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IMPROVED SENSITIVITY OF DIRECT MICROSCOPY FOR ACID FAST BACILLI: SEDIMENTATION AS AN ALTERNATIVE TO CENTRIFUGATION FOR CONCENTRATION OF TUBERCLE BACILLI

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ABSTRACT

Tuberculosis is a global health problem so there is a need for cost effective as well as rapid diagnostic methods to address this perennial problem. We aimed to compare the efficiency of concentration of AFB from NaOCl treated samples by centrifugation with that by sedimentation with the direct smear. 138 Sputum samples from patients suspected with tuberculosis were subjected to Ziehl-Neelsen staining before concentration and NaOCl centrifugation & NaOCl sedimentation techniques and the results are tabulated. Concludes there was significant increase in positivity rate of sputum smears for acid fast bacilli after NaOCl centrifugation method and NaOCl sedimentation method. In these centrifugation showed the highest rate (26.81%) and where as sedimentation showed (25.36%). By comparing these two, there is no statistically significant difference between the concentration methods. Overnight sedimentation significantly increases the sensitivity of microscopy and this method could be alternative for diagnostic centers that are not equipped with a centrifuge.

KEY WORDS

Acid-fast bacilli (AFB), sodium hypochlorite (NaOCl).

INTRODUCTION

Tuberculosis is a global health problem, for every 3 minutes 2 persons die in our country[1]. There is a need for cost effective as well as rapid diagnostic methods to address this perennial problem. Sputum smear microscopy is less sensitive, culture is technically complex and slow, determination of drugs susceptibility is even more technically complex and slower, yet chest radiography is non-specific and tuberculin skin testing is imprecise the results which are often non-specific. In view of these limitations there is a need for less complicated and more accurate tests. Developing new diagnostics is one of the six elements of the global plan to stop TB strategy. Of the intensive TB control programs, case finding plays a pivotal role and remains the cornerstone for effective control [2]. Without the right diagnostic tools; we cannot stop the TB epidemic.

There are two basic approaches for the diagnosis of tuberculosis. The 'direct' approach includes detection of *Mycobacteria* or its products and the 'indirect' approach includes the measurements of humoral and cellular responses of the host against tuberculosis [3].

The major objective of TB control programs are to identify and treat patients with infectious pulmonary TB, the diagnosis of which relies on bacteriological examination of sputum. Culture of *Mycobacteria* is the reference method for detection of tubercle bacilli but it is prohibitively slow and requires special safety procedures in laboratories. Serological techniques are not useful in control programs due to lack of sensitivity and specificity [4]. Among the new approaches for rapid diagnosis of TB, the nucleic acid amplifications methods are the most promising [5] but the technology is not applicable to control programs in developing countries [6].

Microscopy of direct smear for acid-fast bacilli (AFB) is the most commonly used method for diagnosis but its major disadvantage is discouragingly low sensitivity when used in control programs. In one study by ABER.., et al. (1980) the sensitivity of direct microscopy ranged from 8.8% to 46.4%. Consequently, there is an urgent need to improve the lab diagnosis of pulmonary TB [7,8].

Previous studies have shown that liquefaction of sputum with sodium hypochlorite (NaOCl) and concentration of bacilli through centrifugation will significantly increase the sensitivity of direct microscopy [6,8,9].

MATERIAL & METHOD

This prospective study was conducted after Institutional Ethics Committee clearance has been taken in the department of microbiology Kamineni institute of medical sciences, Narketpally, Nalagonda. 138 Sputum samples from patients suspected with tuberculosis were subjected to Ziehl-Neelsen staining before concentration and NaOCl centrifugation & NaOCl sedimentation techniques.

Specimen collection:

At least 2 sputum samples were collected from the single patient. Good quality of sputum was collected in a sterile, disposable, clean, wide mouthed, unbreakable, leak proof, provided with tight-fitting lid ,container. Deep coughing was encouraged so that exudative material is obtained from the lungs. If the patient is unable to expectorate a satisfactory sample, deep coughing was induced by inhalation of an aerosol of warm, hypertonic (5-10%) saline. The specimen was properly labeled [10,11] .

Preparation of slide, Direct microscopy, Staining procedures followed as explained in the Monica Cheesbrough[11]

Concentration methods:

1. **Centrifugation method**
2. **Sedimentation method**

Procedures followed as explained in the Allwood M D M, Lee Y C [12]

RESULTS

One thirty eight samples were collected from suspected pulmonary tuberculosis and were subjected to direct microscopy (ZN staining), using direct smears, after centrifugation & sedimentation the results are tabulated.

Table no.1 shows the number of positive and negative samples with their percentage of the three methods-direct smear microscopy, centrifugation method and sedimentation method

In table 2 By NaOCl+sedimentation method 35 AFB positive cases were detected were as in direct microscopy 16 samples were AFB positive. By NaOCl+centrifugation method 37 AFB positive cases were detected

Table 3 shows that smear positivity for AFB more in males 36 compared to females 17.

Table 4 shows acid fast bacilli (AFB) prevalence in extremes of age groups.

DISCUSSION

Grading of AFB smears [11]

Examination	Result	Grading	No. of fields examined
More than 10 AFB per oil immersion field	Positive	3+	20
1-10 AFB per 1oil immersion field	Positive	2+	50
10-99 AFB per oil immersion field	Positive	1+	100
1-9 AFB per 100 oil immersion fields	Positive	Scanty-8 (record the actual number of AFB seen)	100
No AFB per 100 oil immersion fields	Negative	-	100

Table no.1 shows the number of positive and negative samples with their percentage of the three methods-direct smear microscopy, centrifugation method and sedimentation method

S.no	Method	Number of samples positive for AFB (n=138)	Number of samples negative for AFB (n=138)
1	Direct smear	16(11.60%)	122(88.40%)
2	Centrifugation method	37(26.81%)	101(73.19%)
3	Sedimentation method	35(25.36%)	103(74.64%)

In table 2 By NaOCl+sedimentation method 35 AFB positive cases were detected were as in direct microscopy 16 samples were AFB positive. By NaOCl+centrifugation method 37 AFB positive cases were detected.

S.no	Method	Number of positive slides(n=138)
1	Direct smear Vs NaOCl + sedimentation	16 Vs 35
2	Direct smear Vs NaOCl + centrifugation	16 Vs 37
3	NaOCl + sedimentation Vs NaOCl + centrifugation	35 Vs 37

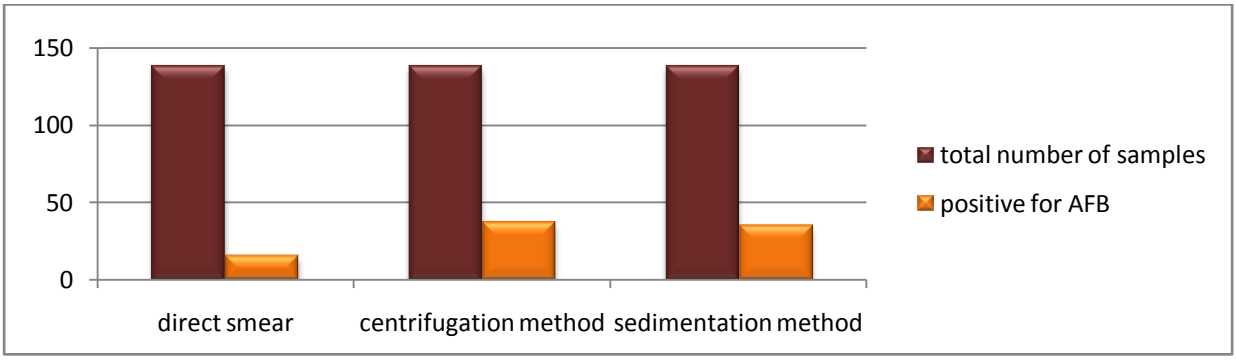
Table 3 shows that smear positivity for AFB more in males 36 compared to females 17.

S.No	Sex determination	Total number of samples	Positive for AFB	Negative for AFB
1	Females	38	17(44.73%)	21(55.24%)
2	Males	100	36(36%)	64(64%)

Table 4 shows acid fast bacilli (AFB) prevalence in extremes of age groups.

S.no	Age group	Total Number of samples	Positive for AFB	Percentage (%)
1	0-14	2	-	-
2	15-30	28	2	71.42%
3	31-59	76	32	42.10%
4	≥ 60	32	19	59.37%

DIAGRAM shows positivity of three methods: Direct microscopy, Centrifugation method, Sedimentation methods



There are evidences that increase the positivity by sodium hypochlorite (NaOCl) centrifugation and sedimentation method. Hakan Mioner, et al, documented that the liquefaction of sputum with sodium hypochlorite followed by concentration of bacilli through overnight sedimentation significantly increases the sensitivity of direct microscopy, and this method could be an alternative for diagnostic centers that are not equipped with a centrifuge.

Some studies were based on the comparison of individual patients and some on individual samples, as different definitions of positive tests were used this review deals only with the bleach method. Other concentration methods have been studied [19,20,21,22] but none of them shows better performance and none disinfects the sample. The bleach method is, moreover, the most widely studied method in low- and middle income countries.

The bleach method has some built-in disadvantages. First, a bleach-treated sample cannot be used for Mycobacteria culture, as the NaOCl kills *M.tuberculosis.* In case Mycobacterial culture is asked for, another sample must be requested. It must be recognized, however, that if safety cabinets are not available, the bleach should be mixed with the sample in the container in which it has been deposited—to avoid aerosol formation one should not pour potentially infected sputum into a tube outside a safety cabinet. The concentration and exposure times in the reviewed papers (i.e., about 2% for about 15 min) should therefore be sufficient to effectively disinfect the specimens.

Second, the bleach method requires more work and more equipment, particularly if concentration is performed by centrifugation. Bleach itself is inexpensive and readily available almost everywhere.

The half-life of NaOCl is about 12 months; it is likely to be reduced by 1 month if the bottle is opened and by about 3 months if the ambient temperature is high (around 30°C)[23]. Need for screw tapped tubes can be bypassed as the sample is disinfected. If one bleach sample can be used instead of three direct smears [12] or if the three samples are pooled before liquefying with NaOCl [24] the workload might even be reduced using the bleach method compared to the direct method. Another aspect in favor of the bleach technique is that the lack of debris on the microscopy slide makes reading simpler, and hopefully less time consuming than the 5 min recommended for reading the direct smear.

Third, prolonged exposure to NaOCl (≥60 min) gradually reduces the possibility of detecting acid-fast bacilli [14]. This factor must be acknowledged, especially as in peripheral settings laboratory technicians usually have tasks other than sputum microscopy and it might sometimes be difficult to keep track of time.

Finally, the bleach sediment due to the reduction of debris can be difficult to see macroscopically on the slide. This might explain Wilkinson and Sturm's results where many samples that were positive with the direct method were negative with the bleach method [25]. It may be necessary to clearly mark on the slide where the bleach sediment has been put to avoid this problem.

Conclusion:

There was significant increase in positivity rate of sputum smears for acid fast bacilli after NaOCl centrifugation method and NaOCl sedimentation method. In these, centrifugation showed the highest rate (26.81%) and where as sedimentation showed (25.36%). By comparing these two, there is no statistically significant difference between the concentration methods.

The average numbers of bacilli per microscopy field indicate that centrifugation is more efficient for concentration of tubercle bacilli. These two concentration methods increase the sensitivity of direct microscopy. There was statically difference between direct microscopy and concentration methods of sodium hypochlorite centrifugation method and sedimentation method.

The applicability and effectiveness of these techniques as clearly demonstrated. We recommended prompt adaption, especially in laboratories where Mycobacterial culture not performed.

Overnight sedimentation significantly increases the sensitivity of microscopy and this method could be alternative for diagnostic centers that are not equipped with a centrifuge.

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