ORIGINAL RESEARCH

Assessing the sensitivity of sputum smear examination by Pot's technique and Sodium hypochlorite concentration technique

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ABSTRACT

Background: In developing nations, the microbial diagnosis of Pulmonary Tuberculosis (PTB) is crucial for standard treatment and tuberculosis control initiatives. The present study was conducted to assess the sensitivity of sputum smear examination by Pot's technique and Sodium hypochlorite concentration technique. **Materials & Methods:** 70 sputum samples of pulmonary tuberculosis patients of both genders were first processed by routine Ziehl-Neelsen (ZN) staining. Sputum smear negative was subjected to Pot's technique and Sodium hypochlorite concentration technique to evaluate the efficacy of these concentration techniques. **Results:** A total of 5/97 (5.1%) sputum samples were positive for AFB by routine RNTCP staining whereas 7/97 (7.21%) samples were reported to be positive by observers by sodium hypochlorite concentration technique. Sensitivity was 100%, specificity- 98%, PPV-80% and NPV- 100%. The difference was significant (P< 0.05). Of the 94 specimens from analysed using Pot's technique, 8/97 (8.2%) were sputum positive. Sensitivity was 100%, specificity- 97%, PPV-86% and NPV- 100%. The difference was significant (P< 0.05). **Conclusion:** The concentration technique can greatly enhance the yield of sputum smear microscopy for diagnosing PTB, particularly in areas where HIV prevalence is high, as co-infection results in pauci-bacillary sputum.

Keywords: Pulmonary Tuberculosis, Pot's technique, Sodium hypochlorite

INTRODUCTION

In developing nations, the microbial diagnosis of Pulmonary Tuberculosis (PTB) is crucial for standard treatment and tuberculosis control initiatives. In countries where tuberculosis is among the most common causes of chronic cough, the proportion of smear-positive cases among suspected tuberculosis cases falls within the anticipated 5-20% range.[1] It is estimated that sputum smear examination is used to diagnose 60-70% of all PTB cases. Despite its lower sensitivity relative to fluorescent and LED microscopy, bright field microscopy is low-cost and quick, making it appropriate for peripheral laboratories in resource-limited settings and developing countries.[2]

Various enhancements have been proposed to boost the quantity of microscopic detection, such as fluorescent microscopy using auramine or rhodamine staining, serial examination of sputum specimens, and chemical fluidization of sputum with concentration achieved through sedimentation or centrifugation. The Zeihl Neelsen (ZN) stain is widely employed globally for smear examination, but it can only detect bacilli when their concentration is approximately 105/ml of sputum.[3] The sensitivity is diminished because, given the volume of sputum material examined under oil immersion field, the likelihood of overlooking the organism is elevated. A significant portion of TB transmission can happen prior to the time when the sputum concentration reaches a critical level at diagnosis.[4]

A negative smear cannot rule out tuberculosis, given that approximately 55% of global PTB cases are reported to have a low bacillary load.[5] Furthermore, it has been determined that sputum smear microscopy shows reduced sensitivity in cases of HIV–TB co-infection, where the sputum smear is often negative. Additionally, pre-stained smears created from positive

sputum samples are still infectious and potentially dangerous.[6]The present study was conducted to assess the sensitivity of sputum smear examination by Pot's technique and Sodium hypochlorite concentration technique.

MATERIALS & METHODS

The study was carried out on 70 sputum samples of pulmonary tuberculosis patients of both genders. All gave their written consent to participate in the study. Data such as name, age, gender etc. was recorded. All samples were first processed by routine Ziehl-Neelsen (ZN) staining. Sputum smear negative was subjected to Pot's technique and Sodium hypochlorite concentration technique to evaluate the efficacy of these concentration techniques.Results thus obtained were subjected to statistical analysis. P value < 0.05 was considered significant.

RESULTS

Table I Comparative evaluation of sodium hypochlorite concentration method against RNTCP method

	RNTCP positive	RNTCP negative	P value	
Sodium hypochlorite positive	5	2	0.01	Sensitivity- 100% Specificity-98% PPV-80% NPV- 100%
Sodium hypochlorite negative	0	90		
Total	5	92		

Table I shows that a total of 5/97 (5.1%) sputum samples were positive for AFB by routine RNTCP staining whereas 7/97 (7.21%) samples were reported to be positive by observers by sodium hypochlorite concentration technique.Sensitivity was 100%, specificity-98%, PPV-80% and NPV- 100%. The difference was significant (P< 0.05).

Table II Comparative evaluation of Pots method against RNTCP method

	RNTCP positive	RNTCP negative	P value	
Pots positive	5	3	0.01	Sensitivity- 100% Specificity-97% PPV-86% NPV- 100%
Pots negative	0	89		
Total	5	92		

Table II shows that of the 94 specimens from analysed using Pot's technique, 8/97 (8.2%) were sputum positive. Sensitivity was 100%, specificity-97%, PPV-86% and NPV- 100%. The difference was significant (P< 0.05).

DISCUSSION

Bright field microscopy of Ziehl-Neelsen stained smears offers a rapid, cost-effective, and highly specific means of detecting tubercle bacilli. The main downside of this technique is its sensitivity, which is discouragingly low.[7] Therefore, a more effective approach that is economical and provides a high bacillary yield and sensitivity is needed. Only a centrifuge machine is needed for pretreatment with sodium hypochlorite, making it easy to perform.[8] The sputum pre-treatment with 5% sodium hypochlorite solution (NaOCl) serves to disinfect the sputum and guarantees its liquefaction in 30 minutes, all while preserving the acid fastness of Mycobacterium tuberculosis.[9]Test sensitivity is increased by sodium hypochlorite, and it makes samples safer to handle. Bleach is cheap and can be found almost everywhere. NaOCl has a half-life of approximately 12 months. This duration is likely to decrease by one month when the bottle is opened and by about three months at elevated ambient temperatures (around 30°C).[10]The present study was conducted to assess the sensitivity of sputum smear examination by Pot's technique and Sodium hypochlorite concentration technique.

We found that a total of 5/97 (5.1%) sputum samples were positive for AFB by routine RNTCP staining whereas 7/97 (7.21%) samples were reported to be positive by observers by sodium hypochlorite concentration technique.Sensitivity was 100%, specificity-98%, PPV-80% and NPV- 100%. Singhania PRet al[11]evaluated the sensitivity of sputum smear examination by Pot's technique and Sodium hypochlorite concentration technique over conventional Revised National Tuberculosis Program (RNTCP) method. A total of 94 samples received from 47 patients were analysed. 04/94 (4.25%) samples were positive for AFB by routine RNTCP staining. With use of Sodium hypochlorite concentration method and Pots method, 05/94 (5.31%) and 06/94 (6.38%) samples were positive indicating a rise of 1.06% and 2.1% respectively over conventional. Additional two cases were detected compared to RNTCP, amounting to 100% rise in case detection which otherwise would have been missed.

We found that of the 94 specimens from analysed using Pot's technique, 8/97 (8.2%) were sputum positive. Sensitivity was 100%, specificity-97%, PPV-86% and NPV- 100%. Kaore NM et al[12] in their study a total of 591 sputum samples from 219 patients were included in the study with 168 males (76.71%) and 51 females (23.28%).A total of 77 samples (13.02%) from 34 patients were positive by routine method whereas by concentration method 119 samples (20.13%) from 49 patients were found positive diagnosing 15 additional patients. This rise of 7.11% in sputum positivity over routine is highly significant

(P=0.001021, $\chi(2)=10.78$) with 44.11% increase in diagnosed cases.There is a statistically significant rise in smear positive cases after concentration with 5% sodium hypochlorite solution. Considering its low cost, decontaminating and liquefaction properties with better sensitivity, this method is safe and can be of vital importance; at least for smear negative cases.

Das S et al[13]evaluated four variants of carbol fuchsin solution by the pot method and compared the results with ZN staining, taking culture as gold standard.Five hundred sputum samples from presumptive tuberculosis cases were homogenised and divided into two parts. One part was subjected to routine ZN staining and culture on solid medium, the other was equally distributed into four pots. Equal quantities of the basic fuchsin (BF) variant were added to each pot. Variant I contained 2% BF with 10% phenol and 4% ammonium sulphate (PhAS), while Variant II had 0.6% BF with PhAS; Variants III and IV contained respectively 2% and 0.6% BF with 10% phenol only. Variant III gave excellent results compared to ZN (κ = 0.97), with sensitivity, specificity, and positive and negative predictive values similar to those of ZN, taking culture as gold standard. Pot contents were negative for Mycobacterium tuberculosis culture.

The shortcoming of the study is small sample size.

CONCLUSION

Authors found that the concentration technique can greatly enhance the yield of sputum smear microscopy for diagnosing PTB, particularly in areas where HIV prevalence is high, as co-infection results in pauci-bacillary sputum.

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