



Gautam Prabesh^{*1}, Alam Fahad², Rijal Shikha³

¹Department of Pathology, Hulhumale hospital, Huvandhumaahigun, Maldives.

²Department of Pathology, National Medical College, Birgunj, Nepal.

³Department of Paediatrics, Nobel Medical College & Teaching Hospital, Biratnagar, Nepal.

Date of Submission : March 02, 2019

Received in Revised Form : March 17, 2019

Date of Acceptance : April 11, 2019

Date of Publishing : July 30, 2019

ABSTRACT

Background:

In developing countries, tuberculous lymphadenitis (TBLN) is one of the most common causes of lymphadenopathy (30-52%). Diagnosis of TBLN still faces many challenges as a full proof evidence of TBLN can only be made by detection of acid-fast organism as the cause for granulomatous inflammation. Ziehl-Neelsen staining (ZN) method for acid-fast bacilli (AFB) plays a key role in the diagnosis but its sensitivity is not optimal, ranging from 9-46%. The need for newer, improved, low-cost techniques cannot be overemphasized. Objective of the present study was to introduce the newer technique and determine its sensitivity for detection of AFB as compared to the conventional ZN method.

Methods:

The study included patients with clinical suspicion of tubercular lymphadenitis presenting to the Department of Pathology, Nepalgunj Medical College Teaching Hospital, Banke over a period of one year. 100 cases were included and FNA was performed using a 22G needle. Smears from the aspirates were processed for routine cytology and conventional ZN method. The residual aspirated material in the needle was subjected to liquefaction with 2ml of 5% sodium hypochlorite solution in a test tube for 30 minutes followed by adding 2ml of distilled water. The material was centrifuged for 15 minutes at 3000 rpm. Smears were made from the sediment on a

clean glass slides, followed by ZN, mounted and screened.

Results:

In the present study, 69 cases were finally diagnosed as TBLN. AFB was positive by ZN method in 41 cases which increased to 62 cases by bleach method. Thus, sensitivity of bleach method was 89.85% whereas the sensitivity of conventional ZN method was 59.42%.

Conclusion:

The bleach method was more sensitive and safer than routine ZN method. As the background of the smear was clear, the bacilli were easily visible and the screening time was shorter. The implementation of bleach method improves the microscopic detection of AFB, reduces laboratory-acquired infections and can be a useful contribution to routine cytology.

Keywords: Acid-fast bacilli; Bleach method; Fine needle aspiration; Ziehl-Neelsen stain.

***Corresponding author:** Dr. Prabesh Gautam
Department of Pathology, Hulhumale Hospital,
Huvandhumaahigun, Maldives
Email: iamprabesh123@gmail.com

INTRODUCTION

In developing countries, tuberculous lymphadenitis (TBLN) is one of the most common causes of lymphadenopathy (30-52%).¹ Lack of awareness, illiteracy and limited facilities for

investigation make the diagnosis of TBLN a major problem in a developing country like Nepal. Diagnosis of TBLN still faces many challenges as a full proof evidence of TB can only be made by detection of acid-fast organism as the cause for granulomatous inflammation. The clinical parameters for the diagnosis of TBLN are neither specific nor their absence excludes Tuberculosis (TB). The only practically available method for diagnosing extra-pulmonary tuberculosis (EPTB) is direct smear microscopy for tubercle bacilli of the sample from the lesion. Ziehl-Neelsen (ZN) method for acid-fast bacilli (AFB) plays a key role in the diagnosis but its sensitivity is not optimal, ranging from 9-46% when used in TB control programs, as the minimum number of tubercle bacilli necessary to produce a positive smear results has been estimated to be minimum 5,000 to 10,000 per ml.^{2,3}

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.³ Newer molecular techniques, such as polymerase chain reaction (PCR), are costly to be routinely used in developing countries where tuberculosis is prevalent.⁴ Microscopy has many advantages when it comes to speed and feasibility, and if its sensitivity could be improved, it has the potential to become an even more valuable tool for tuberculosis control program around the world. The need for newer, improved, low-cost techniques cannot be overemphasized. The bleach concentration method is one of the safest concentration methods for improving the sensitivity of direct microscopy for the detection of AFB.

In the present study, we used the bleach concentration method on fine needle aspirates and compared the sensitivity of this technique in detecting AFB with that of routine ZN staining on FNA smears. This study has been taken up to validate the studies done earlier.⁵⁻¹⁰

MATERIALS AND METHODS

The study included 100 patients with clinical suspicion of tubercular lymphadenitis presenting to the Department of Pathology Nepalgunj Medical College Teaching Hospital, Banke from 1st May 2013 to 1st May 2014. Patients presenting with lymphadenopathy were subjected to brief clinical examination. Data regarding age, sex, duration, and description of swelling like size,

number, site and association with HIV were documented for each patient. FNA was performed with a 22-gauge needle and a 10-mL disposable syringe. Following lymph node aspiration, approximately 5–6 smears were made on clean glass slides. One or two smears were fixed in 95% ethanol and stained with Papanicolaou stain. Air-dried smears were stained using May-Grünwald-Giemsa stain (MGG) and the routine ZN method. The residual aspirated material in the needle was flushed out and subjected to liquefaction with 2ml of 5% sodium hypochlorite (NaOCl, Hypo, bleach) solution in a test tube at room temperature for 30 min followed by adding an equal amount of distilled water (2ml). The material was then centrifuged for 15 min at 3000 rpm. The supernatant was discarded and smears were made from the sediment on clean glass slides, followed by ZN staining. The slides were mounted and screened. Smears from the aspirates were processed for routine cytology and the conventional Ziehl-Neelsen method.

RESULTS

This study was carried out on 104 patients with clinically suspected TBLN, referred to the Department of Pathology, Nepalgunj Medical College. Of these 104 cases 4 cases were eliminated from the study because 2 cases were diagnosed as malignancy and 2 cases were taking anti-tubercular therapy.

Table 1: Correlation of cytomorphological diagnosis with the bleach method and the conventional Z-N method

Cytomorphological diagnosis	Bleach method		Conventional Z-N method		Total
	Negative for AFB	Positive for AFB	Negative for AFB	Positive for AFB	
RLN	18	2	20	0	20
SPLN	13	8	18	3	21
TBLN	7	52	21	38	59
Total	38	62	59	41	100

Correlation of significance (Bleach method):
($\chi^2=45.152$, $df=2$, $p=0.0001$)

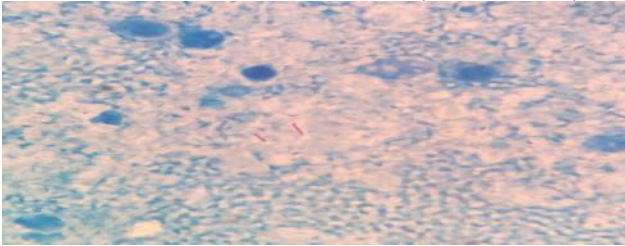
Figure 1: Microphotograph showing AFB by bleach method (Z-N x 1000)



Figure 2: Microphotograph showing clumps of AFB by bleach method (Z-N x 1000)



Figure 3: Microphotograph showing AFB by conventional Z-N method (Z-N x 1000)



DISCUSSION

In the present study, 100 aspirations were done and the results were compared with various studies. Among the 100 lymph-nodes studied, the maximum numbers of aspiration were performed from cervical region- 56 cases (56%). Similar findings were also observed by Annam et al.⁶, Patel et al.⁸ and Dwivedi et al.¹⁰ Female preponderance was noted in our study, accounting for 55% of the cases. However, Annam et al.⁶ and Dwivedi et al.¹⁰ found male preponderance in study. Khubnani et al. 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 Z-N staining and 70.90% cases positive for AFB by the bleach method.² Annam et al. studied 99 cases of lymph node aspirate, among which TB was diagnosed in 41.94% on cytology, conventional Z-N staining for AFB was positive in 33.33% and bleach method for AFB was positive in 63.44%.⁶ Gangane et al. found 23% cases diagnosed as TB on cytology, conventional Z-N staining for AFB was positive on 27% and bleach method for AFB was positive in 72%.⁵ Chandrashekhar et al. studied 112 cases of lymph node aspirate, among which TB was diagnosed in 60.7% on cytology, conventional Z-N staining for AFB was positive in 12.5% and bleach method for AFB was positive in 63.44%.⁹ Dwivedi et al. studied 200 aspirates from lymph nodes, 52% (104/200) were indicative of TBLN cytologically, conventional Z-N method detected AFB in 35.5% (71/200) and the smear positivity increased to 68% (136/200) when modified bleach was used.¹⁰ In the present study 69 cases were finally diagnosed as TBLN, AFB was

positive by Z-N staining method in 41 cases which increased to 62 cases by bleach method. So, thus sensitivity of bleach method was 89.85% whereas the sensitivity of conventional method was 59.42%.

The discrepancies between cytomorphological diagnosis and bleach method in the present study occurred in 10 cases. Of the 10 cases, two were RLN and eight were SPLN, but all of them were positive for AFB. The possible explanation for the diagnosis of RLN on cytology but positive for AFB by the bleach method may be due to the loss of scattered epithelioid cells among the polymorphous population of lymphoid cells.¹¹ Among the 21 specimens diagnosed as SPLN, eight cases were positive for AFB by the bleach method while only three cases were positive by the conventional Z-N method, the probable reason could be loss of the bacilli among the necrotic debris. Also, seven specimens diagnosed as TB on cytology and negative by the bleach method may be due to a decrease in the density of the bacilli.⁶ 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.¹¹ Thus, the preparation of samples by the bleach method reduces the time required for examination of the slides to detect AFB.

CONCLUSION

The present study validates the literature on the utility of the concentration of AFB by modified bleach method for detection of tubercular bacilli in lymph node aspirates and is more sensitive than the conventional ZN method. In the present study, we were able to establish AFB positivity in 62% of cases with the bleach method. This detection rate is much better than routine Z-N staining. It was also observed that by routine Z-N staining, most of the aspirates had scant AFB positivity and searching for them was a tedious, time-consuming exercise compared to the bleach method. By the bleach method, the majority (80.64%) of positive cases showed grades of AFB positivity that were above “scanty,” making them easily visible and detectable. Similarly, the bacilli were seen in

clumps in a thin background, making the screening process easier, faster and less strenuous on the eye.

Thus, the bleach method for detection of tubercle bacilli in LN aspirate is more sensitive than the conventional Z-N method. Moreover, the bleach method is safe, inexpensive and easy to perform and requires no additional equipment and also reduces laboratory-acquired infections. It improves the microscopic detection of acid-fast bacilli and can be useful contribution to routine cytology

REFERENCES

1. Gupta AK, Nayar M, Chandra M. Critical appraisal of fine needle aspiration cytology in tubercular lymphadenitis. *Acta cytological*. 1992;36:391-4.
2. Khabnani H, Munjal K. Application of bleach method in diagnosis of extra-pulmonary tuberculosis. *Indian Journal of Pathol Microbiol*. 2005;49(4):546-50.
3. Daniel TM. Rapid diagnosis of tuberculosis: laboratory techniques applicable in developing countries. *Rev Infect Dis*. 1989;2:S471-8.
4. Savic B, Sjobring U, Alugupalli S, Larsson L, Miorner H. Evaluation of polymerase chain reaction, tuberculostearic acid analysis, and direct microscopy for the detection of *Mycobacterium tuberculosis* in sputum. *J Infect Dis*. 1992;166:1177-80.
5. Gangane N, Anshu, Singh R. Role of modified bleach method in staining of acid-fast bacilli in lymph node aspiration. *Acta Cytol*. 2008;52:325-8.
6. Annam V, Mohan HK, Rekha BP. Improved microscopical detection of acid-fast bacilli by the modified bleach method in lymph node aspirates. *Indian J Pathol Microbiol*. 2009;52:349-52.
7. Khabnani H, Munjal K. Application of bleach method in diagnosis of extra-pulmonary tuberculosis. *Indian Journal of Pathol Microbiol*. 2005;49(4):546-50.
8. Patel M, Patel K, Italiya SL, Kaptan KR. Improved diagnosis of tuberculosis in lymphnode cytology by bleach method for detection of acid fast bacilli in comparison to conventional Ziehlneelsen staining method. *Int J Med Sci Public Health*. 2013;2:935-9
9. Chandrasekhar B.P, Aruna K. Utility of concentration method by modified bleach technique for the demonstration of acid-fast bacilli in the diagnosis of tuberculous lymphadenopathy. *J Cytol*. 2012;29(3):165.
10. Dwivedi G, Mathur C. Modified bleach method-improving microscopic detection of acid fast bacilli in fine needle aspiration of smears of lymph nodes. *JMSCR*. 2103;1(4):176-83.
11. Cebre N, Karlsson U, Jonsson G. Improved microscopical diagnosis of pulmonary tuberculosis in developing countries. *Trans R Soc Trop Med Hyg*. 1995;89:191-3