Orginal Article

Light Emitting Diode (LED) Fluorescent Microscopy: a Milestone in the Detection of Paucibacillary Mycobacterium in Case of Pulmonary Tuberculosis.

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Abstract

In Bangladesh with a large number of pulmonary tuberculosis cases and financial constraints with high HIV risk, evaluation of scanty i.e paucibacillary cases has great importance. To study the efficacy of Light Emitting Diode fluorescent microscopy in the diagnosis of pulmonary tuberculosis specially paucibacillary cases in comparison to conventional fluorescent microscopy, Ziehl-Neelsen staining and culture of sputum samples from patients suspected of pulmonary tuberculosis. 150 sputum samples collected from the patients suspected of pulmonary tuberculosis were processed by the Petroff's method, and subjected to Ziehl-Neelsen staining (ZN), which were examined by both LED and conventional fluorescent microscope (CFM) and culture on Lowenstein-Jensen media (gold standard) for detection of Mycobacterium tuberculosis. In this study, out of 150 patients 14.67%, 8.67%, 4% cases were detected as paucibacillary (Scanty) cases by LED, CFM, ZN respectively. LED fluorescent microscopy is more effective in the detection of paucibacillary cases of pulmonary tuberculosis than other methods of microscopic examination.

Keywords: Paucibacillary, LED, CFM, Culture, Pulmonary Tuberculosis.

Introduction

Tuberculosis is a major public health problem in Bangladesh. According to present estimation, approximately 320,000 people fall ill of tuberculosis each year and 64,000 die to tuberculosis.[1] By the end of 2007, NTP Bangladesh achieved 91 percent treatment success rate and over 71 percent case detection rate. Generally, the lifetime risk of developing active TB is around 10 percent while for TB/HIV co-infection the risk may be rise 60 percent. Globally it is estimated that 11 percent of 8.8 million newly diagnosed TB patients are co-infected with HIV[1]. It is also a major public health problem in Bangladesh and ranks sixth among the 22 highest burden tuberculosis countries in the world.[2] Nearly 2.14% of the population become infected every year.[3]

For developing countries with a large number of cases and financial constraints, evaluation of rapid and less expensive diagnostic methods like demonstration of acid-fast bacilli (AFB) in smears has great importance.[4,5] Direct microscopy for AFB is widely used method for diagnosis and confirmation of pulmonary tuberculosis and when positive, defines the more infectious cases.[6] This method is highly specific, faster and cheaper for detection of AFM in sputum. But, there are several drawbacks of this method. First of all, ZN stain has low sensitivity relative to fluorescent stain[7] and culture.[8] Secondly, it take more time to scan at least 300 fields. Thirdly, it needs experienced pathologist. Finally, it often miss the paucibacillary tuberculosis and when the patient is co-infected with HIV. Technical error is also more common in case of ZN stain, in which heated carbol fuchsin is very much important.[9]

The new light emitting diode (LED) fluorescent microscope is cheaper and with more life expectancy (10,000 hours) than conventional fluorescent microscope (CFM) (300 hours)[10] LEDs excite auramine without producing UV light.[10] It also produces minimal hat and contains no hazardous materials. Also there is no need of dark room for LED fluorescent microscope like others. Moreover, as LED needs low power consumption, it can be operated by portable battery.[11] It gives us the opportunity to diagnose TB easily at earliest time with more comprehension.

If we can spread the applicability of FM, it will minimize the time to detect TB-bacilli, initiation of therapy and decrease the burden of TB in our country. Therefore, it will be generally accepted

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that the LED fluorescent method should be given preference over the ZN methods.

Materials and Methods

This cross section study was carried out in the Department of Clinical Pathology, Department of Microbiology and Immunology, BSMMU, Dhaka and National Tuberculosis Reference laboratory (NTRL) of National Institute of Diseases of Chest and Hospital (NIDCH), Mohakhali, Dhaka. The main objective of the study was to compare the findings of auramine stained sputum smear by LED fluorescent microscopy with conventional fluorescent microscopy and to compare with Ziehl-Neelsen (ZN) stained sputum smear by bright field microscopy for the diagnosis of pulmonary tuberculosis.

The validity of different discrimination indices were evaluated by calculation their sensitivity, specificity, accuracy, PPV and NPV as follows:

Sensitivity= True positive/ (true positive + false negative)

Specificity= True negative/ (true negative + false positive)

Accuracy= (True positive + True negative)/ (True positive + false negative+ true negative + false positive)

PPV=True positive/ (True positive + false positive)

NPV=True Negative/ (True negative + false negative)

Smear reporting is done according to table1

Table 1: Grading Scales for bright field (Ziehl-Neelsen) and Fluorescence microscopy (WHO, IUATLD, 2007)

Union / WHO Scale x1000 field=HPF Result	Bright fiels (x1,000 magnification; 1 length=2cm =100 HPF)	Fluorescence (x200-250 magnification; 1 length=30 fields =300 HPF)	Fluorescence (x400 magnification; 1 length=40 fields =200 HPF)
Negative	Zero AFB/100 HPF	Zero AFB/1 Length	Zero AFB/1 Length
Scanty	1-9 AFB/100 HPF	1-29 AFB/1 Length	1-19 AFB/1 Length
1+	10-99 AFB/100 HPF	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	5-50 AFB/1 field
3+	>10 AFB/1 HPF (on average 20 HPF)	>100 AFB/1 field	>50 AFB/1 field

HPF=high-power field; AFB=acid-fast bacilli

Results

Out of 150 cases 67 positive cases were detected by LED of which 22 cases were scanty. Out of 53 positive cases detected by CFM, 13 cases were scanty. On the other hand, ZN stain detected only 06 cases as scanty among the 39 positive cases. Here scanty cases were include in paucibacillary cases.

Table 2: Comparison of positive for mycobacterium case from Auramine staining by LED, Conventional Fluorescent Microscope (CFM) and ZN staining.

	LED	CFM	ZN
Scanty	22(14.67)	13(8.67)	06(4)
1+	45(30)	40(26.66)	33(22)
Total	67(44.67)	53(35.33)	39(26)

Figure in parenthesis indicate percentage. Grading scale was done according to table 1

Table 3: Sensitivity, Specificity, Accuracy, Positive and Negative predictive values of sputum examination by ZN stain (Bright field Microscope), Conventional (CFM) and LED fluorescent microscope by auramine stain (n=150).

	ZN	CFM	LED
Sensitivity	56.06	68.18	95.38
Specificity	97.61	90.47	94.11
Accuracy	79.33	80.66	94.66
PPV	94.87	84.90	92.53
NPV	73.87	78.35	96.38

(Sensitivity, Specificity, Accuracy, Positive and Negative predictive values are expressed in percentage)

Discussion

Tuberculosis (TB) is a major public health problem in Bangladesh since long. Estimates suggest that daily about 880 new TB cases and 176 TB deaths occur in the country.[4]

Despite all the advances made in the treatment and management, still tuberculosis is a public health problem in Bangladesh with adverse social and economic consequences. Current recommendations for the control of tuberculosis emphasize case detection so as to allow treatment of patients and thereby limit the transmission of the bacilli. The mainstay for its control is the rapid and accurate identification of the infected individuals.[12] The estimated detection limit of microscopy is 10^4 bacilli/ml of sputum.[13]

A number of alternative diagnostic tests that use molecular and immunological methods have been developed. While molecular methods overcome the insensitivity of smear method and the time required for culture, they depend upon retrieval of a specimen from the site of infection and require a well-prepared laboratory and well-trained personnel. This simplest rapid method is the detection of acid-fast bacilli by microscopy. In developing countries, microscopy of sputum is by far the fastest cheapest and more reliable method for diagnosis of pulmonary tuberculosis. The estimated detection limit of microscopy is 10^4 bacilli/ml of sputum.

In case of immunocompromised patients like HIV infected cases has a major impact on tuberculosis on the pathogenesis of tuberculosis. It directly attacks the critical immune mechanisms involved in protection against tuberculosis. In the early stages of HIV infection, when CMI is only partially compromised, pulmonary tuberculosis presents typically as upper lobe infiltrates and cavitations with high bacillary load in the sputum, whereas in the late stages, primary tuberculosis like pattern with diffuse interstitial and infiltrates, little or no cavitation is seen resulting in paucibacillary picture of sputum. It makes more difficult to diagnose the cases though they are smear positive and infections and contribute substantially to the transmission of disease.

In this study on evaluation of the microscopic techniques by comparing them with the gold standard culture technique, it was found that only 06(4%) cases by ZN and 13(8.67%) cases by CFM, whereas LED detected 22(14.67%) "scanty" cases. These were detected as paucibacillary cases, in which 16 cases were missed by ZN and 09 cases were missed by CFM. This proves that AO stain examined by LED is a better method for its close comparability to the gold standard technique. These were almost comparable with other several studies.[14-18]

Sputum culture is widely regarded to be the most sensitive and specific test for the detection of pulmonary TB, but its routine use in resource-limited settings is hampered by excessive cost, slow turnaround times, and the need for adequate laboratory infrastructure. In practice, improvements in direct sputum sample evaluation that result from improved sensitivity and/ or improved access to decentralized diagnostic services remain highly relevant.

In conclusion, immunocompromised patients like HIV co-infected with TB posses paucibacillary picture due to little or no cavitation. The need for rapid smear results and effective treatment of the most infection TB cases remains paramount. The efficacy of LED fluorescence microscopy proved to be much higher than conventional fluorescent

microscopy and bright field microscopy and comparable to that of culture. In this study, Auramine O (AO) stained sputum smear has been found to improve significantly the sensitivity, predictive value of negative test, percentage of false negative and efficiency. So, LED microscopy of sputum by AO staining can be used effectively along with ZN stain for the diagnosis of pulmonary tuberculosis instead of doing difficult and time consuming culture method in the peripheral health centre of our country.

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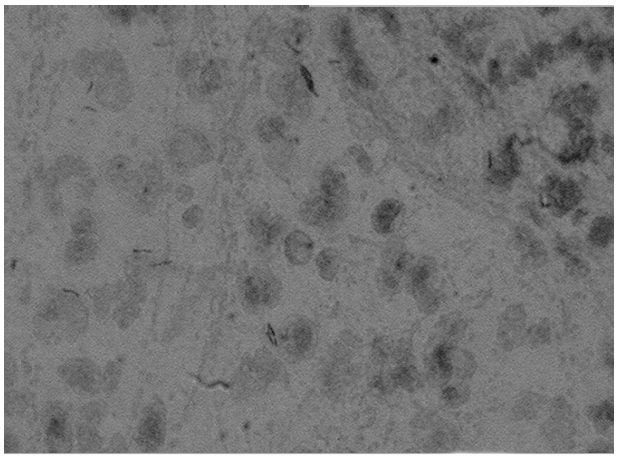

Fig-1: Photomicrograph of Ziehl-Neelsen stain sputum smear showing TB bacilli (AFB +++) (x1000)

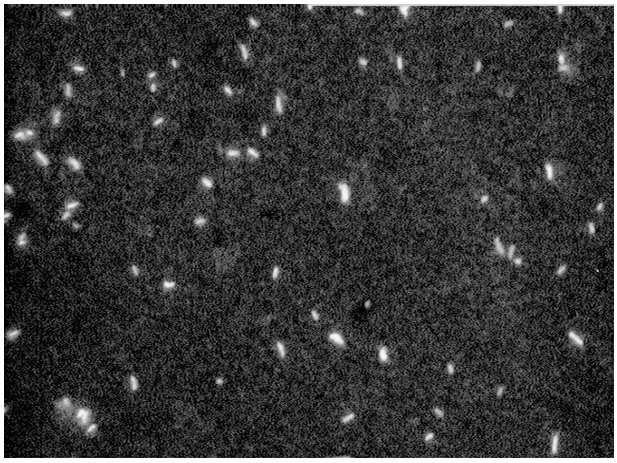

Fig-2: Photomicrograph of Auramine O stain sputum smear showing TB bacilli (AFB +++) by Conventional Fluorescent Microscopy (x200)

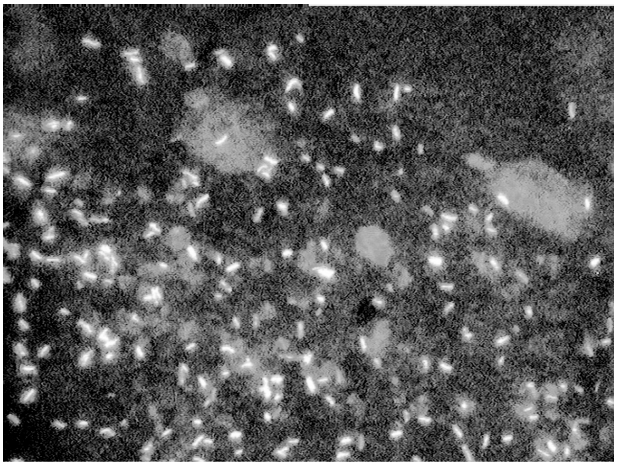

Fig-3: Photomicrograph of Auramine O stain sputum smear showing TB bacilli (AFB +++) by Light Emitting Diode (LED) Fluorescent Microscopy (x200)

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