



A study to assess the Utility of modified bleach method in detection of acid fast bacilli in sputum smears.

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Abstract

Background: Tuberculosis claims over two million lives and causes an economic drain of over three billion rupees a year in India. The success rates of properly applied DOTS (directly observed treatment, short course) exceeds 80-85 % in most countries of south east Asia, however the primary obstacle to complete control over the spread and incidence of the disease is poor case finding, which still remains much less than 70 % in some south east Asian countries such as India (61.3 %). When more cases are identified, more cases are cured and less transmission occurs, reducing the incidence of tuberculosis. Case detection is currently done using direct microscopy on three sputum samples, according to the recommendations of the Revised National Tuberculosis Control Programme (RNTCP). This method is known to have a sensitivity of 50-70 %. This study was done to evaluate the efficacy of modified bleach concentration method in comparison with conventional Ziehl-Neelsen. **Materials and Methods:** A cross sectional study was conducted in BLDEU's SBMPMC from August to September 2013. A total of 100 smears were stained by both conventional ZN stain and Modified Bleach Method and observed for AFB bacilli. **Results and Discussion:** Out of 34 samples were positive by conventional ZN staining and the same were also positive by modified bleach concentration method, in additional 3 samples which were negative by conventional ZN staining was positive by modified bleach concentration method. There was an absolute increase in the number of acid fast bacilli per slide using the bleach-concentration technique compared to the direct method. The bleach method is simple, inexpensive, potent disinfectant and is one of the safest concentration methods for improving the sensitivity of direct microscopy for the detection of AFB.

Keywords: afb, tuberculosis, bleach, zeil neelsen.



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INTRODUCTION

Tuberculosis (TB) is a major cause of death worldwide caused by Mycobacteria, belongs to family Myco bacteriaeae of the pathogenic species belonging to the Mycobacterium tuberculosis complex and is the most common cause of tuberculosis in humans. More than 5.8 million new cases of TB (all forms both pulmonary and extra pulmonary) were reported to the World Health Organization (WHO) in 2009; 95% of cases were reported from developing countries. However because of insufficient case detection and incomplete notification, reported case represents only 63% (range 60-67%) of total estimated cases.¹

So approximately one third of the world's population has been infected with Mycobacterium tuberculosis and two to three million deaths reported each year.²

Case detection is currently done using direct microscopy on two sputum samples for acid fast bacilli using ZN Stain as per the recommendations of the Revised National Tuberculosis Control Programme (RNTCP). This method is known to have a sensitivity of 50-70 %. The United Nations Millennium Development Goals (MDGs) suggest that to halve the prevalence rate by 2015, TB control programmes must reach global targets for detection (70%) and treatment success (85%), this is also the target of the RNTCP. The Global Plan to "Stop TB 2006-2015" recognizes the limitations of sputum smear direct microscopy and recognizes the need for research into new tools that will improve diagnostic detection of M. tuberculosis and shorten the time to diagnosis.³

Microscopy has many advantages when it comes to speed and feasibility, and if its sensitivity could be improved, it has the potential to become and even more valuable for tuberculosis control programmes around the world. The need for new,

improved, low-cost techniques cannot be over emphasised. The bleach concentration method is of the safest concentration methods for improving of direct microscopy for detection of AFB . Hence this study was conducted to know the utility of modified bleach concentration method in comparison with conventional Ziehl– Neelsen staining technique.

Objectives :

To evaluate the efficacy of modified bleach concentration method in comparison with conventional Ziehl–Neelsen staining technique in detecting acid fast bacilli in sputum smears for diagnosing tuberculosis.

MATERIALS AND METHODS

Study design: Correlation study.

Type of study: Hospital based prospective study Duration of study: August 2013 to September 2013 Sample size: 100.

Study site: All clinically suspected tuberculosis patients attending OPD and wards at department of Medicine of BLDEU Shri.B.M.Patil Medical College,Hospital& Research Centre were taken for the study.

Methodology: A prospective study of 100 patients satisfying the inclusion and exclusion criteria, attending clinical department of BLDEU Shri B.M. Patil Medical College, Hospital &RC, Bijapur, Karnataka was studied. A detailed history of included patients was elicited. A complete general physical examination and systemic review of the patients was undertaken.

Procedure:

Approximately 100 patients in two months duration with clinically suspicion as tuberculosis (TB) were taken. Each sputum sample was aliquoted. The first aliquot, was used for direct microscopy, smears stained with conventional ZN staining technique .4

There are four distinctive methods for sputum processing with bleach .

1. Centrifugation.
2. Overnight sedimentation,
3. Timed smear.
4. Mixed smear [bleach processed (overnight) smear is added on top of a direct smear].

The second aliquot of the specimen was transferred into to centrifugation tubes for bleach centrifugation method.

Modified bleach concentration method: 3

Specimen was transferred into centrifugation tube then equal amount of sodium hypochlorite solution (5%) was added to it. This was incubated for 10 minutes at room temperature. Tube was shaken at each minute interval. After 10 minutes of incubation, 8 ml distilled water was added to the tube. Then, tube was centrifuged for 3000g for 10 minutes. After centrifugation supernatant was decanted and smear was prepared from the deposit. Smear was air dried, heat fixed & stained with ZN stain for the examination. Smears prepared from the both conventional ZN staining technique and modified bleach technique were scanned under oil immersion for acid fast bacilli.3

Patients who were on anti tubercular drugs within the previous 3 months were excluded from the study.

RESULTS

Out of the 100 samples tested in both groups , 34 and 37 were tested positive by conventional ZN stain and Modified bleach method respectively.

Table 1 : Acid Fast Bacilli status of smears

ZN stain for AFB	Conventional ZN stain	Modified bleach concentration method
Negative	66	63
Positive	34	37
Total	100	100

Table 2 : Grading of Acid Fast Bacilli Positive

		AFB(MODIFIED ZN STAIN-BLEACH METHOD)					
AFB (CONVENTION AI ZN STAIN)		NEG	1+	2+	3+	4+	TOTAL
	NEG	63	3	0	0	0	66
	1+	0	0	5	7	1	13
	2+	0	0	0	5	9	14
	3+	0	0	0	0	5	5
	4+	0	0	0	0	2	2
	TOTAL	63	3	5	12	17	100

$$X^2 = 160 \quad df = 16 \quad p < 0.000$$

All the positively stained smears from both conventional ZN stain and Modified Bleach Concentration Method were observed under oil immersion microscope for the bacteriological grading of the AFB bacilli as per RNTCP guidelines. The 3 samples which were reported as negative in conventional ZN stain but showed positive in modified bleach concentration method were graded as 1+. Out of 13 samples which showed 1+ grading on conventional ZN stain were increased to grade 2+ in 5 samples & grade 3+ in 8 samples and on modified bleach concentration method. Out of 14 samples which showed 2+ grading on conventional ZN stain were increased to grade 3+ in 4 samples & grade 4+ in 10 samples and on modified bleach concentration method. Out of 5 samples which showed 3+ grading on conventional ZN stain were increased to grade 4+ in all 5 samples. Two samples showed grade 4+ in conventional ZN stain also showed grade 4+ in modified bleach concentration method. So there is marked discrepancy in grading in modified bleach concentration method as compare to conventional ZN staining method in which there is increase in detection rate of smear positive patients. The association was found to be statistically significant on applying chi square test.

DISCUSSION

In developing countries, microscopy of sputum is by far the fastest, cheapest and most reliable method for the diagnosis of pulmonary tuberculosis. While a positive smear will alert the physician to a probable AFB infection, a false negative smear may lead to a false sense of security. Thus although simple, rapid and economical, the estimated detection limit of microscopy is 104 bacilli/ml of sputum. Smear sensitivity may be influenced by a variety of factors including type of specimen, efficiency of decontamination and concentration procedures, the type of staining procedures and experience of microscopists. Wah Wah Aung 2 also reported that the use of NaOCl method increased the numbers of samples positive for acid fast bacilli by more than 100%.

The findings of our study are correlating with Annam V Set al who studied 93 fine needle aspiration specimens from lymph nodes, cyto morphological 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% (39/43) cases. There was a statistically significant correlation ($X^2 = 8.29$, $df=3$, $P<0.05$) between cytomorphological diagnosis, results of smears prepared by the conventional ZN method and the bleach method.

In Chandrashekar et al 6 study, routine ZN staining detected 14 (12.5%) cases of AFB positivity and the bleach method detected 68 (60.7 %) cases of AFB positivity. Hence the modified bleach method detected 54 more cases than were detected by routine ZN staining.

In Aung WW 2study, nine hundred and forty-eight patients were examined, 248 (26.2%) had AFB positive smears using the direct Ziehl-Neelsen (ZN) staining. After household bleach (NaOCl) treatment and centrifugation, the number of smear positive patients was increased to 293 (30.9%). All AFB positive smears by direct ZN method were also positive by household bleach concentration method. Forty-five AFB negative samples by direct ZN method were positive by household bleach concentration method. The increase in numbers of AFB positive samples by household bleach method was significant ($p=0.02$, $2=5.24$). Similar conclusion were also drawn by various authors in different studies.7,8,9.

CONCLUSION

The limits of the bleach method have been tested considerably over the last decade. It is a safe, cheap, applicable, easy to use and effective method applicable even in patients with low sputum bacillary load. This method is simple, affordable, and appropriate for laboratories that already perform microscopy. The bleach-concentration method for sputum smear samples significantly increased the detection rate of smear positive patients compared to the direct method. Thus, a shift from direct sputum microscopy to the bleach-concentration technique should be considered.

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