

## Determination of efficacy of modified bleach method in the detection of tuberculosis in lymph node aspirates in comparison to conventional Ziehl Neelsen method

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### Abstract

**Objective:** To determine the efficacy of modified bleach method in the detection of tuberculosis in lymph node aspirates in comparison to conventional Ziehl Neelsen method.

**Materials and Methods:** FNAC was performed on the 65 patients, clinically suspected as having tubercular lymphadenopathy, over a period of one year. The aspirated material was used to make 3-4 smears on clean glass slides. 95% ethyl alcohol was used as a fixative to fix one or two smears which were then stained by Papanicolaou stain. The rest of the smears were air dried and then stained with May Grunwald Giemsa stain and conventional Ziehl Neelsen (ZN) stain. Smears were prepared by Modified bleach method and further stained with ZN method.

The data was entered in Microsoft Excel spreadsheet and it was analyzed by Kappa statistics.

**Results:** Out of the 13 cases of granulomatous lymphadenitis, 3 cases were positive for acid fast bacilli (AFB) by both ZN and modified bleach method, one case was positive by ZN method but was negative with bleach method and one case which showed bleach positivity showed ZN negativity. Out of 6 cases of acute suppurative lymphadenitis, 2(33.3%) cases showed both ZN and bleach positivity. Out of 34 cases of reactive lymphadenitis, 2(5.88%) cases showed only bleach positivity while none of the cases showed ZN positivity.

**Conclusion:** Bleach method is as efficacious as ZN method in the diagnosis of tubercular lymphadenitis with higher grade of AFB positivity. This method is safe.

**Keywords:** Modified bleach method, ZN method, Granulomatous lymphadenitis.

### Introduction

Tuberculosis is one of the major health issues in the world and India accounts for highest number of cases of tuberculosis in the world. According to a report by World Health Organisation (WHO), in 2015, new tuberculosis cases accounted for 10.4 million cases. In this, men accounted for 5.9 million and that of women and children accounted for 3.5 million and 1 million respectively. There were also 1.4 million TB deaths (1 million among HIV-negative people and 0.4 million among HIV-positive people). In the year 2015, the incidence of tuberculosis in India was 2.8 million of which 1.9 million were men and 0.9 million were female.<sup>1</sup>

Tuberculosis (TB) is highly infectious disease caused by various strains of Mycobacteria, especially Mycobacterium tuberculosis. Both pulmonary TB and extra pulmonary TB are important in terms of health burden to the society. Extrapulmonary tuberculosis accounts for about 15% of the cases. Early detection and treatment is very important in minimizing the spread of tuberculosis and also in reducing the morbidity and mortality. There are various diagnostic tools to detect tubercular lymphadenopathy such as fine needle aspiration cytology (FNAC), serological tests, Ziehl Neelsen (ZN) staining for acid fast bacilli (AFB), culture and newer molecular tests such as Cartridge based nucleic acid amplification (CB-NAAT), Polymerase chain reaction (PCR) and others. ZN method plays a significant role in the diagnosis and prognosis of tuberculosis. The major drawback of ZN method is that it has low sensitivity

that is 20-43%. Culture requires lot of time and needs lot of safety measures to prevent contamination in laboratories. Serological tests like enzyme-linked immunosorbent assay (ELISA) have low sensitivity and specificity. Wide variety of newer molecular tests are quick but expensive and are not widely available.<sup>2</sup>

Modified bleach method is simple, quick, safe and cost-effective method with high sensitivity.<sup>3</sup> Hence, this study is aimed at determining the efficacy of bleach method in comparison with the conventional ZN method.

### Materials and Methods

65 patients clinically suspected as having tubercular lymphadenopathy, over a period of one year, were taken into the study after obtaining ethical committee clearance and informed consent of the patients.

Patients clinically suspected as having tubercular lymphadenopathy, referred to FNAC, were included in the study. Patients who were within three months of initiation of treatment for tuberculosis were excluded from the study.

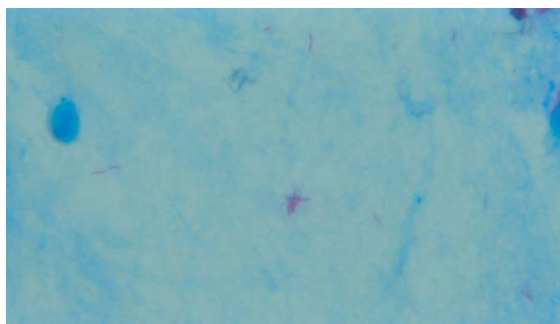
Fine needle aspiration was performed with 23 gauge sterile needle attached to 10 ml syringe.<sup>4</sup> The aspirated material was used to make 3-4 smears on clean glass slides. 95% ethyl alcohol was used as a fixative to fix one or two smears which were then stained by Papanicolaou stain. The rest of the smears were air dried and then stained with May Grunwald Giemsa stain and conventional Ziehl Neelsen (ZN) stain. Smears were prepared by Modified bleach method and further stained with ZN method.

**Modified Bleach Concentration Method for Staining Acid Fast Bacilli**

5ml sterile screw capped tube was taken. The remaining aspirate in the needle hub or syringe was rinsed into this tube using 1ml normal saline. To this tube, 2ml of 5% sodium hypochlorite (NaOCl) was added. Then, the tube was incubated for 15 minutes at room temperature and the mixture was shaken regularly. The tube was centrifuged at 3000rpm for 15minutes after addition of 2ml of distilled water. 1-2 smears were prepared from the sediment after discarding the supernatant. The slides were air dried and they were fixed by heating. Further the smears were stained by routine ZN method (Fig. 1).<sup>2</sup>

In order to make cytomorphological diagnosis, Pap stained and MGG stained smears were observed under low power (10x) and high power (40x) of the microscope. ZN stained smears were observed for tubercle bacilli under high power (40x) and oil immersion (100x). Tubercle bacilli appear as beaded red rods seen either singly or in clumps.<sup>5</sup> The major advantage of smears made by bleach method is that the background is clear. So, the identification of tubercle bacilli becomes easier.

ZN stain was defined as positive if one or more AFB was seen and negative if no AFB was seen in 100 oil immersion fields.



**Fig. 1: Acid fast bacilli by Bleach method (x1000)**

**Table 1: Reporting scheme for AFB on smear as per WHO**

0 AFB /100 fields	Negative
1-9/100 fields	Scanty
10-99/100 fields	1+
1-10/field in 50 fields	2+
>10/field in 20 fields	3+

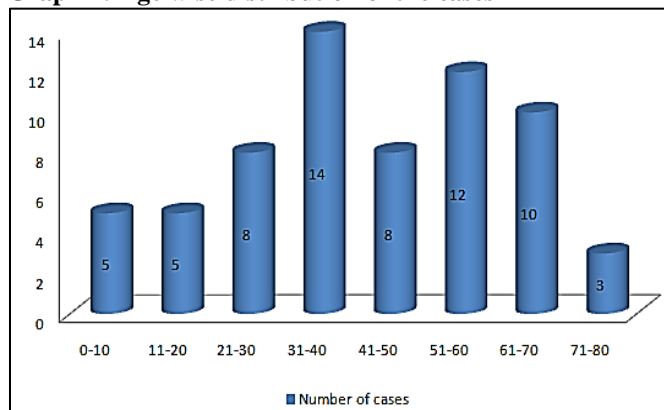
**Statistical Analysis Plan**

Detection rate of tuberculosis by two methods and the degree of agreement between them was analysed using Kappa statistics.

**Results**

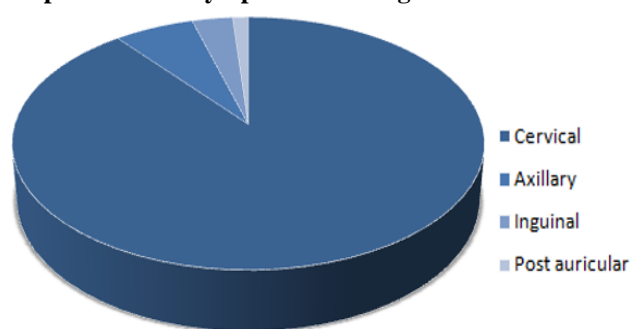
Of the 65 cases of lymph node enlargement, the maximum cases were observed in the age group of 31-40 i.e. 14(21.5%) [Graph 1].

**Graph 1: Age wise distribution of the cases**



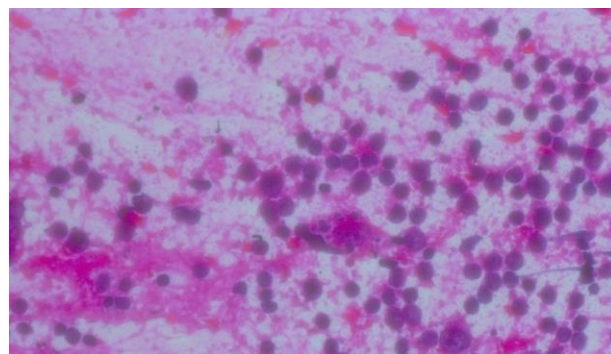
Males (56.9%) were more commonly affected than females. Of the 65 cases, majority of cases (86.1%) presented with cervical lymph node enlargement [Graph 2].

**Graph 2: Sites of lymph node enlargement**



Majority of patients (95.4%) consulted to clinicians within first five months of lymph node enlargement. Loss of appetite was the commonest associated symptoms and was observed in 18.4% of cases followed by fever and weight loss which was seen in 15.3% of cases respectively.

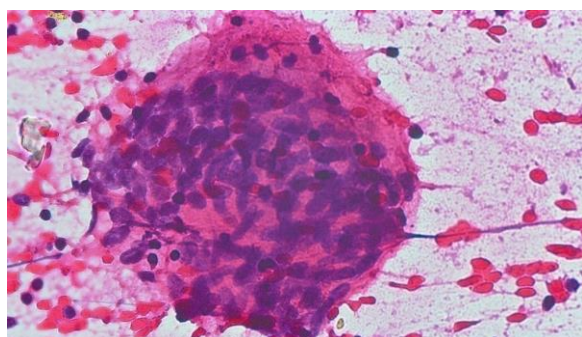
Cytomorphological diagnosis of reactive hyperplasia was based on the presence of mixed population of lymphoid cells like small lymphocytes, centroblasts, centrocytes, immunoblasts, plasma cells with fair number of tingible body macrophages (Fig. 2).<sup>6</sup>



**Fig. 2: Reactive lymphadenitis showing tingible body macrophage (x400, Pap stain)**

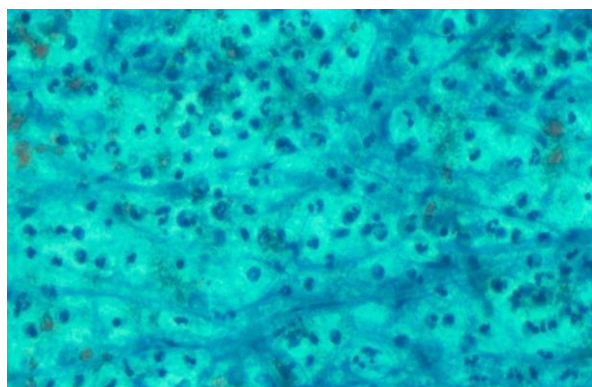
The criteria of cytomorphological diagnosis of tubercular lymphadenitis (Fig. 3) were:

1. Purulent with caseation
2. Only caseation
3. Epithelioid granulomas with caseation
4. Non caseating with epithelioid cells<sup>7</sup>



**Fig. 3: Tubercular lymphadenitis showing Langhans' giant cell (x400, Pap stain)**

The criteria used to diagnose a case of acute suppurative lymphadenitis was based on the presence of sheets of neutrophils along with macrophages and presence of necrosis (Fig. 4)



**Fig. 4: Acute suppurative lymphadenitis comprising of sheets of neutrophils (x400, Pap stain)**

In our study, the commonest diagnosis in clinically suspected cases of tuberculosis was reactive hyperplasia (52.3%) followed by granulomatous lymphadenitis (20%).

Both ZN method and modified bleach method detected 30.76% of AFB positivity in granulomatous lymphadenitis. Both the methods detected 33.3% of AFB positivity in acute suppurative lymphadenitis. Modified bleach method showed 5.88% AFB positivity in reactive hyperplasia where as none of the cases of reactive hyperplasia showed positivity with routine ZN method (Table 2).

**Table 2: Correlation of cytomorphological diagnosis with ZN and modified bleach method**

Cytomorphological diagnosis	ZN Positive	ZN Negative	Bleach Positive	Bleach Negative	Total
Reactive lymphadenitis	0	34	2	32	34
Granulomatous lymphadenitis	4	9	4	9	13
Acute suppurative lymphadenitis	2	4	2	4	6
Chronic lymphadenitis	0	9	0	9	9
Metastatic lymph node	0	3	0	3	3
Total	6	59	8	57	65

**Kappa statistical analysis showed higher degree of agreement between Modified Bleach method and conventional ZN method**

Bleach method showed higher grade of AFB positivity in smears when compared to ZN method in 4 cases (Table 3)

**Table 3: Quantification of AFB on smears**

	ZN	Bleach
0	9	9
Scanty	4	4
1+	2	2
2+	0	2
3+	0	0

**Discussion**

Extrapulmonary tuberculosis accounts for about 15% of the cases. The last decade has witnessed the sudden resurgence of tuberculosis. Early detection and treatment is very important in minimizing the spread of TB and also in reducing the morbidity and mortality.

In a study which was conducted in Beirut, Lebanon, by Shikhani et al, it was observed that the commonest group affected was of age 21-30 years.<sup>8</sup> In the present study, the age group 30-39years was most commonly affected.

A study done by Thompson et al<sup>9</sup> revealed that cervical lymphadenopathy was the commonest site of involvement. In the present study, we also observed that cervical nodes were most commonly involved. Cervical nodes were more commonly affected as tonsils, adenoids and Waldeyer's ring provide easy portal of entry to Mycobacteria and also that 300 out of 800 lymph nodes of the body are located in neck region.<sup>10</sup>

Jones and Campbell divided tubercular lymphadenitis into following 5 stages<sup>11</sup>

- Stage 1: Enlarged, mobile, discrete nodes showing non-reactive hyperplasia
- Stage 2: Periadenitis leading to matted lymph nodes
- Stage 3: Central softening due to abscess formation
- Stage 4: Collar stud abscess
- Stage 5: Formation of sinus

All of the cases of the present study were of Stage 1 of Jones and Campbell staging.

Majority of patients (67.70%) visited hospitals between 1-5 months of lymph node enlargement. Most of the patients who had no other symptom other than lymph node enlargement did not consult the hospital immediately. This is because of lack of awareness among the people. Only when they had some other symptom along with lymphadenopathy did they present to the hospital.

A study was conducted by Annam V et al in the year 2009 in Karnataka, India, on 93 patients. Among the 93 aspirates, 41.94% showed cytological features of tubercular lymphadenitis out of which 33.33% cases showed AFB positivity with ZN method and 63.44% showed AFB positivity with bleach method.<sup>2</sup>

In our study, out of 13 cases of granulomatous lymphadenitis, 3 cases showed both ZN positivity and bleach positivity. One case showed ZN positivity but bleach negativity and the other case which was bleach positive showed ZN negativity. Of the 6 cases that were diagnosed as acute suppurative lymphadenitis, 2 (33.3%) cases showed ZN and bleach positivity. Out of 34 cases of reactive hyperplasia, 2 (5.88%) cases showed only bleach positivity and none of the cases showed ZN positivity.

Few cases that were initially diagnosed as reactive hyperplasia on cytology turned out to be AFB positive. The reason behind this could be due to scattering of epithelioid cells amidst the dense polymorphous population of cells of lymphoid series.<sup>12</sup>

A study done by Prasoon D, revealed that the possibility of finding AFB increases if pus or caseous material is aspirated.<sup>13</sup>

Sodium hypochlorite brings about alteration in the chemical properties of AFB like charge and hydrophobicity. This chemical agent also leads to denaturation of the aspirate resulting in increased sedimentation of AFB. So, all these factors enhance the yield of bacilli per microscopic field. In addition to this, there is a substantial reduction of debris, leaving a clear field for microscopy.<sup>3</sup>

In a study done by Padma Srikanth et al, they concluded that the concentration of sodium hypochlorite decreases with time with the decrease in free chlorine concentration of 2-5% at the end of 60 days. There is no definitive agreement on the concentration or duration of bleach to be used. Another possible disadvantage of bleach is that it has low sensitivity when the volume is low. Bleach digests the proteins that aid the smear to stick on to the slide. Hence, the smears are fragile and are difficult to identify on a slide.<sup>14</sup>

Modified bleach method is as efficacious as conventional ZN method. Modified bleach method has added advantage in that it renders the sample safe to staff handling it. NaOCl is inexpensive. But the sample treated with NaOCl is not suitable for culture.

## Conclusion

In our country where tuberculosis is so prevalent, there is a need of safe, simple and cost effective method of

diagnosis of tuberculosis. FNAC with Modified bleach method is safe and shows higher grade of AFB positivity when compared to conventional method. It decreases the background disturbance facilitating easy identification of AFB on the smears. So, Modified bleach method can be employed as a useful diagnostic method for the early diagnosis of extra pulmonary tuberculosis.

**Conflict of Interest:** None.

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