



Original Research Article

Comparative Evaluation of Two Staining Techniques for Detection of Tubercular Bacilli in Lymphnodal Aspirates

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ABSTRACT

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

Objective: Study was conducted to compare standard Ziehl-Neelsen (ZN) staining technique with Bleach method of concentration for demonstrating tubercular bacilli in lymph node aspirates of suspected tubercular lymphadenitis.

Results: FNA smears from 125 patients suspected clinically of tubercular lymphadenopathy were studied. Of 125 cases, 111 were diagnosed as tuberculosis and 14 as suppurative lymphadenitis. Among 111 cases of tuberculosis, 47 showed pattern 1 (Epithelioid cell granulomas with caseous necrosis); 41 showed pattern 2 (Epithelioid cell granuloma without caseous necrosis) and 24 showed pattern 3 (necrosis alone) on cytomorphology. In pattern 1, 38% were positive by ZN stain and 49% were positive by Bleach method. Out of 14 cases of suppurative lymphadenitis all were negative for tubercular bacilli on ZN stain but 3 showed positivity on Bleach method. On ZN stain smear, positivity for AFB was 22.4% but it increased to 28.8% on Bleach method.

Conclusion: Bleach method for detection of tubercular bacilli in lymphnode aspirates is more sensitive than the conventional ZN method. It is simple, inexpensive, affordable method that requires little additional expertise than required for conventional ZN staining. Moreover, this method effectively kills the tubercular bacilli, rendering the specimen safe to handle and reducing the risk of laboratory infection.

Keywords: Tuberculosis, Ziehl-Neelson stain, Bleach method

INTRODUCTION

Tuberculosis remains a worldwide public health problem despite the fact that the causative organism was discovered more than hundred years ago. ^[1] Lymphadenitis is the most frequent presentation of extra-pulmonary tuberculosis. ^[2] Early and

accurate detection of active cases remains an important objective for appropriate treatment and reduction in the spread of the disease. The diagnosis traditionally depends upon identifying the infective organism in secretions or tissues of diseased individuals. The gold standard for diagnosis of

tuberculosis is culture on L.J. medium. However, it is time consuming and requires specialized safety procedures in laboratories. Sensitivity and specificity of serological techniques is unsatisfactory. Newer molecular techniques such as Polymerase Chain Reaction (PCR), although rapid and sensitive, are costly to be routinely used in developing countries. Due to these limitations, most programmes use Ziehl-Neelsen (ZN) staining method and microscopy for the detection of Acid fast bacilli, but the technique has low sensitivity ranging from 20% to 40%. Various concentration methods which help to clear the necrotic background of smears exist for improving sensitivity of direct microscopy for detection of Acid fast bacilli. [3,4] Bleach concentration method for detection of Acid fast bacilli has been recently described for sputum and other extra-pulmonary specimen. [5,6,7] 2-5% concentration of Sodium hypochlorite (NaOCl) digests the necrotic material and inactivates the mycobacteria without altering their structure, so that even when they are killed, they can be stained and observed. Demonstration of tubercle bacilli by bleach method is a simple technique which requires no expertise and is inexpensive. [8,9,10] The present study is being undertaken to compare the standard Ziehl-Neelsen direct staining technique with Bleach method of concentration for demonstrating tubercular bacilli in the lymph node aspirates of suspected tubercular lymphadenitis.

MATERIALS & METHODS

One hundred twenty five patients suspected clinically of having tubercular lymphadenopathy referred for Fine needle aspiration cytology (FNAC) to the cytology section of Postgraduate department of Pathology were included in the study. The lymph nodes involved by primary tumours and secondary tumours deposits were

excluded from the study. Relevant haematological, biochemical and radiological investigation details were reviewed in these patients. All the aspirates by FNAC were processed for routine cytology and conventional ZN staining and compared with the findings of the Bleach method. The cytological examination was performed by fixing the smears in 95% ethyl alcohol followed by staining with papanicolaou stain. The air dried smears were stained with May-Grunwald Giemsa stain and conventional ZN stain.

For Bleach method, aspirate from the needle was rinsed with 2 ml of 5% sodium hypochlorite (Bleach). After thorough mixing, the mixture was incubated for 15 minutes at room temperature with frequent mixing. An equal volume of distilled water was added and mixed thoroughly and then centrifuged at 3000 rpm for 15 minutes. The supernatant was discarded and smear was prepared using one drop of the sediment, air dried, heat fixed and stained by Ziehl-Neelsen technique. Smears stained with conventional ZN stain as well as Bleach method were scanned under high power lens for the presence of Acid fast bacilli. Positive smears were examined under oil immersion(x1000) lens.

RESULTS

A total of 125 fine needle-aspiration specimens from the lymph nodes were included in the study. Seven patients were HIV positive. The age ranged from 1 to 82 years, with a mean age of 28.4 years. A slight male preponderance was noted, accounting for 52% of the cases. The most commonly involved lymph node group was Cervical i.e. 79(63.2%) lymph nodes, followed by Axillary 13(10.4%), Supraclavicular 13(10.4%), Submandibular 7(5.6%), Generalised 7(5.6%), Inguinal 3(2.4%) and Submental 3(2.4%) lymph nodes.

Out of 125 cases, 89 % (111/125) cases were cytomorphologically diagnosed as Tuberculosis and 11% (14/125) as suppurative lymphadenitis. The diagnosis of suppurative lymphadenopathy was based on aspirated purulent material and presence of degenerated polymorphs and cellular debris against a necrotic background.

On cytomorphology, tuberculous lymph node was diagnosed using following criteria:

- 1) Epithelioid cell granulomas with caseous necrosis seen in 47(37.6%) cases (pattern 1);
- 2) Epithelioid cell granulomas without caseous necrosis seen in 41(32.8%) cases (pattern 2);
- 3) Necrosis alone without epithelioid cell granulomas seen in 23(18.4%) cases (pattern 3).

Cytological Diagnosis	ZN Method		Bleach Method		Total
	+	-	+	-	
Pattern 1	18	29	23	24	47(37.6%)
Pattern 2	1	40	1	40	41(32.8%)
Pattern 3	9	14	9	14	23(18.4%)
Suppurative	0	14	3	11	14(11.2%)
Total	28	97	36	89	125(100%)

Table 1: Correlation of cytomorphological diagnosis with the Conventional ZN stain and the Modified Bleach method

Out of 111 cases diagnosed cytologically as tuberculous lymphadenitis, 47(37.6%) cases showed pattern 1, (*Table no. 1*) of which 38% (18/47) were positive by conventional ZN stain (Figure 1) and 49% (23/47) were positive by modified Bleach method (Figure 2). The positivity increased from 38% to 49%. 41(32.8%) cases showed pattern 2 on cytology. Only one case was positive for AFB by both routine ZN stain and modified Bleach method. The positivity was 2.13%. Pattern 3 (caseous necrosis only) was found in 23(18.4%) cases, out of which 39% (9/23) were positive for routine ZN stain as well as modified Bleach method. 14(11.2%) cases aspirated pus and were diagnosed as acute suppurative lymphadenitis on cytology and

were negative for tubercle bacilli on routine ZN stain (Figure 3). However, 3 out of 11 (27.3%) cases were positive with modified Bleach method for ZN stain (Figure 4).

The smear positivity for AFB on conventional ZN stain was 28(22.4%) while the positivity increased to 36(28.8%) on Bleach method. There was concordance/ agreement between both methods for staining of Acid fast bacilli in diagnosing tuberculosis as calculated by Kappa=0.7383 (0.597-0.879). There was highly significant correlation between cytomorphological diagnosis and the two methods of staining for acid fast bacilli. [as calculated by Correlation co-efficient Kendal's tau-b=0.776 (p<0.001)]

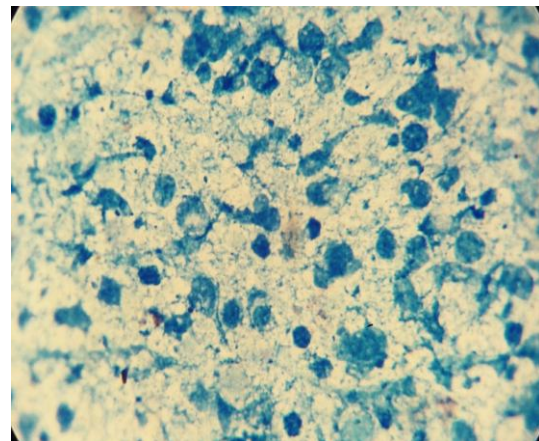


Figure 1: Photomicrograph from a case diagnosed cytologically as tuberculous lymphadenitis but negative for tubercular bacilli with routine ZN stain (1000 X).

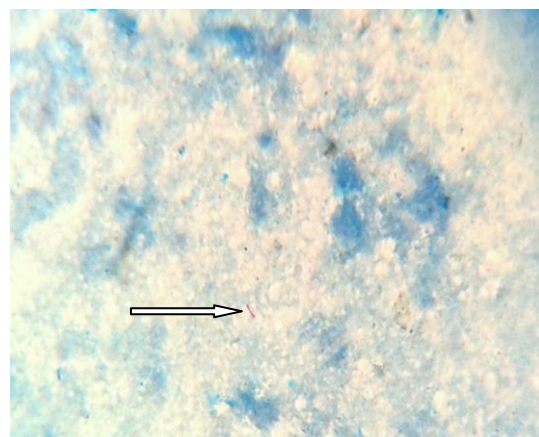


Figure 2: Photomicrograph from the above case showing tubercular bacillus (arrow) when stained with modified bleach method for ZN stain (1000 X).

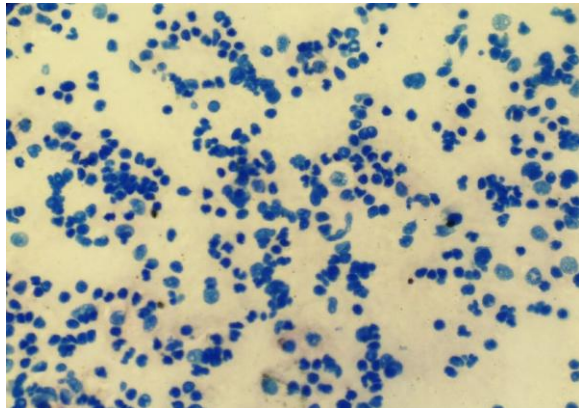


Figure 3: Photomicrograph from a case diagnosed cytologically as acute suppurative lymphadenitis but negative for tubercular bacilli with routine Z N stain (1000 X).

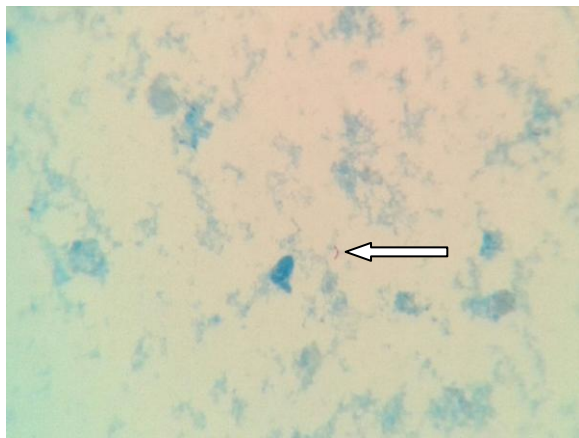


Figure 4: Photomicrograph from above case showing tubercular bacilli (arrow) with dissolution of inflammatory cells after staining with modified bleach method for Z N stain (1000 X).

DISCUSSION

Tuberculous lymphadenitis is the most common type of extra pulmonary tuberculosis. The diagnosis of tuberculosis is easy and simple when the disease is florid or disseminated but localized involvement of extra pulmonary organ or tissue may at times pose a diagnostic problem. 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 the community. In developing countries, microscopy of the specimen is by far the fastest, cheapest and most reliable method for the detection of Acid Fast Bacilli.

In the present study, the cytomorphological features observed were tuberculous lymphadenitis in 89% (111/125) cases and suppurative lymphadenitis in 11% (14/125) cases. Out of 111 cases diagnosed cytologically as tuberculous lymphadenitis, 47(37.6%) cases showed pattern 1, of which 38% (18/47) were positive by routine ZN stain and 49%(23/47) were positive by modified Bleach method. The positivity increased from 38% to 49%. This increase in positivity is due to digestion of granulomas and lymphoid follicular cells in the smears which obscures the visibility of Acid fast bacilli. The Bleach method, by clearing these cellular elements, provides a clear background and easier detection of Acid fast bacilli in the smears.

41(32.8%) cases showed pattern 2 on cytology. Only one case was positive for Acid fast bacilli by both routine ZN stain and modified Bleach method. The positivity was quite low (2.13%). Similar findings were observed by Prasoona D et al. [11] who found an inverse relationship between the presence of granuloma and of Acid fast bacilli. This is due to the ability of epithelioid cells (activated macrophages) to phagocytose and kill the micro-organism.

Pattern 3(caseous necrosis only) was found in 23(18.4%) cases, out of which 39% (9/23) were positive for routine ZN stain as well as modified Bleach method. There is no increase in positivity of Acid fast bacilli by Bleach method as compared to routine ZN stain. This is due to greater density of bacilli in necrotic areas and an already clearer background of the smears as compared to granulomatous pattern.

Aspiration of pus from a lymph node involves a differentiation between acute suppurative lymphadenitis and tuberculous lymphadenitis. The cytologic pictures in both these conditions may, at times, mimic each other showing numerous neutrophils

lying in an abundant necrotic background, especially when scattered epithelioid cells are lost among abundant neutrophils. Differentiation between these two conditions depends upon the presence or absence of Acid fast bacilli. In our study, 14(11.2%) cases aspirated pus and were diagnosed as acute suppurative lymphadenitis on the basis of cytology along with negativity for tubercle bacilli on routine ZN stain. However, 3 out of 11 (27.3%) cases were positive with modified Bleach method for ZN stain. The Acid fast bacilli were missed probably because of the presence of abundant neutrophils in the smear. The digestion of neutrophils and cellular debris resulted in clear background where Acid fast bacilli can be readily identified.

Conventional ZN stain detected 28(22.4%) cases of Acid fast bacilli positivity and Bleach method detected 36(28.8%) cases of Acid fast bacilli positivity. Hence, the Bleach method detected more cases than Conventional ZN staining. Also, there was a statistically significant correlation between cytomorphological diagnoses, results of smears prepared by conventional ZN stain and Bleach method. ($Kappa=0.7383$).

Out of 125 cases, 7 cases were HIV positive. The predominant morphological pattern seen in these cases was caseous necrosis (4/7 cases). 2 cases showed epithelioid granuloma with caseous necrosis (pattern 1), suppurative lymphadenitis was seen in 1 case. Out of 7 cases, 2 cases were positive for Acid fast bacilli with conventional ZN stain and Bleach method as well.

The discrepancies between cytomorphological diagnosis and Bleach method in the present study occurred in 3 cases which were diagnosed as suppurative lymphadenitis on cytomorphology but all these cases were positive for Acid fast bacilli by modified Bleach method. The

probable reason could be loss of bacilli among the necrotic debris in conventional ZN stain whereas Bleach method by providing a clear background, aids in finding Acid fast bacilli in such cases.

The discrepancies were also noted in 76 cases which were negative for Acid fast bacilli by both methods but diagnosed as tuberculous lymphadenitis on cytology; and 2 cases which were positive by conventional ZN staining but negative by Bleach method. These discrepancies may be due to decrease in the density of the bacilli in the given specimen.

The findings of present study are in consonance with those of other studies done by Gangane N et al. who studied 100 cases of tuberculous lymphadenitis and reported a high diagnostic accuracy of modified Bleach method with an Acid fast bacilli positivity rate of 72.0 % as compared to 27% of conventional ZN staining.^[8] Annam V et al. studied 93 cases of lymphadenitis and reported diagnostic accuracy of modified Bleach method with an Acid fast bacilli positivity rate of 63.44% while positivity rate for Acid fast bacilli on conventional ZN method was only 33.33%.^[7] Chandrasekhar B et al. studied 112 cases of tuberculous lymphadenitis. Routine ZN stain detected Acid fast bacilli in 12.5% of cases and the modified Bleach method in 60.7%. Modified Bleach method showed Acid fast bacilli positivity in additional 54 cases where routine Acid fast bacilli staining was negative.^[12] In the study conducted by Patel M et al. among the 115 aspirates, 59.13% were indicative of tuberculosis on cytology, 27.83% were positive for Acid fast bacilli on conventional ZN stain and the smear positivity increased to 61.74% on Bleach method.^[13]

The percentage of Acid fast bacilli positivity in the present study was lower (28.8%) compared to that reported in previous studies. The reason probably is

more number of Pattern 2 cases in the present study.

We have demonstrated that liquefaction of the aspirated specimen with NaOCl followed by centrifugation significantly increased the yield of Acid fast bacilli. This finding is of considerable interest in developing countries where smear-negative tuberculosis has become increasingly common. The improved recovery of Acid fast bacilli after treatment with NaOCl might be due to changes in the surface properties of the Acid fast bacilli (i.e., charge and hydrophobicity). Also, the increased smear positivity by the bleach method is attributable to the reduction of debris, leaving a clear field for microscopy. Thus, the preparation of samples by the Bleach method reduces the time required for examination of the slides to detect Acid fast bacilli which makes the screening procedure easier, faster and less strenuous.

Sodium hypochlorite effectively kills tubercular bacilli. This makes the specimen safe to handle. With the occurrence of multidrug-resistant tuberculosis, the risk of laboratory infection has become a major concern. Use of the Bleach method would definitely lower the risk of laboratory infection.

In conclusion, the Bleach method for detection of tubercular bacilli in lymph node aspirate is more sensitive than the conventional ZN method. This would be of benefit to the patients to receive an early and effective treatment. Moreover, the Bleach is inexpensive, affordable, and the method is simple, safe and requires little additional expertise, workload or time beyond that required for conventional direct smear microscopy.

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