THE VALUE OF FLUORESCENCE MICROSCOPY OF AURAMINE STAINED SPUTUM SMEARS FOR THE DIAGNOSIS OF PULMONARY TUBERCULOSIS

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Abstract. Laboratory diagnosis of pulmonary tuberculosis rests on the bacteriological examination of sputum smears stained by the Ziehl-Neelsen (ZN) method for acid fast bacilli (AFB). In the present study, we have compared light microscopy of ZN stained smears with that of fluorescence microscopy of sputum smears stained by auramine-phenol flurochrome dye for detection of AFB in sputum specimens. Sputum specimens from a total of 2,600 clinically suspected and diagnosed cases of pulmonary tuberculosis were examined by both the methods. Sputum specimens from a total of 1,104 patients were found to be positive for AFB. These included sputa from 975 (37.5%) patients positive for AFB by both ZN and auramine staining methods and sputa from an additional 129 (4.96%) patients positive for AFB by auramine staining only. Thus auramine staining of sputum smears in comparison to that of ZN staining is a better method of sputum microscopy for demonstration of AFB in sputum specimens. Fluorescence microscopy is relatively more sensitive and has the added advantage of allowing a large number of sputum specimens to be examined in a given time, in laboratories equipped with a fluorescent microscope.

INTRODUCTION

Tuberculosis is one of the most significant health problem in developing countries like Nepal. The condition causes more preventable deaths than any other infectious disease (Raviglion *et al,* 1995). According to WHO estimation, approximately one third of the world population is infected with *Mycobacterium tuberculosis* and 20 million people have an active disease (Kochi, 1991). In Nepal, there are about 80,000 people with active tuberculosis, every year about 50,000 people develop the disease and over 15,000 people die (Sharma and Smith, 1996). Therefore accurate diagnosis of the condition is essential both for treatment and overall control of the disease.

Light microscopy of Ziehl-Neelsen stained smear is the method most commonly used in developing countries for demonstration of acid fast bacilli (AFB) in the sputum for diagnosis of pulmonary tuberculosis. Nevertheless, microscopy of ZN stained smears has the disadvantages of being of low sensitivity, tedious and time consuming. Therefore, there is a need for an improved method for detection of AFB in a large number of sputum specimens received in a hospital for diagnosis of tuberculosis (L'herminez, 1993)

In the present study, we examined sputum specimens from 2,600 cases, clinically suspected or diagnosed to be suffering from tuberculosis. From sputum specimens of each patient a pair of smears was made. The smears were examined independently; one by conventional light microscopy after staining by the ZN method, and the other by fluorescence microscopy after staining by the auramine-phenol method. The main objective was to assess the efficacy of each technic in the demonstration of AFB in sputum specimens. In this study also we cultured sputum specimens form 205 patients, in addition to both ZN microscopy and fluorescence microscopy. The purpose was to compare the efficacy of these smear techniques with that of culture in the diagnosis of tuberculosis.

MATERIALS AND METHODS

Clinical specimens

This study was carried out in the Department of Microbiology, BP Koirala Institute of Health Sciences, Dharan (Nepal). All patients with clinically suspected and diagnosed tuberculosis, who pre-

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sented to the general outpatient department, the department of chest and tuberculosis and other outpatient and inpatient departments of the institute were included in the study.

From each patient 3 specimens were collected as per the guidelines of the National Tuberculosis Control Program of Nepal. The patients were provided with wide mouthed containers. On the first day when the patients presented, a first spot sputum specimen was collected, on the second day an early morning specimen and a second spot specimen was collected.

Direct microscopy

All three sputum specimens from each patient were pooled from which sputum smears were made for ZN and auramine staining.

The smears were prepared from the purulent part of the sputum on a clean glass slide. From each pooled sputum specimen two smears were made by swab. One smear was air dried, heat fixed, and stained by Ziehl-Neelsen technic. Another smear was stained by auramine-phenol technic with modification (Cheesbrough, 1984). The smears from sputum specimens were air dried and alcohol fixed by covering with a few drops of 70% (v/v) ethanol for 2-3 minutes. The alcohol fixed smears were flooded with auramine phenol stain for 10 minutes and the excess stain was then washed off with clean water. The smear was decolorized by covering it with 1% v/v acid alcohol for 5 minutes. The slides were washed again with clean water. After washing, the slides were covered with 0.5% potassium permanganate for 30 seconds to 1 minute followed by rinsing with clean water and air drying. The stained smears were examined by a fluorescence microscope first under low power objective (25x) followed by a high power objective (40x). In a positive smear, acid fast bacilli were seen as white yellow rods glowing against a dark background.

If any AFB were present in the sputum smear, the smear was then considered positive. Depending on the number of AFB present, positive sputum smears were graded as follows: scanty (1-9AFB/100 oil immersion fields), + (10-100 AFB/100 oil immersion fields), ++ (1-10 AFB/field) or +++ (> 10 AFB/oil immersion field). The number of AFB seen in the smears were recorded according to the recommendations of the National Tuberculosis Control Program of Nepal.

Culture

Sputum culture in Lowenstein-Jensen (LJ) medium for acid fast bacilli was carried out with the sputum specimens from 205 patients. For culture, the remaining sputum specimens of each patient after making smears were concentrated by Petroff's method. It was carried out by decontaminating sputum specimens with 4% sodium hydroxide and subsequently neutralizing with hydrochloric acid (N/10).

The concentrated specimen was cultured in tubes containing L-J medium. The cultures were incubated at 37°C for 8 weeks and checked once a week for mycobacterial growth. Growth of mycobacterial species on L-J medium was identified by its rate of

Table 1

Comparison of results of microscopy of sputum smears stained by Ziehl-Neelsen and auramine staining.

	Auramine stained smear		Total
	Positive	Negative	
ZN stained smear			
Positive	975 (37.5%)	00 (00%)	975
Negative	129 (4.96%)	1,496 (57.53%)	1,625
Totals :	1,104 (42.46%)	1,496 (57.53%)	2,600

Table 2

Comparison of sputum culture with microscopy of ZN and auramine stained smears for the diagnosis of pulmonary tuberculosis.

	Culture		Total
	Positive	Negative	
ZN stained (a) smear			
Positive	57	5	62
Negative	63	80	143
Totals	120	85	205
Auramine stained (b) smear			
Positive	68	5	62
Negative	52	80	143
Totals	120	85	205

a. Sensitivity 57/120 = 47.5%; Specificity 80/85 = 94.1%; Positive predictive value 57/62 = 91.9%; Negative predictive value 80/143 = 55.9%

b. Sensitivity 68/120 = 56.66%; Specificity 80/85 = 94.1%; Positive predictive value 68/73 = 93.15%; Negative predictive value 80/132 = 60.6%

growth, colony morphology and acid alcohol fastness of the the bacteria after smear staining. The culture tubes showing colonies identified as *M.tuberculosis* were considered as positive.

RESULTS

Sputum specimens from a total of 1,104 cases were found to be positive for AFB. These included sputa from 975 (37.5%) patients positive for AFB by both ZN and auramine staining methods, and sputa from additional 129 (4.96%) patients positive for AFB by auramine staining only (Table 1).

Culture of sputum for *M. tuberculosis* was positive in 120 out of 205 cases. Of these 120 culture positive cases, AFB could be demonstrated in the sputum of 57 cases in both ZN and auramine stained smears.

In additional 11 cases, AFB were demonstrated in sputum by auramine method only. Comparison of results of microscopic examination of smear with that of culture in the diagnosis of tuberculosis is presented in Table 2.

DISCUSSION

Sputum microscopy is still the most reliable, economical and fastest method for demonstration of AFB for the diagnosis of pulmonary tuberculosis in a developing country like Nepal. Early detection of infectious pulmonary cases on the basis of sputum smear examination is one of the most important component of National Tuberculosis Control Program in Nepal (Bam, 1996).

At the moment in the developing coutries like Nepal, where tuberculosis is a major health problem, sputum microscopy is carried out widely by microscopic examination of sputum smears stained by the ZN method. Even though the microscopy of ZN stained smears is considered to be a reliable method for detection of AFB in sputum smears, there are many problems associated with this method. These are : (a) Microscopy of ZN stained smears is not very sensitive; sensitivity of direct sputum microscopy of ZN stained smears has variably been reported from a minimum 8.8% to a maximum 46.4% in several studies carried out elsewhere (Aber *et al,* 1980). (b) It is tedious and considerable time is required for examination of

large number of smears, (c) it is difficult for technical staff to examine a large number of smears at a given time.

These present a major problem particularly in an over-burdened laboratory receiving a large number of sputum specimens for examination of AFB. In such a situation, it becomes increasingly difficult to maintain a high level of performance and quality control in reporting of the smears. Therefore, there is a need for alternate strategies for improving sputum microscopy for AFB.

Results of the present study show that fluorescence microscopy of auramine stained smears relatively is more sensitive than that of microscopy of ZN stained smears in the detection of AFB in sputum. Fluorescence microscopy detected all sputum smears which were positive for AFB by light microscopy of ZN stained smears and in addition, the method could demonstrate AFB in 129 more cases which were negative by ZN stain (Table 1). Also, in culture positive cases it could detect an additional 11 cases (Table 2). Another advantage which was noted in the study was the rapidity of the method using auramine stained sputum smears. In this method, a large number of sputum smears could be examined at ease in a short time. This is particularly important because in a laboratory like ours which is situated in an area endemic for tuberculosis, and receives on average 30-40 sputum specimens in a day for examination for AFB, the technical staff examining the auramine-phenol stained smears found easier while examining a large number of smears in a given time with less strain to their eyes. Another added advantage of this method is that when only a few bacilli are present in the sputum specimens, they could still be detected by their fluorescence, while in ZN stained smears these are frequently missed. Therefore, results of the study shows that fluorescence microscopy has potential as a more sensitive, dependable and reliable method for examination of sputum specimens for AFB in a laboratory like ours receiving a large number of specimens routinely for diagnosis of tuberculosis.

However, the main disadvantage of fluorescence microscopy which may restrict against the wider use is the relative high cost of a fluorescence microscope and its maintenance. The cost of fluorescence bulb is also high and has a definite shelf life. Moreover, fluorescence microscopy in comparison to that of light microscopy requires more technical skill.

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