

Original Article

Role of Bleach Sedimentation to Improve the Accuracy of Sputum Smear Microscopy for Diagnosing Tuberculosis

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ABSTRACT

Objective: To compare the sensitivity of smear microscopy obtained with smears made directly from respiratory specimens to those from concentrated specimen.

Place and duration: The study was conducted at department of Pathology Khawaja M Safdar Medical College/ Abdul Sattar's Lab, Sialkot from July 2014 to December 2014.

Materials & Methods: Sputum samples were collected aseptically. Direct smears were prepared by taking a small portion of purulent part of the sputum with a sterile loop. Concentrated smear was prepared by taking purulent part of sputum and mixing with equal volume of 5% sodium hypochlorite. It was then kept for 15 minutes, centrifuged and sediment used for staining. Both smears were air dried, heat fixed and stained by Ziehl-Neelsen staining technique. The stained slides were examined under oil immersion lens with positive and negative controls. Acid fast bacilli were graded according to International Union against tuberculosis and lung diseases guideline. Cutoff values for Positive AFB were 1-9/100F = 1+, 1-9/10F = 2+ and 1-9/F = 3+.

Results: Out of total 189 samples, 53 samples were positive by direct smear and 63 samples were positive by concentrated smear.

Conclusion: Concentration with bleach increased the sensitivity of sputum smear microscopy. This method is very effective in resource limited areas like ours.

Key words: Acid fast bacilli, Bleach sedimentation, Tuberculosis

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Introduction

Tuberculosis remains the leading chronic infectious disease throughout the world.¹The estimates of 2011 show nine million new cases and 1.4 million TB deaths.² Despite the availability of many highly sensitive diagnostic tools and highly efficacious treatment for decades, tuberculosis (TB) remains a major public health threat worldwide. In developing countries it is more threatening. There were an estimated 8.8 million new cases of tuberculosis globally in 2010 and 1.1 million deaths. This makes tuberculosis the second leading cause of death among the infectious diseases.³ Pakistan is currently at 8th position among high TB

burden countries. Low case detection rate observed in eastern Mediterranean region is mainly because of low detection rate in Pakistan and Afghanistan. If this remains undetected, then a major outbreak of TB can be expected in Pakistan.⁴

Early diagnosis of tuberculosis is crucial both for clinical and epidemiological purposes. Proper and early identification of cases and their good treatment is essential to limit its transmission and achieve successful TB control. Although the gold standards test for TB diagnosis is culture of specimen on liquid media. However due to lack of access to culture facilities and the long turn- around time involved with culture, most laboratories use direct Ziehl -Neelson (ZN) microscopy

for detection of acid fast bacilli (AFB) as their main diagnostic tool. In this method, sputum samples are smeared directly on the slides without any processing and subject to ZN staining. Accurate and reliable laboratory diagnosis of *Mycobacterium tuberculosis* in resource limited settings always remains a challenge.⁵ Sputum concentration by homogenization with different chemicals and microscopic examination of the sediment can increase the detection rate of AFB. Many concentration techniques using sedimentation or centrifugation have been reported.⁶ First time bleach sedimentation was used in 1909 as mucolytic agent before centrifuging sputum samples.⁷ Bleach homogenization followed by overnight sedimentation can significantly increase detection of AFB as compared to direct microscopy.⁸ The systemic review of this technique found an increase in sensitivity of 33% in one of four studies where culture was used as gold standard and mean incremental yield of 6% in four studies in which culture was not used as gold standard.⁹ We planned a study to compare direct microscopy with bleach concentration method.

Materials and Methods

This study was conducted at department of pathology Khawaja M Safdar Medical College/ Abdul Sattar's Lab, Sialkot from July 2014 to December 2014. The permission from institutional ethical & research committee was taken for research purpose. A total of 189 sputum specimens were collected from patients who were suspected to have pulmonary TB disease on the basis of their presenting symptoms. A suspect was defined as an individual if he/she had persistent cough for more than three weeks, and/or evening rise of temperature for more than two weeks. Specimens were scored as saliva or sputum on the basis of visual examination. Suspects were requested to give another specimen in case of saliva specimens. Three sputum samples were collected on three consecutive days. Few patients given only two/one samples.

All the specimens were mixed and direct smears were prepared by taking a small portion of the purulent part of the sputum with a sterile loop. Purulent portion of this mixed specimen is then mixed up with equal volume 5% sodium hypochlorite. Centrifugation of the mixed sample was done at 3000 RPM for 10 minutes. Sediment is used for ZN staining.¹⁰ Positive and negative controls were stained along with test sample. Microscopy was done by two lab technicians and the confirmed by

consultant microbiologist. Acid fast bacilli were graded according to International Union against tuberculosis and lung diseases guideline. Cutoff values for Positive AFB were 1-9/100F = 1+, 1-9/10F = 2+ and 1-9/F = 3+.¹¹ Material for research purpose was provided by the institute. There were neither conflict of interest of authors with the material provider companies nor any financial and other gains were obtained from the companies.

Results

A total number of 189 samples were processed among them 53 samples were positive by direct smear microscopy. After processing the samples with bleach concentration method, positivity increased to 63 samples. (Table I) Statistical analysis was done by using SPSS-15. Descriptive statistics was applied to calculate percentages of different variables. It showed the improvement of 18.9% i.e. $(63-53)/189 \times 100$ by concentrated methods compared to direct smear microscopy. Mean age of the patients was 32.5yrs. Male to female ratio was 1.8.

Number of sputum samples	Total samples	Positive cases	Negative cases
1	26	08	18
2	08	02	06
3	155	53	102
Total	189	63	126

Discussion

Improved smear microscopy is likely the only diagnostic test that can be widely implemented in the short term to improve TB case finding. Several methods have been proposed to optimize smear microscopy, including fluorescence microscopy, overnight sedimentation and bleach processing.¹² Sodium hypochlorite (bleach) is an ideal chemical processing agent for use in developing countries. It is widely available, inexpensive and its disinfectant properties could improve infection control in laboratories lacking adequate biosafety facilities.¹²

In our study we found promising improvement by bleach concentration method as compared to direct microscopy of 18.9%. These results are comparable with the study conducted by Hepple P et al at Pool Region, Republic of Congo which shows 12.4% increase with concentration method.⁶ Study conducted by Bonnet M et al at Kenya,

which used both bleach concentration and light emitting diode fluorescence microscopy showed 30% increment in results. But a study by Barez et al showed that sensitivity was almost similar by both methods.³ Results of another study by Peterson et al are consistent with our results which showed 93% with concentrated method and 82% by direct method.¹³ Similar study was done by Pingle et al in India showing an increment from 11.6% to 41.96%.¹⁴ We observed another finding that smears made from bleach concentrated sputum were read more rapidly than direct smear microscopy (8.4 versus 11.5 minutes respectively). Chew R et al also noted the similar findings in his study.¹⁵

Despite many evidences that the concentrated method may be superior to the direct method, it is yet not being performed in most of the TB laboratories in developing countries like Pakistan due to following reasons; availability of centrifugation facility with irregular power

supply, limited financial and human resources, lack of training facilities and inadequate biosafety arrangements. In developing countries like Pakistan where TB is endemic, most of the TB centers are using direct microscopy which has low sensitivity for TB diagnosis. As our study showed promising findings with concentrated method, it is recommended that direct smear microscopy may be replaced by concentrated method, to achieve better diagnostic accuracy and ensure greater success of TB control programs.

Conclusion

Concentration with bleach significantly increased the sensitivity of sputum smear microscopy. It reduces the time of reading slides as compared to direct microscopy. This method is very effective in resource limited areas like Pakistan to control tuberculosis.

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