



DIAGNOSTIC EFFICACY OF FLUORESCENT COMPARED TO ZEHL NEELSEN (ZN) METHOD FOR MYCOBACTERIUM TUBERCULOSIS IN PULMONARY TUBERCULOSIS PATIENT AT A TERTIARY CARE CENTRE.

Microbiology

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ABSTRACT

Tuberculosis continues to be a major health problem in developing country and is the single largest cause of loss in healthy life year in the productive age group. There are various methods for bacteriological diagnosis of tuberculosis. Smear examination is believed to be simple, cheap, quick and practicable and effective case finding method for developing countries. The present study was undertaken to compare the diagnostic efficacy of fluorescent staining with Zeihl-neelsen(ZN) staining in the diagnosis of pulmonary tuberculosis. 150 cases of suspected pulmonary tuberculosis were included in the study. 300 sputum samples(2 from each patient) were screen for Acid Fast Bacilli (AFB) by Z-N & Fluorescent staining methods. Positive samples detected by fluorescent stain were 39(13%) when compared to Zn stain 26(8.66%).

Conclusion: Compared to Z-N stain (8.66%) fluorescent staining was found to be more efficient (13%) in AFB detection from cases of Pulmonary Tuberculosis.

KEYWORDS

Pulmonary tuberculosis, fluorescent stain, Zeihl-neelsen(ZN) stain, Acid Fast Bacilli.

INTRODUCTION

Rapid identification and treatment of new cases is the keystone of tuberculosis control worldwide.⁽¹⁾ Microscopical diagnosis of Mycobacterium tuberculosis occupies the highest priority in tuberculosis control, which also forms a part of follow up of treatment.⁽²⁾

In developing countries like India, microscopy of the specimen obtained from suspected tuberculosis cases is by far the fastest, cheapest, and most reliable method for the detection of AFB. Most commonly used methods include Zeihl Neelsen (ZN) staining and fluorescent staining. A significant difference in the sensitivity of the two methods has been reported⁽²⁾.

ZN staining is the most extensively used procedure for the demonstration of Mycobacterium tuberculosis in smear.⁽³⁾ It is an inexpensive, rapid and highly specific but has poor sensitivity. Fluorescent microscopy has been proposed as a technique to increase the sensitivity of smear examination.⁽¹⁾ ZN stain can detect bacilli when they are in the order of 10^5 /ml of the sputum whereas a more sensitive fluorescent staining can detect in the order of 10^4 /ml of sputum⁽⁴⁾.

Fluorescent staining by Auramine is an alternative method of staining.⁽⁵⁾ It enters the wall of mycobacteria and makes them glow yellow white, rice like bacteria against dark background in UV light under low power objective of fluorescent microscope.⁽⁶⁾ fluorescent staining has been added in Revised National Tuberculosis Control Program (RNTCP) because of more sensitive and rapid results and its use in field areas⁽⁷⁾. The present study was aimed to check the efficacy of Fluorescent microscopy for diagnosis of pulmonary tuberculosis in comparison to Ziehl-Neelsen staining and culture.

MATERIALS AND METHODS^(8,9,10)

This comparative study was conducted in the Department of Microbiology, Dr V M G M C Solapur Maharashtra, India, from May 2017 to July 2017. Patients attending OPD and having cough for more than 3 weeks with sputum, fever, night sweats, loss of appetite, loss of weight, chest pain, haemoptysis and/ or radiological evidence of tuberculosis were included in study.

Sample collection:

One early morning and one spot sputum samples were collected from each patient in leak proof, wide mouth containers. Instructions were given to patients before collection. The processing of the sample was carried out in a biosafety cabinet. Each sample was subjected to Ziehl-Neelsen (ZN) staining and fluorescent Auramine-O (AO) staining along with culture on Lowenstein-Jensen media.

For ZN staining, slide was seen under 100x magnification of light microscope, scanning at least 100 oil immersion fields before reporting a smear as negative. Grading of smear was done according to RNTCP guideline.

For Auramine O staining, slide was observed under 40 x magnifications of fluorescent microscope⁽¹¹⁾ and smear was graded as per the International Union against Tuberculosis guidelines.⁽¹²⁾

If both the samples were positive on staining then any one sample was subjected to culture on LJ medium and incubated at 37°C.

If both the samples were negative then both samples were cultured.

Even if one is positive and one is negative both the samples were cultured

All culture on L J medium were incubated at 37°C and were examined for growth after 4 days (for rapidly growing Mycobacteria, fungi and contaminant bacteria) and at least twice weekly thereafter. A negative report was given if no growth was seen after 8-12 weeks. Any growth seen is smeared and tested by ZN staining.

RESULT:

During the study period of 3 months a total of 150 patients suspected of having pulmonary tuberculosis based on history, clinical examination and chest x-ray were included in the study. 48 (32%) participants were females and 102 (68%) were male.

Out of 300 sputum samples, the smear positivity for AFB on the conventional ZN method was 8.66% (26 /300) while the positivity increased to 13% (39 /300) on the fluorescent staining. Out of 300 samples, 43 were culture positive, 26 samples were both fluorescent and ZN positive and 13 samples were Fluorescent positive and ZN negative. Among 300 sample, 261 were both fluorescent and ZN negative, Whereas none of the sample was ZN positive and fluorescent negative. Table no 2 shows that scores are definitely higher by fluorescent microscopy positivity (39) compared to ZN staining positivity (26).

Table 1: Comparison of smear examination result by Ziehl-Neelsen (ZN) staining and fluorescent staining

	ZN- POSITIVE	ZN- NEGATIVE	Total
Fluorescent positive	26	13	39
Fluorescent negative	0	261	261
Total	26	274	300

Table 2: Grade wise Correlation of ZN staining and fluorescent staining.

Grading	ZN Positive	FLOURESCENT Positive
Scanty	2	7
1+	4	9
2+	8	11
3+	12	12
Total	26	39

DISCUSSION:

On evaluation of the microscopic techniques by comparing them with the gold standard culture technique, it was found that the positivity of ZN (8.66 %) was much lower than that of fluorescent staining (13 %) which gives evidence that large number of cases missed out by ZN stain were easily detected by fluorescent staining.⁽¹³⁾ Results of present study were in agreement with other studies.^(14,15,16) According to our study, fluorescent staining was a more sensitive method of microscopic demonstration of AFB in sputum specimen, compared to Ziehl-Neelsen staining. Similar findings was reported by Steingart et al.⁽¹⁷⁾

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 Ziehl Neelsen staining as ZN stain can detect bacilli when they are in the order of 10^5 /ml of the sputum whereas a more sensitive AO stain can detect in the order of 10^7 /ml of sputum⁽⁶⁾. Which gives evidence that a large number of cases missed out by ZN stain are easily detected by fluorescent staining⁽¹³⁾

Fluorescent stain was more efficient over ZN stain in detecting tubercle bacilli in sputum as screening was done under low power of magnification (400X) due to which it take less time compared to ZN method were screening was done on oil immersion (1000X) objective. The average reading time taken to evaluate a slide using LED-Fluorescent microscope represented 38% of the time required by conventional ZN staining.⁽¹⁷⁾ The 62% time saving in smear reading could be related to the lower magnification lens used for LED-FM⁽¹⁸⁾ and the quicker scanning of each field as a result of the better visibility of the yellowfluorescent rods against contrast.⁽¹⁹⁾ Studies have shown that a significant number of ZN smears are reported as 'negative' due to insufficient examination time. Recommended time for ZN smear evaluation in busy laboratories is around 5–10 min.⁽²⁰⁾ The reduction in examination time observed with LED-FM could therefore be of tremendous benefit for overburdened laboratories in TB-endemic countries.

Present study reveals that sputum stained by the florescent method is useful and reliable for pulmonary tuberculosis but fluorescent microscopy is costly some laboratories cannot afford to buy it, so in these laboratories Ziehl-Neelson staining is most employed⁽²¹⁾

CONCLUSION

Fluorescent Microscopy was the best method for rapid diagnosis of Pulmonary Tuberculosis as it is sensitive and less time consuming than conventional Ziehl-Neelson method.

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Conflict of interest: None**REFERENCES**

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