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**Original Article**

# Comparison between Light Emitting Diode (LED) Fluorescence Microscopy and Conventional Light Microscopy in the Diagnosis of Pulmonary Tuberculosis

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**Abstract:**

**Background:** Diagnosis of pulmonary tuberculosis is confirmed by sputum microscopy. Sputum can be stained by Ziehl-Neelsen (Z-N) staining and examined by conventional light microscopy. Again it can also be stained with Auramine O stain and examined by light emitting diode (LED) fluorescence microscopy. This study was planned to find the most sensitive, specific and feasible technique for the diagnosis of pulmonary tuberculosis.

**Materials and Method:** This cross-sectional analytical study was conducted in the department of Medicine in collaboration with the department of Microbiology, Sylhet M.A.G. Osmani medical college, Sylhet from 1st January, 2019 to 30th June, 2019. All clinically suspected patients with pulmonary tuberculosis attending both outpatient and inpatient department of Medicine Sylhet M.A.G Osmani medical college hospital, Sylhet during the study period were the study population. Total 380 patients were recruited as study sample after fulfilling the inclusion and exclusion criteria by purposive sampling method. All the patients were referred to department of Microbiology, Sylhet M.A.G Osmani medical college for sputum for AFB examination. All the samples were divided in to two portion and then one portion was marked as group-A- and another portion as group-B. In group-A, conventional Ziehl Neelsen (Z-N) staining with light microscopy and in group-B, Auramine staining with LED fluorescent microscopy were done.

**Result:** Among 380 patients, 47 (12.4%) patients and 52(13.7%) patients were diagnosed as pulmonary tuberculosis by Z-N method and LED fluorescence microscopy respectively but this difference was not significant (*Z=-0.532; p>0.05). Paucibacillary (scanty and 1+) cases were observed more in LED 34 (8.9%) method in comparison to Z-N 31 (8.2%) method. But this difference again did not reach the level of significance (*Z=0.345; p>0.05). But the time required to read the smear by LED method (3.01 ± 0.27 minutes) was significantly shorter than that of Z-N method (6.30 ± 0.33 minutes) (t=561.146; *p<0.001).

**Conclusion:** LED fluorescence microscopy is better than conventional light microscopy in consideration of time taken to finalize result.

**Keywords:** LED fluorescence microscopy, Light microscopy, Pulmonary tuberculosis.

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## Introduction:

Tuberculosis (TB), a potentially fatal contagious disease, caused by Mycobacterium tuberculosis complex, is preventable and curable.[1]

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Along with Mycobacterium tuberculosis it may be occasionally caused by other species of Mycobacterium tuberculosis complex including M. africanum , M. canettii, M. bovis, M. caprae, M. microti, and M. pinnipedii. All are considered as acid fast bacilli (AFB), non-spore forming and non-motile slow growing bacterium.[2] According to World Health

Organization (WHO) report of Bangladesh in 2015 the annual estimated incidence for all cases is 225 per 100,000 populations per year. The prevalence (all cases) is estimated to be 434 per 100,000 populations. The estimated TB mortality is 45 per 100,000 population per year.[3] Pulmonary variety is the commonest form of tuberculosis and occurs in about 80% of cases.[4] Sputum smear microscopy is considered as the most reliable diagnostic test available even in areas having large number of cases and financial constraints. Fluorescent microscopy enhances the diagnostic ability in sample having a low density of acid fast bacilli, which is at times missed by Ziehl-Neelsen (Z-N) staining. Auramine O staining is advantageous over Z-N staining as it is simpler, cost effective and can be visualized even at low magnifications than Z-N (40x/100x). Z-N staining has high specificity but fluorescent microscopy is more sensitive and time taken for the examination of the slide with fluorescent microscope is quiet less as compared to with Z-N staining. Fluorescent microscope may take up to 75% less time than a conventional microscope.[5] However, the implementation of this technique was difficult due to higher cost associated with purchase of the microscope with a mercury vapor lamp. Replacement of mercury vapor lamp with LED illumination system decreases the cost and increases the life span.[6] In Bangladesh Ziehl-Neelsen staining with light microscopy is the most widely used method for the diagnosis of presumptive TB patients. Now Auramine staining with fluorescence microscopy is replacing conventional Z-N method in some centers for detection M. tuberculosis in sputum samples. But there are limited data regarding the sensitivity, specificity, feasibility and cost effectiveness between two methods of smear microscopy in context to our country. There is only one cross sectional study which 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.[7] However, there has been no such comparative study so far to address this issue at Sylhet M.A.G. Osmani medical college hospital, Sylhet. So, this study was planned to help to find the most sensitive, specific and feasible technique for the diagnosis of pulmonary tuberculosis and thus reduce the morbidity and mortality regarding tuberculosis.

**Materials and Method**

This cross-sectional analytical study was conducted in the department of Medicine, Sylhet M.A.G. Osmani medical college, Sylhet in collaboration with the department of Microbiology, Sylhet M.A.G. Osmani medical college, Sylhet from 1st January, 2019 to 30th June, 2019. All clinically suspected patients of pulmonary tuberculosis attending both outpatient and inpatient department of Medicine of Sylhet M.A.G Osmani medical college hospital, Sylhet during the study period were the study population. Clinically suspected pulmonary tuberculosis patient having history of cough with expectoration for more than 2 weeks, low grade fever with or without weight loss, anorexia, haemoptysis ± radiological evidence of pulmonary tuberculosis were the inclusion criteria. Cases of suspected extra pulmonary tuberculosis and those below 18 years of age were excluded from the study. Purposive sampling method was applied in selecting sample and total 380 cases were taken as study sample. Informed written consent was taken from each patient or guardians before taking any interview. An approval of the study protocol was obtained from institutional ethical committee of Sylhet M.A.G Osmani Medical College, Sylhet before commencement of the study.

All the patients were referred to department of Microbiology, Sylhet M.A.G Osmani Medical College for sputum for AFB examination. Sputum samples were collected from all the patients 1st time on spot and 2nd time on coming morning. All the samples were divided in to two portion and then one portion was marked as group-A and another portion as group-B. In group-A, conventional Ziehl Neelsen staining with light microscopy and in group-B, Auramine staining with LED fluorescent microscopy were done. The results were recorded using Grading chart [8] and average time for the procedure including preparation of slide, examination and detection of negative case was recorded. Relevant data from history, physical

examination and investigations was recorded in predesigned questionnaire. Data was processed manually and analyzed with the help of SPSS (Statistical package for social sciences) version 22.0. Quantitative data were expressed as mean and standard deviation; and comparison was done by paired t test.

Qualitative data was expressed as frequency and percentage thus comparison carried by Z-test of proportion. A probability value (p) of less than 0.05 was considered to indicate statistical significance.

**Results:**

Table-1 shows that out of 380 patient's majority were female that is 192 (50.5%) and rest 188 (49.5%) were male. In both the groups, the highest 62(33%) and 69 (35.9%) patients belonged to 31-40 years age group. Table-2 shows that among 380 patients the highest 380 (100.0%) were suffering from low grade fever and cough with expectoration for more than 2 weeks. Table-3 shows that scanty AFB was found in 9(2.4%) cases, 1+ in 22 (5.8%), 2+ in 11(2.9%), 3+ in 5(1.3%) and negative in 333(87.6%) cases by Z-N method. Table-4 shows that scanty AFB was found in 6 (1.6%) cases, 1+ in 28 (7.4%), 2+ in 12(3.2%), 3+ in 6 (1.6%) and negative in 328 (86.3%) cases by LED fluorescent microscopy. Figure-1 shows that among 380 patients total 47 (12.4%) patients were diagnosed as pulmonary tuberculosis by Z-N method; whereas using LED fluorescence microscopy 52 (13.7%) patients were diagnosed as pulmonary tuberculosis. Though case detection by LED fluorescence microscopy was higher than that of Z-N methods but did not reach the level of significance (*Z=-0.532; p>0.05). Figure-2 shows paucibacillary (scanty and 1+) cases were observed more in LED 34 (8.9%) method in comparison to Z-N 31 (8.2%) method. Though paucibacillary case detection by LED fluorescence microscopy was higher than that of Z-N methods but did not reach the level of significance (*Z=0.345; p>0.05). Figure-3 shows that among 380 patients, the average time spent to finalize results of group A (Z-N method) was 6.30 ± 0.33 minutes whereas the same for group B (LED method) was 3.01 ± 0.27 minutes. Here it was evident that the time required to read the smear by LED method is significantly shorter than that of Z-N method (t=561.146; *p<0.001).

**Tables and Figures**

**Table-1: Distribution of suspected patients according to age (n=380)**

| Age | Male (n=188) | Female (n=192) | Total (n=380) |
|---|---|---|---|
| 18 – 30 years | 46 (24.5%) | 49 (25.57%) | 95 (25.0%) |
| 31 – 40 years | 62 (33.0%) | 69 (35.9%) | 131(34.5%) |
| 41 – 50 years | 43 (22.9%) | 37 (19.3%) | 80 (21.1%) |
| 51 – 60 years | 12 (6.4%) | 15 (7.8%) | 27 (7.1%) |
| >60 years | 25 (13.3%) | 22 (11.5%) | 47 (12.4%) |
| Mean | 41.43 ± 15.19 | 40.71 ± 14.62 | 41.07 ± 14.89 |

**Table-2: Distribution of suspected patients according to clinical features (n=380)**

| Clinical features | Frequency | Percentage |
|---|---|---|
| Symptoms: | | |
| Cough with expectoration (>2 weeks) | 380 | 100.0 |
| Low grade fever | 380 | 100.0 |
| Weight loss | 213 | 56.1 |
| Loss of appetite | 139 | 36.6 |
| Haemoptysis | 26 | 6.8 |
| Radiology: | | |
| X-ray findings of PTB | 141 | 37.1% |

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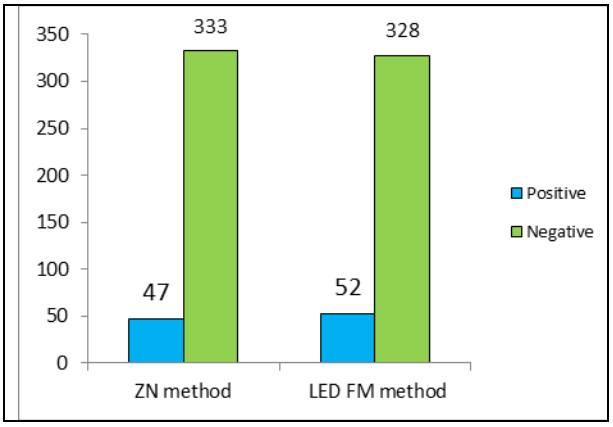
**Table-3: Detection of AFB on sputum examination by Z-N microscopy (n=380)**

| AFB by Z-N microscopy | Frequency | Percentage |
|---|---|---|
| Scanty | 9 | 2.4 |
| 1+ | 22 | 5.8 |
| 2+ | 11 | 2.9 |
| 3+ | 5 | 1.3 |
| Negative | 333 | 87.63 |

**Table-4: Detection of AFB on sputum examination by LED fluorescent microscopy (n=380)**

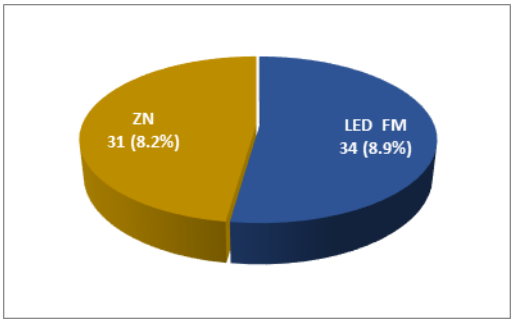
| AFB by LED fluorescent microscopy | Frequency | Percentage |
|---|---|---|
| **Scanty** | 6 | 1.6 |
| **1+** | 28 | 7.4 |
| **2+** | 12 | 3.2 |
| **3+** | 6 | 1.6 |
| **Negative** | 328 | 86.31 |

**Figure-1: Distribution of patients according to the confirmatory diagnosis of pulmonary tuberculosis by Z-N and LED method (n=380)**



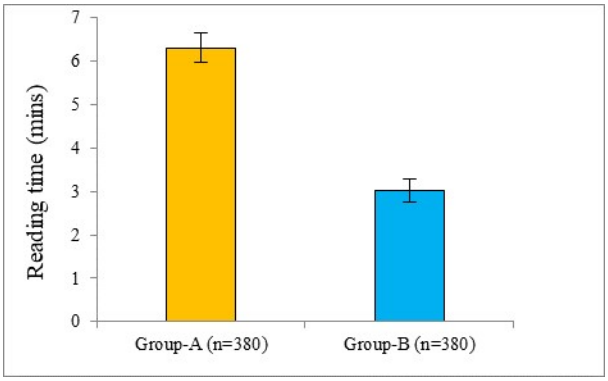

*Z-test of proportion was applied to analysed the data. Though case detection by LED fluorescence microscopy was more than that of Z-N methods but did not reach the level of significance (*Z=-0.532; p>0.05).

**Figure-2: Comparison of paucibacillary case detection by Z-N and LED method (n=380)**




*Z-test of proportion was applied to analysed the data. Though paucibacillary case detection by LED fluorescence microscopy was more than that of Z-N methods but did not reach the level of significance (*Z=0.345; p>0.05)

**Figure-3: Mean time required to read the smear (n=380)**




*p-value was calculated by paired t test. Here it was evident that the time required to read the smear by LED method is significantly shorter than that of Z-N method (t=561.146; *p<0.001).

**Discussion:**

Current recommendations for the control of tuberculosis emphasize early case detection so as to allow treatment of patients and there by limit the transmission of the bacilli. In developing countries, microscopy of sputum is by far the fastest, cheapest and more reliable method for diagnosis of pulmonary tuberculosis.[9] LED based fluorescent microscopy with Auramine stain takes less time in comparison to conventional microscopy with Z-N stain. Even small number of organisms in the smear can be picked up by the LED based

fluorescent microscope. In Z-N staining method, we have to search for the bacilli carefully, but in fluorescence staining because of fluorescence light bacilli are recognized quickly. Additional advantage is that colour blind person can also use the LED based fluorescent microscopy method without difficulty.[10,11] Number of sputum samples collected and methodology of collection exerts its influence on the rate of isolation. Seyoum et al.[12] have used three consecutive samples while Narang et al.[13] used two samples. In our study we have used two samples, one spot and another coming morning sample.

In our study, 380 suspected patients of pulmonary tuberculosis were enrolled. Among them 50.5% and 49.5% were female and male respectively. This findings are comparable to a previous study.[4] In our study majority of the patients belonged to the age group 31-40 years where it was 131 (34.5%) cases. These data were supported by a previous Ethiopian study where they showed highest 86(35.89%) out of 248 patients belonged to similar age group.[4] This indicates that in both developing and underdeveloped countries middle aged population are affected more by tuberculosis.
Our study findings showed that the Z-N light microscopy determined 12.4% positive cases whereas LED FM method determined 13.7% positive cases. Here LED FM method showed better efficacy than Z-N light microscopy but did not reach the level of significance ($p>0.05$). Similar findings, were also observed in other previous studies.[13] The smears stained by Z-N method can detect bacilli when the concentration of bacilli is $10^5$/ml of sputum, whereas a more sensitive staining technique like FM stain detects the bacilli when the bacillary load is $10^4$/ml of sputum.[14]

In this study, we have observed that the average time required to read the smear by LED method is approximately two times shorter than of Z-N method which was statistically significant ($p<0.001$). This finding was agreed by a previous study where they also found LED method required far shorter time to read a smear than that of Z-N method which was also statistically significant ($p=0.01$).[15] Using fluorescent microscopy, the tubercle bacilli when examined under ultra-violet illumination, the bacilli appeared as a bright rod against a dark back ground. Since there was a contrast, the bacilli were readily seen and therefore in very less time large area could be examined with less eye-strain.[16]

In our study by using LED fluorescent microscopy, out of 380 patients 52 was found to be positive of whom 6 patients, 28 patients, 12 patients and 6 patients were scanty, 1+, 2+ and 3+ respectively. On the other hand by using Z-N microscopy, out of 380 patients 47 was found to be positive of whom 9 patients, 22 patients, 11 patients and 5 patients were scanty, 1+, 2+ and 3+ respectively. Minion et al.[13] and Marais et al.[17] reported LED fluorescent microscopy was very effective in identification of scanty and 1+ cases than Z-N microscopy. Similar findings were also observed in our study that paucibacillary cases detection was higher by LED FM method. In fact, 34 (8.9%) cases were diagnosed as paucibacillary by LED FM method whereas 31 (8.2%) paucibacillary cases were diagnosed by Z-N method.

This study shows that LED based fluorescent microscopic staining of sputum is a better method for detecting tubercle bacilli than conventional microscopy with Z-N staining. As our study was done on less number of specimens so it failed to prove statistically significant differences. But it can be safely said that LED microscopy offers a good alternative with better performance to ZN microscopy. Ideally both the staining procedures should be done on the same smear for comparison. Doing both the staining procedures on the same smear (i.e. Z-N staining after Auramine-O staining) can affect the result of the staining procedure as seen in previous studies.[16,17] However, in our study we used different slides for the both the Ziehl- Neelsen staining and LED fluorescence staining in spite of the fact that different smears made from same sample could be variable.

**Conclusion:** This study revealed that case detection by LED fluorescence microscopy was non-significantly more than that of ZN methods ($p>0.05$). Paucibacillary (scanty and 1+) case

detection was also non-significantly higher in LED method compared to ZN method ($p>0.05$). But the mean time required to finalized result was significantly shorter in LED method than that of ZN method ($p<0.001$). In conclusion Light-Emitting Diode Fluorescence Microscopy (LED-FM) is a better option in detecting tubercle bacilli in sputum of suspected of pulmonary tuberculosis.

**Limitations of The Study**

1. It was a cross-sectional study.
2. This was a single centered study.
3. Duration of study was short.
4. Sample size was small.
5. Sensitivity and specificity could not be determined due to absence of culture facilities in this center.
6. Does not proclaim the scenario of whole country.

**Recommendations**

1. Light-Emitting Diode Fluorescence Microscopy (LED-FM) method of sputum examination has been advocated to be method of choice where the large number of sputum smears are to be examined.
2. LED FM method of sputum examination should be adopted in all DOT centers for effective diagnosis of pulmonary tuberculosis
3. However a multicenter study involving larger sample size should be conducted to reach a justifiable conclusion and recommendation

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