis, actinomycosis, cat scratch disease, foreign body granuloma, sinus histiocytosis, malignant lymphoma etc. In Nepal, however incidence and prevalence of tuberculosis being very high, FNAC finding epithelioid cell granuloma is highly suggestive of tuberculosis.

Total AFB positivity in this study was 28.10% which is similar to another study conducted in Nepal by Bhatta S et al showed AFB positivity in 34.92%.¹² Hemlatha A et al, Masilamani et al, Das et al have noted AFB positivity 54%, 55.7% and 52% respectively.^{13,16,21} AFB positivity may be low because 10,000-100,000 mycobacteria / ml of sample should be present for smear AFB positivity. Maximum number of cases in this study showed epithelioid granuloma without necrosis where organisms are scanty. Yield of AFB positivity can be increased by repeat FNAC and culture.¹⁶ In this study, maximum AFB positivity was seen in pattern C (73.3%) followed by pattern B (45.7%) and pattern A (0.74%) which is also in agreement with studies by Bhatta S et al, and Das et al.^{12,21} Intergroup comparison of cytomorphological pattern and AFB positivity was statistically significant ($p < 0.01$).

Granuloma formation suggests good immune response within the host. Smears showing epithelioid granuloma without necrosis show very low AFB positivity as cell mediated immunity of the patients elicits secretion of Th1 cells, cytokines (Y interferon) and tumor necrosis factor-alpha by activated macrophages. Aggregated macrophages form granuloma and effectively neutralize mycobacteria with minimal tissue destruction.¹³ Caseous necrosis is seen as necrotic material in central part. When immune response of the host is low, defective granulomatous response leads to tissue destruction. Smears show extensive necrosis without granuloma and abundant acid fast bacilli.¹³

Limitation of this study are lack of PCR testing, histopathology and culture to further evaluate FNAC findings.

CONCLUSION

FNAC safe, efficient and cost effective tool in diagnosis of tubercular lymphadenitis. In patients with tubercular lymphadenitis, most common FNAC finding was epithelioid granuloma with or without caseous necrosis. AFB positivity was maximum in FNAC smears with extensive caseous necrosis without granuloma and least in epithelioid granuloma without necrosis.

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