

Efficacy of Fluorescent Method over Conventional ZN Method in Detection of Acid Fast Bacilli among Various Cytomorphological Patterns of Tubercular Lymphadenitis

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Abstract: ***Background:** Lymphadenopathy is one of the most common extrapulmonary manifestations of Tuberculosis. Early detection and management of extrapulmonary Tuberculosis prevents mortality and morbidity. Hence, the present study on various cytomorphological patterns and also to study the efficacy of fluorescent method over conventional Ziehl Neelsen [ZN] method in detection of acid-fast bacilli in lymphnode aspirates among tubercular lymphadenitis was performed. **Methods:** Among 201 consecutive patients with clinical suspicion of tuberculosis presenting with lymphadenopathy, fine needle aspirations were performed. Smears were prepared from the aspirates and processed for routine cytology, ZN method and fluorescent method. The efficacy of fluorescent method was analyzed using Chi-Square test. **Results:** Out of 201 aspirates, 197 were studied and remaining 4 were excluded from the study due to diagnosis of malignancy in 1.99% and inadequate aspiration in 0.49%. Among the 197 aspirates, tubercular lymphadenitis was diagnosed by cytology in 50.76% (100/197). In 100 cytological diagnosed tubercular lymphadenitis, positive for acid fast bacilli on ZN method was 24% (24/100). The smear positivity increased to 38% (38/100) on fluorescent method [p value - < 0.001]. The most common cytomorphological sub-type of TB lymphadenitis observed was type 3 pattern. **Conclusions:** The cytomorphological subtyping in tubercular lymphadenitis helps in assessing the stage of the disease. Due to paucibacillary in early stage of the disease, detection of AFB is difficult by either method. As stage of the disease advances, detection of AFB by fluorescent method was found to be more effective as compared to ZN method.*

Keywords: Fluorescent method, Cytology, Tuberculosis, Lymphadenitis.

1. Introduction

Tuberculosis (TB) still remains a global health problem affecting one third of world population. Early diagnosis of TB prevents transmission in the household and community. Lymphadenopathy is one of the most common extrapulmonary manifestation accounting to around 30-40% of TB [1,2]. The clinical findings for the suspicion of TB in lymph nodes are neither specific nor do their absence exclude TB involvement [3]. Although Mycobacterial culture is the gold standard method for diagnosis of TB, it is time consuming and requires specialized facilities in the laboratory. Serological techniques and Molecular techniques like PCR are costly with lack of specificity. Fine needle aspiration cytology (FNAC) can be an important tool in suspecting TB lymphadenitis based on identifying epithelioid granulomas and caseous necrosis. However, conventional Ziehl-Neelsen (ZN) method for detection of acid-fast bacilli (AFB) is simple and rapid but lacks sensitivity ranging from 20% to 43% [4]. Hence, fluorescent method for detection of AFB has proven more effective than ZN method in paucibacillary cases. Thus, the present study was undertaken.

2. Materials and Methods

Two hundred and one consecutive patients with significant lymphadenopathy (irrespective of age and sex) and clinical suspicion of TB; referred for FNAC to the department of cytopathology were included in the study. Informed consent was taken from all the patients. Exclusion criteria consisted of patients without informed consent and insufficient or

hemorrhagic aspirate. The additional investigations like complete hemogram, Chest Roentgenography were reviewed in the patients.

FNAC was performed from the enlarged lymphnode and aspirates were ejected on 5 slides and smears were prepared. One smear was alcohol fixed and stained by Papanicolaou [5] for cytological examination directly. The remaining slides were stained for Giemsa [6], ZN [7] and Fluorescent [8] stains respectively. With Fluorescent staining method, positive controls of known sputum positive smear were used.

Depending upon the cytomorphological appearances, TB lymphnode was subdivided further into six patterns [9]: (i) Epithelioid granulomas with Langhan's giant cells and caseous necrosis [Figure. 1A]. (ii) Only numerous epithelioid cells and granulomas in a reactive background [Figure. 1B] (iii) Caseous necrosis with few epithelioid cells (iv) Caseous necrosis with few lymphocytes and histiocytes. No epithelioid cells. [Figure. 2A] (v) Only caseous necrosis without any other cell types [Figure. 2B] (vi) Tubercular abscess showing predominantly neutrophils along with epithelioid cells [Figure. 2C].

The fluorescent method using Auramine – Rhodamine stain and conventional method using ZN method were performed as per the documented procedure [7, 8]. The AFB under fluorescent method appeared as yellow to orange slender, rod shaped bacilli under fluorescent microscope [Figure. 3A & 3B]. In conventional ZN method, the pink coloured rod shaped bacilli were examined directly for AFB under oil-immersion (X 1,000) using light microscope.

The data were processed using test of association (Chi square test).

3. Results

Of the 201 aspirates, 197 were evaluated and the remaining 4 were excluded from the study due to diagnosis of malignancy in 1.99% and inadequate aspiration in 0.49%. The age ranged from 03-75 years, with the mean age of 11 years. The Male: Female ratio was 1.1:1 indicating slight male preponderance.

Among the 197 lymph nodes studied, aspirates were frequently from, cervical (n=126) followed by supraclavicular (n=20), submandibular (n=18) and axillary (n=16) groups.

The cytomorphological features observed were reactive lymphadenitis in 36.5% (72/197) cases, suppurative lymphadenitis in 12.7% (25/197) cases and TB lymphadenitis in 50.7% (100/197) cases.

The criteria for diagnosis of reactive lymphadenopathy was established based on mixed population of lymphoid cells without any malignant findings and a considerable number of tingible body macrophages [8]. The cytomorphological diagnosis of suppurative lymphadenitis was based on the aspirated purulent material showing abundant neutrophils with macrophages containing ingested necrotic debris in a necrotic background.

Of the 100 aspirates diagnosed as TB lymphadenitis by cytology, the smear positivity for AFB on conventional ZN method were 24% (24/100) while the positivity increased to 38% (38/100) by fluorescent method. The correlation between conventional ZN method and fluorescent method [Refer Table.1] was statistically significant ($P < 0.001$).

Based on cytomorphological appearance, TB lymphnode was further subdivided into six patterns and compared with ZN method and fluorescent method [Refer Table.2]. The commonest cytomorphological pattern of TB lymphadenopathy herein AFB was observed in both ZN method and Fluorescent method was pattern III [Figure. 1C], followed by pattern V and pattern IV.

4. Discussion

TB lymphadenitis is one of the most common mode of clinical presentation for extra-pulmonary tuberculosis. Though cytological features of TB lymphadenitis are well described, confirmatory diagnosis requires demonstration of AFB or a positive bacterial culture. In our study, the commonest age group of TB lymphadenopathy was between 20-30 years, which was observed in other studies. However there was slight male preponderance with M:F ratio of 1.1:1. Most frequent group of lymph nodes involved were cervical group and this finding is in concordance with that reported in literature. Discrete lymph node involvement was more common than multiple lymph node involvement. Matting of lymph nodes was observed in only 12% of cases reported as tubercular on cytomorphology as compared to 21% reported by Gangane et al [9] and 26.8% reported by Agarwal et al [10].

Like most of these studies, we also report a significantly high positivity of AFB detection by Fluorescent method [38%] as compared to ZN method [24%] in cytologically diagnosed tubercular lymphadenitis. The increased positivity by fluorescent method as compared to ZN method can be attributed to the fact that fluorescent stain is a simple staining procedure, reveals a microscopic picture of great brilliance and marked contrast even under low power objective with considerable reduction of scanning time. The case which was reported as positive by ZN but negative by A.R can be either because of absence or scanty yield of bacilli in that particular smear or because of interpretation of true bacillary fluorescence as non specific fluorescence. Notably, all the cases which were positive for bacilli by either or both the stains, were diagnosed as TB on cytomorphology. Another advantage which we observed for fluorescent method has been the increase in grade of AFB positivity in cases reported as scantily positive or negative by Z.N stain. This finding is in concordance with the findings of Kumar et al [11] and Ba F et al [12]. Amongst the various cytomorphological patterns described for TB lymphadenitis, we observed pattern III in 27% of aspirates, closely followed by pattern II and pattern I with 25% and 24% cases respectively. Bailey et al [13] reported pattern III as commonest whereas Gangane et al [9] observed pattern I frequently in his study. On comparing cytomorphology with both ZN and fluorescent method, detection of AFB was more frequently seen in patterns III, V and IV. The AFB positivity was higher in Fluorescent method as compared to ZN method.

However, the results of our study are limited by the fact that bacteriological confirmation by culture for all the TB patients have not been obtained.

5. Conclusion

We conclude that fluorescent method is a simple, rapid and easy staining method which reduces smear examination considerably in hospitals with increased work load. The advantage of fluorescent method is in diagnosis of paucibacillary cases of TB. Fluorescent method in combination with cytomorphological patterns of TB lymphadenitis can help in assessing the staging of extrapulmonary TB.

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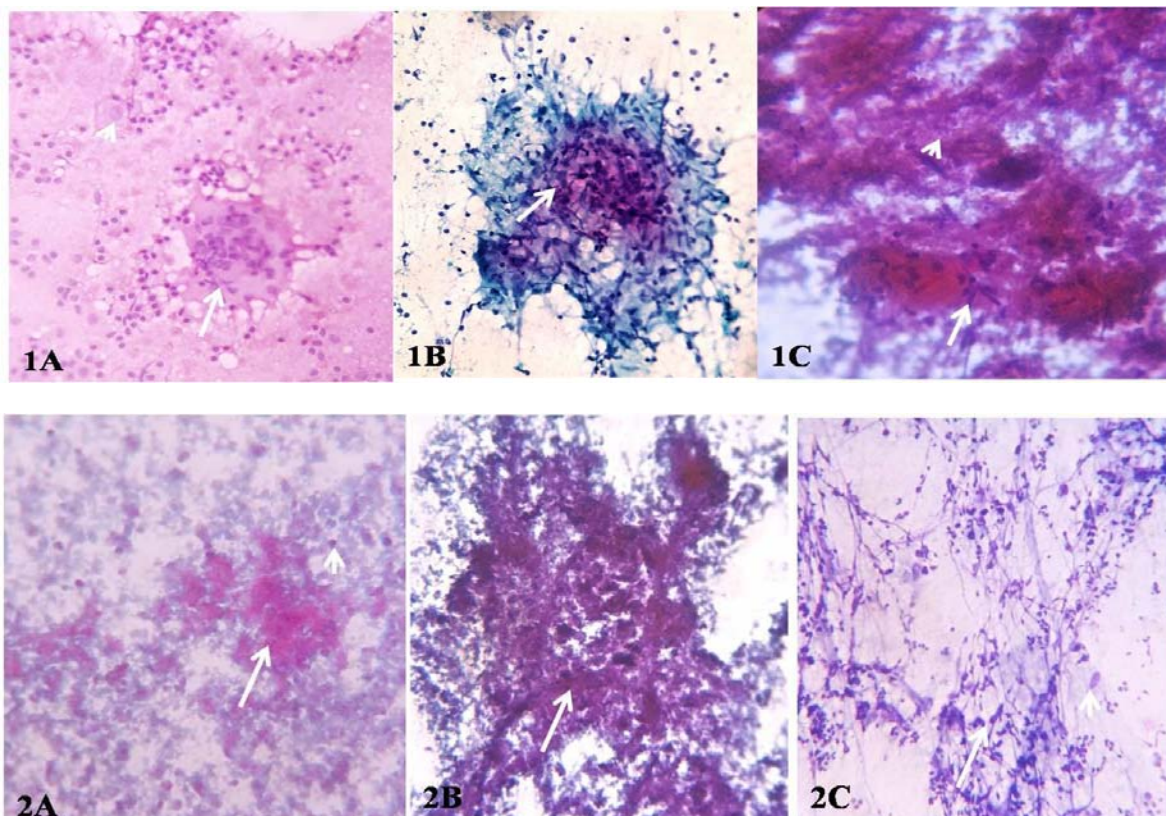
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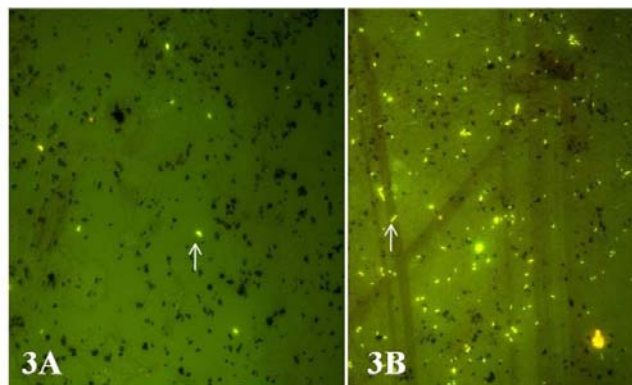
Table 1: Comparison of ZN method with fluorescent method for the detection of AFB in cytologically diagnosed tubercular lymphadenitis

Conventional ZN method	Fluorescent method		Total
	Positive	Negative	
Positive	23	1	24
Negative	15	61	76
Total	38	62	100
$\chi^2 = 44.83, df = 1, P < 0.001^*$			

Table 2: Correlation of Cytomorphological patterns with fluorescent method and ZN method

Cytomorphological patterns	Fluorescent method.		Conventional ZN method		Total
	Positive	Negative	Positive	Negative	
Pattern I	02	22	01	23	24
Pattern II	02	23	01	24	25
Pattern III	16	11	09	18	27
Pattern IV	06	04	05	05	10
Pattern V	09	01	06	04	10
Pattern VI	03	01	02	02	04
Total	38	62	24	76	100





Legends to Figures

Figure.1A: Epithelioid granulomas with epithelioid cells [Short Arrow], Langhan's giant cells [Long Arrow] and background of caseous necrosis (H&E, X400).

Figure.1B: Only numerous epithelioid cells and granulomas [Arrow] in a reactive background (PAP, X400).

Figure.1C: Caseous necrosis [Short Arrow] with epithelioid cells [Long Arrow]. (PAP, X400).

Figure.2A: Caseous necrosis [Long Arrow] with few lymphocytes [Short Arrow] and histiocytes. No epithelioid cells (PAP, X400).

Figure.2B: Only caseous necrosis [Arrow] without any other cell types (PAP, X400).

Figure.2C: Tubercular abscess showing predominantly neutrophils [Long Arrow] along with epithelioid cells [Short Arrow] (PAP, X400).

Figure.3A: Paucibacillary smear showing scant slender yellow to orange rod shaped bacilli (AR, X400).

Figure.3B: Multibacillary smear showing plenty of slender yellow to orange rod shaped bacilli (AR, X400).