

A Study of Modified Bleach Method in Comparison to Direct Ziehl-Neelsen Staining for Diagnosis of Pulmonary and Extrapulmonary Tuberculosis In a Tertiary Care Hospital

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ABSTRACT

Introduction: Tuberculosis is a global health burden especially in the developing world and presents in pulmonary and extra pulmonary forms. Among the various conventional and molecular methods available for detection of tuberculosis, Microscopy still remains the most popular and widely used technique. Conventional Ziehl Neelsen staining has a disadvantage of poor sensitivity. Modified bleach methods offers better visualisation of smears with increase in sensitivity.

Materials and Methods: The present study was carried out to compare the two techniques. A total of 115 patients suspected of pulmonary tuberculosis and 53 patients suspected of having tubercular lymphadenitis were enrolled in the study. The specimens obtained were stained by these two methods and observed under oil immersion lens.

Results: Of the 115 sputum specimens, 96 were positive for acid fast bacilli in modified bleach method as compared to 67 by conventional ZN staining. Modified bleach method detected more cases of tubercular lymphadenitis (39) as compared to ZN staining (17).

Conclusion: Modified bleach method can be preferred over direct ZN staining as it is cheap, safe and has better sensitivity.

Keywords: Tuberculosis, Modified Bleach Method, Ziehl Neelsen, Fine Needle Aspiration Cytology.


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INTRODUCTION

Tuberculosis is a major health problem in India which accounts for the major burden of this disease worldwide. India accounts for maximum number of both TB and MDR TB cases. The disease presents itself in pulmonary and extra pulmonary sites and results in significant morbidity and mortality resulting in great economic burden on the country. Many techniques are used for diagnosis of pulmonary and extrapulmonary tuberculosis that includes microscopy (Ziehl Neelsen staining and fluorescent staining), line probe assay, nucleic acid amplification technique (NAAT) and culture.¹⁻³ Microscopy remains the most popular and widely applied method of detection of tuberculosis. Presence of acid fast bacilli in ZN staining method offers a speedy diagnosis and also helps in assessing infectiousness. In developing countries, microscopy offers an advantage of being cheap and rapid method.⁴⁻⁶

However ZN staining has its own disadvantages. It has low sensitivity, particularly for cytological specimens and in paediatric and HIV cases. There is risk to the laboratory worker if a biosafety cabinet is not available.^{2,5,6} The presence of cellular debris may also hinder the screening of the smears resulting in missing the

positive cases. Many studies have proposed liquefaction and concentration of sputum before ZN staining to increase the diagnostic sensitivity. Liquefaction results in efficient release of bacilli. Liquefaction with chemicals like N acetyl L cysteine-sodium hydroxide (NaLC-NaOH), sodium hypochlorite, chitin, chymotrypsin have been proposed by various authors. However cost of these reagents is a limiting factor in resource constrained setting.

Modified bleach method using the sodium hypochlorite offers many advantages. It is simple, cheap, economical, more sensitive; and also disinfects the specimen because of action of hypochlorite.⁵⁻⁸

MATERIALS AND METHODS

This prospective study was carried out in department of microbiology in association with department of pathology from March 2013 - January 2014. A total of 115 patients suspected of pulmonary tuberculosis and 53 patients suspected of having tubercular lymphadenitis were enrolled in the study. Informed consent was taken from each patient.

Inclusion Criteria

- i. Patients clinically suspected of pulmonary tuberculosis presenting in outpatient department / inpatients of the hospital
- ii. Patients clinically suspected of having tubercular lymphadenitis

Exclusion Criteria

1. Treatment for Tuberculosis within the previous 3 months
2. Initiation of antitubercular therapy before sampling
3. Non cooperative patients

Collection of Sputum Specimens⁹: Sputum specimens were collected as per RNTCP guidelines. Spot specimens were collected from the patients. The sputum specimens were analysed macroscopically and only the specimens with purulent discharge were accepted. In case patients were unable to expectorate, early morning sputum specimen was collected from them.

Collection of Lymph Node Aspirates: The lymph node aspirates were collected under aseptic conditions with sterile disposable

syringe by FNAC technique. Two smears were made, one for Giemsa staining and the other for ZN staining. The remaining aspirated material was used for modified bleach method.

Modified Bleach Method^{10,11}

The specimen (sputum / lymph node aspirate) was taken in sterile screw capped tube. An equal amount of household bleach (5%NaOCl) was added to it. The tube was then shaken for thirty seconds. The tube was kept at room temperature for half an hour with intermittent shaking. Then equal amount of distilled water was added and centrifuged at 3000 rpm for 15 min. The supernatant was discarded and the pellet was used for making the smear followed by ZN staining.

RESULTS

Tables 1 and 2 show comparison between routine ZN staining and modified bleach method staining of sputum specimens and lymph node aspirates.

Table 1: Comparison between direct ZN staining and modified bleach method in sputum specimens

ZN staining	Bleach method	Bleach method	Total
	AFB positive	AFB Negative	
AFB Positive	67	0	67
AFB Negative	29	19	48
Total	96	19	115

Table 2: Correlation of cytological findings and staining techniques (modified bleach method and direct ZN staining) in lymph node aspirates

Cytological features	Direct ZN staining		Modified bleach method	
	AFB Positive	AFB Negative	AFB Positive	AFB Negative
▪ Granulomatous lymphadenitis (27)	7	20	19	8
▪ Caseation necrosis only (11)	5	6	9	2
▪ Suppurative lymphadenitis (14)	5	9	11	3
▪ Reactive lymphadenitis (1)	0	1	0	1

DISCUSSION

In developing country like India, tuberculosis remains a challenge, a great health hazard and associated with social stigma. Diagnosis of pulmonary and extrapulmonary tuberculosis can be achieved by various methods. Conventional culture methods take weeks while molecular methods are costly and not easily available. Microscopy still remains commonest method for diagnosis of tuberculosis. However, 10⁴ bacilli/ml sputum must be present to be able to be detected by conventional ZN staining. Also other factors that affect the sensitivity are the specimen quality, individual interpretation, presence of cellular debris. Similarly detection of acid fast bacilli is difficult in aspirates and tissues by conventional ZN staining. Many studies have reported increase in sensitivity by prior sputum/aspirate digestion using various chemicals followed by concentration by centrifugation. Sodium hypochlorite (household bleach) being cheap and easily available is such an agent.^{12,13}

In our study, majority of the patients were from the rural background. Seven patients were having HIV coinfection. Modified bleach method detected more AFB positive cases (96 vs 67 and

39 vs 17) as compared to routine ZN staining of sputum specimens and lymph node aspirates respectively. All the seven HIV infected patients were sputum smear negative in routine ZN staining but modified bleach method was able to detect acid fast bacilli. This corroborates the finding of other authors from different parts of the world.¹⁰⁻¹⁶

As compared to ZN staining, modified bleach method was found to be more sensitive in detection of acid fast bacilli in the specimens in our study. It was observed that there was higher density of bacilli per microscopic field as compared to conventional staining. The background was more clear resulting in better visualisation of the slide and saving time as smears were scanned faster. There was reduction in cellular debris on smears on microscopic slides as compared to direct stained smears. This is in accordance with studies conducted by other authors.^{6-8,10,12}

The only drawback is that the smears are thin and can be washed off during the staining procedure. Also during heating of the smear crystals may form. However this can be overcome if the smear preparation is done carefully.

CONCLUSION

Detection of acid fast bacilli by modified bleach method is greatly increased as compared to conventional ZN staining. It offers a great advantage in detection of tuberculosis especially in cytology specimens where sensitivity of direct method is particularly low. Being a simple, cost effective method with decreased turnaround time, this method can be routinely employed in all diagnostic laboratories for efficient and more effective detection of TB cases which might escape diagnosis otherwise.

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