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UNRAVELING THE CYTOMORPHOLOGY OF TUBERCULOUS LYMPHADENITIS - INSIGHTS THROUGH ZIEHL-NEELSEN STAINING

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ABSTRACT: -

Introduction: In developing nations, compelling display of extrapulmonary tuberculosis is peripheral lymphadenopathy. In Southeast Asia, tuberculous lymphadenitis accounts for a significant proportion of cases of enlarged neck lymph nodes. We aim to appraise the cytomorphological patterns in cases of tubercular lymphadenitis and associate acid fast bacilli positivity with various cellular morphology and cytology patterns.

Methods: Prospective cross sectional study design was exercised in a six-month span from July to December 2023. Of the 239 patients exhibiting lymphadenopathy, 150 cases were diagnosed with tuberculous lymphadenitis and underwent cytomorphological evaluation using Hematoxylin & Eosin, Giemsa, and Ziehl-Neelsen stained smears. The personal and clinical data of these patients were also recorded in a predetermined pro forma.

Results: Our study noted a male preponderance, with the highest incidence in the third decade. The majority of cases involved cervical lymph nodes (118, 78.66%). On cytomorphological examination, patterns A, B, and C were noticed in 28 (18.67%), 73 (48.67%), and 49 (32.67%) cases, respectively. AFB grading revealed a hefty number funneled to Grade 3+ [87 cases (58.00%)], seconded by Grade 1+ [37 cases (24.67%)], and trailed by Grade 2+ [26 cases (17.33%)]. Overall AFB positivity was 55.33%.

Conclusion: Although Fine Needle Aspiration Cytology is a relatively painless, economical procedure with high sensitivity for diagnosing tuberculous lymphadenitis, our study concludes that the diagnostic yield can be enhanced by examining the cytomorphological patterns amalgamated with acid-fast bacilli detection by ZN staining.

Keywords: - Acid fast Bacilli, Cytology, Fine Needle Aspiration, Lymph node, Tuberculosis, ZN staining

INTRODUCTION: -

In ancient times, diseases were often attributed to supernatural forces, and early cultures believed that illnesses were punishments from gods, curses from enemies, or the result of possession by evil spirits(1). Indeed, *Mycobacterium tuberculosis* (*M. tuberculosis*) is one of the most remarkable and persistent pathogens in human history, infecting humans for over 70,000 years and estimated global prevalence of about 1/4 of global citizenry(2). Tuberculosis (TB) indeed caused by the bacillus *M. tuberculosis*, have an airborne transmission of spread. The Southeast Asia and Western Pacific regions bear a significant portion of the global TB burden and along with the African region; is responsible for morbidity rates amounting to 95%, with alarming levels of mortality rates of 98%(3).

In middle-income countries, TB lingers to attract a momentous public well-being issue, and tuberculous lymphadenopathy is considered the prevalent form of extrapulmonary TB (EPTB). Extrapulmonary manifestations account for about 15–20% of all TB cases with affliction to extrapulmonary sites constituting GIT, dermal appendages, LN, MSK system, CNS, and reproductive system(4). Of all the TB cases without HIV co-infection, EPTB accounts for approximately 15-20% and this proportion can rise significantly to around 40–50% due to their weakened immune systems in HIV affected individuals. Among the various forms of EPTB,

tuberculous lymphadenopathy is most commonly reported(5). Cervical lymphadenopathy is certainly the prevalent form of extrapulmonary TB, accounting for 60%–90% of cases, which is commonly referred to as scrofula(6). Tuberculous lymphadenitis shows a distinct demographic pattern as against that of pulmonary sickness. Tuberculous lymphadenitis is greatly prevailing in younger women between 20 and 40 years, that contrasts with pulmonary tuberculosis, which tends to be more prevalent in older men(7,8).

Fine Needle Aspiration Cytology (FNAC) is considered a practical, quick, and safe demonstrative tool concerning tuberculous lymphadenitis, especially in high-burden regions where resources are limited. However, the gold standard tool being culture for unambiguous scrutiny, FNAC is recognized for its high sensitivity and specificity, and is an minimally invasive outpatient procedure(9). The diagnostic efficacy of FNAC in tuberculous lymphadenitis is reported to be comparable to that of histopathology in many studies(10).

We intended at assessing cytomorphological patterns of tubercular lymphadenitis using FNAC and correlating the decisions with ZN staining to detect acid-fast bacilli (AFB), and at gauging the overall AFB positivity in tuberculous lymphadenitis cases.

METHODOLOGY: -

A prospective cross-sectional study was adopted in six months (July–December 2023) in the Cytopathology Department at our hospital. Institutional Ethical Committee approval was accomplished. Patients showing off with peripheral lymphadenopathy were subjected to FNAC. In a predetermined pro forma, demographic characteristics with relevant clinical symptoms and physical findings were entered.

All cases subjected to FNAC were cytologically numbered into three groups. FNAC specimens stained with May-Grünwald Giemsa and Hematoxylin & Eosin were postulated for cytomorphological interpretation. Based on the classification by Das et al(11), FNAC smears was classified toward 3 patterns: Pattern A - Epithelioid granuloma without necrosis, pattern B - Epithelioid granuloma with necrosis, and pattern C - Necrosis without epithelioid granuloma.

FNAC samples subjected to Ziehl-Neelsen stain to assess acid-fast bacilli positivity and graded according to the classification by Kumar et al(12), positive smears were contained into 3 grades: Grade 1+: occasional bacilli observed, Grade 2+: Singly scattered AFB present, Grade 3+: Numerous bacilli packed in bundles.

The data were analyzed using SPSS 21.0 for Windows. Descriptive statistics summarized the demographic and clinical characteristics of the study population. Frequencies and percentages were calculated for diagnostic categories and cellular patterns observed in FNAC specimens.

RESULTS: -

Participant attributes

In this study period, 239 FNAC procedures were performed on patients with lymphadenopathy in the department. Of the 239 cases of lymphadenopathy, tuberculous lymphadenitis [150/239 (62.76%)] was the predominant lesion comprising of cases, followed by reactive lymphoid hyperplasia [67/239 (28.03%)] cases, acute suppurative lymphadenitis [13/239 (5.44%)] and chronic inflammatory lymphadenitis [9/239 (3.77%)], as illustrated in figure 1.

The patients' ages ranged between 28 and 67 years, with a mean age of 36.46 years (SD = 11.4) The current research constituted 99 males (66%) and 51 females (34%). Predominantly affected age group was 31 - 40 (47/150, 31.33%), followed by 21 - 30 (39/150, 26.15%) and 41 - 50 (28/150, 18.67%). A predominance of male cases was realized, with a gender distribution of 1.94:1, as illustrated in table 1 and figure 2.

In this study, all patients presented with peripheral lymph node swelling [150/150 (100%)] with the majority of the cases having fever [109/150 (72.67%)] followed by cough [83/150 (55.33%)] and weight loss [44/150 (29.33%)]. The cervical nodes [118/150 (78.66%)] were the most frequently involved group whereas inguinal lymphadenopathy [6/150 (4.00%)] was least common (Table 2).

Aspirate Characteristics

Based on the aspirate image, the majority of cases had blood-mixed aspirate [125/150 (83.33%)], followed by purulent/pus material [19/150 (12.67%)] and caseous or cheesy material [6/150 (4.00%)], as

displayed in table 2. Three-fourths of blood-mixed aspirates exhibited patterns A and B combined, while only one-fourth of purulent/pus and caseous/cheesy aspirates showed pattern C.

Cellular morphology & cytological features, AFB positivity and smear grading

Among the 150 sampled, pattern B topped the findings in 73 cases (48.67%), stalked by pattern C [49 cases (32.67%)], and tailed by pattern A [28 cases (18.67%)], as portrayed in figure 3. AFB positivity was established in 4 (4.82%) cases of pattern A, 49 (59.04%) of pattern B, and 30 (36.14%) of pattern C, as illustrated in table 3. The highest AFB positivity (65.32%) was recognized in aspirates consisting of purulent substances. Overall, the AFB positivity was seen in 55.33% (83/150) of cases.

AFB grading revealed a hefty number funneled to Grade 3+ [87 cases (58.00%)], seconded by Grade 1+ [37 cases (24.67%)], and trailed by Grade 2+ [26 cases (17.33%)].

Figure 1 - Distribution of cases of lymphadenitis

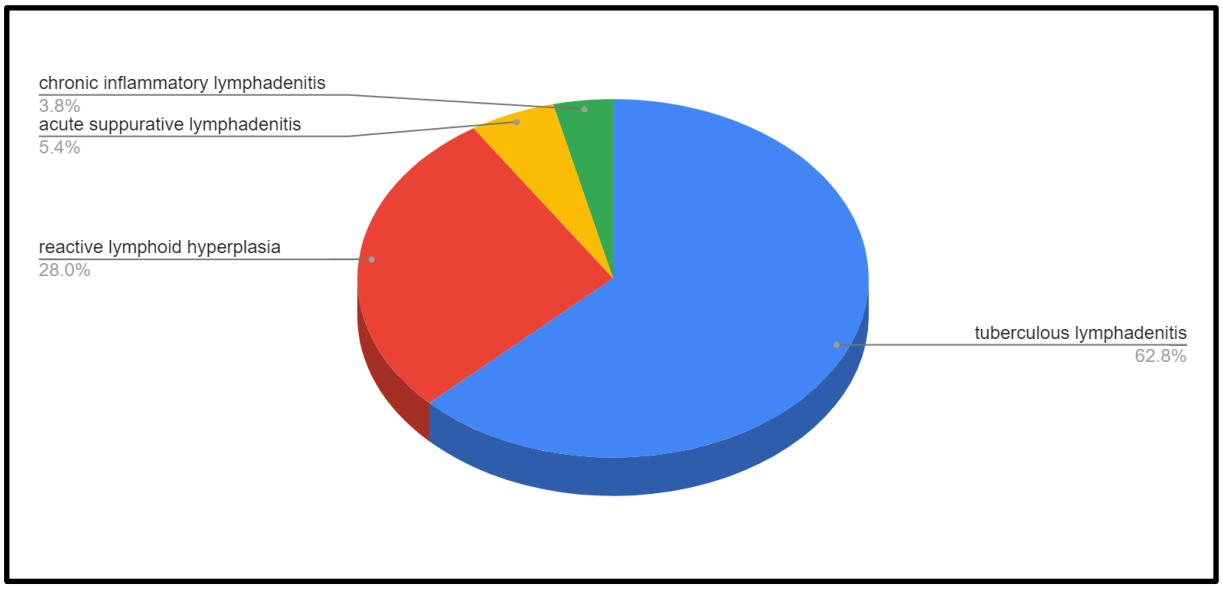


Table 1 - Baseline features of study subjects

Age group (years)	Male No. (%)	Female No. (%)	Total No. (%)
21 - 30	27 (27.27)	12 (23.53)	39 (26.15)
31 - 40	33 (33.33)	14 (27.45)	47 (31.33)
41 - 50	16 (16.16)	12 (23.53)	28 (18.67)
51 - 60	14 (14.14)	8 (15.69)	22 (14.67)

61 - 70	9 (9.10)	5 (9.80)	14 (9.33)
Total	99 (66.00)	51 (34.00)	150 (100)

Table 2 - Clinical characteristics of the study population

Parameter	Category	No. (%)
Clinical features	Lymph node swelling	150 (100)
	Fever	109 (72.67)
	Weight loss	44 (29.33)
	Cough	83 (55.33)
Anatomical site	Cervical	118 (78.66)
	Axillary	7 (4.67)
	Supraclavicular	9 (6.00)
	Inguinal	6 (4.00)
	Submandibular	10 (6.67)
Nature of aspirate	Purulent	19 (12.67)
	Blood mixed	125 (83.33)
	Cheesy	6 (4.00)

Figure 2 - Gender distribution of tuberculous lymphadenitis cases

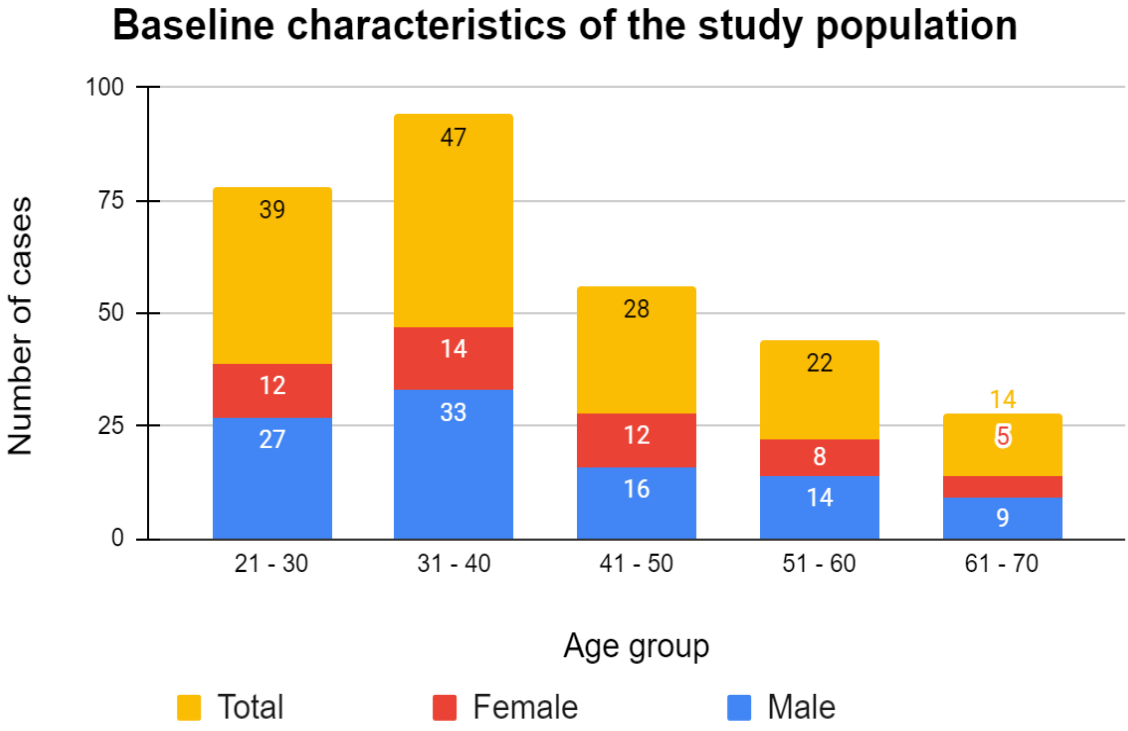
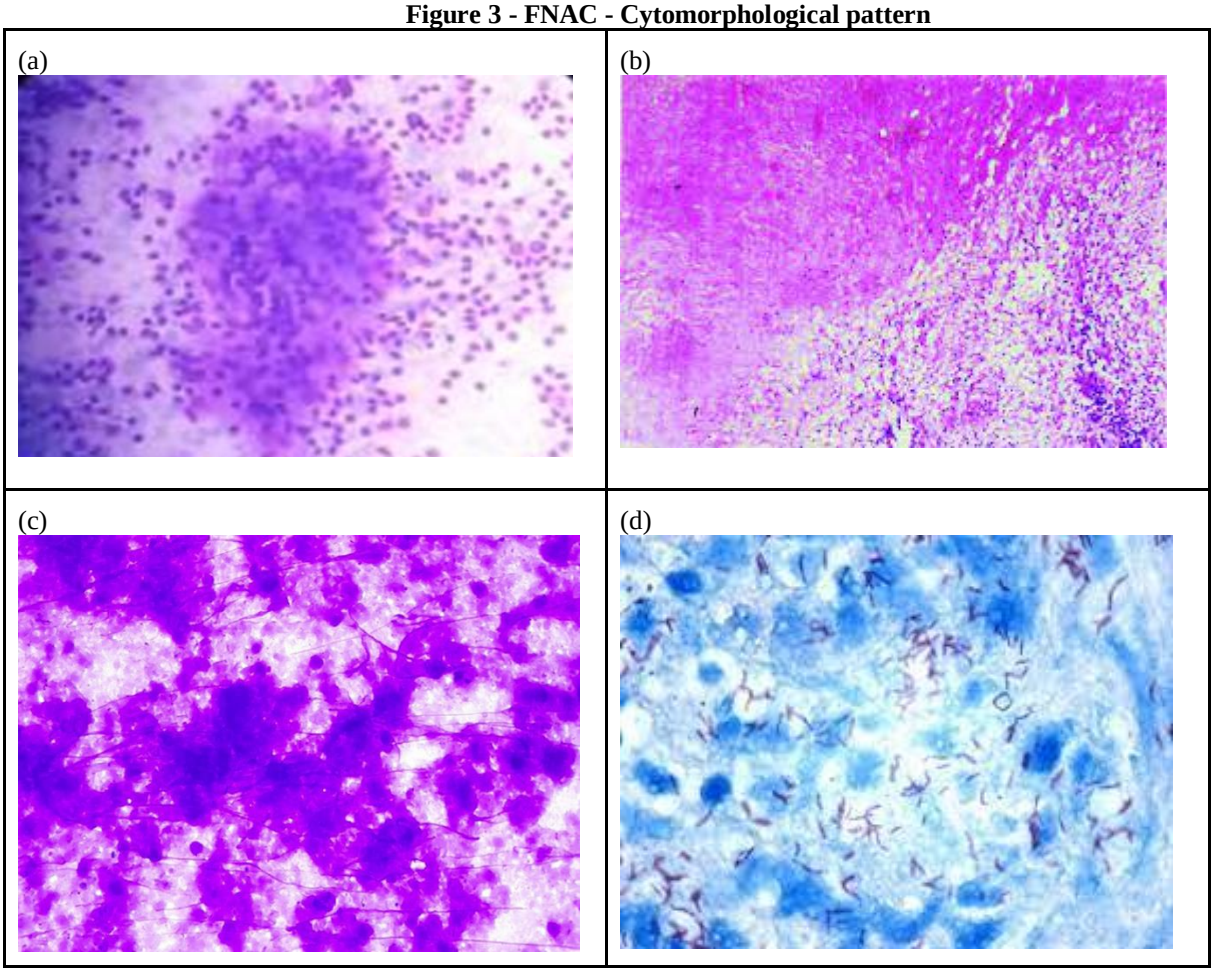


Table 3 - Patient distribution based on cytology & AFB positivity

Cytopathological pattern	No. (%)	AFB +ve cases No. (%)	AFB -ve cases No. (%)
Pattern A	28 (18.67)	4 (4.82)	24 (35.82)
Pattern B	73 (48.67)	49 (59.04)	24 (35.82)
Pattern C	49 (32.67)	30 (36.14)	19 (28.36)
Total	150 (100)	83 (55.33)	67 (44.66)

Figure 3 - FNAC - Cytomorphological pattern



(a) FNAC smear revealing Pattern A (Leishman stain ×400). (b) FNAC smear detailing Pattern B (Leishman stain ×400). (c) FNAC smear anecdote Pattern C (Leishman stain ×400). (d) AFB in ZN stain (Ziehl-Neelsen stain ×1000, oil immersion).

DISCUSSION: -

FNAC provides an immediate preliminary diagnosis in the investigation of lymphadenopathy, with minimal trauma, fewer complications, and a significantly lower cost compared to surgical biopsy(13). While several studies(14–16) have highlighted FNAC as an precise and decisive initiatory tool for critiquing lymphadenopathy, its understanding exigencies considerable expertise.

In the current study, the peak cases were funneled in the 31–40 age consort (31.33%), seconded by the 21–30 age bracket (26.15%). This aligns with studies by Rao(17), Chand et al(18) and Selvaraju et al(19). The study also showed a male preponderance, with a gender distribution of 1.94:1, aligning to findings in previous studies(20–22). While the male bias varies by geographic location and year, the overall gender distribution is typically 1.8:1(23), which is in agreement to our findings.

In our study, the cervical group (78.66%) affliction was mostest remark, which is consistent with findings in several recent studies(6,24,25).

In this study, appertaining to the nature of aspirate, the major chunk had blood-mixed aspirates (83.33%), second in order had purulent aspirates (12.67%) and a minimalist had cheesy aspirates (4.00%). These findings are in agreement with work by Thakur et al(26) and Vimal et al(1).

AFB positivity for tuberculous lymphadenitis by ZN staining varies between 35.6% and 55.2%(27). Our observation is an overall AFB positivity of 55.33%, which is comparable to findings in earlier studies(18,28).

In our research, the considerable recognition was pattern B (48.67%), consistent with findings reported by Gupta et al(29) and Hemalatha et al(27), and higher than those reported by Chand et al(18) and Nidhi et al(14). Pattern C was contemplated substantially in 32.67% of cases with second in order by pattern A in 18.67% of cases, which aligns with the work by Nidhi et al(14) and Hemalatha et al(27).

CONCLUSION: -

FNAC, amalgamated with ZN staining for acid-fast bacilli, officiates tools for investigating lymphadenitis by cause of tuberculosis. It is a reliable, quick, and cost-effective diagnostic tool in the interest of tubercular lymphadenitis. The diagnostic yield can be improved by examining both the cytomorphological patterns and ZN staining. Regardless of whether granulomas are present, ZN staining should be performed whenever a suspicion of infectious etiology is considered.

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