

Research Article

Bleached smear microscopy provides higher yield in diagnosing pulmonary tuberculosis

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Received: 04 April 2016

Accepted: 09 May 2016

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ABSTRACT

Background: Tuberculosis (TB) remains one of the major deadliest communicable diseases throughout the world. Worldwide, 9.6 million people are estimated to have fallen ill with TB in 2014, India accounts for 23% of total global cases. The study evaluated the performance of direct sputum smear versus bleach concentration smear in diagnosis of pulmonary tuberculosis.

Methods: Patients more than 10 years of age who presented with history of cough for >2 weeks duration were included. One spot specimen and another early morning specimen were collected on two consecutive days. All the sputum smears prepared from direct and bleach concentration were examined under microscope after Ziehl-Neelsen staining.

Results: A total of 290 specimens were examined by microscopy, among which 128/290 (44.1%) were positive on direct smear, additionally 72/290 (24.8%) were positive on bleached smear, and therefore the gain% in the diagnostic yield was 24.8% (P<0.0001). The order of yield was mucoid specimen (28.6%)>blood stained (26.74%) >purulent specimen (20.8%) (p>0.05) and early morning specimens (33%)>spot specimen (22%) (p>0.05), However, these findings were not statistically significant.

Conclusion: Microscopic examination of bleached smear examination has shown a significant yield over conventional method in the detection of pulmonary Tuberculosis. Such superiority was independent of type of sputum and timing of collection of sputum.

Keywords: Bleach concentration microscopy, Pulmonary tuberculosis, Direct microscopy, Diagnostic yield

INTRODUCTION

Tuberculosis (TB) remains one of the major deadliest communicable diseases throughout the world. Worldwide, 9.6 million people are estimated to have fallen ill with TB in 2014, India accounts for 23% of total global cases. India is said to be one among the countries with high TB burden associated with HIV and MDR-TB.¹ Microscopy of direct smears for acid-fast bacilli is the most commonly used method for diagnosis of

tuberculosis.² Apart from low sensitivity, this method also has other disadvantages including the increased risk of infection transmission to technicians.³ A systematic review analyzed the results of bleach microscopy method and found a significant improvement in case detection of tuberculosis which ranged from 7-253%.⁴ Furthermore, as a disinfectant, household bleach (sodium hypochlorite) has the advantage of lowering the risk of laboratory infection.⁵ Many studies have reported that bleach digestion of sputum prior to smear preparation has

been reported to increase the yield of microscopy for diagnosing pulmonary tuberculosis.⁶⁻⁸ The study evaluated the performance of direct smear versus bleach concentration smear in diagnosis of pulmonary tuberculosis. Additionally, we also compared the diagnostic yield from spot sample and early morning sample in diagnosis of pulmonary tuberculosis.

METHODS

The present study comprises of 200 patients attending the outpatient clinics of Pulmonology and General medicine of Narayana Medical College during Oct 2013 to September 2013. Patients who presented with history of cough for >2 weeks duration were included. Patients who were on anti-tuberculosis treatment or quinolones 2 weeks before or having extra pulmonary tuberculosis were excluded from the study.

The participant's demographic data which includes age, gender, treatment history of antibiotics, risk factors like smoking, alcoholism, coexisting lung disorders, history of previous tuberculosis, HIV and clinical history such as fever, chest pain, loss of weight and appetite were noted.

Participants were given instructions and explained on how to produce a good quality specimen.⁹ One spot specimen and another early morning specimen were collected on two consecutive days.

Specimens were discarded if they contain only saliva. Smears were made from each specimen for direct smear microscopy, stained by Ziehl-Neelsen [ZN] staining and graded as per RNTCP guidelines and then specimen was concentrated by Bleach method as described by Gebre N

et al prior to smear preparation, stained by ZN staining and graded.^{10,11}

Quality control

All the smears were read by two experienced RNTCP technicians who were blinded to their results. Randomly some of the direct and bleach smears were read by other technician on same day and by same technician on 2nd day respectively.

Ethical clearance

The ethical clearance for the study was given by Institutional ethical committee of the Narayana Medical College.

Data analysis

All the data of the participants was entered and analyzed using SPSS software version 20.0. Positive smear specimens and detection rates were compared between direct smear and bleach smears. The gain of bleach smears was calculated as increase in positive smears by bleach concentration compared with direct smear microscopy, divided by the total number of positive smears by microscopy. The positivity was also compared between type of specimens and timing of collection.

RESULTS

A total of 200 patients were recruited in the study. Out of them, 144 were males (72%), 56 females (28%). The mean age was 47.1±19.29 years. Only 10% of the cases were known HIV positive.

Table 1: Comparison of increase in diagnostic yield among type of specimen, timing and grading after bleach concentration.

Smear	DMP	DPBP	DNBP	Gain%	P value
Specimens (n=290)	128/290 (44.13%)	200/290 (68.96%)	72/290 (24.8%)	24.8%	P<0.0001
Type of Specimen					
Purulent (n=120)	50/120 (41.67%)	75/120 (62.5%)	25/120 (20.8%)	20.8%	p>0.05
Mucoid (n=84)	37/84 (44.05%)	61/84 (72.62%)	24/84 (28.6%)	28.6%	
Blood Stained (n=86)	41/86 (47.67%)	64/86 (74.42%)	23/86 (26.74%)	26.74%	
Time of Specimen					
Spot (n=218)	98/218 (44.95%)	146/218 (66.97%)	48/218 (22.01%)	22.01	p>0.05
Early Morning (n=72)	30/72 (41.6%)	54/72 (75%)	24/72 (33.3%)	33.3	

SP- Spot smear positive, EMP- Early morning smear Positive, DMP- Direct Smear Positive, DPBP- Direct smear positive and Bleach smear positive, DNBP- Direct smear negative and Bleach smear positive, Gain-Increase in number of positive smears after bleach concentration

From participants a total of 290 specimens were examined by microscopy, among them 128/290 (44.1%) were positive on direct smear, additionally 72/290 (24.8%) were positive on bleached smear, and therefore

the gain% in the diagnostic yield was 24.8%. When we analyzed, we found only a marginal % gain (p>0.05) among different type of specimens, we found that the order of yield was mucoid (28.6%)>blood stained

(26.74%)>purulent specimen (20.8%). Additional exploratory analysis revealed that the yield was more in early morning specimens (33%) than with spot specimens (22%), however such a finding was not statistically significant. (Table 1)

DISCUSSION

Direct smear examination under microscope is the gold standard method for establishing infection with tuberculosis. We observed a 44.14% positivity by ZN staining and such an observation was higher than 18.2% in Kashyap B et al report 12.20% in Iqqbal S et al report, 5.38% in Mutha A et al report and 12.5% Angaw B et al report.^{6,12,13,15}

The bleached smear microscopy method is safe, cheap, easy and sensitive. The implementation of bleach method clearly improves case detection and can be a useful contribution in the national tuberculosis control program. In our study, after examining the bleached smears, we were able to detect *Mycobacterium tuberculosis* in 24.83% of cases which were not detected by direct method.

This finding is in contrast to studies of Mutha A et al, (9.09%) and almost similar to Mindloi PB et al (23.14%), Angaw B et al (23.2%) and Pingle et al (28.57%).¹⁴

This increase in case detection may be due to the bleached smear which provides clear microscopic field by digestion of mucoid debris and improved visualization leading to higher density of bacilli per field.

Our study demonstrated a gain of 33.3% in detection of *Mycobacterium tuberculosis* in early morning specimens when compared to spot specimens. Findings in our study coincides with the findings of Pingle P et al¹⁴, Makunde WH et al¹⁷. In the overall study significant positivity was observed after bleach concentration when compared to direct staining.

Limitations

In this study we did not attempt culturing of the specimen as bleach renders the specimen unsuitable for cultures. Specimens from extra pulmonary cases, gastric lavage and Broncheo-alveolar lavage were also not analyzed due to technical difficulties.

CONCLUSION

Microscopic examination of bleached smear has shown a significant yield over conventional method in the detection of pulmonary Tuberculosis. Such superiority was independent of type of sputum and timing of collection of sputum. All resource poor TB centers should consider the use of bleach concentration technique in diagnosis of pulmonary TB.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Kandati J, Boorsu SK, Pathalapati R, Buchineni M. Bleached smear microscopy provides higher yield in diagnosing pulmonary tuberculosis. *Int J Res Med Sci* 2016;4: 2193-6.