

Improved Diagnosis of Pulmonary Tuberculosis using bleach Microscopy Method

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ABSTRACT

Background: The bacteriological diagnosis of tuberculosis (TB) is largely dependent on the Ziehl-Neelsen (ZN) microscopy. This method has a low sensitivity, which can be improved by the concentration of the specimen with sodium hypochlorite (NaOCl), followed by centrifugation before doing acid fast staining (AFB).

Aim: To study the improvement in the sensitivity of the sputum smear by the bleach method.

Setting and Study Design: This study was conducted in BLDEU's Shri B.M. Patil Medical College, Hospital and Research Centre, Bijapur, Karnataka, India. Eighty five patients who visited Shri B.M. Patil Medical College, Hospital and Research Centre between January 2012 to December 2012 were investigated. On spot, morning and second on spot samples were collected from each patient. Direct smears were prepared and they were stained with the hot ZN technique and the remaining samples were concentrated by using 5% NaOCl, followed by centrifugation and staining with ZN stain. The improvement in the sensitivity following the bleach method was studied.

Statistical Analysis: The Fisher's exact test was used.

Results: A total of two hundred and fifty five specimens from eighty five patients were included in this study; each patient produced three specimens. AFB was detected in twenty five direct smears and in eighty four bleach smears. A statistically significant ($p < 0.001$) increase in the positivity with the use of the bleach method was detected as compared to that with the use of the direct method. The ZN sensitivity, specificity, positive predictive value (PPV), and the negative predictive value (NPV) were 29%, 99%, 96% and 74% respectively with a 95% confidence interval, with the use of the 5% NaOCl method.

Conclusion: The bleach method has advantages over the direct ZN method, as it is simple and as it does not require any additional expertise beyond that which is required for the conventional direct smear microscopy. The materials and the reagents are also affordable and they are available locally.

Key words: Tuberculosis, Ziehl-Neelsen method, Acid fast bacilli, Sodium hypochlorite

INTRODUCTION

Tuberculosis (TB) remains a worldwide public health problem despite the fact that the causative organism was discovered more than 100 years ago [1]. TB is a major public health problem, with an estimated two billion people being infected with tubercle bacilli world wide. The estimated global prevalence of the disease is 139 per 1, 00,000 population [2].

The major objective of the TB control programmes is to identify and to treat the patients with infectious pulmonary tuberculosis, the diagnosis of which relies on a bacteriological examination of the sputum. The culture of Mycobacterium is the reference method for the detection of the tubercle bacilli, but it is prohibitively slow and it requires special safety procedures in laboratories. Serological techniques are not useful in the control programmes, due to a lack of sensitivity and specificity. Among the new approaches which are used for a rapid diagnosis of TB, the nucleic acid amplification methods are the most promising, but the technology is not applicable to the control programmes in the developing countries [3].

Several improvements have been suggested to increase the yield of the microscopic detection: a serial sputum specimen examination, fluorescent microscopy with auramine or rhodamine staining and chemical fluidization of the sputum with concentration by sedimentation or centrifugation [4-6]. Sodium hypochlorite

(NaOCl) or bleach has been used for over a century in this application. The concentrations of 2-5% of NaOCl digest the sputum products and they inactivate the mycobacteria without altering their structures, so that even when they are killed, they can still be stained and observed. This provides a greater security for laboratory use. Further centrifugation or sedimentation concentrates the acid fast bacilli (AFB) in the mixture and it increases the rate of the positivity [7].

MATERIAL AND METHOD

This study was conducted from January 2012 to January 2013 in the Department of Microbiology, Shri B.M. Patil Medical College, Hospital and Research Centre, Bijapur, Karnataka, India.

According to the WHO recommendation, all the patients with more than fifteen years age group who attended the Out Patients Department (OPD) and the inpatients who were admitted with cough which lasted for more than two weeks, were included in this study [Table/Fig-1]. The exclusion criteria were the initiation of the treatment for tuberculosis before the sampling was performed. A total of 255 sputum samples were collected from 85 patients. Each patient produced three sputum specimens.

The specimen collection and processing: An oral consent was taken from the patients and instructions were given for the sputum

production. The collection of the first sample was supervised by a TB nurse. The samples which lacked any purulent material were rejected and the patients were asked to try again. The patients were given a sputum container for expectoration at home the following morning. When the second sample was brought to the laboratory, it was examined macroscopically. If no purulent material was present, the technician at the laboratory supervised the collection of the replacement sample. A third container was provided for expectoration the following morning. Smears were made from each specimen for direct microscopy.

Each smear was heat fixed and it was stained by using the hot ZN method. After the direct smears were made, the remainder of each sample was processed for the bleach method. An equal amount of household bleach (5% NaOCl) was then added to the sputum sample in a screw cap tube and the tube was shaken for 30 seconds. Then, the tube was left on the table top for 10-15 minutes at room temperature and it was hand shaken for 30 seconds, every five minutes. An equal amount of distilled water was then added and the tube was centrifuged at 3000 rpm for fifteen minutes. The supernatant was discarded and the pellet was suspended in a few drops of the remaining fluid. Smears were prepared from the suspended sediment.

The Sodium hypochlorite (Naocl) solution: The "Rheachem" bleach was purchased, in which the stated chlorine concentration was 5%. To prevent the reduction of the chlorine activity due to a repeated exposure to air, each 5 L bottle was decanted after it was opened into a 25ml brown glass bottle for daily use and the remaining solution which remained unused at the end of the day was discarded. A ZN smear was made from each new bottle to ensure no contamination of mycobacteria.

All the direct smears were read by one microscopist and the bleached smears were read by another microscopist and at the end of the study, the collection smears were swapped between the two microscopists. The microscopist who read the direct smear first, now read the bleached smear and the other microscopist who read the bleached smear first, now read the direct smear. The results were compared by one of the investigators who examined the discordant slides and discussed with both the microscopists to reach a consensus. The data was analysed by using a statistical method (Fisher's exact test).

RESULTS

A total of 90 patients were recruited, of which 5 patients were excluded from the analysis due to incomplete data. A total of 255 specimens from 85 patients were selected, and each patient produced 3 specimens. The male/female ratio was 3.3: 1.

A dual reading lead to disagreement in 7 samples, 2 for the direct smears and 5 samples for the bleach smears. This was resolved by rereading and a third microscopist examined the discordant slides.

AFB was detected in 25 direct smears and in 84 bleach smears [Table/Fig-2]. A statistically significant difference ($p < 0.001$) gave an increase in the positivity with the bleach method, especially for paucibacillary taking ATS (American Thoracic Society) staging method [Table/Fig-3]. The percentage gain from the bleach was more (10.5%) in stage 1 (paucibacillary). All the samples which were positive for the direct smears were positive by the bleach method also, except one sample.

	Patients (N = 85)	Sputum samples (N = 255)
Sex		
Male	65	195
Female	20	60
Age group (In Years)		
15-24	12	36
25-34	15	45
35-44	13	39
45-54	19	57
55-64	12	36
> 64	14	42
Total	85	255

[Table/Fig-1]: Origin of sputum samples

Stages	Direct smear	Bleach smear	Percentage gain for bleach smear
I	8 (32%)	29 (34.5%)	2.5%
II	6 (24%)	21 (25%)	1%
III	7 (28%)	24 (28.6%)	0.6%
IV	4 (16%)	10 (11.9%)	-4%
Total	25	84	

[Table/Fig-2]: Case detection: Number of smear positive patients (ATS staging and percent gain by bleach method compared to direct smear)

Quantified Result	Interpretation by ATS Scale (Threshold at 1/100 fields)
Negative	Negative
1-2 AFB/300 fields	Doubtful
1-3 AFB/100 fields	Positive 1+
4-9 AFB/100 fields	Positive 1+
1-9 AFB/10 fields	Positive 2+
1-9 AFB/field	Positive 3+
>10 AFB/field	Positive 4+

[Table/Fig-3]: ATS (American Thoracic Society) scale

Method	NaOCl (In %)	% Sensitivity (95% CI)	% Specificity (95% CI)	% of PPV (95% CI)	% of NPV (95% CI)
Centrifugation	5%	29%	99%	96%	74%

[Table/Fig-4]: Sensitivity, Specificity, Positive and Negative Predictive value and 95% confidence interval using 5% sodium hypochlorite concentration technique

DISCUSSION

In the developing countries, the microscopy of the specimen is by far the fastest, cheapest and the most reliable methods for the detection of AFB. In the late 1940s, sputum liquefaction with NaOCl (which is readily available at low costs as household bleach) and then its concentration by centrifugation before acid fast staining was implemented to improve the smear positivity for the detection of AFB [8]. In our study, all the samples which were positive for the direct smears were positive by the bleach method also, except for one sample which was positive by the direct smear but negative by the bleach method. This may have to do with the mechanism of the method of digestion and flocculation of the substances (which may have been due to the protein from the pus) which were present in the sputum, that make AFB co-precipitate during centrifugation.

The results showed that there was a significant increase in the sensitivity with the use of 5% NaOCl [Table/Fig-4]. The increase in the 23.14% smear positivity with the use of 5% NaOCl with the centrifugation method was very encouraging as compared to that of the direct smears.

This suggests that NaOCl digests the sputum, which when followed by the concentration of bacilli by either centrifugation or sedimentation, greatly increases the number of bacilli per microscopic field, which explains the increase in the sensitivity [9].

Several investigators have used 5% NaOCl [9-12]. A study in Ethiopia which used 5% NaOCl, showed an increase in the sensitivity from 54.2% to 63.1% in HIV negative patients and a sensitivity of 38.5% to 50% in HIV positive patients [11]. In these studies, the bacilli were concentrated by the centrifugation method. Other studies which used 5% NaOCl showed sensitivities of 70%[9] to 100% [10].

Several methodological parameters may explain such a wide range of sensitivities: the target population, the number of patients and the samples which were collected.

CONCLUSION

The bleach method has advantages and disadvantages. The advantages; it is simple and it does not require additional expertise beyond that which is required for the conventional direct smear microscopy. The materials and reagents are affordable and they are available locally in the countries where TB is endemic. The specimen preparation does not require extra work load, and time which is required for the drying of the slides involves no extra labour time.

The disadvantages are that the bleach method can result in fragile smears; because of the smears getting washed out. The smears can be washed off during slide staining, and care is required to avoid this problem. Overheating of the slides may result in the formation of crystals of hydroxide, which may put a compromise on the readings. Another drawback is the poor stability of the bleach when it is stored in suboptimal conditions.

This study suggests that the bleach method can significantly improve the yield of the microscopy for the TB diagnosis, especially in the settings with a high prevalence of HIV.

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