

Utility of Modified Bleach Method Technique for the Demonstration of Acid Fast Bacilli in the Diagnosis of Tuberculous Lymphadenopathy in Comparison to Routine Ziehl-Neelsen Staining

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ABSTRACT

Background: There is major role of fine needle aspiration in diagnosis of tubercular lymphadenitis as it is a less invasive procedure and Ziehl-Neelsen (ZN) method for acid-fast bacilli (AFB) plays a key role in diagnosis and also for the monitoring of treatment in tuberculosis. The bleach concentration method is a technique with many benefits over routine ZN staining as it improves the sensitivity of direct microscopy for the detection of AFB.

Methods: A total of 129 cases of tuberculous lymphadenitis diagnosed by fine needle aspiration cytology (FNAC) were categorized into six cytomorphological patterns. The acid-fast bacilli positivity by routine staining was correlated with modified bleach methods of ZN staining. Sensitivity of routine ZN and modified bleach concentration was compared. Grading of acid fast bacilli and time taken to detect bacilli was also compared.

Result: The classic cytomorphological pattern of tuberculosis of epithelioid granulomas and caseous necrosis was seen in 21.1% of cases. Routine ZN staining detected AFB in 58.9% of cases and the modified bleach method in 89.1%. The modified bleach method showed AFB positivity in all cases where routine ZN staining was positive. The mean time taken for detection of AFB by modified bleach method was 8.91 minutes and by routine ZN staining method was 23.36 minutes.

Conclusion: The modified bleach method was more sensitive and safer than routine ZN staining. As the background was clear, the bacilli were easily visible and the screening time was shorter.

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Introduction

India accounts for nearly one third of the global burden of TB and it is the most important public health problem. India alone accounts for 26% (2.0-2.4 million) of global cases. [1] Every day more than 20,000 people become infected with tubercle bacillus, more than 5000 develop the disease and more than 1000 die from Mycobacterium tuberculosis infection. [2] Lymphadenopathy is the most common form of extra pulmonary TB. [3,4,5]

FNAC provides an alternative to excision biopsy for lymph nodes and is an easy procedure for collection of material for cytomorphological and bacteriological examination. There is major role of fine needle aspiration in diagnosis of tubercular lymphadenitis as it is a less invasive procedure and Ziehl-Neelsen (ZN) method for acid-fast bacilli (AFB) plays a key role in diagnosis and also for the monitoring of treatment in tuberculosis.

In spite of great utility, the major disadvantage of ZN staining is low sensitivity, ranging from 9 to 46%. [6,7] The bleach concentration method is a technique with many benefits over routine ZN staining as it improves the sensitivity of direct microscopy for the detection of AFB, can effectively kill mycobacterium tuberculosis which makes the specimen safe to handle and is inexpensive & easy to perform and requires no additional equipment.

Materials and Methods

In a time course of 1 year, total of 129 cases suspected of tuberculosis were taken. In each case smears were stained by Giemsa stain, pap stain and routine ZN staining. In rest of the left out material, modified bleach technique was applied. In this method the aspirated material was subjected to liquefaction with 5% sodium hypochlorite (NaOCl, bleach) solution at room temperature for 30 minutes, followed by centrifugation at 3000 rpm for 15 mins. Smears were made from the deposit and were stained by routine ZN staining.

The spectrum of cytomorphological features observed on Papanicolaou and MGG stained smears was broadly categorized into six patterns – 1) Epithelioid granuloma with langhans giant cells and caseous necrosis (pattern 1); 2) Epithelioid cells in a reactive background (pattern 2); 3) Caseous necrotic material with epithelioid cells (pattern 3); 4) Caseous necrotic material with a few lymphocytes and histiocytes, with no epithelioid cells (patter 4); 5) Smears with predominantly neutrophils, degenerating epithelioid cells and necrotic material (pattern 5). Smears stained by both the methods were thoroughly screened for presence of AFB under 100X oil immersion.

After identifying AFB in the routine ZN slide and modified bleach technique grading of the AFB was done on the slide and comparison between the both staining system was done. (table 1) This grading system was created on the basis of pilot study done in our department with the help of grading system done on the sputum smear.

Result

All 129 cases of suspected tubercular lymphadenitis were screened for AFB by routine ZN stain and modified bleach method. The age range of the cases varied from 1 to 85 years. There was female preponderance accounting 55.9% of total cases. Most patients were in the age group of 16 – 30 years constituting 48.8%, followed by age group of 31-45 years constituting 23.7%. The youngest patient was 1 year old. The oldest patient was 81 years old.

Among the 129 lymphnodes studied, maximum cases involved cervical lymphnodes (71.3%), followed by axillary region (7.7 %). Least number of patients presented with preauricular and posterior occipital region lymphadenopathy.

The smear positivity for AFB on conventional ZN method was 58.9% (76/129) while the positivity increased to 89.1% (115/129) by bleach method. The comparison between conventional ZN method and bleach method showed statistical significance. (table 2)

The mean time taken for detection of AFB by modified bleach method was 8.91 minutes and by routine ZN staining method was 23.36 minutes. The time difference between two methods was statistically significant. (table 3)

There was a significant difference between the grading of AFB positivity by modified bleach method and routine Z-N staining (p value <0.001) with modified bleach method showing higher grades of positivity. (table 4)

Discussion

Tuberculosis (TB) is an ancient infection that has plagued humans since times immemorial and still continues to remain a major public health problem especially in developing countries like India. Hence, early diagnosis of tuberculosis and initiating optimal treatment would not only enable cure of an individual patient but will significantly reduce the transmission of infection and disease to others in the community.

Diagnostic modalities must also be tailored according to the needs of the population and epidemiology of TB in that region. These include improved microscopy, usage of liquid culture for childhood and extrapulmonary TB, chemical and physical detection of mycobacterial antigens in paucibacillary condition, antigen capture,

antibody detection, cellular immune recognition, nucleic acid amplification and phage assay. However all these investigations are time consuming and expensive.

In the present study smears suggestive of tubercular pathology were divided in to six patterns depending upon cytomorphological findings. The most common pattern (n=35, 27.1%) was pattern 3 and least common (n=16, 12.4%) was pattern 5. However in a study by chandrashekhar et al in the year 2012, pattern 1 was seen in 42 (38%) cases, pattern 2 in 27 (24%), pattern 3 in 8 (7%), pattern 4 in 17 (15%), and pattern 5 in 16 (14%)

cases which was inconsistent with the distribution pattern of the present study.^[9]

Majority of the AFB positive cases by modified bleach method showed more number of bacteria present in clean thin background making them easily visible.

The present study validates the literature on the utility of concentration of AFB by modified bleach method for detection of tuberculous bacilli in lymph node aspirates. The comparison of percentage of smears positive with routine ZN staining and modified bleach method in several other studies is shown in the table no 5.

Table 1: Grading of AFB on FNAC smears.

| NUMBER OF AFB | GRADE | NUMBER OF FIELDS EXAMINED |
|------------------------------------|--------|---------------------------|
| >10 per oil immersion field | 3+ | 20 |
| 1-10 per oil immersion field | 2+ | 50 |
| 10-99 per 100 oil immersion fields | 1+ | 100 |
| 0-9 per 100 oil immersion fields | Scanty | 200 |

Table 2: Comparison of routine ZN staining and modified bleach technique in detection of AFB

| | | AFB – BLEACH TECH | | Total | Chi-square value | p-value |
|-----------------|----------|-------------------|-----------|------------|------------------|---------|
| | | Positive | Negative | | | |
| AFB-ZN STAINING | Positive | 76 | 0 | 76 | 16.33 | <0.001 |
| | Negative | 39 | 14 | 53 | | |
| Total | | 115 | 14 | 129 | | |

TABLE 3: Comparison of time taken for detection of AFB by routine ZN staining and modified bleach technique by applying paired t test.

| Paired Samples Statistics | | | | | |
|---------------------------|-------------|-------|-----|----------------|---------|
| | | Mean | N | Std. Deviation | p-value |
| Pair 1 | TIME ZN | 23.36 | 129 | 6.26 | <0.001 |
| | TIME Bleach | 8.91 | 129 | 6.94 | |

TABLE 4: Comparison of grading of AFB positivity in routine ZN staining and modified bleach technique.

| | | GRADING BY BLEACH METHOD | | | | Total | Chi-square value | p-value |
|------------------------|---------|--------------------------|-----------|-----------|----------|-----------|------------------|---------|
| | | Scanty | Grade 1 | Grade 2 | Grade 3 | | | |
| GRADING BY ZN STAINING | Scanty | 7 | 20 | 10 | 2 | 39 | 43.13 | <0.001 |
| | Grade 1 | 0 | 16 | 12 | 2 | 30 | | |
| | Grade 2 | 0 | 0 | 4 | 1 | 5 | | |
| | Grade 3 | 0 | 0 | 0 | 2 | 2 | | |
| Total | | 7 | 36 | 26 | 7 | 76 | | |

TABLE 5: Comparison of AFB positivity in different studies by ZN & modified bleach technique.

| AUTHORS | ROUTINE-ZN STAINING | MODIFIED BLEACH TECHNIQUE |
|--|---------------------|---------------------------|
| Khubani et al ^[9] (2005) | 21.8% | 71.9% |
| Gangane et al ^[10] (2008) | 51.27% | 72.0% |
| Annam et al ^[11] (2009) | 33.33% | 63.44% |
| Chandrashekhar et al ^[9] (2012) | 12.5% | 60.7% |
| Patel M M (2013) ^[12] | 21.3% | 61.74% |
| Present study | 58.9% | 89.1% |

In the current study, mean time taken by the routine ZN staining in detecting AFB was 23.36 minutes and by modified bleach method it was 8.91 minutes. Paired T test was applied and significant difference was noted in time taken by modified bleach method (mean time 8.91 min) and routine ZN staining (mean time 23.36 min), resulting in the p value of <0.001 which was significant. Chandrashekhar et al in the year 2012, in his study also suggested reduction in time taken for detection of AFB by modified bleach method, although they did not quantify the difference of time in their study.^[9]

In the present study the number of bacilli per oil immersion field in routine ZN staining and modified bleach method was graded. Numbers of bacilli were found to be more per oil immersion field in modified bleach method. There was significant difference in positivity grade of bacilli in two methods with higher grade in bleach method (p value < 0.001).

The difference in time for detection of AFB and grading of bacilli on smear by two methods is compared in this study. However this comparison was not done in earlier studies.

Conclusion

The bleach method for AFB is simple, safe and cost-effective. The results would be more accurate if the concentration by bleach solution and incubation time is strictly followed. 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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Competing Interests

None

Reference

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