ORIGINAL ARTICLE

AN EVALUATION STUDY OF THE SPUTUM SMEAR CONCENTRATION TECHNIQUE FOR THE LABORATORY DIAGNOSIS OF PULMONARY TUBERCULOSIS

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ABSTRACT

The microbial diagnosis of Pulmonary Tuberculosis plays a key role in routine treatment and Tuberculosis control Programmes in developing countries. Many patients have presented with signs and symptoms of pulmonary tuberculosis, of which consecutive direct sputum smear microscopy have given negative results for Acid-Fast Bacilli (AFB). Microscopy of smears made directly from sputum has a low sensitivity and there is an urgent need for improved methods. This study was carried out at the Yaoundé University Teaching Hospital and is aimed at evaluating the sputum smear concentration technique in the laboratory diagnosis of pulmonary TB. Sputum samples were collected in screw-cap tight containers and evaluated by both the direct and concentrated methods. Microscopy of direct smears of sputum after liquefaction with 5% sodium hypochlorite (NaOCl) solution; and concentration of the organisms by centrifugation were compared and evaluated. Results showed an increase in sensitivity from 18.27% to 25% with a specificity of 90.95%. The tuberculosis prevalence was 25%. 13.33% belongs to the age range 20-40 years and 11.67% to the age range >40 years. The positive predictive value was 73%. We concluded that the use of sodium hypochlorite (NaOCl) in the concentration of acid-fast bacilli (AFB) in sputum significantly improves the laboratory diagnosis of pulmonary tuberculosis
Keywords: Sputum smear concentration, Laboratory diagnosis, Pulmonary tuberculosis

INTRODUCTION

Tuberculosis(TB) is a contagious disease caused by *Mycobacterium tuberculosis* (Koch's bacillus) which kills many victims than any infectious disease. It continues to be the major cause of disability and death worldwide [1]. The human host serves as the only natural reservoir for *M. tuberculosis*, but the ability of the organism to effectively establish latent infection has enabled it to spread to nearly one-third of the world's population. From this reservoir, eight million new TB cases occur each year and about three million die from it. In 1993, because of the serious public health threat posed by TB, the World Health Organization (WHO) declared it a "Global Emergency" [2].

The emergence of HIV/AIDS has had grave consequences for TB [3]. HIV infection markedly increases the susceptibility for new TB infection to develop into active disease, which can be rapidly progressive. Consequently, a large number of epidemics of TB have been reported from facilities in which HIV infected people are concentrated. In one out break, the entry of an index case into an HIV residence facility was followed by

11 patients developing TB over a five months period [4]. Since these are epidemiologically linked, they must be tackled in tandem [5]. The very large increase in the number of tuberculosis cases yearly and the reactivation of latent infection worldwide is a called for concern for early diagnosis and initiation of effective chemotherapy. Bacteriological diagnosis of tuberculosis is largely dependent on Ziehl Neelsen (ZN) microscopy.

Many patients continue to present with signs and symptoms of pulmonary tuberculosis of which consecutive direct sputum smear technique have proven negative for acid-fast bacilli (AFB) by Ziehl Neelsen staining. As such more sensitive diagnostic techniques are needed which can be readily available and affordable especially in less developed countries. The diagnosis of tuberculosis in some cases is simply based upon the clinical presentation; including the history and the patient's physical signs and symptoms that relate to the site of the disease. In many other cases, diagnosis relies on the bacteriological examination of sputum. However microscopy   of   smears   made

directly from sputum has a low sensitivity and there is an urgent need for improved methods [6], hence this tudy was carried out to find out whether the concentration sputum smear technique could significantly improve the laboratory diagnosis of pulmonary tuberculosis over the direct sputum smear technique.

Materials and Methods
Study Area
This study was carried out at the University teaching hospital, Yaoundé from March to July, 2009. Yaoundé is the administrative capital of Cameroon with a population of **143,000,000** inhabitants. It is centrally located and made up of immigrants from all the regions of Cameroon. The outpatient clinic of the Yaoundé Central Hospital is in the heart of the city.

Study Subjects and Sampling:
The study population consisted of in and out patients who came to receive tuberculosis treatments or those who were hospitalized for tuberculosis at the university teaching hospital Yaoundé.

Study Type .
The study consisted of one part which is prospective study of patient's samples that were hospitalized or coming for consultation for tuberculosis or to complete their tuberculosis treatment. This was a cross sectional study with an analytical component. Consecutive adult patients with a productive cough of more than three weeks participated in the study. One spot and two early morning sputa were screened for AFBs.

Specimen Analysis The specimens where all analyzed using two techniques   Direct sputum smears technique. Concentrated sputum smears technique.

Macroscopy
Analysis was done with sputum samples which appear purulent, mucopurulent, mucoid, or mucosalivery.
Purulent: green looking, mostly pus.
Mucopurulent: green looking with pus and mucus.
Mucoid: mostly mucus.
Mucosalivary: mucus with a small amount of saliva.
Salivary specimens were rejected as this will be of no diagnostic significance of pulmonary tuberculosis.
Laboratory Procedure The first, second and third sputum samples of patients who had consented were pooled and divided into two. The first of this pooled sample was smeared directly and stained by the ZN technique. The second of the pooled samples was bleach digested, centrifuged, supernatant discarded' Sediment fixed and stained by the Ziehl Neelsen technique.

Sodium Hypochloride (Naocl) Concentration Technique.
Reagents
Sodium hypochlorite (Na0Cl), 5% Distilled water
Procedure 2ml of sputum was transferred to a test tube (15-20ml capacity). An equal volume (2ml) of concentrated sodium hypochloride (bleach) solution was added and mixed well. And this was left at room temperature for 10 to 15 min, shaking at intervals to break down the mucus in the sputum. 8ml of distilled water was added and mixed well. It was then centrifuged at 3000g for 15 minutes. A plastic bulb pipette was used to discard the supernatant fluid.and the sediment was then transferred to a clean glass slide, spread to make a thin preparation and allowed to air dry. The preparation was heat fixed and stained using the Ziehl Neelsen technique as described elsewhere Acid-Fast Bacilli (AFB) appears red, straight or slightly curved rods.

REPORTING SPUTUM SMEARS FOR AFB.

Number of AFB objective x 100	Note	Answer	Conclusion of bacillus
$0/100$ fields	Negative	No AFB	
$1-9/100$ fields	Nb/100 fields	Nb/100 fields	Rare AFB
$10-99/100$ fields	Nb/100 fields	1+	Fairly numerous AFB
$1-10/100$ fields	Nb/100 fields	2+	Numerous AFB
$>10/100$	Nb/100 fields	3+	Numerous AFB

*WHO (2000), if less than 3AFB/100 fields are seen, this must be considered as Rare AFB

CALCULATION OF ANALYTICAL SENSITIVITY

The Analytical sensitivity was calculated using the formula: *Analytical sensitivity (Direct technique)* = Total number of positive result / total number of specimens examined

Analytical sensitivity result(Concentrated technique) = Total number of positive / total number of specimens examined

The ability of a diagnostic test to indicate when a disease is present or absent is dependent on its quality and is described in terms of sensitivity, specificity and the predictive value.

Predictive value of positive test = _True Positive_ × 100 / All Positive tests

Statistical analysis

Chi Square(X^2) statistical test was used to test for the significance of results obtained

RESULTS

A total of 104 samples where processed by the sodium hypochloride (NaOCl) concentration technique and the direct sputum smear technique for the Ziehl Neelsen staining method. This constituted of 67 males and 37 females. The ages of the patients ranged from 17 to 73 with the mean age being 39 years. The ages were further divided into three groups of; < 20, 20-40, and > 40. Evaluation was also done based on the nature of sputum sample into purulent, mucopurulent, mucoid and mucosalivary.

Table. 2: Distribution by Age group

Age range	Direct technique	Concentrated technique	Percentage
20-40	9	16	55.56%
> 40	10	10	44.44%

Table 3: Distribution by Nature of Specimen

Technique	Purulent	Mucopurulent	Mucoid	Mucosalivary
Direct	8	8	0	3
Concentration	9	8	3	6
Percentage	37.78%	35.56%	6.67%	20%

α=0.01   d.f = 3 X^2 = 5.25   P< 0.05

The Ziehl Neelsen staining had a specificity of 90.59%. The analytical sensitivities were 18.27% for the direct smear technique and 25% for the concentration sputum smear technique. The prevalence of pulmonary tuberculosis was calculated to be 25%. The positive predictive value was 73%. Based on the ages, there was no positive subject for the age range less than 20 years. 55.56% of the study population was positive for the age range 20 to 40 years while a total of 44.44% was positive for the age range greater than 40 years (Table.2). Based on sex, the males were more infected than the females.

DISCUSSION Proper identification of tuberculosis cases is the pillar of tuberculosis control programs

[7]. Currently, this is accomplished mostly by microscopic examination of stained sputum. Thus, technical accuracy and proficiency are of paramount importance [8]. In this study, the specificity of the Ziehl Neelsen technique was 90.59%. The analytical sensitivity of the direct technique was lower than that of the concentrated technique. The sensitivity was increased from 18.27% to 25%.

This is in accordance with the conclusion of Ghazisaeedi *et al.*[9] who found that the concentration smear technique could improve the sensitivity of the direct sputum smear technique. was also done based on the nature of sputum sample into purulent, mucopurulent, mucoid and mucosalivary. The Ziehl Neelsen staining had a specificity of 90.59%. The analytical sensitivities were 18.27% for the direct smear technique and 25% for the concentration sputum smear technique. The prevalence of pulmonary tuberculosis was calculated to be 25%. The positive predictive value was 73%.Based on the ages, there was no positive subject for the age range less than 20 years. 55.56% of the study population was positive for the age range 20 to 40 years while a total of 44.44% was positive for the age range greater than 40 years (Table.2). Based on sex, the males were more infected than the females.

The prevalence of pulmonary tuberculosis was 25%. The positive predictive value was 73%. Since the positive predictive value is high, then the higher is the probability that a positive result means the patient is infected. Based on the nature of specimen being analysed, 37.78% was purulent, 35,56% was mucopurulent, 6.67% was mucoid and 20% was mucosalivary. There was a statistically significan difference between the nature of the specimen and the diagnostic technique being used. Results indicate that age is a risk factor for the prevalence of pulmonary tuberculosis. Therefore the increased incidence of tuberculosis in this age group can be attributed to low accumulated wealth, consumption of unpasteurized milk, diabetes, being unemployed, living in overcrowded conditions, illicit drug use and a history of incarceration, HIV/AIDS infection which are significantly associated with the development of tuberculosis. Among the methods suggested for smear preparation, the N-acetyl-l-cysteine method has been shown to be a sensitive (28 to 87%) and reliable method for microscopy and

culture [7]. However, due to limitations in funds and equipment, this technique is not being performed in TB laboratories of countries with limited resources [10]. In most parts of these countries direct smear microscopy with low sensitivity (25 to 50%) is the only available method for the diagnosis of tuberculosis[11]. The concentration sputum smear technique is appropriate for developing countries and its application would increase the efficiency of tuberculosis control programs. As a potent disinfectant, NaOCl also has the advantage of lowering the risk of laboratory infection. However, in developing countries where most health units lack culture facilities this method could still be utilized to accelerate initiation of treatment since tuberculosis is curable and the drugs are readily available.

CONCLUSION

Within the limits of this study where there was a small sample size we could conclude that: the use of sodium hypochloride (NaOCl) in the concentration of acid-fast bacilli (AFB) in sputum significantly improves the laboratory diagnosis of pulmonary tuberculosis. We therefore recommended that all sputum samples should undergo the concentration sputum smear technique for the laboratory diagnosis of pulmonary tuberculosis, that more sensitive and rapid tests should be adopted and used and education of laboratory personnel on the need to concentrate sputum specimens for tuberculosis diagnosis.

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