

A COMPARATIVE STUDY ON THE ACCURACY OF LIGHT EMITTING DIODE FLUORESCENT MICROSCOPY AND CONVENTIONAL SPUTUM SMEAR MICROSCOPY IN THE DIAGNOSIS OF PATIENTS WITH PULMONARY TUBERCULOSIS

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ABSTRACT

Aims/Objectives: To compare the efficacy of light emitting diode fluorescent microscopy (LED) over conventional sputum smear microscopy in diagnosing Mycobacterium Tuberculosis.

Materials and Methods: This comparative study was conducted in the department of Pulmonology In Chalmeda Anand Rao Institute Of Medical Sciences, Karimnagar from November-2015 to June- 2017. A total of 100 sputum samples were collected from patients suspected of pulmonary TB.

Results: Out of 100 cases which were included in the study, 17 cases showed sputum positivity on AO staining where as only 11 cases showed sputum positivity on ZN staining. Number of paucibacillary cases detected on AO staining were 7, compared to only 2 on ZN staining.

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.

KEY WORDS: Mycobacterium Tuberculosis, Sputum examination, Ziehl Neelsen Staining(ZN staining), Auramine-O Staining (AO staining).

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BACKGROUND

Tuberculosis remains world's leading cause of death from a single infectious agent. Pulmonary tuberculosis is the most common presentation of tuberculosis (TB). For developing countries with a large number of cases and financial constraints, evaluation of rapid and inexpensive diagnostic methods has a great importance.

It continues to be the world's most important

infectious cause of morbidity and mortality among adults. Nearly 9 million people develop TB disease each year, and estimated 1.6 million die from the disease [1].

Microscopy remains the most appropriate method till date, but it suffers from low sensitivity for paucibacillary samples, found more frequently among immune compromised patients co- infected with HIV [2]. This seriously

jeopardizes the quality of Ziehl Neelsen (ZN) microscopy, further reducing its yield [3].

Direct microscopy for Acid Fast Bacilli (AFB) is a widely used method for the diagnosis and confirmation of pulmonary Tuberculosis. First of all, ZN stain has low sensitivity relative to fluorescent stain [4] and culture [5], secondly it takes more time to scan at least 300 field. Thirdly, it needs experienced microbiologists. Fourthly, it often misses the paucibacillary TB cases especially when the patient is co-infected with HIV. Technical error is also more common in cases of ZN staining [6].

The advantages of fluorescence staining procedure are that it is simpler and can be examined at a lower magnification than ZN (40x Vs 100x). It has been estimated that Fluorescent microscope (FM) may take up to 75% less time than ZN [4]. This advantage would be a tremendous benefit for overburdened laboratory system in many low resource settings. We, therefore, performed a study comparing the reliability of LED and ZN stained sputum smears in the diagnosis and sputum conversion of pulmonary tuberculosis.

MATERIALS AND METHODS

This comparative study was conducted in the department of PULMONOLOGY in CHALMEDA ANAND RAO INSTITUTE OF MEDICAL SCIENCES, KARIMNAGAR from November-2015 to June-2017. A total of 100 sputum samples were collected from patients suspected of pulmonary TB. , according to the following inclusion and exclusion criteria. Detailed history of those patients was recorded. Thorough clinical examination was done.

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:

- Cough with sputum (more than 2wks)
- Low grade Fever with evening rise
- 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.

Permission to conduct the study was obtained from Institutional Ethics committee(IEC), CHALMEDA ANAND RAO INSTITUTE OF MEDICAL SCIENCES, KARIMNAGAR. Informed oral consent was obtained from all patients.

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

OBSERVATIONS AND RESULTS

Out of 100 sputum samples collected, 11 and 17sputum samples were found to be positive for AFB by ZN and AO staining respectively. The ZN smear positivity rate and the AO smear positivity rate in this study was 11% and 17% respectively.

Table 1: Showing number of positive cases and their grading by ZN staining.

Grading	Count of ZN STAINING RESULTS
1+	1
2+	4
3+	5
SCANTY	1
NEG	89
Grand Total	100

Out of 100 cases, ZN staining was negative in 89 cases and positive only in 11 cases.

Chart 1: Bar diagram showing number of positive cases and their grading by ZN staining.

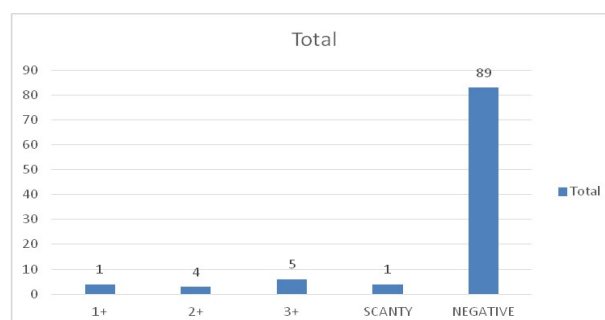


Table 2: Showing number of positive cases and their grading by AO staining.

Grading	Count of A-O STAINING RESULTS
1+	4
2+	3
3+	6
SCANTY	4
NEGATIVE	83
Grand Total	100

Out of 100 cases, AO staining was negative for 83 cases and positive for 17 cases.

Chart 2: Bar diagram showing number of positive cases and their grading by AO staining.

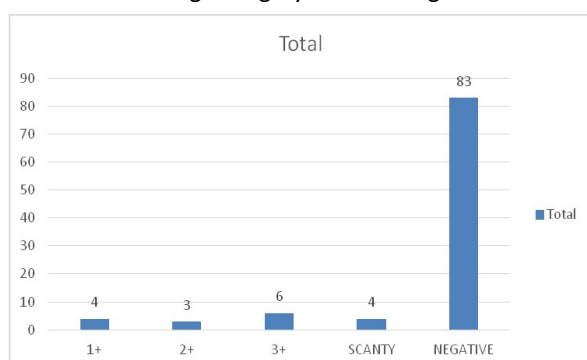


Table 3: Comparison of ZN and Auramine staining reports.

	No. of Positive smears	No. of Negative smears
ZN stain	11 (11%)	89 (89%)
Auramine stain	17 (17%)	83 (83%)

Out of 100 cases , Auramine staining was negative for 83 cases as compared to 89 cases by ZN staining and positive for 17 cases as compared to 11 cases by ZN staining. . Similar results have also been reported by studies done by Suria Kumaret al, 2012 [7]. Whereas higher smear positivity rates were shown by K Prasanthi et al, 2005(50% by ZN, 69% by AO) [8] and Ulukanligil et al (67.6% by ZN, 85.2% by AO) [9].

Chart 3: Bar diagram shows the positive smear results of ZN staining and by Auramine O staining by LED fluorescent microscopy.

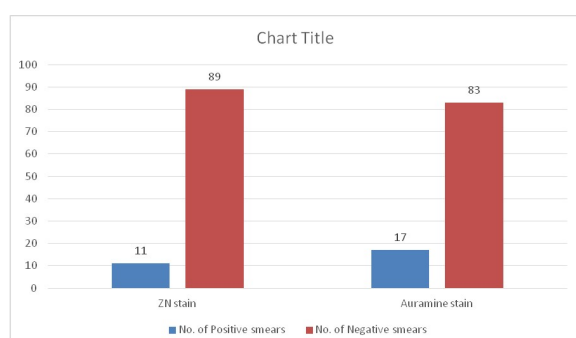


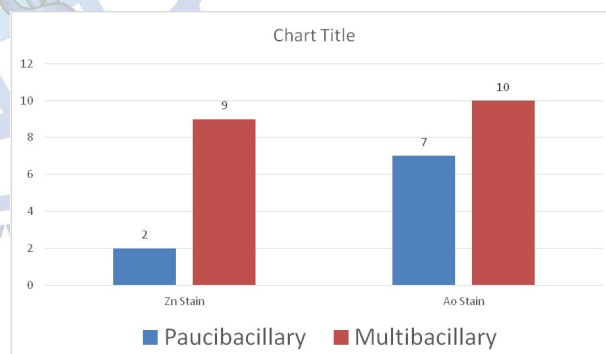
Table 4: Distribution of paucibacillary and multibacillary cases detected by ZN staining and LED fluorescent microscopy.

Staining method	No. of paucibacillary cases	No. of multibacillary cases	Total no. of positive cases detected
ZN staining	2(18.1)	9(81.9)	11
Auramine staining	7(41.1)	10(58.9)	17

Number of Paucibacillary cases detected by Auramine staining are more in number compared to ZN staining.

In study conducted by Hooja S1, Pal N, Malhotra B, Goyal S, Kumar V, Vyas L. 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% [10]. Whereas culture as a standard reference was not made in our study.

Chart 4: Distribution of paucibacillary and multibacillary cases detected by ZN staining and LED fluorescent microscopy.



DISCUSSION

Current recommendations for the control of Tuberculosis emphasize early case detection so as to allow treatment of patients and thereby limit the transmission of bacilli. 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 10^4 /ml of sputum. In the present study out of the 100 samples examined, 11(11%) and 17(17%) TB cases were detected by ZN and AO staining methods respectively.

It was observed that a total no. of 6 sputum smears which were negative by ZN method were

positive by AO staining method and that AO staining with LED Microscopy was more efficient over ZN stain in determining paucibacillary cases have also been proved in this study. AO staining could detect 7 paucibacillary cases, whereas ZN staining detected only 2 of them. This was in concordance with studies done by Laifangbam et al 2009 [11]. Failure to detect and hence to treat paucibacillary cases can be effectively prevented by the use of fluorescent LED microscopy. In the same study conducted by Laifangbam S1, Singh HL, Singh NB, Devi KM, Singh NT et al showed Out of 102 patients, 44.1%, 71.6% and 70% were found positive by ZN, AO and culture 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 Pauci-bacillary cases than ZN. There was more agreement between culture and fluorescence microscopy (95.1%) than with ZN microscopy (69.6%). The percentage of false negative by AO staining was only 2.78% which was in sharp contrast to that of ZN (40.27%)

In other study conducted by BaF, Rieder HL et al showed Concordance was 96.9% and 92.3% with AO and ZN. 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 which was in similar fashion with our study [12].

In a study conducted by Dick van et al., 58 out of the 221 sputum specimens evaluated, 36 (16.3%) were positive for Mycobacterium tuberculosis by culture. Sensitivity and specificity documented for the different modalities were 84.7% and 98.9%, respectively, for the LED assessment; 73.6% and 99.8%, respectively, for the MVP assessment; and 61.1% and 98.9%, respectively, for light microscopy. The mean time to read a negative smear was 1.4 min with fluorescence microscopy and 3.6 min with light

microscopy, reflecting a time savings of 61% with fluorescence microscopy [13].

Table 5: Results of the study by Two Methods.

Diagnostic modality	No. of sputum smear specimens determined to be positive for Mycobacterium tuberculosis		No. of discrepant results
	Microscopist 1	Microscopist 2	
Light microscopy	23	25	10
Fluorescence microscopy Mercury vapor lamp	25	29	30
Light-emitting diode	33	32	7

As evident from the above table, the ability to detect mycobacterium tuberculosis is more on fluorescent microscope compared to light microscope. Among fluorescent microscope Light emitting diode is superior to Mercury Vapor Lamp.

In a study conducted by Jai Kishan Karahyla, staining and LED-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 [14].

In a comparative study on different staining methods for the detection of acid fast bacilli (Mycobacterium tuberculosis) in sputum samples conducted by Dr. Narayan Shrihari et al [15]. The results obtained from ZN stained smears and fluorescent (AO) stained smears were compared together, the result of our study revealed that AO (11.43%) was nearly as sensitive as ZN (12.32%)

In a study titled COMPARATIVE STUDY OF AURAMINE STAINING USING LED FLUORESCENT MICROSCOPY WITH ZIEHL-NEESEN STAINING IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS done by Saroj Golia et al 634 sputum samples collected from suspected cases of pulmonary Tuberculosis were processed and subjected to ZN and Auramine-O (AO) staining for detection of TB. Positive smears were graded according to standard WHO criteria. Out of 634 sputum smears, 10.57% and 16.56% were positive by ZN and AO respectively. AO was found to be superior to ZN in detecting TB cases and also AO staining was able to detect more paucibacillary cases than ZN [16].

CONCLUSION

It can be concluded from our study 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.

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