ABSTRACT

Background: Microscopy detection of acid-fast bacilli (AFB) by Ziehl-Neelsen (ZN) method is a rapid and feasible method but has low sensitivity. Aims: To evaluate the efficacy of bleach method with cytological diagnosis and the conventional method. Methods: Five hundred and seventy-five patients with tubercular lymphadenitis were evaluated. The acid-fast positivity by routine staining was compared with modified bleach method of AFB staining. Result: Among 575 patients, 230 (40%) were positive for AFB on conventional ZN method, 310 (53.9%) on cytology and the smear positivity was increased to 365 (63.5%) by bleach method. Conclusion: The bleach method is simple and inexpensive. It clearly improves microscopic detection of AFB.

Key words: Modified bleach, tubercular lymphadenitis, Ziehl-Neelsen method

INTRODUCTION

Tuberculosis (TB) is a granulomatous inflammatory process consequent to infection by Mycobacterium tuberculosis. TB affects one-third of the world population. The average prevalence of all forms of TB in India is estimated to be 5.05/1000 population.[1] With the improvement in the economic and social condition and the use of effective anti-TB therapy (ATT), there is a decline in pulmonary TB cases. However, extrapulmonary TB is now more common, and lymphadenopathy is the most common form of extrapulmonary TB.[2,3] With early and accurate diagnosis spread of the disease can be reduced. The conventional Ziehl-Neelsen (ZN) method for acid-fast bacilli (AFB) is useful in diagnosis. However, it has sensitivity ranging from 20% to 43%.[4-5] Conventional mycobacterial culture method is sensitive but is time consuming and takes about 6-8 weeks for results. Serological tests are nonspecific,[6] newer molecular techniques such as polymerase chain reactions (PCRs) are costly.

Previous studies have shown that liquefaction of sputum and lymph node aspirate by sodium hypochlorite (NaOCl, bleach) and concentration of bacilli through centrifugation will significantly increase the sensitivity of direct microscopy.[7-10] The aim of present study is...
to evaluate bleach method and compare it with the conventional ZN method.

METHODS

The Ethical Committee permission was granted to conduct this study. The study group included suspected patients of TB, who presented with cervical lymphadenopathy and were referred for fine needle aspiration cytology (FNAC) to Department of Pathology. Exclusion criteria were patients on ATT within previous 3 months and elderly patients with known primary malignancy. A total of 575 patients were included. FNAC was done by 22-24 gauge needle and 10 ml syringe. All the aspirates yielded pus or pus mixed material were processed for conventional ZN stain, routine cytology, and bleach method.

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

The bleach method was performed with the remaining aspirated material in the needle hub or syringe, which was rinsed with 1 ml normal saline and transferred into 5 ml sterile disposable, conical screw-capped tubes. 2 ml of 6% NaOCl was added and the mixture was incubated at room temperature for 15 min by shaking at regular intervals. Centrifugation done at 3000 g for 15 min after adding 2 ml of distilled water to the mixture. The supernatant was discarded, and the sediment was transferred to a clean slide. The slide was air dried, heat fixed and stained by 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. Cytological smears were examined under light microscope. ZN stained smears were examined under oil immersion (×1000) [Figure 1].

RESULT

The present study included 580 cases. Of these 575 cases were evaluated, and five specimens were eliminated because of inadequate sampling. HIV positive cases were 40 in number. The age ranged from 2 years to 80 years, with a mean age of 39 years. Out of 575, on cytomorphological basis, reactive lymphadenitis was seen in 194 (33.9%) cases, acute suppurative lymphadenitis in 71 (12.3%) cases, and tubercular lymphadenitis in 310 (53.9%) cases [Tables 1 and 2].

The criteria for diagnosis of reactive lymphadenopathy were a polymorphous population of lymphoid cells without malignant features and presence of a fair number of tingible body macrophages. Out of 33.9% (194/575) cases diagnosed as reactive lymphadenitis, modified bleach method yielded positivity in 22.8% (43/194) cases while all the cases were negative by the conventional ZN method. Of the 40 HIV-infected patients, the reactive pattern on cytology was seen in 28 cases. Among these, the bleach method was positive for AFB in 22 cases, and all cases were negative by the conventional ZN method.

The morphological 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. Among 12.4% (71/575) cases diagnosed as acute suppurative lymphadenitis, the bleach method was positive in 88.7% (63/71) of the cases while the conventional ZN method identified AFB in only 54.89% (39/71) of the cases.

On cytomorphology, tubercular lymph node was diagnosed using the following criteria [Figure 2]:
- Purulent with caseation.
- Only caseation.
- Caseation with epithelioid cells.
- Noncaseating with epithelioid cells.

Of 53.9% (310/575) TB cases, AFB was identified by bleach method and conventional ZN method in 89.3% cases.

| Cytomorphological diagnosis | Bleach method | Conventional ZN method | Total |
|-----------------------------|---------------|------------------------|-------|
|                             | Positive | Negative | Positive | Negative |                      |
| Reactive LN                 | 43      | 151      | 00       | 194      | 194                  |
| Suppurative LN              | 45       | 26       | 12       | 59       | 71                   |
| TB LN                       | 277      | 33       | 218      | 92       | 310                  |
| Total                       | 365      | 210      | 230      | 345      | 575                  |

LN: Lymph node, TB: Tuberculosis, ZN: Ziehl-Neelsen

Figure 1: Tubercle bacilli in lymph node smears after using bleach technology (Ziehl-Neelsen, ×1000)
Table 2: Comparison of the conventional ZN method with the bleach method for the detection of acid-fast bacilli

|            | Conventional ZN method | Bleach method |
|------------|-------------------------|---------------|
| Positive   | 230                     | 365           |
| Negative   | 345                     | 210           |
| Total cases: 575 | Total cases: 575        |               |

ZN: Ziehl-Neelsen

Table 3: Comparison of different studies done previously with the present study

| Study                  | Total | Tubercular morphology on cytology (%) | Conventional ZN method | Modified bleach method |
|------------------------|-------|--------------------------------------|------------------------|------------------------|
| Khubnani and Munjal    | 55    | 43.44                                | 21.8                   | 70.9                   |
| Gangane et al.[12]     | —     | 23                                   | 20                     | 72                     |
| Chandrashekhar and Aruna[13] | 112   | 60.7                                 | 12.5                   | 63.44                  |
| Annam et al.[9]        | 99    | 41.94                                | 33.3                   | 63.44                  |
| Patel et al.[10]       | 115   | 59.13                                | 29.41                  | 66.18                  |
| Present study          | 575   | 53.9                                 | 40.0                   | 63.5                   |

ZN: Ziehl-Neelsen

DISCUSSION

FNAC has high accuracy in the early diagnosis of tubercular lymphadenopathy. The ZN method for AFB confirms the diagnosis. Methods used to increase the sensitivity of ZN method was the use of NaOCl (bleach) as a mucolytic for sputum samples. Bleach is cheap, readily available, acts as effective disinfectant,[9,15] and gives a higher density of bacilli per microscopic field and reduction of debris resulting in a clear background.[16]

In the present study, 575 aspirations were included. Major age group was 30-39 years. Females contributed 54.0% similar to the study by Patel et al.[10]

In the present study of 575 cases, 310 (53.9 %) cases were suggestive of TB. On cytology 218/310 (70.3%) were positive for AFB by conventional ZN staining and 277/310 (89.3%) for AFB by bleach method.

The reason behind this can be explained by the fact that due to their lipid coat, mycobacteria remain buoyant during centrifugation. Bleach method allows the deposition of bacilli at the bottom of the test tube after centrifugation. Annam et al.[9] explained it due to changes in the surface properties of the bacilli (i.e., charge and hydrophobicity) and denaturation of the specimen leading to flocculation and subsequently increased sedimentation rate of the AFB. As bleach kills bacteria, good results are possible only when the method is to be used within 1 h of a collection of specimen.[13]

194/575 (33.9%) cases show reactive lymphadenitis on cytology, but bleach method gave positivity in 43/194 cases (22.8%) for AFB. This is explained by the loss of scattered epithelioid cells among the polymorphous population of lymphoid cells.[17] Thus, granulomas are easily missed in such cases. Furthermore, a conventional method for AFB could not detect bacilli in any case which was diagnosed as reactive on cytology smears. Thus, bleach method could be of great use in such cases.

Among 71/575 (12.3 %) specimen diagnosed as supplicative lymphadenitis, 39/71 (54.3%) cases were positive by conventional ZN method while bleach method yielded positivity in 63/71 (88.7%) cases, the reason may be loss of the bacilli among the debris.

Thus, the primary outcome of the study is that bleach method is more efficient in demonstrating the presence of AFB on cytology smears in reactive, tubercular and supplicative lymphadenitis. It is also concluded that there are least chances of detection of AFB in reactive lymphadenitis by conventional AFB technique. Thus, if such cases of reactive lymphadenitis are not responding to the routine course of antibiotics than they should definitely be screened for AFB by bleach method.

The other interesting fact noted was that all the HIV positive cases which were diagnosed as tubercular
on cytology smears were positive for AFB on both conventional as well as bleach method. This could be due to high tubercular bacilli load in such patients. Thus in cytologically diagnosed tubercular cases of HIV patients, even conventional method of ZN goes well. The other secondary outcome from this study was that the most common age group for cervical lymphadenopathy is 30-39 years.

Furthermore, all the cases which were negative by a conventional method, but turned out to be positive on bleach method were closely followed-up clinicoradiologically. It was found that all such cases were actually tubercular and responded on ATT.

**Strength of the study**
The study involves the diagnosis of tubercular cases by their clinico-cytological correlation and AFB demonstration.

**Limitations of the study**
The cases of lymphadenitis which turned out to be negative even on screening with bleach method should further be confirmed by molecular methods such as PCR to find out the true efficacy of TB detection by bleach method. In future, other studies are invited in which the efficacy of bleach method of AFB detection can be seen in the PCR confirmed tubercular cases.

**CONCLUSION**
Early and definite diagnosis of TB is essential to treat patients to reduce the spread of disease. Combined method of FNAC and AFB detection by bleach modification increased the diagnostic value of ZN method. This is also very effective in endemic developing countries like India where cost effectiveness of test plays an important role in the treatment of the patient. There are cases in which cases diagnosed as reactive or suppurative lymphadenitis do not respond to antibiotic treatment. In such cases, pathologists should be aware regarding the significance of screening the AFB by Bleach method of ZN staining.

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**Conflicts of interest**
There are no conflicts of interest.

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