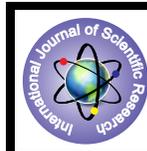


A Study of The Diagnostic Yield of Fluorescent Staining Techniques in The Diagnosis of Pulmonary Tuberculosis At Rohilkhand Medical College and Hospital, Bareilly



Medical Science

KEYWORDS :

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Introduction :-

Pulmonary Tuberculosis is a major Public health problem affecting all the sections of the society .Sputum smear microscopy is the most widely used and rapid test taken for the diagnosis of pulmonary tuberculosis and is now considered as the backbone of the RNTCP .The bacilli in the sputum can be detected by the ZiehlNeelsen or Fluorescent staining technique .

In developing countries like ours with a large number of tuberculosis (TB) cases and limited resources, the diagnosis of TB relies primarily on smear microscopy for Acid Fast Bacilli (AFB) but its sensitivity is limited in paucibacillary cases¹.

The sputum culture is a better considered diagnostic modality with far better sensitivity and specificity but the problem arises in the time consumed for the AFB bacilli to grow on the culture which stands upto 6 weeks. Where workloads are high and the amount of time spent examining smears is low, sensitivity is correspondingly low.

Laboratory infrastructure development is urgently needed, as is the development of more sensitive and rapid TB diagnostics more suitable for peripheral settings. Realizing this fact ,many of researchers have aimed to improve the performance of smear microscopy through new technology and service delivery approaches.it has now been proved that fluorescent stain performs better and allows the detection of more positive smears². The use of LED-FM significantly increased the proportion of smear positive cases among presumptive TB patients under routine program conditions in high workload laboratories⁸.

For developing countries with a large number of cases and financial constraints, evaluation of rapid and inexpensive diagnostic methods has great importance. The bacilli in the sputum can be detected microscopically by ZN stain and fluorochrome stain³.

Auramine staining of sputum smears in comparison to that of ZN staining is a better method of sputum microscopy for demonstration of AFB in sputum specimens. Fluorescence microscopy is relatively more sensitive and has the added advantage of allowing a large number of sputum specimens to be examined in a given time, in laboratories equipped with a fluorescent microscope.⁴Fluorescence microscopy is more sensitive than conventional microscopy.⁵ .Presently, the LED-based fluorescent microscopy technique has been established as the best alternative to ZN-staining. The modifications in sample collection, sample processing methodology and staining techniques can further expand the base of this age-old diagnostic modality⁹.

This study aimed to assess the accuracy of LED-FM for the diagnosis of TB compared to ZN microscopy in a high TB prevalence country at a tertiary health centre.

Materials and Methods :-

The study was conducted on patients visiting Department of Pulmonary Medicine at OPD / IPD clinic in the Bareilly district.Staining was done in the DOTS/ Microscopy centre under the Department of Pulmonary Medicine ,Rohilkhand Medical College , Bareilly .The Institutional Ethical Committee permission was taken .A Comparative study was conducted in 6040 patients

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The smears were stained by Auramine O staining.Auramine O staining technique and smear grading were done as per RNTCP technical manual guidelines as mentioned in the table below.

RNTCP ZN staining grading (using 100x oil immersion objective and 10x eye piece)	Auramine O fluorescent staining grading (using 20 or 25x objective and 10x eye piece)	Reporting /Grading
>10 AFB/field after examination of 20 fields	>100 AFB/field after examination of 20 fields	Positive, 3+
1-10 AFB/field after examination of 50 fields	11-100 AFB/field after examination of 50 fields	Positive, 2+
10-99 AFB/100 field	1-10 AFB/ field after examination of 100 fields	Positive, 1+
1-9 AFB/100 field	1-3 AFB/100 fields	doubtful positive /repeat
No AFB per 100 fields	No AFB per 100 fields	Negative

RESULTS :-Out of 1400 Patients , 227 patients (16.2%) were found to be suffering from the Pulmonary Tuberculosis.

The mean age in 227 patients was 41years .The significance of the data was calculated using Chi's Square value , $\chi^2 = 1.238$.Out of the 227 positive patients.

	Fluorescent Staining
Sputum Grading	
1 Positive	39
2 Positive	43
3 Positive	82
Doubtful/Scanty	63
	227

Discussion :-

Despite recent advances, the smear microscopy remains the corner stone for the diagnosis of PTB in high burden countries like India.

Time taken to detect the AFB bacilli on the slide was reportedly three times faster with LED-FM (mean time = 1 min) compared to data on conventional ZN microscopy (mean time = 3 min).

Therefore, in programmatic conditions, the introduction of LED-FM would significantly reduce laboratory workloads and possibly allow better quality microscopy.

Fluorescent microscopy is the best method to detect cases of bacteriological positive cases of tuberculosis who are a menace to healthy population. WHO has also recommended the sputum microscopy examination to be gold standard in the diagnosis of pulmonary tuberculosis which is the cheapest, cost effective, less time consuming and confirmatory method.

RNTCP has also accepted the sputum microscopy staining method in the detection of new and old cases of pulmonary tuberculosis. Earlier all the sputum microscopy centres were using ZeihlNeelsen staining method but during the last few years the district TB control organization has provided the Fluorescent Microscopy, which has been a corner stone in the field of diagnosis of pulmonary tuberculosis.

Reza LW et al⁸ concluded that the use of LED-FM significantly increased the proportion of smear positive cases among presumptive TB patients under routine program conditions in high workload laboratories. In their study they found that the sputum smear positivity increased by 30% in the sites where the LED microscopy was used.

RituSinghal, Vithal Prasad Myneedu⁹ concluded that sputum smear microscopy for the diagnosis of pulmonary TB has stood the test of time. In resource-limited countries, microscopy will remain the primary means of microbiological diagnosis of TB for the foreseeable future. Presently, the LED-based fluorescent microscopy technique has been established as the best alternative to ZN-staining. The modifications in sample collection, sample processing methodology and staining techniques can further expand the base of this age-old diagnostic modality.

Laifangbam S, Singh HL, Singh NB, Devi KM, Singh NT³ found that Out of 102 patients, 44.1% and 71.6% were found positive by ZN and AO respectively. AO was found to be superior to ZN on several aspects. The difference in their case detection rates was statistically significant ($\chi^2 = 24.93$, $p < 0.001$). AO was also able to detect more paucibacillary cases than ZN. The percentage of false negative by AO staining was only 2.78% which was in sharp contrast to that of ZN (40.27%). The better case detection rates of AO over ZN were comparable to those found by several studies. The efficacy of fluorescence microscopy proved to be much higher than conventional light microscopy.

Singh NP, Parija SC⁴ concluded that fluorescence microscopy is more sensitive than conventional microscopy. Retrospective analysis of directly ZN-stained smears showed 55.6% sensitivity compared to the FM (72.8%), which is also in line with the superiority of fluorescent microscopy.

Hooja S, Pal N, Malhotra B, Goyal S, Kumar V, Vyas L.6 concluded that of 393 clinical samples Mycobacterial growth

was detected in 137 (35.77%) specimen. 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. The sensitivity of AFB smear microscopy increased by 27.41% and was statistically significant ($p < .001$) when both methods were combined. The specificity was 99.19% for both ZN and AO.

Prasanthi, K., Kumari⁷ concluded that the higher sensitivity of FI stain (45%) than that of ZN stain (29%) in detecting the paucibacillary cases associated with HIV co-infection was seen in their study.

The Fluorescent Microscopy found to be more effective in the diagnosis of cases of pulmonary tuberculosis.

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