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## Modified Bleach Method – Improving Microscopic Detection of Acid Fast Bacilli in Fine Needle Aspiration Smears of Lymph Nodes

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### ABSTRACT

*Background:* Ziehl-Neelsen (ZN) method proves to be a good modality of microscopic detection of Acid Fast Bacilli (AFB) in Fine Needle Aspiration (FNA) smears of lymph nodes though has a low sensitivity. It has a potential to become an even more valuable tool if its sensitivity could be increased. *Objective:* To improve microscopic detection of AFB in FNA cytology of lymph nodes using modified bleach concentration method and compare results with cytomorphological diagnosis and conventional ZN method. *Study Design:* In 200 patients with clinical suspicion of tuberculosis presenting with lymphadenopathy, Fine Needle Aspiration Cytology (FNAC) of the lymph nodes was performed. Smears were processed for routine microscopy and conventional ZN method. The remaining material in needle

*hub/syringe was rinsed and kept with bleach in accordance with modified bleach method. The significance of bleach method over the conventional ZN method was analyzed. Results:* Among the 200 aspirates, 52% (104/200) were indicative of tuberculous lymphadenitis cytomorphologically, conventional ZN method detected AFB in 35.5% (71/200) and the smear positivity increased to 68% (136/200) when modified bleach method was used. *Conclusion:* Modified bleach method is easy, inexpensive and improves the microscopic detection of AFB significantly. Moreover it is a potent disinfectant and thus limits the risk of laboratory acquired infections.

**Keywords-** Fine Needle Aspiration Cytology, Acid Fast Bacilli, Lymph Node, Ziehl-Neelsen Stain, Modified Bleach Method.

## INTRODUCTION

India is highest tuberculosis (TB) burden country accounting for one fifth (21%) of global incidence [1]. Despite the availability of highly efficacious treatment for decades, tuberculosis remains a major global health problem. While pulmonary tuberculosis is the most common presentation, extra-pulmonary tuberculosis is also an important clinical problem [2]. Lymphadenopathy is the most common form of extra-pulmonary tuberculosis [3].

The utility of Fine Needle Aspiration Cytology (FNAC) in diagnosis of tuberculous lymphadenitis has been highlighted in a number of studies during the last two decades [4],[5],[6]. In developing countries like India, the only practically available method for diagnosing extra-pulmonary tuberculosis is direct smear microscopy for Acid Fast Bacilli (AFB) of the sample from the lesion using Ziehl-Neelsen (ZN) method but it has a low sensitivity ranging from 9% to 46% [7]. Microscopy has many advantages when it comes to speed and feasibility, and if its sensitivity could be improved, it has the potential to

become an even more valuable tool for tuberculosis control programmes around the world [8]. In the last decade many researchers have suggested that the performance of smear microscopy can be significantly improved if the sample is liquefied with one or other chemical reagents and then concentrated by centrifugation or sedimentation before acid fast staining [9]. There are various concentration methods for

improving sensitivity of direct microscopy for detection of tubercle bacilli in specimen. Among these, the bleach concentration method is one of the safest concentration methods which improve the sensitivity of detection of acid fast bacilli [7]. In the present study, FNAC was used to diagnose cases of tuberculosis. The modified bleach concentration method was used on fine needle aspirates of lymph nodes and the results were compared with that of routine ZN staining on Fine Needle Aspiration.

## MATERIALS AND METHODS

The present study was conducted on two hundred clinically suspected patients of tuberculosis with lymphadenopathy referred for FNAC in department of Cytology. The exclusion criteria were treatment for TB within previous 3 months or initiation of TB treatment before sampling was performed. A detailed history was taken in each case and through clinical examination was done. An informed consent was taken from the patient after explaining the procedure. FNAC was performed and aspirates were processed for:

- a) Cytomorphological features, to arrive at cytological diagnosis.
- b) Direct microscopy for detection of AFB using conventional ZN staining.
- c) Direct microscopy using modified bleach concentration method for detection of AFB.

For cytological examination, smears were prepared directly and air-dried smears were stained with May-Grunwald Giemsa (MGG) and Ziehl-Neelsen (ZN) stains.

The bleach method was performed with the remaining aspirated specimen in the syringe or

needle hub, which was rinsed with 1 ml normal saline and transferred into 5 ml sterile test tubes. To this test tube, 2 ml of 5% Sodium hypochlorite (NaOCl) was added and the mixture was incubated at room temperature for 15 minutes by shaking at regular intervals. The test tube containing the mixture was concentrated by centrifugation at 300 g for 15 minutes after addition of 2 ml of distilled water. The supernatant was carefully discarded and the sediment was transferred with a sterile pipette on to a clean sterile slide. The slide was air-dried, heat fixed and stained by the ZN method. As a control, 2 ml of distilled water was centrifuged and the sediment was stained by ZN staining to rule out any error due to contamination while testing each specimen.

The slide were allowed to dry and was carefully examined under low power (10X), high power (40X), and oil immersion (100X) lens for the presence of tubercle bacilli which appeared as bright red beaded rods singly or in groups against a clear white background. The results were analysed using Chi- Square ( $\chi^2$ ) test.

## RESULTS

Out of 200 patients included in the study, maximum cases (30%) presented between the age group 21-30 years. The age ranged from 5 months to 85 years. The M: F ratio was 1.13:1. A total of 11 patients were Human Immunodeficiency Virus (HIV) positive. 44% (88/200) cases presented in between 1 to 3

months of onset of lymphadenopathy.

Of the 200 cases 116 (58%) had lymphadenopathy along with mild fever. Cough, loss of weight and loss of appetite were other clinical symptoms. Also, 50 (25%) cases presented without any other associated symptoms except lymphadenopathy.

The cervical group of lymph nodes was most predominantly affected in 65.5% (n=131) cases. Other lymph nodes involved were submental (n=6), submandibular (n=18), preauricular (n=3), supraclavicular (n=19) and axillary (n=17). 6 cases showed involvement of cervical together with axillary group. The clinical examination of involved lymph node regions showed that there was discrete nodal enlargement in majority (78%) of cases. Matted lymph nodes present in 34 (17%) cases and sinus formation was noted in 10 (5%) cases.

The cytomorphological features observed were suppurative lymphadenitis in 27.5% (55/200) cases, reactive lymphadenitis in 20.5% (41/200) cases and tuberculous lymphadenitis in 52% (104/200) cases. There was a statistically significant correlation between cytomorphological diagnosis, results of smears prepared by conventional ZN method and bleach method (Table 1 and Table 2).

**Table 1**

**Correlation of cytomorphological diagnosis with conventional ZN method**

Cytomorpholog ical diagnosis	Conventional ZN		Total
	Positive	Negativ	
Suppurative LN	6	4	5
Reactive LN	0	4	4
Tuberculous LN	6	3	104
Total	7	1	200

$$\chi^2=78.1, df=2, p<0.001$$

**Table 2****Correlation of cytomorphological diagnosis with modified bleach method**

Cytomorphological diagnosis	Bleach method		Total
	Positive	Negative	
Suppurative LN	31	2	5
Reactive LN	10	3	4
Tuberculous LN	95	9	104
Total	13	6	200

$$\chi^2=65.30, df=2, p<0.001$$

The diagnosis of suppurative lymphadenopathy was based on aspirated purulent material and presence of degenerated polymorphs and cellular debris against a necrotic background [10]. Of the 27.5% (55/200) cases diagnosed as suppurative lymphadenitis, the bleach method was positive for AFB in 56.36% (31/55) while the conventional ZN method was positive in only 10.9% (6/55) cases.

The criteria for diagnosis of reactive lymphadenitis were presence of mixed population of lymphoid cells with predominance of small lymphocytes and centroblasts, centrocytes, immunoblasts and plasma cells in varying proportions together with presence of tingible body macrophages [11]. Among 20.5% (41/200) cases diagnosed as reactive lymphadenitis, the bleach method could detect AFB in 24.39% (10/41) cases and all cases were negative by conventional ZN method.

On cytomorphology, tuberculous lymph node was diagnosed using following criteria: 1) Epithelioid granulomas with caseous necrosis, 2) Epithelioid granulomas without caseous necrosis and 3) Necrosis alone without

epithelioid granulomas [12]. Of 52% (104/200) cases, AFB were identified by bleach method and conventional ZN method in 91.34% (95/104) and 62.5% (65/104) cases, respectively.

The smear positivity for AFB on conventional ZN method was 35.5% (71/200) while the positivity increased to 68% (136/200) on bleach method. The comparison between conventional ZN method and bleach method showed statistical significance (Table 3).

**Table 3**  
Comparison of the conventional ZN method with the bleach method for detection of AFB

Conventional ZN method	Bleach method		Total
	Positive	Negative	
Positive	71	0	71
Negative	65	6	12
Total	13	6	20

$$\chi^2=51.8, df=1, p=0.000$$

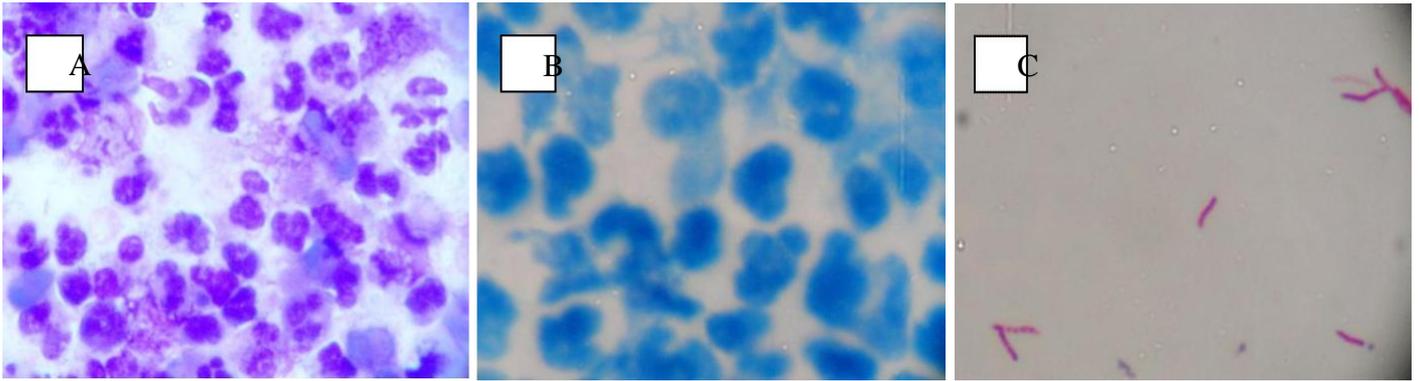


Figure 1: Suppurative lymphadenitis. (A) Cytomorphology showing abundant degenerate polymorphs with macrophages containing ingested debris against a necrotic background (MGG, X400); (B) Smear was negative for AFB by conventional ZN method (X1000); (C) Bleach Method shows few AFB with clear background (X1000)

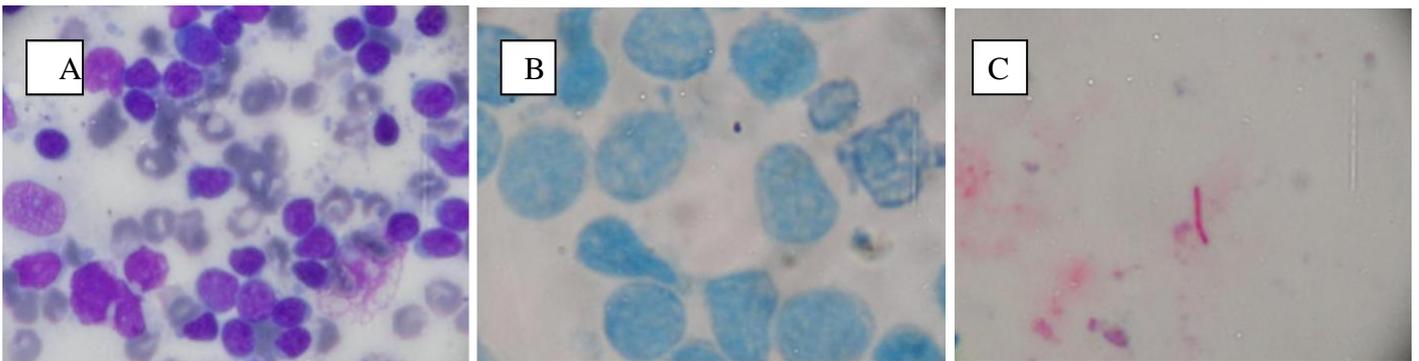


Figure 2: Reactive lymphadenitis. (A) Cytomorphology showing polymorphous population of lymphoreticular cells along with tangible body macrophages against a background of RBCs; (B) Smear was negative for AFB by conventional ZN method (X1000); (C) Bleach Method shows an AFB in center of field with clear background (X1000)

## DISCUSSION

Tuberculosis is an ancient infection that has plagued humans since times immemorial, still continues to remain a major public health problem especially in developing countries like India. The emergence of AIDS has added fuel to the existing fire of tuberculosis. The magnitude of the problem is so huge that it warrants rapid diagnosis to limit its spread [13].

The diagnosis of tuberculosis is easy and simple when disease is florid or disseminated but localised involvement of extra-pulmonary organ or tissue may at times pose a diagnostic problem [13]. The clinical parameters for the diagnosis of tuberculosis in lymph nodes are neither specific nor do their absence exclude tubercular involvement [14]. Early diagnosis of tuberculosis and initiating optimal treatment would not only enable a cure of an individual patient but will curb the transmission of infection and disease to others in community [15]. Diagnostic modalities must also be tailored to needs of population and epidemiology of tuberculosis in that region.

These include improved microscopy, usage of liquid culture for childhood and extra-pulmonary tuberculosis, chemical and physical detection of mycobacterial antigens in paucibacillary condition, antigen capture, antibody detection, cellular immune recognition, nucleic acid amplification and phage assay [15].

In developing countries, microscopy of the specimen is by far the fastest, cheapest and most reliable method for the detection of AFB. In late 1940s, sputum liquefaction with sodium hypochlorite (NaOCl/ bleach) and then concentration by centrifugation before acid fast staining was implemented to improve smear positivity for detection of AFB [16]. In the present study, this technique was slightly modified and applied to fine needle aspirates from lymph nodes.

In present study, the discrepancies between cytomorphological diagnosis and bleach method occurred in 50 cases. Out of these 50 cases, 10 cases were reactive lymphadenitis and 31 cases were suppurative lymphadenitis, but these cases were positive for AFB by modified bleach method. And 9 specimens were negative for AFB by bleach technique but diagnosed as tuberculous lymphadenitis on cytology. The possible explanation for the diagnosis of reactive lymphadenitis on cytology but positive for AFB by bleach method could be due to loss of scattered epitheloid cells among the polymorphous population of lymphoid cells [17].

Among the 31 cases diagnosed as suppurative lymphadenitis positive for AFB by bleach method, the probable reason could be loss of bacilli among necrotic debris. Also, 9 cases diagnosed as TB on cytology and negative by bleach method may be due to decrease in the

density of bacilli. Thus, we have demonstrated that the liquefaction of the aspirated material

with bleach followed by centrifugation significantly increases the yield of AFB.

The findings of present study are in consonance with those of Khubnani and Munjal [7] who studied 55 cases of extra-pulmonary tuberculosis out of which 17 were from lymph node aspirates showed significant rise in the sensitivity of positivity for AFB after bleach method. Out of 17 lymph nodes studied, aspirates were, cervical (13), inguinal (3) and axillary (1). Cytology was suggestive of tuberculosis in 10 cervical lymph node aspirates while conventional ZN staining detected AFB in 8 and bleach method was positive in 11 aspirates. Out of 3 inguinal aspirates studied one was suggestive of Tuberculosis on cytology, negative on conventional ZN staining but positive for AFB on bleach method. The single case of axillary lymphadenopathy was suspected to be tubercular on cytology, negative for AFB by conventional ZN staining but positive by bleach method.

Gangane et al [8] studied 100 cases of tuberculous lymphadenitis and reported a high diagnostic accuracy of modified bleach method with a AFB positivity rate of 72.0 % as compared to 27% of conventional ZN staining.

Annam et al [17] studied 93 cases of lymphadenitis and reported diagnostic accuracy of modified bleach method with an AFB positivity rate of 63.44% while positivity rate

for AFB on conventional ZN method was only 33.33%.

Chandrasekhar and Prayaga [18] studied 112 cases of tuberculous lymphadenitis. Routine ZN staining detected AFB in 12.5% of cases and the modified bleach method in 60.7%. Modified bleach method showed AFB positivity in additional 54 cases where routine AFB staining was negative.

There are many advantages of modified bleach method over conventional ZN technique. Sodium hypochlorite effectively kills tubercle bacilli. This makes the specimen safe to handle. Moreover the bacilli can be easily detected against a clear background which makes the screening procedure easier, faster and less strenuous. However, the smears are thin and not easily visible to naked eye therefore extra care is required in labeling and staining the correct side of slide.

Thus, the modified bleach method is easy, inexpensive and improves the microscopic detection of AFB significantly. It is a potent disinfectant and thus limits the risk of laboratory acquired infections. Moreover it is more sensitive than conventional ZN method in detection of AFB.

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