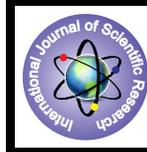


A Comparative Study of the diagnostic yield of Fluorescent and ZiehlNeelsen Staining Techniques with the reference to the diagnosis of Pulmonary Tuberculosis



Medical Science

KEYWORDS : Fluorescent Staining, Ziehl Neelsen Staining , Pulmonary Tuberculosis.

V.K. Tiwari	Professor, Deptt. of Pulmonary Medicine, R.M.C.H., Bareilly
Nishant Gupta	Resident 2nd year, Deptt. of Pulmonary Medicine, RMCH, BAREILLY
Sanjay Bansal	Professor & Head, Deptt. of Pulmonary Medicine, RMCH, BAREILLY
Dr. Ashok Agarwal	Professor, Deptt. of Pediatrics, RMCH, BAREILLY (U.P.)
Dr. Amit Kumar	Assistant Professor, Deptt. of Pulmonary Medicine, RMCH, BAREILLY (U.P.)
Dr. Ankit Khurana	Resident 3rd year, Deptt. of Pulmonary Medicine, RMCH, BAREILLY (U.P.)
Dr. Nadeem Akbar	Resident 3rd year, Deptt. of Pulmonary Medicine, RMCH, BAREILLY (U.P.)

ABSTRACT

Aim :- A Comparative Study of Efficacy of Fluorescent staining Technique in comparison to Ziehl Neelsen staining technique from the same sputum sample .

Materials and Methods :- A Comparative study was conducted on 3020 patients submitting 6040 samples who were suspected of Pulmonary Tuberculosis by the Ziehl Neelsen and Auramine O staining technique respectively.

Results :- Out of 1102 positive patients, 222(3.6%) patients were missed by ZN staining technique, who were detected by Auramine O staining method. Of the 222 patients missed by ZN staining method , 58 of them were scanty positive , 62 were 1+ and 71 were 2+ and 31 cases were 3+.

Conclusion :- However Auramine O is superior to Ziehl Neelsen on several aspects - It is Less time consuming, More slides can be processed in a less time and is more accurate.

Introduction:

Pulmonary Tuberculosis is considered to be a major public health problem worldwide .Sputum smear microscopy is the most widely preferred and rapid test that is widely used for the detection and diagnosis of pulmonary tuberculosis .The bacilli in the sputum can be detected either by either ZiehlNeelsen or Fluorescent staining technique .

In the poor sections of society with high TB burden especially in the developing countries, the laboratory equipments and their infrastructure for the diagnosis of pulmonary tuberculosis is not always available . However, the only diagnostic technique for the detection of Tuberculosis suitable to peripheral levels of health services is sputum smear microscopy. In developing countries like India with a high tuberculosis burden and limited number of adequate resources and infrastructure, the diagnosis of Tuberculosis relies mostly on serial sputum smear microscopy for Acid Fast Bacilli (AFB), however its sensitivity is considered to be low in paucibacillary cases¹.

Sputum smear microscopy is said to have a low and variable sensitivity. In the scenarios with high burden of tuberculosis, the work load is high and therefore the amount of time spent examining smears is low, therefore the corresponding sensitivity is low.

So the adequately resourced laboratory infrastructure development is urgently needed, as is the development of more sensitive and rapid TB diagnostics more suitable for peripheral settings. Recognizing this fact worldwide , many research groups have been constantly working upon to improve the performance of smear microscopy through new advances in technology and rapid techniques.

In many developing countries, diagnosis of tuberculosis is based on ZiehlNeelsen staining . However, it has been recently observed that the fluorescent stain Auramine provides far better

yield and detection of positive smears² In the developing countries like India , with a large number of cases of tuberculosis and variable financial constraints at different levels, evaluation of rapid and inexpensive diagnostic methods has now become of great value and importance . The acid fast bacilli in the sputum samples can be easily detected via microscopy using Ziehl Neelsen staining and fluorescent staining technique³

Auramine staining of sputum smears staining is a better method of sputum microscopy for demonstration of AFB in sputum specimens as compared to that of Ziehl Neelsen staining . In the recent years fluorescence microscopy is found to be relatively far more sensitive with an additional advantage of facilitating the examination of a large number of sputum specimens in a shorter course of time than previously preferred staining techniques⁴.

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 care health centre.

Materials and Methods :-

The study was conducted on patients reporting at the Department of Pulmonary Medicine at OPD / IPD clinic in the Bareilly district. Staining was done in the DOTS/ Microscopy centre of the Department of Pulmonary Medicine, Rohilkhand Medical College, Bareilly .The Institutional Ethical Committee permission was taken. A Comparative study was conducted on 3020 patients submitting 6040 samples i.e. one spot and one morning sample.

After collecting spot sample, individuals were provided with pre-labeled sample containers for the collection of morning sample at home. New unscratched slides labeled with study numbers were used for smear preparation. The smears were stained by Ziehl-Neelsen and Auramine O staining. Ziehl Neelsen and Auramine O staining technique and smear gradings were done as per RNTCP Technical Manual Guidelines as mentioned in the table below*.

LED fluorescent microscopy (400x:1 length =40 fields=200 HPF) RNTCP staining grading	IUALTO/WHO scale (1000x field = HPF) grading Grading	Minimum number of fields to be examined Minimum number of fields to be examined
Zero AFB /length No AFB per 100 oil immersion field	Negative negative	40 100
1-19 AFB /length 1-9 AFB per 100 oil immersion field	Scanty Scanty	40 100
20 -199 AFB /length 10-99 AFB per 100 oil immersion field	1+ 1+	40 100
5- 50 AFB /I field on average 1-10 AFB per oil immersion field	2+ 2+	20 50
>50 AFB /I field on average>10 AFB per oil immersion field	3+ 3+	08 20

*as mentioned in the manual for sputum smear Fluorescent microscopy(RNTCP) .

* The module for Laboratory Technicians(RNTCP) pg no 22 .

RESULTS :-Out of 6040sputum samples, 1102 (18%) were found to be bacteriologically positive for AFB.Of the 1102 positive patients 848(77%) were male and 254(23%)were female.

The mean age in 3020 patients was 38 years.Out of the positive 1102 patients, 880 (14.5%) patients were detected positive by the Ziehl Neelsen staining method, where as 1102 (18.20%) patients were detected positive by the Auramine O staining method. The significance of the data was calculated using Chi's Square value,χ² = 1.198 . The Auramine O was found to be superior to ZN staining technique on several aspects.

Out of the 1102 positive patients, 222(3.6%) patients were missed by ZN staining technique, who were detected by Auramine O staining method. The following was the grading ZN/AFB – scanty (135/77), 1+(165/103), 2+ (387/316), 3+(415/384).

Of the 222 patients missed by ZN staining method , 58 of them were scanty positive , 62 were 1+ and 71were 2 + and 31 cases were 3+ .

The mean reading time of Auramine O technique was three times faster than the ZN technique with very good acceptance by the technicians.

Table 1 : Comparison between the ZeihlNeelsen and Fluorescent Staining technique yield.

	Fluoroscet Staining (n=1102)	Zeihl Neelsen Staining(n=880)	Number of cases missed by Zn Staining method
Sputum Grading			
3 +	415	384	31
2 +	387	316	71
1 +	165	103	62
Doubtful/Scanty	135	77	58
	1102(18.2%)	880(14.5%)	222(3.6%)

Figure 1 : Statistical Comparison between the ZeihlNeelsen and Fluorescent Staining technique yield.

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) resulting in time saving of approximately 2 minutes per slide⁵(66%).

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

Fluorescent microscopy is the best method to detect cases of bacteriological positive cases of tuberculosis who are 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 for the last 3 years ,the microscopy centre has been using the fluoroscent microscope, which has been an important development in the field of diagnosis of pulmonary tuberculosis.

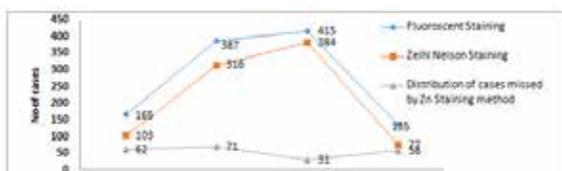
Laifangbam S et al 3 found that out of 102 patients, 44.1% and 71.6% were found positive by ZN and AO respectively. AO was found to be superior to ZN in several aspects. The difference in their case detection rates was statistically significant (Chi's Square value ,χ²= 24.93, p <0.001). AO was also able to detect more pauci-bacillary 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 FM over ZN were comparable to those found in several studies^{2,3,4,6,7}. The efficacy of fluorescence microscopy proved to be much higher than conventional light microscopy ³.

Singh NP ,Parija SC⁴concluded 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 et al 8 concluded that of 393 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% forAO. Direct fluorescent microscopy detected 9.3% paucibacillary sputum samples that were missed on ZN staining. Onconcentration, 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 was99.19% for both ZN and AO.

Prasanthi, K., Kumari⁶ observed that the higher sensitivity of FM stain (45%) than that of ZN stain (29%) in detecting the pauci-bacillary cases associated with HIV co-infection .The higher sensitivity of the sputum smear by FM was also observed by Ziaaem et al (2008)⁹,Cattamanachi A et al (2009)¹⁰, Steingart KR et al (2004)¹¹, with varied specificity . The FM has an advantage of speed , ease of screening , reduces the observers fatigue and better yield in the paucibacillary cases .

Marais BJ et al (2008)¹² observed the similar finding in his



study , where he observed 1.4 minutes for processing a slide by FM as compared to 3.6 minutes with conventional ZN microscopy , reflecting a time saving of 61% with FM .

Thus , the Fluorescent Microscopy is found to be more effective in the diagnosis of cases of pulmonary tuberculosis .

Conclusion :-

The Auramine O is superior to ZiehlNeelsen on several aspects –

It is Less time consuming so more slides can be processed in a shorter duration of time .More accurate diagnostic value as the paucibacillary bacilli can be detected easily and accurately by the Auramine O staining technique . It can be foreseen that Fluorescent microscopy can replace the ZeihlNeelsen microscopy in next 5 to 10 years .

REFERENCE

- 1)Pal N, Malhotra B, Goyal S, Kumar V, Vyas L. Comparison of ZiehlNeelsen&Auramine O staining methods on direct and concentrated smears in clinical specimens. *Indian Journal of Tuberculosis*. 2011 Apr;58(2):72-6 | 2) Hanscheid T . The future looks bright: low-cost fluorescent microscopes for detection of *Mycobacterium tuberculosis*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2008 Jun;102(6):520-1. | 3)Laifangbam S, Singh HL, Singh NB, Devi KM, Singh NT.A comparative study of fluorescent microscopy with Ziehl-Neelsen staining and culture for the diagnosis of pulmonary tuberculosis. *Kathmandu Univ Med J (KUMJ)*. 2009 Jul-Sep;7(27):226 -30 | 4)Singh NP, Parija SC. The value of fluorescence microscopy of auramine stained sputum smears for the diagnosis of pulmonary tuberculosis. *Southeast Asian J Trop Med Public Health*. 1998 Dec;29(4):860-3 | 5) Marais BJ, Brittle W, Painczyk K, Hesselting AC, Beyers N, Wasserman E et al .Use of light-emitting diode fluorescence microscopy to detect acid-fast bacilli in sputum.*Clin Infect Dis*. 2008 Jul 15;47(2):203-7. | | 6) Prasanthi, K , Kumari, A . Efficacy of fluorochrome stain in the diagnosis of pulmonary tuberculosisco-infected with HIV. *Indian Journal of Medical Microbiology*. 2005 , 23(3): 179-185 | 7) Annam V, Kulkarni MH, Puranik RB.Comparison of the modified fluorescent method and conventional Ziehl-Neelsen method in the detection of acidfast bacilli in lymphnode aspirates.*Cytojournal*. 2009 Jul 18;6:13. | 8) Hooja S , Pal N, Malhotra B, Goyal S, Kumar V, Vyas L.Comparison of Ziehl Neelsen & Auramine O staining methods on direct and concentrated smears in clinical specimens.*Indian J Tuberc*. 2011 Apr;58(2):72-6. | | 9) Ziaaem , Namaei M , Khazae M , Azarkar G. A comparison of the value of two different sputum stainings i.e. Zeihl Neelsen and fluorescent for the diagnosis of Acid Fast bacilli . *Archives of Clinical Infectious Diseases*. 2008; vol 3: 2. | 10)Cattamanachi a , Lucian , Davis , J huang l. Sensitivity and specificity of FM for the diagnosis of pulmonary tuberculosis in a high HIV prevalent setting. *Int J Tuberc Lung Dis*. Sept 2009; 13(9): 1130-1136. | 11) Steingart KR, Henry M, Ng V, Hopewell PC, Ramsay A, Cunningham J, Urbanczik R et al . Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review.*Lancet Infect Dis*. 2006;6(9):570-81. | 12) Kivihya Ndugga L E A , M R A van Cleeff , W A Githui , L W Nganga , D K Kibuga , J A Odhiambo . A comprehensive comparison of Ziehl-Neelsen and fluorescence microscopy for the diagnosis of tuberculosis in a resource-poor urban setting. *The International Journal of Tuberculosis and Lung Disease*7(12):1163-71. |