

Role of Light Emitting Diode (LED) Fluorescence Microscopy in the Diagnosis of Smear Negative Pulmonary Tuberculosis.

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Abstract

Light emitting diode (LED) fluorescence microscopy offers well described benefits compared with brightfield microscopy by Ziehl-Neelsen stained sputum, even which are smear negative. We evaluated the diagnostic performance of fluorescence microscopy, using novel light-emitting diode (LED) technology as an alternative to the brightfield microscopy. The objective of this study was the role of LED fluorescence microscopy in diagnosis of smear negative pulmonary tuberculosis. This is a prospective study consisted of 50 smear negative patients, who were clinically suspected cases of pulmonary tuberculosis. All samples were stained by both ZN stain and Auramine stain and as a gold standard all were cultured on Lowenstein-Jensen Media. On evaluation of all sputum samples were found negative by ZN method but by auramine stain 16%, 20%, 20% cases were found positive by conventional fluorescence microscopy (CFM), LED and culture respectively. LED fluorescence microscopy is more useful test to distinguish the smear negative cases. It also provide an effective guideline to make decisions regarding judicious use of antitubercular drug therapy.

Keywords: Smear negative Sputum, LED, CFM, Culture, Pulmonary Tuberculosis

Introduction

Tuberculosis is one of the most significant health problems in developing countries like Bangladesh. The condition causes more preventable deaths than any other infectious disease according to DGHS, WHO estimation, nearly 2.14% of the population became infected every year¹. 300,000 progress to disease and at least 70,000 people die of TB each year. Incidence and prevalence of tuberculosis in Bangladesh are 225/100,000 per year and 391/100,000 per year respectively². It remains world's leading cause of death from a single infectious agent³. It is also a major public health problem in Bangladesh and ranks sixth among the 22 highest burden tuberculosis countries in the world⁴.

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 importance⁵. Direct microscopy for AFB is widely used method for diagnosis and confirmation of pulmonary tuberculosis and when positive, defines the more infectious cases⁶. This method is highly

specific, faster and cheaper for detection of AFB in sputum. But, there are several drawbacks of this method. First of all, ZN stain has low sensitivity relative to fluorescent stain⁷ and culture⁸. Secondly, it takes more time to scan-at least 300 field. 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 important⁹.

Last of all, the new light emitting diode (LED) fluorescent microscope is cheaper and with more life expectancy than Conventional fluorescent microscope (CFM). LEDs excite auramine without producing UV light¹⁰. It also produces minimal heat 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 battery¹¹. 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

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country. Therefore, it will be generally accepted that the LED fluorescent method should be given preference over the ZN methods.

Materials and Methods

This Prospective study was carried out in the Department of Clinical pathology, Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University (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.

50 patients who were smear negative

Pulmonary smear-negative TB (PTB-) can be defined as

* A patient with symptoms suggestive of TB with three sputum specimens negative for AFB; and

* Persisting symptoms after a course of antibiotics; and

* Again three negative sputum specimens for AFB during repeat sputum examination; and

* Chest X-ray abnormalities consistent with active TB and

* Diagnosis made by a physician

Results

Out of 50 patients, all are found ZN negative cases. But 08 (16%) cases were found positive by conventional fluorescent microscopy and 10(20%) were found positive by both LED microscopy and culture.

Table 1

Results of ZN stained microscopy, Conventional fluorescent microscopy (CFM), LED microscopy and culture of sputum for diagnosis of pulmonary tuberculosis (N=50)

Finding	ZN	CFM	LED	Culture for mycobacterium
n=50				
Positive	00(00)	8 (16)	10 (20)	10 (20)
Negative	50(100)	42 (84)	40 (80)	40 (80)

Figure in parenthesis indicate percentage

Table II

Sensitivity, specificity, accuracy and positive and negative predictive values of the LED, CFM and Z-N for identification of pulmonary tuberculosis in Smear negative cases (N=50).

Validity test	Smear negative (n=50)		
	LED	CFM	Z-N
Sensitivity	80.0	60.0	-
Specificity	95.0	95.0	100.0
Accuracy	92.0	88.0	80.0
PPV 80.0	75.0	-	-
NPV 95.0	90.5	80.0	-

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 country⁴. Nearly one-third of the global population, i.e. two billion people, is infected with Mycobacterium tuberculosis and thus at risk of developing the disease. More than nine million people develop active TB every year and about two million die.

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 individuals¹².

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. The 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 sputum¹³.

In this study on evaluation of the microscopic techniques by comparing them with the gold standard culture technique, it was found that in case of ZN stain there was agreement in 80% cases and disagreement in 20% whereas for Auramine-O(AO) stain there was agreement in 100% cases by LED

with culture. In this study by brightfield microscopy with ZN stain 10(20%) cases were missed and detected as false negative. On the other hand by auramine stain conventional fluorescent microscopy missed only 2(4%) and the new lumin attached LED fluorescence microscope detected same cases (20%) as for culture, which was gold standard. 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 studies¹⁴⁻¹⁸.

Sputum culture is widely regarded to be the most sensitive 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.

However, 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 cultures method in the peripheral health centre of our country.

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