

Comparison of Z. N. staining & fluorescent microscopy in detection of M. Tuberculosis bacilli in Fine needle aspiration smears

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Abstract

Aims: To compare ZN stain method with Fluorescent method for detection of tuberculous bacilli in FNA smears in terms of sensitivity & feasibility. **Settings and Design:** A Prospective study was conducted in the department of Pathology at tertiary care center. FNAC done from lymph node lesion in clinically suspected cases of tuberculosis attending the Department of Medicine, Surgery, ENT, TB and Chest. **Methods and Material:** Fine-needle aspiration was performed in the Department of Pathology from January 2016 to February 2017. Out of 409 overall FNAC samples, there are 193 FNAC Lymph node lesions, out of them 65 clinically suspected cases were processed for direct microscopy using conventional ZN staining and routine cytology and compared with the findings of the modified fluorescent method. **Statistical analysis used:** Simple statistical analysis done by using χ^2 test. **Results:** Out of the 65 Tuberculous positive aspirates, the smear positivity for AFB on the ZN method was 43.07% (28/65) while the positivity increased to 87.69% (57/65) on the Auramine-Rhodamine fluorescent method. **Conclusions:** Fluorescent microscopy is rapid, simple, easy method with high detection rate for AFB as compared to ZN method.

Key-words: Tuberculous lymphadenitis, ZN stain, Fluorescent stain.

Key Messages: Fluorescence microscopy is far better than ZN stain due to its high sensitivity and short period of time for detection.

Introduction

Tuberculosis (TB) is a major health problem in developing countries. Lymphadenopathy is the most common presentation of extra pulmonary tuberculosis [1,2]. Fine needle aspiration cytology (FNAC) has assumed an important role in the evaluation of peripheral Lymphadenopathy as a possible minimally invasive alternative to excisional biopsy [3].

The cytological criteria for the diagnosis of possible tubercular lymphadenitis have been clearly defined as epithelioid cell granulomas with or without multinucleated giant cells and caseation necrosis (Figure 1,2). Shows cytological criteria for the diagnosis of tubercular lymphadenitis [4]. Conventional Ziehl-Neelsen (ZN) method for acidfast bacilli (AFB) plays a key role in the diagnosis and the monitoring of

treatment in tuberculosis [5]. Fluorescent microscopy using auramine-rhodamine (AR) staining is rapid, simple, easy method with high detection rate for AFB as compared to ZN method [6,7]. Basically, the study was an attempt to find out cost-effective, rapid, and sensitive technique which can be used routinely in developing countries for early diagnosis and effective treatment of tuberculous lymphadenitis.

The study demonstrated the correlation of the cytomorphological features with various techniques in FNA smears from patients who are suspected of having tuberculous lymphadenitis. We tried to use fluorescent microscopy auraminerrhodamine staining to detect Mycobacterium and to compare it with conventional ZN method on lymph node aspirates in cytology.

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Materials & Method

A Prospective study was conducted at the department of Pathology at GMERS medical college and hospital Junagadh From January 2016 to February 2017. Total 409 number of FNAC samples received, out of those 193 FNAC are from Lymphnode lesion and among them, 65 clinically suspected cases of tuberculous lesions attending the Department of Medicine, Surgery, ENT, TB and Chest medicine at GMERS medical college and hospital were studied.

Sampling method:

All the aspirates by FNAC were processed for direct microscopy using conventional ZN staining and routine cytology and compared with the findings of the modified fluorescent method.

A total of three smears were prepared from each of the FNAC aspirates with the help of 23G needle: one alcohol-fixed wet smear was stained by hematoxylin and eosin (H and E) for cytological examination directly, and second and third air-dried smears were stained with ZN and AR stains, respectively.

Inclusion criteria: Both males and females (>1 year) with well palpable and enlarged peripheral lymph node were included.

Exclusion criteria: Patient with age < 1 year, very small or no palpable lymph nodes, or known cases of malignant, allergic, or skin disorders.

On cytomorphology, the tuberculous lymph node, was diagnosed using the following criteria: (i) purulent with caseation; (ii) only caseation; (iii) caseation with epithelioid cells; and (iv) non caseating with epithelioid cells.

AR stained slides were examined under fluorescent microscope using the blue excitation filter (450–480 nm). Mycobacteria appear as greenish yellow, slender, and slightly curved rod-shaped (Figure 3). ZN stained smears were examined for AFB under oil immersion (1000X) using light microscopy which appeared as pinkish, thin curved rod-shaped bacterium measuring 0.5 to 3 micrometer and sometimes as beaded. Results data are plotted & analyzed by simple statistical method using chi-square test.

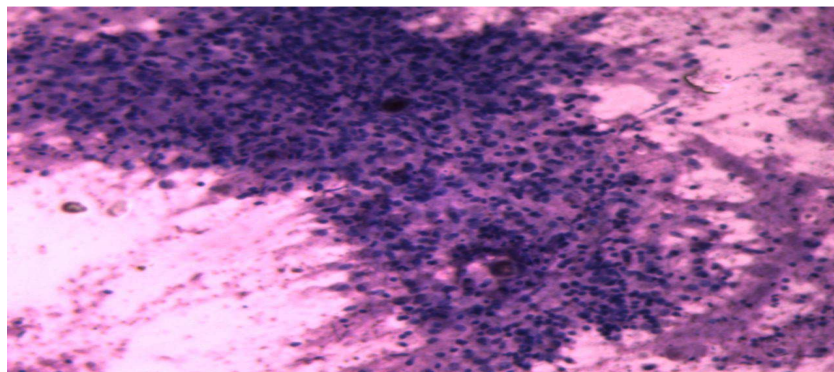


Fig-1: Granuloma formation (H&E x 100)

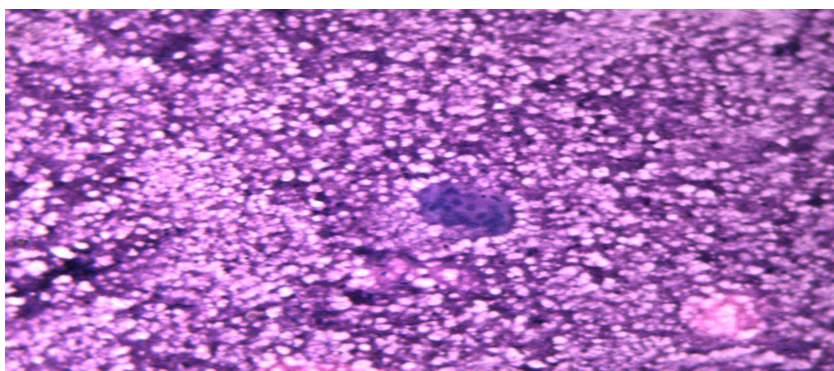


Fig 2: Giant cell reaction (H&E x 100)

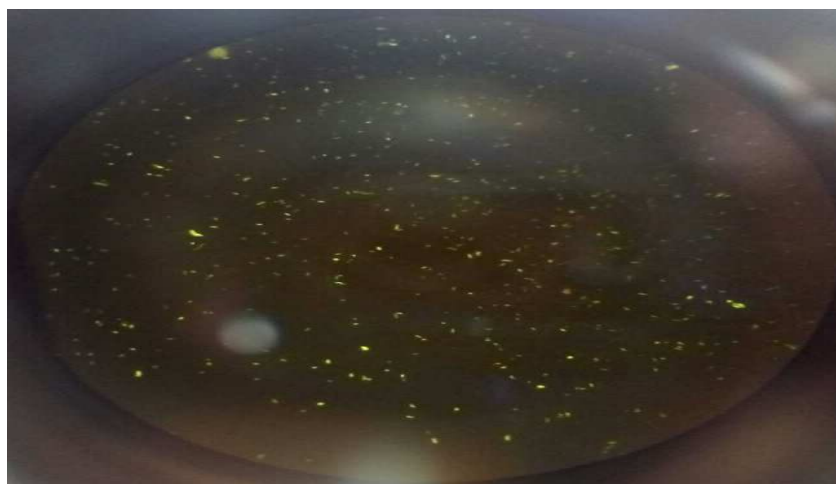


Fig 3: Fluorescent staining Slide Positive for AFB (+3) (AR x 400)

Results

A total of 193 fine-needle aspirated specimens from lymph nodes were included in the study. Out of 193 cases, 65 aspirates were reported as cytomorphology suggestive of tuberculous lymphadenitis. The age ranged from 1 to 70 years, with the mean age of 28.5 years. Male preponderance was noted accounting for 61.53% (40/65) of cases. 56.92% (37/65) of the cases with suggestive cytomorphology of tubercular lymphadenitis were in the range of 21-30 years of age (see Table 1).

Table-1: showing Age and Gender wise distribution of TB lymph node cases.

Age Range	Male	Female	Total	Percentage
0-10	00	01	01	1.53%
11-20	01	01	02	3.07%
21-30	24	13	37	56.92%
31-40	04	04	08	12.30%
41-50	08	04	12	18.46%
51-60	02	01	03	4.61%
61-70	01	01	02	3.07%
	40	25	65	

Age range of patient is from 1 to 70 years, with male predominance of 61.53%.

Most number of cases are from 21-30 years of age group with 56.92%.

Table-2: Distribution of Tuberculous Lymph node cases.

Region of Lymph node	Number of Cases	Percentage
Cervical	37	56.92%
Axillary	18	27.69%
Supraclavicular	04	6.15%
Inguinal	03	4.61%
Submandibular	02	3.07%
Submental	01	1.53%
	65	100%

Out of 65 cases cervical region is most common site of involvement for tuberculosis 37 (56.92%) in present study.

Table-3- I: Results of ZN and AR Staining.

Results	ZN Stain		AR Stain	
	Number	Percentage	Number	Percentage
Positive	28	43.08%	57	87.69%
Negative	37	56.92%	08	12.31%
TOTAL	65	100%	65	100%

P < 0.0001, Statistically Significant

Table-3-II: Sensitivity of ZN and AR Staining.

Results	ZN Stain	AR Stain
Sensitivity	43.08%	87.69%
Specificity	21.27%	55.56%

In present study, the most common site of involved lymph nodes was from the cervical region in 56.92% (37/65) of the cases (Table-2). Out of the 65 Tuberculous positive aspirates, the smear positivity for AFB on the ZN method was 43.07% (28/65) while the positivity increased to 87.69% (57/65) on the AR fluorescent method (Table 3). The correlation between increased sensitivity of test results with use of modified fluorescent method showed statistical significance (P< 0.001).

The cytomorphological features observed were reactive lymphadenitis in 41.45% (80/193) cases, acute suppurative lymphadenitis in 24.87% (48/193) cases and tubercular lymphadenitis in 33.67% (65/193) cases.

The criteria for the diagnosis of reactive lymphadenopathy was established based on polymorphic population of lymphoid cells without malignant features and a considerable number of tingible body macrophages.

The cytomorphological diagnosis of acute suppurative lymphadenitis was based on the aspirated purulent material showing abundant neutrophils with macrophages containing ingested necrotic debris in a necrotic background.

Three patterns were found in tuberculous lymphadenitis: (1) granulomatous lymphadenitis, (2) caseating necrotizing lymphadenitis, and (3) acute inflammation with granulomas.

The cytomorphological features observed were granulomatous lymphadenitis in 60.00% (39/65), caseating necrotizing lymphadenitis 30.76% (20/65), and acute inflammation with granuloma 9.24% (06/65).

Discussion

FNAC is an easy, reliable outpatient procedure for the diagnosis of tubercular lymphadenitis in palpable superficial lymph nodes, and it is ideally suited for use in resource limited settings, especially in developing countries where tuberculosis is a major cause of morbidity and mortality [8].

Since the early 1940s, the comparison of the fluorescent method with the conventional ZN method on sputum smears was implemented to improve the smear positivity for the detection of AFB. The use of a fluorochrome acid-fast stain, such as AR, is recommended because of its increased sensitivity and ease of interpretation compared with the ZN method [9]. The AFB typically fluoresce as golden, slender, rod-shaped bacilli, but they may appear curved or bent.

Also, some individual AFB may display heavily stained areas referred to as beads and/or alternating light and dark areas of stain producing a banded appearance. Although the ability to retain aryl methane dyes, such as auramine O, after washing with alcohol or weak acids is a primary feature of the genus *Mycobacterium*, it is not entirely unique to the genus. Other bacteria, which contain mycolic acids, such as *Nocardia*, can also exhibit this feature.

The exact method by which the stain is retained is unclear but it is thought that the stains become trapped within the cell or may form a complex with the mycolic acids. This is supported by the finding that shorter chain mycolic acids or *Mycobacterium* cells with disrupted cell walls stain weakly acid-fast. A disadvantage is that

there is a more intense binding of the mycolic acids to the fluorochrome dye causing bacilli, which are apparently rendered nonviable by chemotherapy to be acid-fast [10,11]. Laboratory plays a critical role in the diagnosis of TB. In developing countries, FNAC of lymphnode is by far the fastest, cheapest, and definitive method for the detection of AFB. We attempt to use the fluorescent method and compare it with the conventional ZN method on lymph node aspirates (FNAC).

Detection of AFB by conventional microscopy is simple and rapid but lacks adequate sensitivity. False-negative results are possible, especially in paucibacillary cases.

Culture is essential for a definitive diagnosis; however, it takes weeks for identification, and its sensitivity is also relatively low in paucibacillary conditions [12,13]. Its major disadvantages are low sensitivity, time consuming.

In present study maximum cases affected were in the age group of 21-30 yrs, which accounted for 56.92% (37/65) of total cases. The age group most commonly affected by tuberculosis in our study is correlated with other studies like Dagar et al[14] Annam et al[15]. In our study mean age was 28.5 years, Dagar et al[14] has also observed the similar mean age for diagnosis.

Male preponderance was noted in our study as 61.53% (40/65), which is differ from other study like Dagar et al [14] Annam et al [15] which having female predominance.

There were 37/65 (56.92%) cases of cervical lymphadenopathy similar finding has also been observed by Annam et al[15] (72% cervical Lymphnodes), Thakur et al [16] found that there was 83.3% cases of cervical lymphadenopathy.

In present study of 65 samples, the total AFB positivity rate was 43.08% (28/65) cases on ZN stain and was increased to 87.69% (57/65) on fluorescent stain.

This comparison of Ziehl-Neelsen and fluorescent results showed a significant p value of <0.0001 for the presence of bacilli. Similar findings seen in study of Dagar et al[14] (43.08% cases on ZN stain and was increased to 51.3% on fluorescent stain.), Annam et al[15](44.1% cases on ZN stain and was increased to 81.37% on fluorescent stain.). Among various cytomorphological Pattern of Tuberculous lymphadenitis, Granulomatous Lymphadenitis (60%) was the most common pattern in our study which is correlate well with other study like Thakur et al[16].

Conclusion

FNAC of Superficial lymphnode is outpatient procedure & requires little infrastructure and equipment, so ideal for developing countries. In laboratories where there is considerable load of work for detection of AFB,

Fluorescence microscopy is far better than ZN stain due to its high sensitivity and short period of time needed to scan whole smear and slides can be examined under low magnification allowing large areas of smear to be examined. Fluorescent microscopy is rapid, simple, easy method with high detection rate for AFB as compared to ZN method especially in patient with a low density of bacilli that are likely to be missed on ZN stained smears.

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