



A comparative study of ziehl-nelson staining and auramine staining in sputum sample for the diagnosis of pulmonary tuberculosis.

Science

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KEYWORDS:

Introduction

Tuberculosis is worldwide public health problem caused by *Mycobacterium tuberculosis*.¹ Globally, it is estimated that one-third of population is asymptotically infected with tuberculosis.² Mostly the new cases and death occurs in the developing countries where infection is mainly acquired in the childhood.³ India alone accounts for one-fourth of the burden globally.⁴ There are various methods for bacteriological diagnosis of tuberculosis. Currently, radiometric assay allows detection of *Mycobacterium tuberculosis* growth and provides antibiotic sensitivity results more rapidly usually within 10 days. However use of the technique is limited because culture medium contains radioactive carbon. Genetic probes are on the other hand quite easy to use and allow identification of culture bacteria in only a few hours by polymerase chain reactions. This method has not become a routine laboratory technique, particularly due to lack of sufficient specificity and sensitivity. Serological tests are currently not reliable enough for the diagnosis of tuberculosis.⁵ Microscopic examination and culture are still essential elements of the bacteriological diagnosis of tuberculosis in microscopic examination; the diagnosis of tuberculosis is confirmed on the basis of demonstration of tubercle bacilli in the sputum or any other pathological material.^{6,7} Smear examination is believed to be simple, cheap, quick and practicable and effective case finding method for developing countries. Ziehl-Nelson is the most extensively used procedure for the demonstration of mycobacterium tuberculosis in smear.⁸ The requisites for the staining procedures are; basic fuchsin, phenol, absolute alcohol; sulphuric acid and methylene blue. Microscopic examination under oil immersion objective reveals mycobacterium are red bacilli. Fluorescent staining by Auramine is other methods of staining. In this a smear is made from the specimen and stained with fluorescent stain called auramine. The auramine stain enters the wall of *Mycobacterium tuberculosis* bacterial cell and makes them glow against dark background under UV light.⁹ Microscopic examination under low power objective will reveal mycobacteria as glowing yellow white, rice like bacteria in the smear.

The present prospective study was under taken to see the efficacy of Ziehl-Nelson method versus fluorescent staining in the detection of mycobacterium in sputum sample.

AIMS AND OBJECTIVES

To study the sensitivity and benefits of fluorescent microscopy over conventional ZN staining.

MATERIAL AND METHODS

Total of 300 sputum samples irrespective of all age groups were collected from 150 patients suspected with pulmonary tuberculosis including the immunocompromised patients who came to the outpatient department of PGI Rohtak. A comparative study was done from January – March, 2014 in the hospital. Two sputum samples were collected; one spot and the second one early morning fasting sample. It was collected in a sterile, clean; wide mouth container. Each sample was processed. In Ziehl-Nelson staining procedure; sputum slide is heat fixed and carbol fuchsin is poured over the entire slide, which is kept for 5 minutes with gentle heating. It is rinsed properly and decolourised with 25% sulphuric acid, kept for 2-3 minutes.¹⁰ Slide is rinsed again and counterstained with 0.1% methylene blue for further 30 seconds. Lastly slide is washed and dried up and seen under 100x magnification of light microscope.¹¹ In Auramine O staining, flood the

slide with Auramine –phenol and keep it for 7-10 minutes. Wash well and decolorize for 2 minutes with acid alcohol, two times. It is rinsed and counterstained with 0.1% potassium permanganate with 30 seconds. Wash it well and observe under 40 x magnifications of fluorescent microscope.¹² The results were graded as per the International Union against Tuberculosis guidelines.¹³

WHO TABLE

Table 1: Grading chart (WHO, IUATLD, 2007) for fluorescent microscopy

Union /WHO scale x 1000 field =	Bright field x 1000 magnification ; 1 length =2 cm=100 HPF	Fluorescence (x 200-250 magnification ; 1 length =30 field = 300 HPF	Fluorescence (x 400 magnification ; 1 length = 40 fields= 200 HPF
Negative	Zero AFB / 100HPF	Zero AFB / 1 length	Zero AFB / 1 length
Scanty	1-9 AFB / 100HPF	1-29 AFB / 1 length	1-19 AFB/1length
1+	10-99 AFB / 100HPF	30-299 AFB / 1 length	20-199 AFB / 1 length
2+	1-10 AFB / 1 HPF (on average 50 HPF)	10-100 AFB / 1 field on average	5-50 AFB / 1 field on average
3+	>10 AFB / 1 HPF (on average 20 HPF)	>100 AFB / 1 field on average.	> 50 AFB / 1 field on average

RESULTS

Out of 300 sputum samples of 150 patients, 32(10.66%) sputum samples were positive for Ziehl-Nelson and 55(18.33%) sputum samples were positive for Auramine O.

Auramine O stained sputum smear under fluorescent microscope

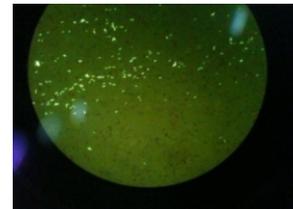


Fig 2. Ziehl-Nelson stained sputum smear under light microscope. Bacilli appear as pink colored rod shape organism

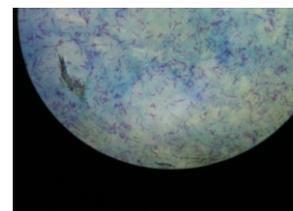


Table 2: Comparison of ZN and Auramine O Staining Reports

Staining method	Positive smears	Negative smears
Ziehl Nelson staining	32	268
Fluorescent staining	55	245

Discussion

In developing countries, microscopy of the specimen is by far the fastest, cheapest, and most reliable method for the detection of AFB.¹⁴ However fluorescent staining has been added in Revised National Tuberculosis Control Program (RNCP) because of more sensitive and rapid results and can be used in field areas. In a study done by Ben *et al* in 2008, showed that out of 221 sputum samples, 33(14.9%) samples were positive with Auramine staining and 24(10.85%) samples were positive with Ziehl-Nelson staining. It demonstrated superior diagnostic results by fluorescent microscopy when compared with conventional light microscopy. Laifangbam *et al* in 2009 revealed a study on 102 suspected patients, where in 44.1% patients were positive with Ziehl Nelson staining and 71.6% patients were positive for Auramine O staining. It was found by this study that Auramine O staining was superior to Ziehl -Nelson staining.

In the present study, we conclude that the Auramine O staining is superior to that of the conventionally used ZN stain. It states that out of 300 sputum samples; 32 (10.66%) samples were ZN positive and 55(18.33%) samples were Auramine O positive. The diagnostic accuracy of fluorescent microscope was found much more superior and much more sensitive than the conventional light microscopy.

The results of present study indicate that Auramine staining of sputum smears is a more sensitive method of sputum microscopy for demonstration of AFB in sputum specimen, compared to Ziehl-Neelson staining. The use of Fluorescent Microscopy greatly improves the diagnostic value of sputum smear especially in patients with low density of bacilli that are likely to be missed on Zeihl Neelson stained smears. The method is economical in both time and expense and recommended for laboratories handling large number of sputum specimens.¹⁵

Conclusion

Sputum examination for the tubercle bacilli is usually conducted for patients clinically and or radiologically suspected of pulmonary tuberculosis. However, the standard method of sputum examination, that is, ZN staining is not sensitive enough and a large number of the suspected cases miss diagnosis. Fluorescent stain is a more efficient over ZN stain in detecting Tubercle bacilli in sputum. Since screening is done under low power of magnification (40X), fluorescence has been found to be less time consuming compared to ZN method (100X) in the diagnosis of tuberculosis. Hence, it has been advocated to be methods of choice where the large numbers of sputum smears are to be examined. The fluorescing bacilli are easily identifiable and cause less eye-strain.

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