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Full Length Research Paper

Comparison between specificity and sensitivity of sodium hydroxide and sodium hypochlorite concentration methods for the diagnosis of AFB

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ABSTRACT

Identification of *Mycobacterium* in the clinical laboratory by microscopy is important for initial diagnosis of Acid Fast Bacilli (AFB) but with low sensitivity and specificity. This work compared specificity and sensitivity of sodium hydroxide (NaOH) concentration method (Control) versus sodium hypochlorite (NaOCl) concentration method (Test) for AFB diagnosis, so that the more specific and sensitive method will improve diagnosis of AFB. Three hundred and five (305) clinical samples of sputum, laryngeal swab, gastric and cerebrospinal fluid from suspected tuberculosis patients were analyzed in Jos University Teaching Hospital. In both control and test methods, specimens were concentrated and deposit smear made on a clean slide and stained with AFB staining technique. A total of 54 samples (17.7%) were positive for AFB with NaOH method while 38 samples (12.5%) were positive with NaOCl method. The NaOH method diagnosed 16 additional patients, which constituted a rise of 5.2%. Moreover, NaOH and NaOCl specificity in detecting AFB were 87.54% and 82.29% respectively while their sensitivity were 100% and 70.37%. This indicated that NaOH concentration method has advantage over NaOCl concentration method even when both are simple, safe, not requiring additional training or expensive reagents.

Keywords: AFB, Sodium hydroxide, Sodium hypochlorite, Sensitivity, Specificity.

INTRODUCTION

The World Health Organization declared tuberculosis a global emergency in 1993, following about a decade of alarming increase in global incidence of the disease. This global increase affected countries where TB has been brought under control. This declaration elevated TB to a status that was hither unprecedented in public health; for no disease had ever before in human history been accorded this distinction or notoriety (WHO, 2000). Moreover there has been a disturbing increase in the number of TB cases that are caused by organisms that are resistant to the most important drugs, isonazid (INH) and rifampicin (RMP) (WHO, 2000). WHO (2000) indicated that there are approximately 10 million new cases of TB per year with 3 million deaths. It estimated that trend continued, deaths per year resulting from TB will rise to 4 million by 2004. India had the highest burden of the disease (30% of all cases). In African, it was then stated that the number of TB cases was likely to double within 10years (2000-2010) due to the spread of HIV. Human Immunodeficiency Virus (HIV) associated with TB was seen in sub-Saharan African Countries (De cock *et al*., 1999). According to WHO (2003), the global incidence rate of TB was growing at approximately 0.40% per year, but much faster in sub-Saharan Africa and in countries of the former Soviet Union. Directly observed Therapy short course (DOTS) programmes notified 2.4 million new TB cases of which 1.2 million were smear- positive, and over 10 million patients had been diagnosed and treated in

DOTS programme since 1995. Over all, DOTS programme (De cock *et al.,* 1998) in 22 high burden countries were not increasing case detection towards the 70% target within designated DOTS areas set to be achieved by 2005. Diagnosis thus becomes problematic since the non-concentrate methods are not highly specific and sensitive (Cheesbrough, 2006). Laboratory diagnosis of *Mycobacterium Tuberculosis* utilizing acid fast staining and culture of processed sputum specimens have been utilized for decades but while rapid and fairly specific, it has sensitivity low enough to be useful only as a presumptive screening test (Date, 2017). Identification of *Mycobacterium* in the clinical laboratory still remains difficult and time consuming. In the present work, the two concentration methods used to concentrate and analyze the samples (CSF Sputum, laryngeal swab, gastric washing for AFB diagnosis were the sodium hydroxide concentration and the sodium hypochlorite concentration methods. In comparing these methods, sodium hydroxide method was used as the control or standard while the sodium hypochlorite method was the test method. The specificity and sensitivity of sodium hypochlorite was compared to know which was higher than the other. Sodium hydroxide was used to concentrate the specimens because it can liquefy the specimens; and Sodium hypochlorite was also used to concentrate the specimens because it can break the mucolytic structure of AFB specimens. Then the one found to be a more specific and sensitive method will improve diagnosis of AFB. The aim of this work was therefore to compare the specificity and sensitivity of sodium hydroxide and sodium hypochlorite methods for Acid Fast Bacilli (AFB) diagnosis.

MATERIALS AND METHODS

Sampled specimen

A total of 305 clinical samples of sputum, laryngeal swabs, CSF, and gastric washings from consenting subjects were studied by comparing concentration with sodium hydroxide to sodium hypochlorite microscopy for AFB diagnosis in September and November 2004, at Jos University Teaching Hospital.

Collection of AFB specimens

Samples of Sputum, laryngeal swabs, CSF, and gastric washings were collected into sterile universal bottles. Each patient's identity and serial number were written on the containers. The sputum was collected by deep coughing. The laryngeal swab was collected by placing the laryngeal swab beneath the tongue. CSF samples were collected from the 3rd and 4th lumbar vertebrate by lumber puncture with lumbar needle. Gastric washing were collected by the introduction of the gastric tube into the stomach and drawing the tube to get the gastric washings.

Macroscopy

Samples were described as purulent, muco purulent, mucoid and mucosalivary. Blood stained samples were also noted.

Procession of samples

Samples were processed by the sodium hypochlorite centrifugation techniques and sodium hydroxide centrifugation technique.

Sodium hypochlorite centrifugation technique to concentrate AFB

Sodium hypochlorite was used to kill m tuberculosis and also liquefies it. According to Saceanu *et al.* (1993), liquefaction and concentration of the samples (sputum, gastric washings, C.S.F., laryngeal swabs) for acid fast staining may be conducted safely on the open bench by first treating the specimen in a safety cabinet with an equal volume of 5% sodium hypochlorite. An equal volume of 5% sodium hypochlorite was added to each of the sample (sputum, gastric washings, CSF, laryngeal swabs) in the universal bottle and homogenized. They were left at room temperature for 10-15 minutes shaking at interval to break the mucus in the samples. About 8ml of distilled water was added and well mixed. The sodium hypochlorite treated sample were centrifuged at 30000 Revolution per minute

(RPM) for 5 minutes. The supernatant fluid was decanted into a disinfectant jar 5% (NaOCl). The deposits (sediments) were well mixed and smears made on a clean slide. They were allowed to air dry (Cheesbrough, 2006).

Sodium Hydroxide centrifugation technique for concentration of AFB

Similarly, Sodium hydroxide was used to concentrate AFB because it is a mucolytic agent (for liquefaction and digestion of mucous) and also kills bacilli. According to Saceanu *et al.* (1993), liquefaction and concentration of samples for acids fast staining may be conducted safely on the open bench by first treating the specimen in a safely cabinet with an equal volume of 5% Sodium Hydroxide. An equal volume of 5% concentrated sodium hydroxide was added to each sample in the universal bottle and homogenized. They were left at room temperature for 10-15 minutes shaking at interval to break the mucus in sputum and other body fluids. About 8ml distilled water was added and well mixed. The sodium hydroxide samples were centrifuged for 5 minutes at 3000 (RPM). The supernatant fluid was decanted into 5% phenol solution. The deposits (sediments) were well mixed and smears made on clean grease, free slide. They were allowed to air dry (Cheesbrough, 2006).

Staining of slides

Smears were heat fixed. The smears were covered with carbol fuchsin stain. The stains were heated until vapour just began to rise (at about 60^{o}C). The heated stain was allowed to act on the slides for about 5 minutes. Slides were washed with water. The smears were flooded with 3% v/v malachite green for 1-2 minutes washed and allow to air dry. They were examined using the x 100 oil immersion objective of the light microscope (Cheesbrough, 2006).

RESULTS AND DISCUSSIONS

Table 1 shows AFB among the studied population by age and gender. A total of 54 AFB positive samples were obtained from the 305 samples using sodium hydroxide concentration method, representing 17.7% positivity. Similarly, 38 positive samples were recorded using sodium hypochlorite method, giving 12.5% positivity.

Table 1 showed that age group 20-29 years had the highest prevalence of AFB. Table 2 also showed positivity of subjects to AFB with sodium hydroxide method by age and gender while Table 3 showed sensitivity and specificity of Control and Test methods to AFB.

Table 1: AFB among the studied population age

Age group (Years)	Subjects examined [no. (%)]	Overall infected with AFB [no. (%)]	
		Sodium hydroxide method	Sodium hypochlorite method
<20	3.9 (12.8)	3 (1.0)	2 (0.7)
20-29	105 (34.4)	23 (7.5)	18 (5.9)
30-39	64 (21.0)	13 (4.3)	9 (3.0)
40-49	45 (14.8)	7 (2.3)	5 (1.6)
50-59	22 (7.2)	4 (1.3)	3 (1.0)
60-69	20 (6.5)	3 (1.0)	1 (0.3)
≥70	10 (3.3)	1 (0.3)	0 (0.0)
Total	**305 (100)**	**54 (17.7)**	**38 (12.5)**

Table 2: Positivity of subjects to AFB with sodium hydroxide method by age and gender

Age group	Males positivity		Females positivity		Overall positivity	
(Years)	Number	Relative %	Number	Relative %	Number	Mean ±SD
<20	2	5.71	1	5.26	3	5.485±0.225
20-29	12	34.28	11	57.89	23	46.085±11.805
30-39	11	31.43	2	10.53	13	20.98±10.45
40-49	7	20.00	0	0.00	7	10.0±10.0
50-59	1	2.86	3	15.79	4	9.325±6.465
60-69	1	2.86	2	10.53	3	6.695±3.835
≥70	1	2.86	0	0.00	1	1.43±1.43
Total	**35**	**100**	**19**	**100**	**54**	**100**

*Positivity (%) = $\frac{\text{Positive in each range}}{\text{Total positive}} x \frac{100}{1}$

Table 3: Sensitivity and specificity of Control (Sodium hydroxide) and Test (Sodium hypochlorite) to AFB

Age (Years)	AFB (+) by Control [No. (Rel. %)]	AFB (+ve) by Test [No. (Rel. %)]
<20	3 (5.56)	2 (5.26)
20-29	23 (42.59)	18 (47.37)
30-39	13 (24.07)	9 (23.68)
40-49	7 (12.96)	5 (13.16)
50-59	4 (7.41)	3 (7.90)
60-69	3 (5.56)	1 (2.63)
≥70	1 (1.85)	0 (0.0)
Total	**54 (100)**	**38 (100)**

Computation of sensitivity and specificity:
a = Total positive by Test (Sodium hypochlorite concentration method) = 38
b = Total positive by Control (Sodium hydroxide concentration method) = 54
c = Difference between *a* and *b* (Test and Control) =16
d = Total negative for AFB by Control = 251 (i.e., 305 – 54)

Sodium hypochlorite Sensitivity = $\frac{a}{a+c} x \frac{100}{1} = \frac{38}{38+16} x \frac{100}{1} = \frac{38}{54} x \frac{100}{1}$ = 70.37%

Sodium hydroxide Sensitivity $= \frac{b}{a+c} x \frac{100}{1} = \frac{54}{38+16} x \frac{100}{1} = \frac{54}{54} x \frac{100}{1}$ = 100%

Sodium hypochlorite Specificity = $\frac{d}{b+d} x \frac{100}{1} = \frac{251}{54+251} x \frac{100}{1} = \frac{251}{305} x \frac{100}{1}$ = 82.29%

Sodium hydroxide Specificity $= \frac{b+d-a}{b+d} x \frac{100}{1} = \frac{54+251-38}{54+251} x \frac{100}{1} = \frac{267}{305} x \frac{100}{1}$ = 87.54%

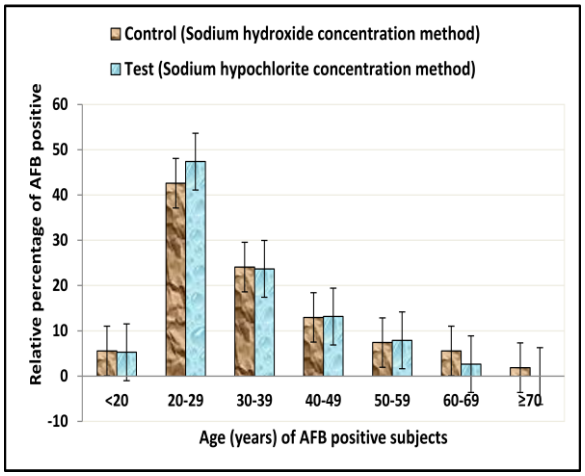

Fig. 1: Comparison of the relative percentages of AFB positives with Control and Test methods.

Figure 1 showed the comparison AFB positives by Control and Test. It suggested that the most active segment of the society – those aged between 20 and 40years - were most vulnerable to TB.

In this study, the 17.7% AFB positive samples is 5% above the findings of Idigbe and Onwujekwe (1983). The advantage of NaOH over NaOCl methods in this work may be due to the fact that sodium hypochlorite does not hydrolyze mycolic acid very well as sodium hydroxide (Cheesbrough, 2006). TB positivity observed more among the age range 20-29 years which is the active sexual age may be due their youthful exuberances, being predisposed to factors like sexually transmitted disease and smoking that could lead to immune deficiency increased susceptibility to AFB infections also reported from Uganda and Ethiopia (Giradi *et al*., 1992). Also, male positivity over that of females (Table 2) may be due to some gender related-habits like smoking identified in a similar study in Cameroon (Girardi et al., 1992).

This study has demonstrated that NaOH concentration method had advantage of diagnosing 16 additional patients, which constituted a rise of 5.2% over NaOCl concentration method even when both could be simple, safe, not requiring additional training or expensive reagents.

Conflicts of Interest

The authors declared no conflicts of interest.

Contributor's Statement

Two contributors were required to complete this study, and each read and approved the final manuscript before submission.

Chukwujekwu, C.R developed the study design, did sample collection, conducted the bench work, statistical analyses of the data, and wrote the manuscript

Elomba, C.C provided input to the study design, interpreted the results, accessed all the data in this study and is responsible their integrity and accuracy, critically edited final version to be published.

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