

Enhancing Sensitivity of Ziehl-Neelsen Stain Using Sodium Hypochlorite Sedimentation Technique for Diagnosis of Undiagnosed Cases of Smear Negative Pulmonary Tuberculosis

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ABSTRACT

Tuberculosis is the chronic infectious disease and for its diagnosis battery of investigations are done. Ziehl Neelsen staining for detection of causative Acid Fast Bacilli is one of them. Positive smears diagnose the disease but negative smears doesn't exclude the disease. We here done attempt to diagnose the undiagnosed cases by modifying the conventional ZN staining method. Patients suspicious of having pulmonary Tuberculosis were included in the study. Sputum examination was done by Ziehl-Neelsen staining method. Acid Fast Bacilli positive sputum samples were excluded from the study. Total 120 confirmed smear negative cases by conventional Ziehl-Neelsen method were studied. Sputum samples were processed for Culture and sodium hypochlorite sedimentation technique. Smears were stained by ZN method after sodium hypochlorite sedimentation technique. Of 120 initial negative cases 8 cases were found to be positive. Culture was positive in 10 cases. Thus this study was an attempt to show the increase in sensitivity of conventional ZN staining with the use of sodium hypochlorite concentration.

Keywords: sodium hypochlorite, Ziehl-Neelsen stain, tuberculosis.

INTRODUCTION

In spite of various efforts for controlling epidemics, tuberculosis remains a problem for public health especially in poorer countries of the world. The problem is mainly with the high HIV prevalent regions (1). Now days one of the main challenge for tuberculosis control programmes is the increasing cases of multidrug resistance tuberculosis and also the alarming increase in HIV associated tuberculosis infections. As the number of tuberculosis cases is rising the increase in smear negative pulmonary tuberculosis is disproportionately large (2), it has been observed that the smear negative pulmonary tuberculosis cases are largely associated with HIV infection. So such undiagnosed cases must be diagnosed by some advanced methods for confirmation of diagnosis of smear negative cases.

There are few studies conducted in the last decades for such modifications in Ziehl-Neelsen (ZN) technique for diagnosis of smear negative cases. Many researchers have suggested that performance of sputum smear microscopy can be significantly improved with the use of sodium hypochlorite (NAOCL) concentration by centrifugation or sedimentation before staining by ZN method (3,4).

Fewer studies are conducted for diagnosis of smear negative tuberculosis cases. This study was carried out to check whether the sodium hypochlorite sedimentation technique has a role in enhancing the diagnosis of smear negative tuberculosis cases.

MATERIALS AND METHODS

Total 120 cases of direct ZN smear negative sputum specimens from new cases suspicious of tuberculosis attending Government Medical College and Hospital Latur, were included in the study. All these samples were double crosschecked by different microscopist for their negative status on ZN smears. Specimens were mixed thoroughly for homogenization. Each sample was labelled and divided in two parts. One part was processed further for culture on Lowenstein Jensen medium using standard method considering culture as gold standard. The second half of the sample was processed for sodium hypochlorite sedimentation technique.

Sodium hypochlorite sedimentation technique: In this technique the sputum sample was mixed with 3.5% sodium hypochlorite and kept for 15 hours at room temperature. Then the supernatant was carefully pipette off. Smears were prepared from the sediment, which then air dried, heat fixed and further stained with ZN technique. Considering culture as a gold standard, sensitivity and specificity of sodium hypochlorite sedimentation method was determined.

The staging of positive smears was done as per criteria's given by American Thoracic Society Scale. The criteria's are as follows:

Grade I is 1-9 Acid Fast Bacilli/100 oil immersion fields,

Grade II is 1-9 Acid Fast Bacilli/10 oil immersion fields,

Grade III is 1-9 Acid Fast Bacilli/oil immersion fields and

Grade IV is > 10 Acid Fast Bacilli/oil immersion fields.

RESULTS

120 initially confirmed smear negative specimens by conventional ZN staining were included in the study. Ages of the patients were ranging from 10 – 80 years and average age being 45 years. There was predominance of males over females. M: F ratio was 1.62.

Out of 120 samples which were reported negative by conventional ZN staining method, 8 (6.66%) samples were found to be positive when smears were repeated after processing with sodium hypochlorite sedimentation technique. Culture for Acid Fast Bacilli was positive in 10 (8.33%) cases but culture was positive in seven cases which were positive after sodium hypochlorite sedimentation technique.

Table I: Results of microscopy and culture among 120 smear negative cases.

Microscopy Result	Culture Results	
	Culture Positive Cases	Culture Negative Cases
Positive Microscopy after Sodium Hypochlorite sedimentation	07	01
Negative Microscopy after Sodium Hypochlorite sedimentation	03	109
Total	10	110

The culture method was considered as gold standard and accordingly microscopy after sodium hypochlorite sedimentation method had sensitivity and specificity of 70 % and 99 % respectively. The smears were graded with reference to American Thoracic Grading System and the detail of grading profile of positive smears is as follows.

Table II: American Thoracic Society Grading Profiles of eight positive smears after sodium hypochlorite sedimentation method.

Stage	Number of Positive smears	Percentage
I	05	62.5
II	02	25
III	01	12.5
IV	00	0
Total	08	100

DISCUSSION

Conventional method of ZN staining is routinely performed for diagnosis of tuberculosis as it is rapid and inexpensive method with high

specificity. The major disadvantage of this method is its low sensitivity. As the sensitivity of ZN method is low, there is need for some modifications in this method for increasing its sensitivity. Present study was an attempt made for the same.

Of the 10 samples which were culture positive, 07 samples were positive for Acid Fast Bacilli on microscopy after sodium hypochlorite concentration. Thus the sensitivity was 70 %. This signifies the reliability of the method. Our study showed the improvement of 6.66% in smear positivity by this method. This is consistent with study conducted by Peerapur et al (5), who showed significant increase in the sensitivity using 3.5% sodium hypochlorite with 6.77% smear positivity.

WA Githui et al (6) also showed increase in sensitivity by this method with 8.7% smear positivity. Gebre et al (7) showed that meticulous preparation and examination of smears made directly from sputum gives sensitivity of 55% compared with the culture, whereas the sensitivity of the sodium hypochlorite method is close to 70%. To increase the sensitivity of ZN technique attempts were made by many researchers with the use of different concentrations of sodium hypochlorite which subsequently followed by either sedimentation or centrifugation. For instances, study carried by B.V. Peerapur et al (5) used 3.5% sodium hypochlorite and they found 75% sensitivity. In another study carried out in Ethiopia in 2003, 5% of sodium hypochlorite was used followed by centrifugation for laboratory diagnosis of pulmonary tuberculosis (8).

Another study conducted in Ghana, 1% sodium hypochlorite sedimentation and sodium hypochlorite xylene flotation method. They compared the improvement in the sensitivity by both these methods. 77.2% sensitivity was found in the method using 1% of sodium hypochlorite while 71.8% sensitivity found in in method using sodium hypochlorite-xylene flotation (9).

Most of these studies haven't carried in smear negative specimens. However they showed 1% and 5% of sodium hypochlorite concentration improved the sensitivity, but was not satisfactory. Peerapur et al (5) and Matu S et al (10) studied smear negative samples. Matu S et al used 5% and 3.5% of sodium hypochlorite concentration followed by centrifugation. They compared the results and found increased detection rate of smear positivity with the use of 3.5% sodium hypochlorite. In most of these studies the bacilli were concentrated using centrifugation method. Centrifugation method needs centrifuge machine, which may not be available in small peripheral laboratories. Our study used simple and inexpensive sedimentation making it feasible to do in any simple laboratory. The initial smear negative 8 cases were positive after sodium hypochlorite sedimentation method, thus this method

has potential to increase the sensitivity of conventional ZN method. There are also few other benefits of this method. This method shows the clear background on microscopy which helps seeing bacilli easily. This clear background is due to digestion of other cells and debris by sodium hypochlorite. It also reduces the time for screening the stained smears for a microscopist. With these many advantages of sodium hypochlorite, it is also cheap and easily available in the market.

CONCLUSIONS

Diagnosis of undiagnosed cases is important in tuberculosis control programmes to reduce the morbidity and mortality. 3.5% sodium hypochlorite sedimentation method has potential to diagnose smear negative cases. Hence it can be helpful for improving diagnostic services in resource limited settings. So it can be recommended in such settings and also in settings where ZN microscopy is core diagnostic tool and in areas with high tuberculosis and HIV prevalence. However more studies are needed for evaluation of this method.

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