A Comparative Study on the Accuracy of Light Emitting Diode Fluorescent Microscopy and Conventional Sputum Smear Microscopy in the Diagnosis in Patients with Pulmonary Tuberculosis

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Abstract: 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 bacilli. Ziehl Nelson( ZN) stain can detect bacilli when they are in the order of 10^5/ml of the sputum, where as a more sensitive Auromine O (AO) stain can detect in the order of 10^4/ml of sputum. In the present study out of the 500 samples examined, 55(11%) and 85(17%) TB cases were detected by ZN and AO staining methods respectively.

Keywords: pulmonary tuberculosis, LED microscopy, conventional microscopy, Ziehl Nelson’s stain, AO stain.

INTRODUCTION
Tuberculosis continues to be the world’s most important infectious cause of morbidity and mortality among adults. The best estimate is that there were 1.7 million TB deaths in 2016, including 0.4 million deaths resulting from TB disease among HIV-positive people [1]. For developing countries with a large number of cases and financial constraints, evaluation of rapid and inexpensive diagnostic methods has a great importance.

- A study conducted by Ba F, Rieder HL et al. [3] showed Concordance was 96.9% and 92.3% for diagnostic and follow-up examinations, respectively. The yield was similar with both techniques for specimens with at least 10 bacilli per 100 fields, but higher with fluorescence microscopy in those with fewer than 10 bacilli per 100 fields. The mean time required by fluorescence microscopy before declaring a slide as negative with the same magnification was 3 minutes 34 seconds, compared to 7 minutes 44 seconds with the Ziehl-Neelsen technique.
- In a study conducted by Jai Kishan Karahyla, Kamlesh Kumari and Pritpal KauAtwal [4], ZN staining and LED- Fluorescent Microscopy (FM) both samples were positive in 98.48% cases and 96.24% cases respectively, however Sputum with ZN staining and LED-FM staining showed very narrow difference respectively. But the present study showed a significant advantage of led microscopy in diagnosing PTB cases, over ZN microscopy.
- In study conducted by Hooja S, Pal N, Malhotra B, Goyal S, Kumar V, Vyas L [5] by title Comparison of Ziehl Neelsen & Auramine O staining methods on direct and concentrated smears in clinical specimens. The results are Using culture as the reference method; the sensitivity of direct staining was 55.55% for ZN and 71.85% for AO. Direct fluorescent microscopy detected 9.29%
paucibacillary sputum samples that were missed on ZN staining. On concentration, the sensitivity increased by 6.67% for ZN and 11.11% for AO. Whereas culture as a standard reference was not made in our study.

OBJECTIVES
To compare the efficacy of light emitting diode fluorescent microscopy (LED) over conventional sputum smear microscopy in diagnosing Mycobacterium tuberculosis, in 500 clinically suspected tuberculosis cases for early detection of paucibacillary cases of pulmonary tuberculosis.

MATERIALS AND METHODS
This comparative study was conducted in the department of respiratory medicine in government chest diseases and tuberculosis hospital, hanamkonda. A total of 500 sputum samples were collected from patients of presumptive pulmonary TB, according to the following inclusion and exclusion criteria. Detailed history of those patients was recorded. Thorough clinical examination was done. Ethical clearance was obtained from Kakatiya Medical College Ethical Committee.

Inclusion criteria
• All patients with symptoms suggestive of pulmonary tuberculosis with or without past history of pulmonary tuberculosis
• Age group of 12yrs to 80yrs

Exclusion criteria
• Sputum mixed with blood
• Sputum mixed with food particles
• Sputum which is more of saliva

Methods
• During the study the spot and early morning sputum samples of patients those who come to the pulmonology department with the following complaints was collected
  A) Cough with sputum (more than 2weeks)
  B) Low grade Fever with evening rise
  C) Associated with or without loss of weight

Two sputum samples were collected on two consecutive days from each patient (One spot and one early morning sample) in clean, sterile, heat proof, wide mouth containers. The processing of samples was carried out in a biosafety cabinet. Each sample was then subjected to ZN staining and Fluorescent Auramine –O (AO) staining.

RESULTS AND DISCUSSION
• 85 patients were diagnosed as pulmonary tuberculosis by the AO stain and 55 patients were diagnosed as pulmonary tuberculosis by the ZN stain out of the 500 suspects.
• Total yield sputum positivity was 17% for AO stain and 11% for ZN stain in the diagnosis of pulmonary Tuberculosis.
• In this study proportion of the paucibacillary cases in the total positive cases is 18.1% (10 out of 55) by ZN stain and 41.1% (35 out of 85) by AO stain.
• AO staining with LED is more efficient over ZN stain in detecting Tuberculosis bacilli in sputum, especially in the diagnosis of paucibacillary PTB cases.

CONCLUSION
Hence our study concludes that AO staining with LED is more efficient over ZN stain in detecting Tuberculosis bacilli in sputum, especially the paucibacillary cases in the diagnosis TB. Also sensitivity of LED microscopy is greater than ZN staining.

REFERENCES
1. TB India. RNTCP annual status report. 2017