Indian Journal of Clinical Practice, Vol. 31, No. 8, January 2021

Efficacy of Modified Bleach Concentration Method for Demonstration of Acid-fast Bacilli in Fine Needle Aspiration of Lymph Nodes with Clinical Suspicion of Tuberculosis

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ABSTRACT

Background: Tuberculous lymphadenitis is the most common form of extrapulmonary tuberculosis. Fine-needle aspiration cytology of lymph node for its diagnosis is simple and safe. Conventional Ziehl-Neelsen (ZN) method for acid-fast bacilli plays a key role in the diagnosis; however, it has variable sensitivity due to low bacterial load. We evaluated the role of bleach concentration method before performing ZN method for the detection of mycobacterium in clinically suspected cases of tuberculous lymphadenitis. **Method:** A total of 103 samples of fine-needle aspirates were collected from clinically suspected cases of tuberculous lymphadenitis as part of routine diagnosis. All the samples were processed for cytology, conventional ZN staining, bleach concentration followed by ZN staining. **Results:** As per cytomorphological diagnosis of aspirates, 50.50% cases were categorized as reactive hyperplasia, 43.68% cases as tubercular lymphadenopathy and 5.82% cases of suppurative lymphadenitis. The detection rates of conventional ZN method and bleach concentrated ZN method were 28.15% and 33%, respectively. The bleach method has 100% sensitivity and specificity while conventional ZN method showed 85.29% and 100%, respectively. **Conclusion:** Bleach concentrated method can be done before conventional ZN staining for detection of tubercle bacilli, as it has a higher case detection rate than that of the conventional ZN method.

Keywords: Bleach method, ZN staining, tubercular lymphadenitis, fine-needle aspiration cytology

Tuberculosis (TB) is one of the top 10 causes of death and the leading cause from a single infectious agent (above human immunodeficiency virus/acquired immune deficiency syndrome [HIV/AIDS]). Overall, 1.4 million people died from TB in 2019 (including 2,08,000 people with HIV) globally. Major suffering due to TB is attributable to appearance of virulent strains, resistance to multiple drugs and steady increase in HIV infection. When TB is bacteriologically confirmed or clinically diagnosed in other parts of the body other than the lung, such as the abdomen, meninges, genitourinary tract, joints, bones, lymph nodes and skin, it is classified as extrapulmonary tuberculosis (EPTB). The most common extrapulmonary sites of TB infection include the lymph nodes, the pleura, the genitourinary system, the gastrointestinal tract, the bones and the central nervous system. Tuberculous lymphadenitis is seen in nearly 35% of EPTB. The methodology for diagnosis of EPTB can divided into: (a) Primary diagnostic studies and (b) Ancillary diagnostic studies. The primary diagnostic studies are fine-needle aspirate cytology (FNAC), lymph node biopsy, culture and molecular tests which are based on nucleic acid amplification for detecting *Mycobacterium tuberculosis* namely polymerase chain reaction, line probe assays. The ancillary diagnostic studies are tuberculin skin test and interferon-gamma release assays. Molecular methods are rapid and sensitive but expensive for routine use in the developing countries. The usefulness, priority and scope of various techniques used in TB diagnosis depend on the epidemiological situation prevailing in individual countries and on the resources available. In our setup, the only practically available

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bacteriological method for diagnosing EPTB is direct smear microscopy of material obtained from FNAC. FNAC of lymph node offers early availability of results since the test is simple, safe, quick and causes minimal trauma for the diagnosis of lymph node TB. The collection of material by FNAC is suitable for the patients as it is relatively painless procedure. Cytomorphology and mycobacterial visualization of smears using Ziehl-Neelsen (ZN) staining method plays a key role in the diagnosis and monitoring of treatment in TB. However, the sensitivity of this technique is low (22-43%). If the sensitivity of detection of acid-fast bacilli (AFB) by any staining method can be improved, it has the potential to become the most valuable tool for TB detection and control programs around the world. Over the years, several improvements have been done to increase the microscopic detection of AFB in sputum specimens. In late 1940s, sputum liquefaction with household bleach, i.e., sodium hypochlorite (NaOCl) and concentration by centrifugation prior to ZN staining was done to improve the AFB detection. Several studies showed that the use of bleach, in concentrations of 2-5%, digests the sputum and inactivates the bacteria without altering its structure. This method is simple, requires no expertise and cost-effective. Majority of the studies have been done on sputum and body fluid samples for detecting TB bacilli by using bleach method, but very few of them are there on lymph node aspirate samples.

The present study has been undertaken to find out the efficacy of modified staining using bleach technique in comparison to conventional ZN staining technique for detection of AFB on lymph node aspirates of patients suspected to have tubercular lymphadenitis in our hospital.

MATERIAL AND METHODS

A prospective study was conducted in the Dept. of Pathology, Punjab Institute of Medical Sciences, Jalandhar. The proposed study duration was 1 year, from April 2016 to March 2017. The 103 patients of clinically suspected tuberculous lymphadenitis belonging to all the age groups, who were referred for FNAC, were included in this study. Exclusion criteria were the patients on antitubercular drugs within previous 3 months and elderly patients with known primary malignancy.

FNAC was done by using 22-gauge needle and 20 mL syringe. All the aspirates yielded whitish, pus or pus-mixed material, which was expressed on to the glass slide. Smears were made from the expressed material. The remaining material in the hub was washed with 1 mL normal saline and collected in conical test tube and subjected to centrifugation. Three smears were prepared, of which, 2 were air-dried and one was subjected to wet fixation. One of the air-dried smear was stained with Giemsa stain for routine cytology and another smear was stained with conventional ZN stain. The wet fixed smear was stained with hematoxylin and eosin stain.

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 conical screw-capped test tube and mixed with 2 mL of 5% NaOCl. After thorough mixing, the mixture was incubated at 37°C for 15 minutes by shaking at regular intervals. An equal amount of distilled water was added, mixed and then centrifugation done at 3,000 g for 15 minutes. 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 while testing each specimen. Cytological smears were examined under light microscope. The smears, one with conventional ZN technique and the other with bleach concentration techniques, were examined under oil immersion lens for the presence of AFB. At least 100 fields were scanned for AFB, which is a standard procedure. The data were processed and the sensitivity, specificity and positive and negative predictive values were calculated.

RESULTS

The present study was undertaken to emphasize the role of bleach concentration method over the conventional ZN direct smear microscopy for the detection of tubercle bacilli in FNAC material of lymph nodes. In the 1 year duration, a total of 103 patients were evaluated. As per sex distribution of patients, 59.22% were female patients and 40.78% were male patients. Male-to-female ratio was 1:1.45. The age range of the suspects was between 1 and 70 years with a mean age of 26.43 years. As per age group, 41% patients were in the age group of 21-40 years and 0-20 years each, respectively, 15% were in the age group of 41-60 years and 3% were above 60 years of age. The most common lymph node group involved was cervical 66.99% (69/103), followed by axillary 7.76% (8/103), supraclavicular and submandibular 6.79% (7/103) each, inguinal and submental 4.85% (5/103) each and postauricular 1.94% (2/103). Enlarged single lymph node was the most common mode of presentation seen in 72.81% (75/103) cases.

According to cytomorphological diagnosis of lymph node aspirate, reactive hyperplasia was seen in 52 (50.49%) cases, followed by granulomatous inflammation with necrosis in 31 (30.1%), granulomatous inflammation without necrosis in 09 (8.73%) cases, nonspecific inflammatory pathology in 6 (5.82%) cases and necrosis alone without granuloma formation in 5 (4.85%) cases (Table 1).

Among the 45 cases suggestive of tuberculous lymphadenopathy, the cytological patterns were as follow:

- Pattern 1: Granulomatous inflammation with necrosis 68.88% (31)
- Pattern 2: Granulomatous inflammation without necrosis 20% (09)
- Pattern 3: Necrosis alone without granuloma formation 11.11% (05) (Table 2).

In Pattern 1, 24/31 (77.41%) were positive for AFB by conventional ZN technique, while bleach method detected AFB in 26/31 (83.87%) cases. Hence, 2 cases missed by the conventional ZN staining method were picked up by the bleach method. In Pattern 2, 03/09 (33.33%) were positive for AFB by conventional ZN staining, while, the bleach method detected AFB in 04/09 (44.44%) cases. Here also, 1 case missed by the conventional ZN method was picked up by the bleach method. In Pattern 3, positivity for AFB with both the conventional ZN method and bleach method was 40% (02/05). Out of 06 nonspecific inflammatory pathology smears tested for AFB, none was AFB positive by conventional ZN method but 2 cases were positive for AFB by bleach method. All the cases cytologically diagnosed as reactive hyperplasia were negative for AFB by both the methods (Table 3).

The smear positivity for AFB on conventional ZN staining method was 28.15% (29/103), while the positivity increased to 33.01% (34/103) when the bleach method was used. Thus, the bleach method detected AFB in additional 5 cases, which was statistically significant.

The direct smear microscopy for ZN staining showed sensitivity 85.29%, specificity and positive predictive value (PPV) 100% and negative predictive value (NPV) 95.15%, whereas bleach method showed sensitivity, specificity, PPV and NPV 100%, respectively (Table 4).

Table 1. Cytomorphological Pattern Observed on Lymph Node Aspirates

Cytomorphological pattern	Number of cases (n = 103)
Reactive hyperplasia	52
Granulomatous inflammation with necrosis	31
Granulomatous inflammation without necrosis	09
Nonspecific inflammatory pathology	06
Necrosis alone	05

Table 2. Pattern of Cases in Various Cytomorphological Tubercular Lymphadenopathy

Pattern	Types of tubercular lymphadenopathy	Number of cases (n = 45)
Pattern 1	Granulomatous inflammation with necrosis	31 (68.88%)
Pattern 2	Granulomatous inflammation without necrosis	09 (20%)
Pattern 3	Necrosis alone without granuloma formation	05 (11.11%)

Table 3. 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 hyperplasia	Nil	52	Nil	52	52
Nonspecific inflammatory pathology	02	04	Nil	06	06
Granulomatous inflammation with necrosis	26	05	24	07	31
Granulomatous inflammation without necrosis	04	05	03	06	09
Necrosis alone	02	03	02	03	05
Total	**34**	**69**	**29**	**74**	**103**

Table 4. Comparison of Sensitivity, Specificity, Positive and Negative Predictive Values in Conventional ZN Method and Bleach Method

Values	ZN method (%)	Bleach method (%)
Sensitivity	85.29	100
Specificity	100	100
Positive predictive value	100	100
Negative predictive value	95.15	100

DISCUSSION

In developing countries like India, the diagnosis for tubercular lymphadenitis mainly relies on FNAC and aspirate direct smear microscopy. The 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. The increased sensitivity by bleach method is probably due to the fact that NaOCl removes debris and leaves the microscopic field free for easy examination. The present study was carried out on lymph node aspirates to know and compare the sensitivity of bleach method over conventional ZN staining.

In our study, the patients showed a wide age group ranging from 1 to 70 years with the mean age being 26.43 years. Most of the patients (41%) were in the age group of 21-40 years and 0-20 years. The male-to-female ratio was 1:1.45. Our study is in accordance with the study done by other workers that reported maximum number of cases (36.65%) in the age group of 21-30 years and male-to-female ratio of 1:1.21. However, other workers reported male predominance.

In the present study, most common lymph node group involved was cervical (66.99%) followed by axillary (7.76%), supraclavicular and submandibular (6.79% each), inguinal and submental (4.85% each) and postauricular (1.94%), and enlarged single lymph node was the most common mode of presentation (72.81%). These findings are almost similar with a study done by other workers, which also reported greater involvement of cervical lymph node and supraclavicular lymph nodes (66% and 8.6%, respectively) and 61% of enlarged single lymph nodes.

The cytomorphological features were analyzed based on the nature of aspirate and microscopy. In the present study, as per cytological pattern, 43.68% cases (45/103) were diagnosed as tubercular lymphadenopathy.

In this study, all reactive hyperplasia lymph node aspirates were negative for AFB by both routine ZN staining and bleach method. Whereas other workers reported AFB in few cases of reactive hyperplasia by bleach method which were initially negative for AFB by ZN staining, 24.4% and 22.2%, respectively.

In our study, a total of 6 (5.82%) cases showed nonspecific inflammatory pathology (suppurative lymphadenitis). None of these cases were positive for AFB by conventional ZN staining. Later on, 2 cases (33.33%) of suppurative lymphadenitis were diagnosed as tuberculous lymphadenitis based on the detection of AFB in bleach method. Similar findings were reported by Bhardwaj et al. They reported 11.2% cases of suppurative inflammation and all of these cases were negative for AFB by conventional ZN staining, whereas 21.4% positivity was reported by bleach method. Other workers reported 42.8% positivity by conventional method and 82.1% positivity by bleach method in the suppurative lymphadenitis. The diagnosis of nonspecific inflammatory pathology was based on cytological picture showing numerous degenerated polymorphs, lymphocytes and plasma cells along with cellular debris. Two of these cases had foci of necrosis.

In our study, cytomorphological features of TB were seen in 45 (43.68%) cases. It was further categorized under 3 patterns as shown in Table 2. Three cases which were initially negative for AFB by the routine ZN smear in Pattern 1 and 2 were then reported positive for AFB by the bleach method. This increase in positivity could be due to increased number of the bacilli per field and clean background due to digestion of cellular elements by bleach method. The morphology of AFB also appeared to be better preserved, and they were thicker and longer than the routine ZN smears. This could probably be due to swelling of bacilli in the solution. The above mentioned observations were also noted by other workers in their studies. In the present study, we observed that bleach method has 100% sensitivity and specificity compared to conventional ZN method, which showed sensitivity and specificity of 85.29% and 100%, respectively.

CONCLUSION

The use of bleach method prior to ZN staining helps in liquefaction of lymph aspirate and concentration of

bacilli by centrifugation helps in increased positivity of direct microscopy, making the screening process easier, faster and less laborious. The implementation of the bleach method can be a useful contribution to routine cytology examination for detection of AFB in FNAC aspirate.

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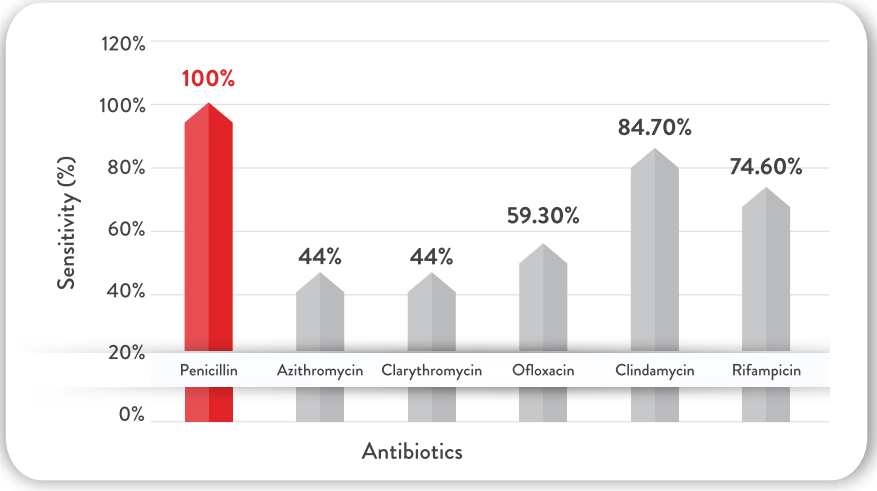

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