
ORIGINAL ARTICLE**Sodium Hypochlorite Sedimentation Technique: A Simple Way to Enhance Sensitivity of Ziehl-Neelsen Stain in Diagnosis of Smear Negative Pulmonary Tuberculosis**

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Abstract:

Background: With the looming global HIV pandemic, the problem of tuberculosis tiptoes as a perpetual companion. Smear negative pulmonary tuberculosis which pose substantially a challenge for diagnosis, hoots this combination into noxious health problem. There is, therefore, an urgent need to establish more sensitive, safe and fairly rapid methodologies that could confirm diagnosis particularly in smear negative pulmonary tuberculosis patients. **Aim:** This study was carried out to establish whether, 3.5% sodium hypochlorite overnight sedimentation method improves the sensitivity of Ziehl-Neelsen (ZN) stain in samples declared as smear negative. **Material and Methods:** A total of 605 direct ZN smear negative sputum specimens were examined after concentration with 3.5% sodium hypochlorite overnight sedimentation method. **Results:** Forty one (6.77%) samples were found to be positive when smears were repeated after performing sodium hypochlorite sedimentation technique. Sensitivity and specificity of this method was found to be 76.31% and 97.88% respectively. **Conclusion:** 3.5% Sodium hypochlorite sedimentation technique has the potential to improve the diagnosis in tuberculosis in smear negative pulmonary tuberculosis cases especially in resource poor countries.

Keywords: Smear Negative Tuberculosis, Sodium Hypochlorite, Sputum Concentration Methods.

Introduction

Despite renewed efforts to control the epidemics, tuberculosis remains a public health emergency predominantly affecting the poorest countries of

the world, especially in high HIV prevalence regions [1]. With the problem of multidrug resistance and an alarming increase in HIV associated tuberculosis infection, the magnitude and severity of tuberculosis epidemics are increasing. This dramatic increase has been attributed largely to HIV infection and rising poverty levels. Along this increase in tuberculosis cases, the increase in smear negative pulmonary tuberculosis has been disproportionately large [2]. Smear negative tuberculosis occurs more frequently in HIV infected patients than in non-HIV patients. There is, therefore, an urgent need to establish more sensitive, safe and fairly rapid methodologies that could confirm diagnosis particularly of smear negative pulmonary tuberculosis patients.

In the last decade, many researchers have suggested that the performance of sputum smear microscopy can be significantly improved if sputum is liquefied using sodium hypochlorite and then concentrated by centrifugation or sedimentation prior to staining [3,4]. Sodium hypochlorite (NaOCl) in concentrations of 2-5% of NaOCl digest the sputum products and inactivate the mycobacteria without altering their structures, so that even when they are killed, they can still be stained and observed [5]. However, majority of the studies focused on centrifugation. Furthermore, no studies have addressed the effect of sodium hypochlorite on specimens that are classified as smear negative tuberculosis.

There are reports of improved sensitivity of direct microscopy for acid-fast bacilli by sedimentation as an alternative to centrifugation for concentration of tubercle bacilli [6]. But further studies are needed for the evidence to support implementation of sodium hypochlorite sedimentation methods [3].

Accordingly, this study involves only negative smears treated with 3.5% sodium hypochlorite and left overnight for 15 hours and then air-dried, heat fixed and stained using ZN staining technique. This study was carried out to establish whether sodium hypochlorite overnight sedimentation method specifically improves diagnosis of smear negative tuberculosis.

Material and Methods:

A total of 605 direct ZN smear negative sputum specimens from new tuberculosis suspects attending Raichur District Hospital were included in the study. Sputum smears were prepared from all the specimens and stained with ZN technique to confirm their status. Only smear negative sputum specimens were included in this study. The specimens were homogenized using a vortex mixture.

The specimens were aliquoted into two equal portions. One portion was processed for culture on Lowenstein-Jensen medium using standard methods as culture is considered as gold standard. The other portion was treated with 3.5% of sodium hypochlorite, and left overnight at room temperature for 15 hours after which the supernatant was carefully pipetted off. Smears were made from the sediment, air-dried, heat fixed and then stained with Ziehl-Neelsen technique. The sensitivity and specificity of sodium hypochlorite sediment smear method was determined using the conventional culture as the gold standard. Staging of the positive smears was done using American Thoracic Society Scale (ATS) accordingly Grade-I is 1-9

Acid Fast Bacilli/100 oil immersion fields, Grade-II is 1-9AFB/10 oil immersion fields, Grade-III is 1-9AFB/oil immersion field and Grade IV is > 10AFB/oil immersion field.

Results:

Six hundred and five (605) initially confirmed direct Ziehl-Neelsen smear negative sputum specimens were included in the study. Age of the patients was ranging from 1-90years and the average age being 46.4years. Male: Female ratio amongst the study group was 1.52. Out of 605 samples which were reported negative by conventional Ziehl-Neelsen staining method, 41(6.77%) samples were found to be positive when smears were repeated after performing sodium hypochlorite sedimentation technique. Culture for acid fast bacilli was positive for 38(6.28%) samples and the details of which is given in (Table 1).

Table 1: Results of Microscopy and Culture among 605 Smear Negative Patients

Microscopy Results	Culture Results	
	Culture Positive cases	Culture Negative cases
Positive microscopy after sodium hypochlorite sedimentation	29	12
Negative microscopy after sodium hypochlorite sedimentation	09	555
Total	38	567

Considering culture as gold standard test, microscopy after sodium hypochlorite sedimentation had the sensitivity and specificity of 76.31% and 97.88%. Positive and negative predictive values were 70.73% and 98.40% respectively. The grading of the smears was also done according to American Thoracic Society (ATS) grading system and grading profile of positive smears is detailed in (Table 2).

significant increase in the sensitivity using 3.5% sodium hypochlorite with 8.7% smear positivity. A meticulous preparation and examination of smears made directly from sputum gives a sensitivity of 55% compared with the culture whereas the sensitivity of the NaOCl method is close to 70% [8].

In an attempt to increase the sensitivity of Ziehl-Neelsen smear microscopy, many previous research-

Table 2: American Thoracic Society Grading Profiles of 41 Positive Smears after Sodium Hypochlorite Sedimentation

Stage	Number of Positive Smears	Percentage	Culture Positive Cases*	Percentage
I	26	63.14	15	51.72
II	12	29.26	11	37.93
III	3	07.31	3	10.34
IV	0	00.00	0	00.00
Total	41	100.00	29	100.00

*Total number of culture positive cases were 38 of which 29 were smear positive and 09 were smear negative.

Discussion:

Direct microscopy of Ziehl-Neelsen stained smears is rapid, inexpensive and highly specific method for detection of tubercle bacilli. The major disadvantage of this technique is a discouragingly low sensitivity. Hence an improved method which is cost effective with high bacillary yield and high sensitivity is required. This study was an attempt made of obtaining the same. Of the 38 samples which were culture positive 29 were positive for microscopy after sodium hypochlorite concentration thus the sensitivity was 76.31%. This proves the reliability of the technique and is a very encouraging finding. Our study has shown the improvement of 6.77% in smear positivity by this method. This is consistent with study conducted in Nairobi by WA Githui *et al* [7] who showed

chairs have used different concentrations of sodiumhypochlorite followed by either sedimentation or centrifugation. Nevertheless these studies have not documented the use of 3.5% sodium hypochlorite and direct Ziehl-Neelsen smear negative specimens together. For instance, in a study carried out in Ethiopia in 2003, 5% sodium hypochlorite was used followed by centrifugation for laboratory diagnosis of pulmonary tuberculosis. In this study the sensitivity was found to be 75%. [9] In another study in Ghana, 1% sodium hypochlorite sedimentation and sodium hypochlorite-xylene floatation was used to compare the improvement in the sensitivity. The method using 1% sodium hypochlorite was found to more sensitive with the sensitivity of 77.2% while sodium hypochlorite-xylene sensitivity was

71.8% [10]. These studies however have not addressed the aspect of smear negative specimens and although 1% and 5% sodium hypochlorite concentrations improved the sensitivity encouragingly, it was not satisfactory. Another study in Kenya in 2007, involved smear negative samples with the use of 5% and 3.5% sodium hypochlorite concentrations followed by centrifugation and the results were compared. It was found that 3.5% sodium hypochlorite with centrifugation indicated higher yield [11].

Furthermore, in all these studies, bacilli were concentrated using centrifugation method only. Centrifugation has serious limitations because it requires access to a centrifuge, which may not be available in many peripheral laboratories in most of the resource limited countries. But simple sedimentation is used in this study making it inexpensive and can be done in any simple laboratory.

Taking into consideration that between 1000-5000 bacilli/ml of sputum is required for a smear to become positive [7], findings from this study in which initially smear negative samples had yielded stage-I and stage-II smear positivity in 38(92.68%) of 41 smear positive samples (Table-2) after sedimentation with 3.5% sodium hypochlorite clearly indicates that this method has the potential to increase the sensitivity of Ziehl-Neelsen staining technique when lower number of acid fast bacilli are present in smears, thus, improving detection rates in smear negative tuberculosis cases.

Sodium hypochlorite overnight sedimentation method has several advantages. These include the

ease of seeing the bacilli against a clear background under the microscope since the other cells and debris in the sputum are digested and decontaminated by sodium hypochlorite and cleared during staining process leaving only the acid fast bacilli with few pus cells. Sodium hypochlorite is ideal as it is cheap and easily available in the market. Digestion of sputum and sedimentation with sodium hypochlorite increases the recovery rate of mycobacteria. This might be attributable to changes in the surface properties of the mycobacteria and/or denaturation of the sputum constituents leading to flocculation and subsequent increased sedimentation rate of the bacteria [12]. There is significant reduction in the time for diagnosis of ZN smear negative tuberculosis cases that usually takes long process to ascertain, thereby, lowering the rate of transmission within the community and eventually reducing mortality and morbidity due to tuberculosis [7, 12].

Conclusion:

Early diagnosis of tuberculosis is pivotal in initiating effective therapy by Tuberculosis Control Programmes whose core element is to reduce individual morbidity and mortality. Smear negative tuberculosis pose a considerable challenge in this regard. 3.5% sodium hypochlorite sedimentation technique has the potential to improve smear positivity thereby improving diagnostic services in resource limited settings hence can be recommended in settings with high tuberculosis or HIV prevalence areas where ZN microscopy is a core diagnostic tool for diagnosis of tuberculosis.

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