ABSTRACT

**Background:** Extra pulmonary tuberculosis (TB) is prevalent in developing countries and its diagnosis is often delayed, thus increasing the morbidity and mortality. Bleach method is cost effective, sensitive and safe method for demonstration of Acid fast bacilli (AFB) and is very valuable in diagnosing a case of tuberculous lymphadenitis. This simple procedure would benefit the patients to receive an early and specific treatment.

**Aims & Objective:** The aim of our study was early diagnosis of tuberculosis in lymph node Fine needle aspiration cytology (FNAC) by bleach method for detection of AFB in comparison to conventional Ziehl–Neelsen (ZN) method.

**Material and Methods:** Total 115 cases clinically suspected as tuberculous lymphadenitis in one year duration were included in study. All the aspirates by FNAC were processed for routine cytology, ZN staining and bleach method. The significance of the bleach method over the ZN method was analyzed using the $\chi^2$ (chi-square) test.

**Results:** Among the 115 aspirates, 59.13% (68/115) were indicative of TB on cytology, 27.83% (32/115) were positive for AFB on conventional ZN method and the smear positivity increased to 61.74% (71/115) on bleach method.

**Conclusion:** The implementation of the bleach method clearly improves microscopic detection of AFB over ZN method. The bleach method can be easily performed and reduce chances of laboratory acquired infections.

**Key-Words:** Lymph Node Cytology; Ziehl Neelsen (ZN) Method; Bleach Method; Tuberculosis; Acid Fast Bacilli (AFB)

Introduction

Tuberculosis is most common and important infectious cause of death in our country. Tuberculosis can involve any organ system of our body while pulmonary TB is most common presentation, extra pulmonary tuberculosis is also equally important problem.[1] Extra pulmonary tuberculosis often manifests as peripheral lymphadenopathy or palpable intra-abdominal masses and can be diagnosed by fine needle aspiration cytology (FNAC).[2]

Cure of the disease is possible only with correct diagnosis and appropriate treatment. Early and accurate detection of active cases remains an important objective for reduction in the spread of the disease. The diagnosis traditionally depends upon identifying the infective organism in secretions or tissues of diseased individuals. There are, however, several limitations to the traditional techniques used. Staining by the Ziehl-Neelsen technique though useful can detect bacilli only when there are more than 10,000 organisms per ml of sputum. Conventional culture methods are sensitive and can detect 10-100 organisms per sample. However, they are time consuming and take 6 to 8 weeks for the results. Furthermore, viable organisms are needed for culture.[3] Serological techniques have the disadvantage of lack of sensitivity and specificity. Newer molecular techniques such as polymerase chain reaction, although rapid but costly and required more expertization. So, can't be used routinely in developing countries where most TB cases occur.[4]

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.[5] Detection of tubercular bacilli by bleach concentration method is a simple technique which requires no expertise and is inexpensive.[4] The aim of present study was to determine the efficacy
Materials and Methods

The study was conducted from January 2011 to December 2011 after obtaining ethical committee clearance and after taking informed consent of patients. Total 115 cases clinically suspected as tuberculous lymphadenitis were included in study. Aspiration was done by using 22-24 gauge disposable needle and 10 ml syringe. All the aspirates by FNAC were processed for direct microscopy using conventional ZN staining and routine cytology and compared with the findings of the bleach method. For cytological examination, smears were prepared; wet-fixed smears (in absolute alcohol) were stained by Papanicolaou stains and hematoxylin and eosin while air-dried smears were stained with May–Grunwald Giemsa and ZN stains. Smears were reported by experienced pathologists after correlating the clinical data and other investigations.

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 disposable, conical screw-capped tubes. To this conical tube, 2 ml of 5% NaOCl was added and the mixture was incubated at room temperature for 15 min by shaking at regular intervals. The conical tube containing the mixture was concentrated by centrifugation at 300 g for 15 min 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. Slides prepared with the drop of centrifuged sediment, stained by the ZN method and examined using oil-immersion in light microscope. FNAC smears directly stained by the conventional ZN method were also examined. The 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 of smears. The data were processed using test of association (χ² test).

Results

A total of 115 fine needle-aspirated specimens from the lymph node were included in the study. The age range of patients was from 2-70 years. The maximum numbers of cases were in the age group of 21-30 years (35.65%). Female preponderance was noted, accounting for 54.78% (63 /115) of the cases. A total 16 patients were human immunodeficiency virus (HIV) positive. Among the 115 lymph nodes cases were studied, the maximum numbers of aspiration were performed from cervical region (72.17%) followed by generalized lymphadenopathy (16.53%), axillary (10.43%) and inguinal (0.87%). The cytological diagnosis of reactive lymphadenitis was given on the basis of polymorphous lymphoid population along with lymphocytes in varying stages of maturation and variable number of tangible body macrophages. Of the 16.52% (19/115) cases diagnosed as reactive lymphadenitis, the bleach method was positive for AFB in 15.78% (3/19) cases and all the cases were negative by the conventional ZN method. [Table 1&2]. Of the 16 HIV-infected patients, reactive pattern on cytology was seen in one case in which the bleach method was positive for AFB but negative by the conventional ZN method.

The cytological diagnosis of acute suppurative lymphadenitis was based on the aspirated purulent material showing abundant neutrophils in a necrotic background. Among 24.35% (28/115) cases diagnosed as acute suppurative lymphadenitis, the bleach method was positive in 82.14% (23/28) of the cases while the conventional ZN method identified AFB in only 42.85% (12/27) of the cases. [Table 1&2]. Of 16 HIV-infected patients, suppurative lymphadenitis on cytology were seen in 6 cases. 5 cases were positive by bleach method while only 3 cases were positive by conventional ZN method.

Aspiration from tuberculous lymphadenitis show three major cell patterns: Epithelioid granulomas without necrosis, epithelioid granulomas with necrosis and necrosis without epithelioid granuloma. Of 59.13% (68/115) cases of
tuberculous lymphadenitis reported on cytology, AFB were identified by bleach method in 66.18% (45/68) and conventional ZN method in 29.41% (20/68) cases. [Table 1 & 2]. Of 16 HIV-infected patients, tubercular pattern on cytology were seen in 9 cases. All the cases were positive by bleach method but only 5 cases were positive by conventional ZN method.

The smear positivity for AFB on conventional ZN method of all cases was 27.83% (32/115) while the positivity increased to 62.60% (72/115) on bleach method. The comparison between conventional ZN method and bleach method showed statistical significance [Table 2]. (Chi-square test $\chi^2 = 26.48$, degree of freedom (df) = 1, $P < 0.001$).

Table-1: Correlation of Cytological Diagnosis with Conventional ZN Method and Bleach Method

| Cytological Diagnosis | ZN Method | Bleach method | Total (%)
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Reactive LN</td>
<td>00</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>Suppurative LN</td>
<td>12</td>
<td>16</td>
<td>05</td>
</tr>
<tr>
<td>TB</td>
<td>20</td>
<td>48</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>83</td>
<td>115</td>
</tr>
</tbody>
</table>

Table-2: Comparison of the Conventional ZN Method with the Bleach Method for the Detection of Acid-Fast Bacilli

| ZN Method | Bleach method | Total (%)
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>00</td>
</tr>
<tr>
<td>Negative</td>
<td>40</td>
<td>43</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>43</td>
</tr>
</tbody>
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Discussion

The diagnostic dilemma posed by extra pulmonary tuberculosis in resource-limited developing countries can be curtailed by FNAC, a relatively cheap and less invasive procedure with a high diagnostic accuracy. This will ensure prompt treatment and thus reduce attendant morbidity and mortality. The Ziehl Neelsen stain for AFB of tuberculosis confirms the diagnosis in the presence of inflammatory exudates. AFB positivity is higher in untreated patients and HIV positive cases.[12]

The use of bleach can be traced back to 1909, where sodium hypochlorite (bleach) was used as a mucolytic before centrifuging sputum samples and then processed by direct microscopy after staining with the Ziehl Neelsen method. The bleach method has undergone several modifications and diversifications and has recently received more attention with the advent of the HIV epidemic. Bleach is cheap, readily available and improves the sensitivity of the direct microscopy method considerably. Bleach is also an effective disinfectant, and therefore helps reduce the infection risk to laboratory personnel.[6,7]

The NaOCl method has a special advantage in overburdened control programs, where the technicians, because of the large workload, cannot endure or afford to spend the required 5 to 10 min on the examination of one slide. A meticulous preparation and examination of smears made directly from sputum gives a sensitivity of 55% compared with culture, whereas the sensitivity of the NaOCl method is close to 70%. The technology is appropriate for control programs, and NaOCl is readily available at low cost as household bleach. The increased sensitivity of the NaOCl method is attributable to the significantly higher density of bacilli per microscopic field obtained by this method and reduction of debris, leaving a clear field for microscopy.[5]

In present study, 115 aspirations were done and the results were compared with various studies. Among the 115 lymphnodes studied, the maximum numbers of aspiration were performed from cervical region-83 cases (72.17%). Similar findings were also observed by Annam et al.[4] Female preponderance was noted in our study, accounting for 54.78% (63/115) of the cases. However, Annam et al.[4] found male preponderance in study. Khubnani et al.[8] studied 55 cases of extra pulmonary TB, among which 43.36% cases were suggestive of TB on cytology, 21.8% cases positive for AFB by conventional ZN staining and 70.90% cases positive for AFB by the bleach method. Annam et al.[4] studied 99 cases of lymph node aspirate, among which TB was diagnosed in 41.94% on cytology, conventional ZN staining for AFB was positive in 33.33% and bleach method for AFB was positive in 63.44%. Gangane et al.[9] found 23% cases diagnosed as TB on cytology, conventional ZN staining for AFB was positive in 27% and bleach method for AFB was positive in 72%. Chandrareshkhar et al.[10] studied 112 cases of lymph node aspirate, among which TB was diagnosed in 60.7% on cytology.
conventional ZN staining for AFB was positive in 12.5% and bleach method for AFB was positive in 63.44%. In the present study of 115 cases of lymph node aspirate, 59.13% cases were suggestive of TB on cytology, 29.41% cases positive for AFB by conventional ZN staining and 66.18% cases positive for AFB by the bleach method.

The possible explanation for the diagnosis of reactive lymphadenitis on cytology but positive for AFB by the bleach method in both HIV-positive and negative cases may be due to the loss of scattered epithelioid cells among the polymorphous population of lymphoid cells.[11] Among the 28 specimens diagnosed as suppurative lymphadenitis, 23 cases were positive for AFB by the bleach method while only 12 cases were positive by conventional ZN method, the probable reason could be loss of the bacilli among the necrotic debris. Also, five specimens diagnosed as TB on cytology and negative by the bleach method may be due to a decrease in the density of the bacilli.[14] Liquefaction of the aspirated specimen with NaOCl followed by centrifugation significantly increases the yield of AFB. This finding is of considerable interest in developing countries where smear-negative AFB has become increasingly common. The improved recovery of AFB after treatment with NaOCl might be due to changes in the surface properties of the AFB (i.e., charge and hydrophobicity) and/or denaturation of the specimen leading to flocculation and subsequent increased sedimentation rate of the AFB.[12] Also, the increased smear positivity by the bleach method is attributable to the higher density of bacilli per microscopic field obtained by this method and 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 AFB.[15]

With the occurrence of multidrug resistant TB, the risk of laboratory infection has become a major concern. Use of the bleach method would definitely lower the risk of laboratory infection. Because NaOCl kills the mycobacterium, this method cannot be used on samples intended for culture, but the method is strongly recommended for all laboratories that perform direct microscopy.[4]

**Conclusion**

The continuously increasing number of tuberculosis (TB) cases in recent years and the emergence of strains with multidrug resistance present a public health problem that requires rapid intervention. Early identification and isolation of TB patients is of utmost importance in minimizing the risk of further epidemic spread. Combination of fine needle aspiration cytology with acid fast staining is highly valuable for routine diagnosis of tuberculosis. The bleach method is more sensitive and safer than routine ZN staining. As the background is clear, the bacilli are easily visible and the screening time becomes shorter. Other ancillary investigations like mycobacterial culture, lymph node biopsies and polymerase chain reaction can be reserved for cases, in which there is a strong clinical suspicion with equivocal result of FNAC and acid-fast staining.

**References**

10. Chandrashilkar B, Aruna K. Utility of concentration method by modified bleach technique for the demonstration of acid fast bacilli in the diagnosis of


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