Original Article

Improved microscopical detection of acid-fast bacilli by the modified bleach method in lymphnode aspirates

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ABSTRACT

Objectives: To improve the smear microscopy for detection of acid-fast bacilli (AFB) in fine needle aspiration cytology (FNAC) of lymph node using the bleach method and also to compare this with cytological diagnosis and the conventional Ziehl–Neelsen (ZN) method. **Study Design:** In 99 consecutive patients with clinical suspicion of tuberculosis (TB) presenting with lymphadenopathy, FNACs were performed. Smears from the aspirates were processed for routine cytology and the conventional ZN method. The remaining material in the needle hub and/or the syringe was used for the bleach method. The significance of the bleach method over the conventional ZN method and cytology was analyzed using the χ^2 test. **Results:** Of 99 aspirates, 93 were studied and the remaining six were excluded from the study due to diagnosis of malignancy in 4.04% (4/6) and inadequate aspiration in 2.02% (2/6). Among the 93 aspirates, 33.33% (31/93) were positive for AFB on conventional ZN method, 41.94% (39/93) were indicative of TB on cytology and the smear positivity increased to 63.44% (59/93) on bleach method. **Conclusion:** The bleach method is simple, inexpensive and potent disinfectant, also limiting the risk of laboratory-acquired infections. The implementation of the bleach method clearly improves microscopic detection and can be a useful contribution to routine cytology.

KEY WORDS: Bleach method, cytology, lymphnode

DOI: 10.4103/0377-4929.54991

INTRODUCTION

Extrapulmonary tuberculosis (TB) continues to be a major health problem in developing countries. Lymphadenopathy is the most common form of extrapulmonary TB.[1,2] The clinical parameters for the diagnosis of TB in lymphnodes are neither specific nor does their absence exclude TB involvement.[3,4] However, the conventional Ziehl–Neelsen (ZN) method for acid-fast bacilli (AFB) plays a key role in the diagnosis and also for the monitoring of treatment in TB. Its major disadvantage is low sensitivity, ranging from 20% to 43%.[5,6] Mycobacterial culture is the reference method for detection of tubercle bacilli, but it is time consuming and requires specialized safety procedures in laboratories. Serological techniques have the disadvantage of lack of sensitivity and specificity.[5] Newer molecular techniques such as polymerase chain reaction, although rapid, are costly to be routinely used in developing countries where most TB cases occur.[7] Also, previous studies have shown that liquefaction of sputum by sodium hypochlorite (NaOCl, bleach) and concentration of bacilli through centrifugation will significantly increase the sensitivity of direct microscopy.[8,9] Thus, the present preliminary study of using the bleach (NaOCl) method in fine needle aspiration cytology (FNAC) of lymphnodes was performed under the light of previous literature.

MATERIALS AND METHODS

Ninety-nine consecutive patients suspected clinically of having TB with lymphadenopathy referred for FNAC to the department of cytology were included in the study. Exclusion criteria were treatment for TB within the previous 3 months or initiation of TB treatment before sampling was performed. Relevant investigation details like hematology and chest radiogram were reviewed in these patients. 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 directly and wet-fixed smears (in absolute alcohol) were stained by hematoxylin and eosin and Papanicoloau stains while air-dried smears were stained with May–Grunwald Giemsa and ZN stains, respectively.

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.

Smears stained by the conventional ZN method directly were examined for AFB under oil-immersion (×1000) using a light microscope. Slides prepared with the drop of centrifuged sediment and stained by the ZN method were scanned for AFB at ×100. Positive smears were confirmed at oil-immersion (×1000). The data were processed using test of association (χ^2 test).

RESULTS

A total of 99 fine needle-aspirated specimens from the lymphnode were included in the study. Of these, 93 specimens were evaluated and the remaining six specimens were eliminated because four aspirates identified malignancy and two aspirates were inadequate. A total of eight patients were human immunodeficiency virus (HIV) positive. The age ranged from 2 to 70 years, with a mean age of 23.4 ± 13.5 years. Male preponderance was noted, accounting for 54.8% (51 /93) of the cases. Among the 93 lymphnodes studied, aspirates were from cervical (n = 72), inguinal (n = 9) and axillary (n = 12) groups.

Of these 93 aspirates, the cytomorphological features observed were reactive lymphadenitis in 29.03% (27/93) cases, acute suppurative lymphadenitis in 29.03% (27/93) cases and tubercular lymphadenitis in 41.94% (39/93) cases. There was a statistically significant correlation (χ^2 = 8.29, df = 3, P < 0.05) between cytomorphological diagnosis, results of smears prepared by the conventional ZN method and the bleach method [Table 1].

The criteria for diagnosis of reactive lymphadenopathy were established based on the polymorphic population of lymphoid cells without malignant features and a considerable number of tingible body macrophages.[10] Of the 29.03% (27/93) cases diagnosed as reactive lymphadenitis [Figure 1a], the bleach method was positive for AFB in 22.22% [Figure 1c] (6/27) cases and all the cases were negative by the conventional ZN method [Figure 1b]. Of the eight HIV-infected patients, reactive pattern on cytology were seen in five. Among these five, the bleach method was positive for AFB in four cases and all the cases were negative by the conventional ZN method.

Table 1: Correlation of cytomorphological diagnosis with the bleach method and the conventional ZN method

Cytomorphological diagnosis	*Bleach method*		*Conventional ZN method*		*Total*
	Positive	*Negative*	*Positive*	*Negative*	
Reactive LN	06	21	00	27	27
Suppurative LN	16	11	03	24	27
TB LN	37	02	28	11	39
Total	59	34	31	62	93

Correlation of significance: c^2 = 8.29, df = 3, P < 0.05

The cytomorphological 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 [Figure 2a]. Among 29.03% (27/93) cases diagnosed as acute suppurative lymphadenitis, the bleach method [Figure 2c] was positive in 59.25% (16/27) of the cases while the conventional ZN method [Figure 2b] identified AFB in only 11.11% (3/27) of the cases.

On cytomorphology, tuberculous lymphnode was diagnosed using the following criteria: (a) purulent with caseation, (b) only caseation, (c) caseation with epithelioid cells and (d) non-caseating with epithelioid cells.[11] Of 41.94% (39/93) cases, AFB were identified by bleach method and conventional ZN method in 94.87% (37/39) cases and 71.79% (28/93) cases, respectively. Of the eight HIV-infected patients, tubercular pattern on cytology were seen in three cases. All the cases were positive by both conventional ZN method and bleach method.

The smear positivity for AFB on conventional ZN method was 33.33% (31/93) while the positivity increased to 63.44% (59/93) on bleach method. The comparison between conventional ZN method and bleach method [Table 2] showed statistical significance (c^2 = 24.48, df = 1, P < 0.001).

DISCUSSION

In developing countries, microscopy of the specimen is by far the fastest, cheapest and most reliable method for the detection of AFB. In the late 1940s, sputum liquefaction with NaOCl (readily available at low cost as household bleach) and then concentration by centrifugation before acid-fast staining was implemented to improve the smear positivity for the detection of AFB.[12] This method was slightly modified and applied in cytology (i.e., only on lymph node aspirates).

The discrepancies between cytomorphological diagnosis and bleach method in the present study occurred in 24 specimens. Of the 24 specimens, six specimens were reactive lymphadenitis and 16 specimens were acute suppurative lymphadenitis, but these specimens were positive for AFB by the bleach method, and two specimens were negative for AFB by the bleach method but diagnosed as TB on cytology. 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.[13] All the patients

Table 2: Comparison of the conventional ZN method with the bleach method for the detection of acid-fast bacilli

Conventional ZN method	*Bleach method*		*Total*
	Positive	*Negative*	
Positive	31	00	31
Negative	28	34	62
Total	59	34	93

Statistical significance: χ^2 = 24.48, df = 1, P < 0.001

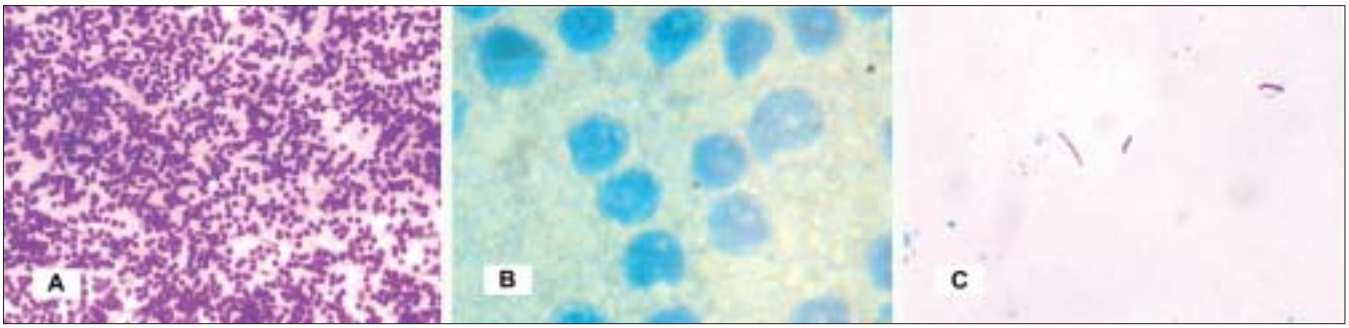

Figure 1: Reactive lymphadenopathy. (a) Cytomorphology showing polymorphous population of lymphoid cells without malignant features and a considerable number of tingible body macrophages (H & E, ×100); (b) Smear was negative for acid-fast bacilli (AFB) by the conventional Ziehl–Neelsen method (×400); (c)Bleach method shows few AFB at the center of the field with clear background (×400)

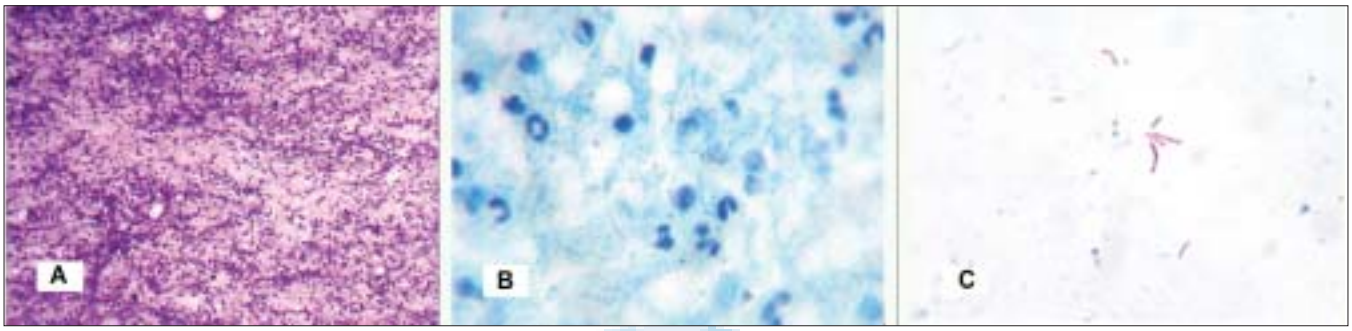

Figure 2: Acute suppurative lymphadenitis. (a) Cytomorphology showing abundant neutrophils with macrophages containing ingested necrotic debris in a necrotic background (H & E, ×100); (b) Smear was negative for acid-fast bacilli (AFB) by the conventional Ziehl–Neelsen method (×400); (c) The bleach method shows AFB at the center of the field with clear background (×400)

responded well for the anti-tubercular therapy. Among the 16 specimens diagnosed as suppurative lymphadenitis positive for AFB by the bleach method, the probable reason could be loss of the bacilli among the necrotic debris. Also, two specimens diagnosed as TB on cytology and negative by the bleach method may be due to a decrease in the density of the bacilli.

Khubnani *et al*.[14] studied 55 cases of extrapulmonary TB, which included 18 aspirates from body fluids, 18 from abscesses drained from various body sites, 17 from lymph nodes and two from skin scrapings. It was found that an overall 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. In the present study of 99 cases, which included only lymphnode aspirates, 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%.

We have demonstrated that 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.[15] 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.[16] Thus, the preparation of samples by the bleach method reduces the time required for examination of the slides to detect AFB.

Acid-fast smear examination by the bleach method does not discriminate between tubercle bacilli and other mycobacteria. However, this is not a major problem in developing countries: Firstly, because the vast majority of patients with AFB has TB and, secondly, because other mycobacteria are usually not present in sufficient concentration to be detected by direct microscopy.[17]

Mycobacteria have a low specific gravity and may remain buoyant during centrifugation.[17] 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 only.

A relative centrifugal force (RCF) of 1800-2400 × g and a centrifugation time of 15-30 min have been recommended for recovering mycobacteria.[18] One major disadvantage of the bleach method is the need for a centrifuge. In the present study, an RCF

of 3000 × g applied for 15 min yielded increased recovery of mycobacteria.

In conclusion, the bleach method for AFB is simple, safe and cost-effective. The results would be more efficient if concentration by bleach solution, RCF and bleach treatment is as per the time schedule and is proportionate. The implementation of the bleach method clearly improves microscopic detection and can be a useful contribution to routine cytology. This would be of benefit to the patients to receive an early and effective treatment.

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Source of Support: Nil, **Conflict of Interest:** None declared.